

*A Prifysgol Caerdydd  
Yr Ysgol Meddygaeth  
Sefydliad Ymchwil Systemau Imiwnedd*

*Cardiff University  
School of Medicine  
Systems Immunity Research Institute*



# Plasticity at the T cell receptor-peptide-major histocompatibility complex class I interface

A thesis submitted to Cardiff University for the candidature of  
Doctor of Philosophy

January 2021

Jade Rhianna Hopkins





## Acknowledgements

The research described in this thesis would not have been possible without the contribution and support of my supervisory team, colleagues, family, and friends. Any attempt at writing would be insufficient to express the extent of my gratitude but I attempt:

Foremost, I would like to thank my supervisor **Professor Andrew Sewell** for allowing me this opportunity, for the continuous support and guidance over the years, and for always finding time for me – especially in these last zoom-filled few months! Thank you to my co-supervisors **Dr David Cole** and **Dr Chris Pudney** for establishing the GW4 collaboration, and your respective protein biochemistry and REES expertise. I would also like to express immeasurable gratitude to **Dr Garry Dolton** for your knowledge, patience, and guidance. Your dedication is inspiring and I cannot thank you enough.

I would like to acknowledge my colleagues and collaborators. Thank you to all of the **T cell modulation group** past and present, but a special mention to **Anna, Thalia, and Mat** for teaching me ‘how to lab’, and to **Tom** for computational help with the directed TCR mutants. Thank you to **Dr Barbara Szomolay** for developing the PS-CPL tool that was vital to this thesis. I would also like to thank all of the **Pudney group** for being so welcoming during my time at Bath, particularly **Dr Dragana Catic** for teaching me how to use the crazy sailor machine. I am grateful to the CBS team, **Dr Catherine Naseriyan** and **Dr Ann Kift-Morgan** for your training and continuing support. Special thanks to **Professor Colin Dayan** and **Professor Susan Wong** for your help with accessing diabetic blood samples.

Thank you to the **Medical Research Council** and the **Wellcome Trust** for funding my PhD, and to all at the GW4 BioMed DTP team for your support. I would like to acknowledge the support of **Professor Ann Ager**, **Professor Phil Taylor**, and **Dr Jason Twohig** for taking the time to read my annual progress reports and always providing helpful advice.

Finally, thank you to **Mum, Dad, Grandma, Ryan, Jess, Hannah, and Annie** for reminding me that the world outside the lab exists. My last, biggest, and most inexpressible thank you to **Aaron** for literally everything, but in particular the endless cups of tea and reassurance, and to my cats **Liam** and **Kevin** for being completely indifferent to the whole thing and offering no help whatsoever.

Diolch o galon i chi gyd.

This thesis is dedicated to **Gloria Jean Richards**.

*“I cannot fix on the hour, or the spot, or the look, or the words, which laid the foundation. It is too long ago. I was in the middle before I knew that I had begun.”*

– Jane Austen, *Pride and Prejudice*.

## Publications

**Hopkins, J. R.**, Crean, R. M., Catici, D. A. M., Sewell, A. K., Arcus, V. L., Van der Kamp, M. W., Cole, D. K., & Pudney, C. R. (2020). Peptide cargo tunes a network of correlated motions in human leucocyte antigens. *The FEBS Journal*, 287(17), 3777–3793.

Madura, F., Rizkallah, P. J., Legut, M., Holland, C. J., Fuller, A., Bulek, A., Schauenburg, A. J., Trimby, A., **Hopkins, J. R.**, Wells, S. A., Godkin, A., Miles, J. J., Sami, M., Li, Y., Liddy, N., Jakobsen, B. K., Loveridge, E. J., Cole, D. K., & Sewell, A. K. (2019). TCR-induced alteration of primary MHC peptide anchor residue. *European Journal of Immunology*, 49(7), 1052–1066.

Whalley, T., Dolton, G., Brown, P. E., Wall, A., Wooldridge, L., van den Berg, H., Fuller, A., **Hopkins, J. R.**, Crowther, M. D., Attaf, M., Knight, R. R., Cole, D. K., Peakman, M., Sewell, A. K., & Szomolay, B. (2020). GPU-Accelerated Discovery of Pathogen-Derived Molecular Mimics of a T Cell Insulin Epitope. *Frontiers in Immunology*, 11. Epub. <https://doi.org/10.3389/fimmu.2020.00296>

Ferrari, M., Baldan, V., Wawrzyniecka, P., Bulek, A., Kinna, A., Ma, B., Bughda, R., Akbar, Z., Srivastava, S., Ghongane, P., Nicholson, A., Sillibourne, J., Jha, R., Lim, W., **Hopkins, J.**, Welin, M., Surade, S., Dyson, M., McCafferty, J., Cordoba, S., Thomas, S., Logan, D., Sewell, A., Maciocia, P., Onuoha, S., and Pule, M. (2021). Structure-Guided Engineering of Immunotherapies Targeting TRBC1 and TRBC2 in T Cell Malignancies (in review at *Science*)

## Summary

**Background** – The interaction between the T cell receptor (TCR) on the surface of  $\alpha\beta$  T cells and the peptide-major histocompatibility complex (pMHC) on the surface of target cells helps  $\alpha\beta$  T cells to defend the host from virtually any foreign pathogen. To achieve such extensive coverage necessitates substantial T cell crossreactivity. While essential for providing complete immune coverage, T cell crossreactivity can also have negative consequences as it is believed to contribute to a large number of autoimmune diseases and can have fatal consequences when T cells are used therapeutically. The therapeutic deployment of the TCR-pMHC interaction therefore requires thorough understanding of the biochemical characteristics that underpin the interaction.

**Results** – I began my studies by defining the crossreactive profile of an autoreactive T cell clone. Using combinatorial peptide library (CPL) screening, I showed that the HLA-A\*0201-restricted, insulin-reactive InsB4 TCR, derived from a patient with type 1 diabetes (T1D) could be strongly activated by peptides arising from three common human pathogens. I further showed that EBV-insulin crossreactivity could be found in some T1D patients but not in healthy controls. The potential for virus-specific T cells to recognise self-antigens in autoimmune patients highlights the importance of understanding the underlying driving features of TCR plasticity. To this end, I set about investigating how two biochemical characteristics can influence the TCR-pMHC interaction, TCR sequence and pMHC flexibility. To explore how TCR *sequence* impacts TCR *function*, I profiled the crossreactive nature of an HLA-A\*0201-restricted, HIV-1-specific TCR and compared its peptide recognition repertoire to mutant versions of the same TCR with CDR3 amino acid substitutions. These studies inadvertently resulted in the discovery that this TCR could also respond to a self-antigen expressed on many HLA-A\*0201<sup>+</sup> cancer cell lines. Recognition of cancer cells, but not the cognate HIV-derived peptide, could be removed by a single CDR3 amino acid substitution. Finally, I studied how the dynamic flexibility of pMHC could be influenced by the peptide cargo by using red-edge excitation shift to examine a collection of well-studied analogues of a preproinsulin-derived peptide in the context of HLA A\*0201.

**Conclusions** – My research highlights both the negative (viral/autoimmunity) and the positive (viral/cancer) aspects of T cell crossreactivity and demonstrates that plasticity in both the TCR and cognate pMHC ligands are likely to play a role in the range of TCR-pMHC interactions that are capable of triggering a T cell response.

## Abbreviations

$\beta_2$ M	Beta 2 microglobin
ABC	Antigen-binding cassette
ACT	Adoptive cell transfer
ADR	Alloimmune defence receptor
AIDS	Acquired immunodeficiency syndrome
AMIGO	Amphoterin-induced protein 2 precursor
APC	Antigen presenting cell
APL	Altered peptide ligand
ATP	Adenosine triphosphate
bNAb	Broadly neutralising antibody
C	Celsius
CAR	Chimeric antigen receptor
Cas9	CRISPR-associated protein-9
CCR5	C-C chemokine receptor type 5
CD	Cluster of differentiation
cDNA	Complementary deoxyribonucleic acid
CDR	Complementarity-determining region
CFSE	Carboxyfluorescein succinimidyl ester
CMV	Cytomegalovirus
COVID	Coronavirus disease
CPL	Combinatorial peptide library screen
CRISPR	Clustered regularly interspaced short palindromic repeats
Cr	Chromium
CSM	Centre of spectral mass
CST	Clonal selection theory
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCR4	C-X-C chemokine receptor type 4
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl-sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EBV	Epstein-Barr virus
EC	Half maximal effective concentration
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assays
ELISPOT	Enzyme-linked immunospot
ER	Endoplasmic reticulum
ERAAP	ER aminopeptidase associated with antigen processing
FACS	Fluorescence-activated cell sorting
FBS	Foetal bovine serum
FITC	Fluorescein isothiocyanate
FPLC	Fast Protein Liquid Chromatography
GAD	Glutamic acid decarboxylase
GBM	Glomerular basement membranes
GFP	Green fluorescent protein

GPU	Graphics processing unit
H	Hour
HeBS	HEPES-buffered saline
HCMV	Human cytomegalovirus
HDX	Hydrogen/deuterium exchange
HER-2	Human epidermal growth factor receptor 2
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSV	Herpes simplex virus
HTLV	Human T-lymphotropic virus
ICAM	Intercellular adhesion molecule
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IMP-2	Insulin-like growth factor 2 mRNA binding protein-2
IPTG	Isopropyl $\beta$ -D-1-thiogalactopyranoside
IRES	Internal ribosomal entry sites
ITAM	Immunoreceptor tyrosine-based activation motif
IU	International units
IV	Influenza virus
$K_D$	Dissociation constant
LB	Lysogeny broth
LCL	Lymphoblastic cell lines
M1	Matrix protein 1
MACS	Magnetic activated cell-sorting
MAGE	Melanoma antigen
MAIT	Mucosal-associated invariant T cell
MART-1	Melan-A/Melanoma antigen recognised by T cells
MBP	Myelin basic protein
MD	Molecular dynamics
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
MIP	Macrophage inflammatory protein
Min	Minutes
mL	Millilitre
MM	Malignant melanoma
MR1	Major histocompatibility class I-related
mRNA	Messenger ribonucleic acid
NEIB	National eye institute bank
NFAT	Nuclear factor of activated T cells
NMR	Nuclear magnetic resonance
NOD	Non-obese diabetic
NR	No response
Ns	Non-significant
NY-ESO	New York esophageal squamous cell carcinoma-1
OD	Optical density
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline



PCR	Polymerase chain reaction
PD-1/2	Programmed cell death protein-1/2
PDB	Protein data bank
PD-L	Programmed death ligand
PE	Phycoerythrin
PerCP	Peridinin chlorophyll protein
PFA	Paraformaldehyde
PHA	Phytohaemagglutinin
PLC	Peptide loading complex
pMHC	Peptide-major histocompatibility complex
PPI	Preproinsulin
RAG	Recombination activating gene
RBC	Red blood cell
rCD2	Rat CD2
REES	Red edge excitation shift
RNA	Ribonucleic acid
RPM	Revolutions per minute
RPMI	Roswell Park Memorial Institute
RSS	Recombination signal sequence
SABR	Signalling and antigen-presenting bifunctional receptor
SARS-CoV	Severe acute respiratory syndrome-associated coronavirus
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SEM	Standard error of the mean
SPR	Surface plasmon resonance
TAA	Tumour-associated antigen
TACE	TNF converting enzyme
TAE	TRIS-acetate-EDTA
TAP	Transporter associated with antigen processing
TAPI-0	TNF processing inhibitor-0
TCR	T cell receptor
TdT	Terminal deoxynucleotidyl transferase
TE	TRIS-EDTA
TGF	Transforming growth factor
TIL	Tumour-infiltrating lymphocytes
TNF	Tumour necrosis factor
Tregs	T regulatory cells
UV	Ultraviolet
V	Volts
VDJ	Variable, diversity, joining region
VZV	Varicella-zoster virus
WBS	Welsh blood service
WPRE	Woodchuck hepatitis post-transcriptional regulatory element
WT	Wildtype

All units of measurement in this thesis follow the International System of Units, unless otherwise specified. Amino acids are referred to by their standard single- or three-letter code.

# Table of contents

<b>1 – Introduction</b> .....	1
1.1 – The immune system .....	1
1.1.1 – Overview .....	1
1.2 – $\alpha\beta$ T cells .....	2
1.2.1 – Overview of T cell types .....	2
1.2.2 – $\alpha\beta$ CD8 <sup>+</sup> T cell function.....	3
1.2.3 – TCR gene rearrangement .....	7
1.2.4 – Maturation and thymic selection.....	9
1.3 – The major histocompatibility complex (MHC) .....	10
1.3.1 – Overview .....	10
1.3.2 – The <i>HLA</i> loci.....	11
1.3.3 – pMHC structure.....	12
1.3.4 – Peptide presentation by MHC.....	15
1.4 – The TCR-pMHC interaction .....	20
1.4.1 – The co-receptors .....	24
1.4.2 – MHC-restriction.....	26
1.4.3 – Specificity and crossreactivity within the TCR-pMHC interaction .....	30
1.4.4 – How is crossreactivity achieved? .....	31
1.5 – Application of the TCR-pMHC interaction.....	32
1.5.1 – The TCR-pMHC interaction in immunity .....	32
1.5.2 – CD8 <sup>+</sup> T cells in autoimmunity .....	33
1.6 – Aims and objectives .....	41
<b>2 – Materials and methods</b> .....	43
2.1 – Protein production.....	43
2.1.1 – Buffers and media used in protein production.....	43
2.1.2 – Vectors and protein sequences.....	45
2.1.3 – Making chemically competent cells .....	46
2.1.4 – Transformation of competent cells.....	47
2.1.5 – Amplifying DNA via mini preparation .....	47
2.1.6 – Expression of constructs and purification of inclusion bodies.....	48
2.1.7 – Refolding soluble protein from inclusion bodies .....	51
2.1.8 – Purification of soluble protein by fast protein liquid chromatography (FPLC) ..	51
2.2 – Biochemical assays.....	53

2.2.1 – Dynamic light scattering to exclude aggregated samples.....	53
2.2.2 – Red edge excitation measurements of HLA-A2 complexes .....	53
2.2.3 – Buffer exchange using a PD-10 desalting column.....	53
2.2.4 – Pressure/temperature dependent fluorimetry.....	54
2.2.5 – Surface plasmon resonance (SPR).....	54
2.3 – Molecular cloning.....	54
2.3.1 – Buffers and media used in molecular cloning and lentiviral production .....	56
2.3.2 – 868 construct and design of mutants .....	56
2.3.3 – Cloning constructs into the pELNS lentiviral vector.....	57
2.3.4 – Sequencing constructs for validation.....	61
2.3.5 – Amplifying DNA via maxi preparation.....	62
2.4 – Lentiviral production and cell transduction .....	63
2.4.1 – Production of lentiviruses in HEK 293T cells.....	63
2.4.2 – Transduction of immortal cell lines.....	64
2.4.3 – Concentration of lentiviral supernatant .....	64
2.4.4 – Transducing primary CD8 T cells .....	64
2.4.5 – Purification based on surface marker expression.....	65
2.5 – Cell culture .....	66
2.5.1 – Buffers and media used in cell culture work throughout this thesis .....	66
2.5.2 – Culturing of adherent cells lines .....	67
2.5.3 – Culturing of suspension cell lines.....	67
2.5.4 – Isolation of CD8 <sup>+</sup> primary T cells from whole blood .....	68
2.5.5 – T cell purification based on multimer-binding specificity.....	70
2.5.6 – Expansion of CD8 <sup>+</sup> primary T cells and subsequent culturing.....	70
2.5.7 – Cell counting.....	71
2.5.8 – Freezing cells for storage in liquid nitrogen.....	71
2.6 – Cell-based functional assays .....	73
2.6.1 – Antibodies used for flow cytometry throughout this thesis.....	73
2.6.2 – Flow cytometry: staining for surface markers .....	74
2.6.3 – Flow cytometry: pMHC multimer staining.....	75
2.6.4 – Enzyme-linked immunosorbent assays (ELISA) of T cell function.....	78
2.6.5 – Combinatorial peptide library (CPL) screens.....	80
2.6.6 – TNF processing inhibitor-0 (TAPI-0) assays .....	80
2.6.7 – Chromium-51 ( <sup>51</sup> Cr)-release cytotoxicity assay.....	81
<b>3 – Insulin-specific T cells isolated from patients with type 1 diabetes strongly recognise common viral epitopes .....</b>	<b>82</b>

3.1 – Introduction .....	82
3.1.1 – Type 1 diabetes .....	82
3.1.1.1 – <i>T1D is a CD8<sup>+</sup> T cell mediated disease</i> .....	83
3.1.2 – T cells are, by necessity, highly crossreactive .....	85
3.1.2.1 – <i>Benefits of T cell crossreactivity</i> .....	86
3.1.2.2 – <i>Drawbacks of T cell crossreactivity</i> .....	87
3.1.3 – Measuring T cell crossreactivity .....	89
3.1.3.1 – <i>CPL screens for measurement of T cell crossreactivity</i> .....	91
3.1.3.2 – <i>CPL screens can identify novel peptide epitopes</i> .....	92
3.1.4 – Aims .....	94
3.2 – Results .....	95
3.2.1 – Work leading up to the project .....	95
3.2.1.1 – <i>The HLA-A2-restricted HLVEALYLV epitope is presented at the surface of pancreatic <math>\beta</math> cells</i> .....	95
3.2.1.2 – <i>CPL scanning identifies the peptide repertoire of the InsB4 T cell clone</i> .....	97
3.2.1.3 – <i>Fungal-derived peptide agonists of the InsB4 T cell clone</i> .....	99
3.2.1.4 – <i>Bacterial-derived peptide agonists of the InsB4 T cell clone</i> .....	101
3.2.2 – Fungal and bacterial peptides act as functional ligands for the InsB4 T cell clone .....	103
3.2.3 – Peptide sequences derived from human viruses act as potent agonists of the InsB4 T cell .....	105
3.2.3.1 – <i>The InsB4 TCR binds to viral epitopes strongly</i> .....	108
3.2.3.2 – <i>Viral epitopes are correctly processed and presented</i> .....	110
3.2.4 – InsB4 recognises EBV-infected HLA-A2 <sup>+</sup> B cells (LCL lines) .....	112
3.2.5 – Insulin-specific T cells in an HLA-A2 <sup>+</sup> T1D patient recognise EBV .....	113
3.3 – Discussion .....	117
3.3.1 – The InsB4 T cell kills human pancreatic $\beta$ cells .....	117
3.3.2 – Molecular mimicry may trigger autoimmune disease .....	117
3.3.3 – InsB4 can be activated by pathogen-derived peptides .....	118
3.3.4 – Viral epitopes strongly activate InsB4 T cells .....	119
3.3.5 – T1D/insulin crossreactivity in T1D patients .....	121
3.3.6 – Summary .....	122
<b>4 – Exploring how mutation of key peptide contact residues in CDR3 impact TCR peptide specificity</b> .....	<b>123</b>
4.1 – Introduction .....	123
4.1.1 – The 868 TCR .....	123
4.1.2 – Probing TCR ‘rules of engagement’ .....	126

4.1.3 – Aims.....	127
4.2 – Results.....	129
4.2.1 – Designing the mutant 868 TCRs.....	129
4.2.1.1 – TCR 3, <i>β</i> T96F.....	134
4.2.1.2 – TCR 6, <i>β</i> V97T.....	134
4.2.1.3 – TCR 22, <i>β</i> D95S.....	134
4.2.1.4 – TCR 24, <i>β</i> D95I T96R V97S.....	134
4.2.2 – Validation of 868 mutants.....	137
4.2.3 – Minimal differences between the recognition footprints of 868, TCR 3, and TCR 6.....	139
4.2.4 – TCR 22 and TCR 24 show differing specificity than the parent 868 TCR.....	142
4.2.5 – Identifying the HLA-A2 self-epitope that the 868 TCR responds to.....	144
4.2.6 – 868 TCR recognises epitopes that are not seen by TCR 22.....	147
4.2.7 – Attempts to validate the lengsin epitope.....	149
4.2.8 – Attempts to isolate lengsin-reactive T cells.....	154
4.2.9 – Preliminary characterisation of two further mutants of the 868 TCR.....	157
4.3 – Discussion.....	163
4.3.1 – TCR transduced T cells.....	163
4.3.2 – TCRs 3 and 6 showed little change in peptide preference.....	164
4.3.3 – TCRs 22 and 24 have altered peptide preferences.....	166
4.3.4 – The 868 TCR may crossreact with a tumour-associated antigen.....	168
4.3.5 – T cells expressing TCRs 9 and 11 might exhibit new specificities.....	171
4.3.6 – Future optimisation to reduce polyclonal background noise.....	171
4.3.7 – Summary.....	172
<b>5 – Peptide cargo modulates pMHC flexibility.....</b>	<b>175</b>
5.1 – Introduction.....	175
5.1.1 – The flexibility of the pMHC is generally overlooked.....	175
5.1.2 – The pMHC as a dynamic molecule.....	175
5.1.3 – The REES phenomenon.....	177
5.1.4 – Aims.....	179
5.2 – Results.....	181
5.2.1 – Producing stable pMHC complexes.....	181
5.2.2 – HLA-A2-ALWGPDPAAA complexes show a significant REES signal.....	183
5.2.3 – $\Delta$ CMS varies with different peptides suggesting the molecular flexibility of each complex is different.....	185
5.2.4 – Peptide cargo modulates energy barriers between conformational states.....	189

5.2.5 – Peptide-MHC contacts might drive global flexibility of the complex .....	191
5.3 – Discussion.....	193
5.3.1 – Evidence for a flexible pMHC .....	193
5.3.2 – REES as a tool for exploring pMHC flexibility .....	194
5.3.3 – Summary .....	195
<b>6 – General Discussion .....</b>	<b>198</b>
6.1 – Understanding the TCR-pMHC interaction .....	198
6.2 – Investigations into the TCR-pMHC interaction .....	198
6.2.1 – Display systems for studying the TCR-pMHC interface.....	199
6.2.2 – Rational design based on knowledge of the TCR-pMHC interaction .....	201
6.2.3 – Further methods for peptide discovery .....	202
6.2.3.1 – <i>T-Scan</i> .....	202
6.2.3.2 – <i>Trogocytosis</i> .....	202
6.2.3.3 – <i>SABR</i> .....	203
6.2.4 – Peptide-MHC plasticity: The other side of the coin .....	203
6.3 –T cell therapy.....	205
6.3.1 – Adoptive cell transfer (ACT) .....	205
6.3.2 – TCR gene transfer.....	207
6.4 – Concluding remarks .....	208
<b>7 – Appendix and supplementary information .....</b>	<b>210</b>
7.1 – Human immunodeficiency virus .....	210
7.2 – Optimised expression of transduced TCRs at the T cell surface .....	217
7.3 – Optimising TCR gene transfer .....	219
7.4 – Supplementary figures .....	222
References .....	232

## List of Figures

Figure 1.1: The structure of the $\alpha\beta$ TCR.....	3
Figure 1.2: V(D)J recombination.....	7
Figure 1.3: The structure of MHC-I.....	13
Figure 1.4: Peptide loading of MHC.....	18
Figure 1.5: The TCR-pMHC interaction.....	22
Figure 1.6: The co-receptors.....	25
Figure 1.7: The advantages and disadvantages of crossreactivity.....	35
Figure 1.8: Molecular mimicry in autoimmunity.....	39
Figure 2.1: Schematic of the pGMT7 plasmid used for protein production.....	46
Figure 2.2: Protein production via <i>E. coli</i> expression systems.....	50
Figure 2.3: Purification of soluble protein.....	52
Figure 2.4: Schematic of surface plasmon resonance.....	55
Figure 2.5: TCR construct design for lentiviral work.....	57
Figure 2.6: pELNS vector for lentiviral work.....	58
Figure 2.7: Representative colony PCR.....	61
Figure 2.8: Typical gating strategy for analysis of T cells.....	74
Figure 2.9: Schematic explanation of tetramer staining.....	76
Figure 2.10: Standard curve for a MIP-1 $\beta$ ELISA.....	78
Figure 3.1: Pathogen-triggered autoimmunity.....	88
Figure 3.2: Typical 9-mer CPL format.....	91
Figure 3.3: An overview of the TCR ligand discovery process.....	92
Figure 3.4: The InsB4 T cell clone recognises an insulin-derived epitope.....	95
Figure 3.5: InsB4 recognises and kills pancreatic $\beta$ cells.....	96
Figure 3.6: Characterising the peptide preferences of InsB4.....	97
Figure 3.7: InsB4 recognises fungal peptides with greater sensitivity than the index peptide.....	99
Figure 3.8: InsB4 recognises bacterial peptides with greater sensitivity than the index peptide.....	101
Figure 3.9: Pure peptide titrations for the top pathogenic peptides.....	102

Figure 3.10: InsB4 TCR binding of pathogen-derived agonist peptides is confirmed by SPR and pMHC multimer staining.....	104
Figure 3.11: InsB4 recognises viral peptides with greater sensitivity than the index peptide.....	106
Figure 3.12: Viral-derived peptides act as strong agonists for the insulin-specific InsB4 T cell clone.....	107
Figure 3.13: InsB4 TCR binding of pathogenic agonists is confirmed by SPR and multimer staining.....	109
Figure 3.14: Putative viral epitopes are efficiently processed and presented to InsB4 T cells in the context of HLA-A2.....	111
Figure 3.15: InsB4 responds to EBV-transformed lymphoblastic cell lines (LCLs) in an HLA-A2 dependent manner.....	112
Figure 3.16: Insulin-specific cells in an HLA-A2 <sup>+</sup> T1D donor can recognise three viral epitopes.....	114
Figure 3.17: EBV/Insulin B chain crossreactivity is not present in healthy donors.....	116
Figure 4.1: The interaction between 868 and HLA-A2- <u>SLYNTVATL</u> .....	130
Figure 4.2: The classification of amino acids based on chemical characteristics.....	132
Figure 4.3: The 868 mutants used in this chapter.....	136
Figure 4.4: Validating expression of the lentiviral constructs.....	138
Figure 4.5: TCRs 3 and 6 show little variation in their ability to recognise the cognate HLA-A2- <u>SLYNTVATL</u> epitope.....	140
Figure 4.6: TCRs 3 and 6 show little variation under CPL screening.....	141
Figure 4.7: 868, TCRs 22 and 24 show differences in peptide preference.....	143
Figure 4.8: CPL screening of TCR 22 and 24 in comparison to 868.....	145
Figure 4.9: 868 and TCR 22 are predicted to have varying peptide preference footprints .....	146
Figure 4.10: Recognition of two putative self-derived epitopes by 868 TCR-expressing cells appears to have been ablated by the $\beta$ D95S mutation in TCR 22.....	148
Figure 4.11: Attempts to validation the lengsin-derived epitope <u>SQLTPPQL</u> .....	151
Figure 4.12: 868-transduced CD8 <sup>+</sup> T cells respond to MOLT-3 cells expressing HLA-A2.....	152
Figure 4.13: 868-transduced CD8 <sup>+</sup> T cells respond to a panel of potential APCs in an HLA-A2-dependent manner.....	153
Figure 4.14: Lengsin-reactive T cells were not found in TIL samples.....	155
Figure 4.15: Potential lengsin-reactive T cells found in healthy PBMC samples.....	156
Figure 4.16: TCR 9 and TCR 11.....	158



Figure 4.17: TCRs 9 and 11 exhibit different peptide preferences than the 868 parental TCR.....	159
Figure 4.18: Recognition of putative epitopes unique to mutant TCRs.....	161
Figure 4.19: Novel specificities in mutant TCRs.....	162
Figure 5.1: Production of pure pMHC complexes.....	182
Figure 5.2: HLA-A2 complexes show a significant REES signal.....	184
Figure 5.3: $\Delta$ CSM varies for pMHC complexes carrying different peptide cargo.....	186
Figure 5.4: Fluorescence intensity spectra for each HLA-A2 complex.....	188
Figure 5.5: $\Delta$ G varies for pMHC complexes carrying different peptide cargo.....	190
Figure 5.6: Peptide-MHC contacts might drive global flexibility of the complex.....	192
Supplementary Figure 7.1: TCR 24-transduced CD8 <sup>+</sup> T cells do not respond to the M1 influenza-derived <u>GILGFVFTL</u> epitope.....	222
Supplementary Figure 7.2: 868-transduced CD8 <sup>+</sup> T cells respond to untransduced HLA-A2 <sup>+</sup> MOLT-3 cells, repeat assay.....	223
Supplementary Figure 7.3: 868-transduced CD8 <sup>+</sup> T cells respond to a range of HLA-A2 <sup>+</sup> immortalised cell lines.....	224
Supplementary Figure 7.4: Pressure/temperature-dependence of HLA-A2- <u>ALW</u> GPDPAAA complex.....	226
Supplementary Figure 7.5: Pressure/temperature-dependence of HLA-A2- <u>YQF</u> GPDPFTA complex.....	227
Supplementary Figure 7.6: Pressure/temperature-dependence of HLA-A2- <u>RQF</u> GPDWIVA complex.....	228
Supplementary Figure 7.7: Pressure/temperature-dependence of HLA-A2- <u>RQW</u> GPDPAAV complex.....	229
Supplementary Figure 7.8: Pressure/temperature-dependence of HLA-A2- <u>MVW</u> GPDPPLYV complex.....	230
Supplementary Figure 7.9: Pressure/temperature-dependence of HLA-A2- <u>RQF</u> GPDPFTI complex.....	231

## List of Tables

Table 2.1: A summary of the buffers and media used during protein production throughout this thesis.....	44
Table 2.2: The buffers and media used for molecular cloning and lentiviral production.....	56
Table 2.3: A summary of the primers used throughout this thesis.....	62
Table 2.4: Buffers and media used for cell culture throughout this thesis.....	67
Table 2.5: A summary of cell lines used in this thesis.....	68
Table 2.6: The antibodies used for flow cytometry throughout this thesis.....	72
Table 3.1: The pathogenic peptides used throughout Chapter 3.....	119
Table 4.1: Contact table for the 868-A2- <u>SLY</u> NTVATL interaction.....	131
Table 4.2: The TCRs used in Chapter 4.....	133
Table 4.3: CPL data was used to screen the 9-mer peptide universe for the top 20 predicted peptide agonists of each TCR.....	160
Table 5.1: The peptides used in Chapter 5.....	181
Table 5.2: The change in CSM over the 292-310 nm excitation range for each protein.....	187
Supplementary Table 7.1 The SASA of the 6 pMHC complexes is similar.....	225

# 1 – Introduction

---

## 1.1 – The immune system

### 1.1.1 – Overview

The human immune system is capable of defending against virtually any foreign antigen by means of a highly specific immune response, despite often never having encountered the offending foreign antigen before (Davis and Bjorkman, 1988). Whilst maintaining this extensive protective coverage against harmful pathogens, the immune system must remain tolerant to self and to non-harmful foreign entities such as ingested food (aberrancies manifest as allergies) and commensal bacteria (Bluestone, 2011).

This sophisticated balance between protection and tolerance is achieved via a series of defensive barriers. Anatomical barriers such as the skin and the oral mucosa provide a physical barrier as the first line of defence against infection (Walker, 2004; Bangert, Brunner and Stingl, 2011). If these barriers are breached the complement system – a group of soluble proteins present in blood plasma – can initiate an immune defence (Jordon, 1982). Complement can be triggered directly by recognition of molecules on the surface of a pathogen (Gialeli, Gungor and Blom, 2018), or indirectly by binding to opsonising antibodies (Jordon, 1982). The triggered complement cascade can result in either the direct lysis of pathogens, via formation of the membrane-attack complex (MAC) (Gialeli, Gungor and Blom, 2018), or can facilitate pathogen removal by recruiting phagocytes that express complement receptors (Jordon, 1982).

In addition to the complement system, a pathogen that manages to breach the anatomical barriers must face numerous other immune defences. These are broadly divided into the innate and the adaptive arms of the immune system (Parkin and Cohen, 2001). The innate immune system, which can be triggered by the complement cascade (Jordon, 1982), consists of immune cells such as macrophages and dendritic cells (Parkin and Cohen, 2001). The response of the innate immune system is extremely rapid following a pathogenic threat (Parkin and Cohen, 2001). The innate immune system has been extensively reviewed elsewhere and will not be discussed further here (Medzhitov and Janeway, 2000; Beutler, 2004; Akira, Uematsu and Takeuchi, 2006; Bangert, Brunner and Stingl, 2011; Riera Romo, Pérez-Martínez and Castillo Ferrer, 2016).

The focus of this thesis is  $\alpha\beta$  T cells which are members of the adaptive immune system. The adaptive immune system, found in jawed vertebrates, evolved after the innate immune system (Pancer and Cooper, 2006). It is populated by lymphocytes known as B and T cells (Parkin and Cohen, 2001). Whilst the humoral immunity provided by B cells is important, the majority of B cells require growth and differentiation factors secreted by T cells (Schimpl and Wecker, 1971, 1972; Hamaoka and Ono, 1986). B cells are reviewed in the following articles (Lebien and Tedder, 2008; Mauri and Bosma, 2012; Hoffman, Lakkis and Chalasani, 2016), this introduction will henceforth centre on the discussion of  $\alpha\beta$  T cells.

## 1.2 – $\alpha\beta$ T cells

### 1.2.1 – Overview of T cell types

T cells express a T cell receptor (TCR). The TCR is a heterodimer; made up of two of four distinct TCR polypeptides;  $\alpha$ ,  $\beta$ ,  $\gamma$ , or  $\delta$ . These polypeptides were first described in the 1980s and primarily form two different heterodimers  $\alpha\beta$  and  $\gamma\delta$  expressed in  $\alpha\beta$  T cells and  $\gamma\delta$  T cells respectively (Kappler *et al.*, 1983; Hedrick *et al.*, 1984; Bank *et al.*, 1986; Brenner *et al.*, 1986; Loh *et al.*, 1987; Davis and Bjorkman, 1988).  $\gamma\delta$  T cells are extensively reviewed (Xiong and Raulet, 2007; Fahl, Coffey and Wiest, 2014; Yazdanifar *et al.*, 2020). ‘Conventional’  $\alpha\beta$  T cells predominate in the human peripheral blood (Davis and Bjorkman, 1988; Zou *et al.*, 2017); their specificity for, and interaction with their target cells is the focus of this thesis.

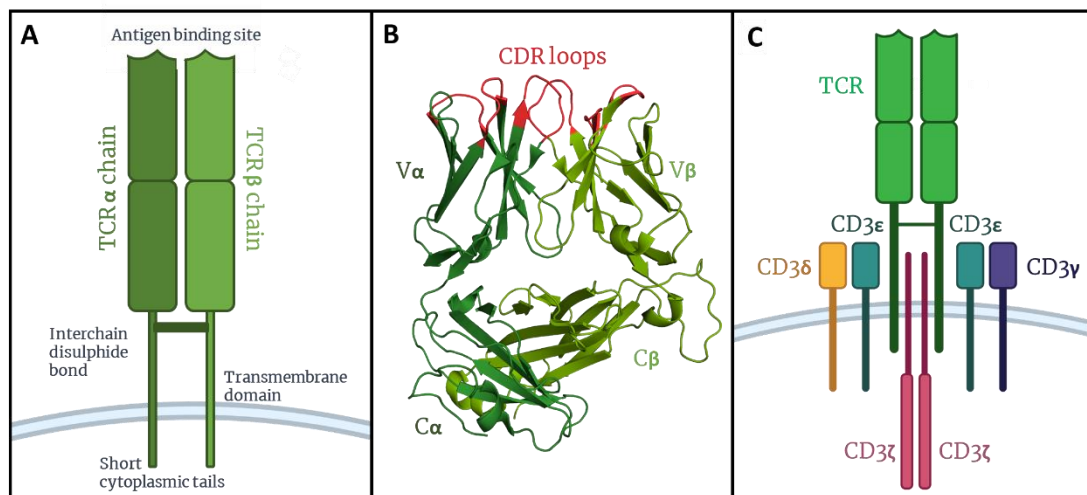
There are thought to be  $\sim 10^{12}$   $\alpha\beta$  T cells in the human body (Arstila *et al.*, 1999). Conventionally,  $\alpha\beta$  T cells are characterised based on their surface expression of one of two glycoproteins called cluster of differentiation (CD)4 and CD8 (Parnes, 1989). As well as being a means of identification and characterisation, these surface glycoproteins are important to T cell function (Miceli, Von Hoegen and Parnes, 1991; Gavichandran and Burakoff, 1994). The CD4 and CD8 glycoproteins, or ‘co-receptors’, help T cells to receive signals (Doyle and Strominger, 1987; Norment *et al.*, 1988; Rosenstein *et al.*, 1989) and can determine how the T cell reacts, whether with direct cytotoxic functions (CD8<sup>+</sup> T cells), or with assistive or regulatory functions (CD4<sup>+</sup> T cells) (Parnes, 1989; Gavichandran and Burakoff, 1994).

CD4<sup>+</sup> T cells can be further subdivided into cells types; those that assist effector arms of the immune system or those that regulate immunity (Tse and Dutton, 1977; Golubovskaya and Wu, 2016), the latter being important in the prevention of autoimmunity, see **Section 1.5.2** (Strelkauskas *et al.*, 1978). In contrast, CD8<sup>+</sup> T cells, often known as cytotoxic T cells, play a more direct role in the removal of pathogens (Nakayama *et al.*, 1979; Zhang and Bevan,

2011). Unorthodoxly, expression of CD4 and CD8 is not always mutually exclusive and CD4<sup>+</sup>CD8<sup>+</sup> T cells have been suggested to play a role in immunity to HIV and cancer (Gallagher, Fazekas de and Miller, 1989; Jiménez *et al.*, 2002; Desfrancois *et al.*, 2010; Frahm *et al.*, 2012).

### 1.2.2 – $\alpha\beta$ CD8<sup>+</sup> T cell function

The structure of the  $\alpha\beta$  TCR is key to understanding  $\alpha\beta$  TCR function. The first TCR crystal structures, published in 1996 (Garboczi *et al.*, 1996; Garcia *et al.*, 1996), and an improved method for refolding soluble forms of the TCR in 2003 (Boulter *et al.*, 2003) led to greater understanding of  $\alpha\beta$  TCR structure. The  $\alpha$  and  $\beta$  polypeptide chains that comprise the  $\alpha\beta$  TCR are themselves comprised of several distinct regions (Kappler *et al.*, 1983; Garboczi *et al.*, 1996; Garcia *et al.*, 1996). Each chain has a large extracellular component, a small hydrophobic domain towards the C-terminus of the chain which forms the single membrane-spanning region, and a short cytoplasmic tail (Bentley and Mariuzza, 1996; Garboczi *et al.*, 1996; Garcia *et al.*, 1996). The  $\alpha$  and  $\beta$  polypeptide chains are covalently linked by disulphide bonds between their extracellular domains, adjacent to the plasma membrane, see **Figure 1.1A** (Garboczi *et al.*, 1996; Garcia *et al.*, 1996).



**Figure 1.1: The structure of the  $\alpha\beta$  TCR.** **A)** a schematic representation of the  $\alpha\beta$  TCR. Showing  $\alpha$  chain (dark green) and  $\beta$  chain (light green) linked by an interchain disulphide bond. Each chain has a transmembrane domain and a short cytoplasmic tail. **B)** 3D crystal structure (868 TCR, PDB: 5NMD) showing the variable and constant domains of the  $\alpha$  chain and  $\beta$  chain. Hypervariable CDR loops within the variable domain shown in red. **C)** The TCR non-covalently associates with a CD3 hexamer to facilitate signalling.

Each TCR chain consists of a variable domain (V) and a constant domain (C), see **Figure 1.1B** (Garboczi *et al.*, 1996; Garcia *et al.*, 1996, 1998; Garcia, Teyton and Wilson, 1999). The constant (C) domains, present on both the  $\alpha$  and  $\beta$  polypeptide chains, are conserved between different TCRs (Garboczi *et al.*, 1996; Ding *et al.*, 1998; Garcia *et al.*, 1998; Garcia, Teyton and Wilson, 1999) (although in the case of the  $\beta$  chain one of two possible, almost identical, constant domains is selected, see **Section 1.2.3** (Rowen, Koop and Hood, 1996; Attaf *et al.*, 2015)). A key feature of the variable (V) domains are the hypervariable loops, known as complementarity-determining regions (CDR), **Figure 1.1B** in red. There are three CDRs on each chain, CDR1-3 (Davis and Bjorkman, 1988; Garboczi *et al.*, 1996; Garcia *et al.*, 1996; Garcia, Teyton and Wilson, 1999). The six CDR loops are responsible for forming the antigen-binding site of the TCR (Garboczi *et al.*, 1996; Ding *et al.*, 1998; Garcia *et al.*, 1998). The  $\alpha\beta$  TCR is generally restricted in that it usually only recognises peptide antigen when they are presented on major histocompatibility complex (MHC) molecules which are expressed on the surface of other cells, this is known as MHC-restriction, see **Section 1.3** (Rosenthal and Shevach, 1973; Zinkernagel and Doherty, 1974; Davis and Bjorkman, 1988).

Successful interaction between an  $\alpha\beta$  TCR and a peptide-MHC (pMHC) can result in T cell activation, and lead to the effective killing of the target cell or the release of soluble immune messengers such as cytokines (Davis and Bjorkman, 1988; Zhang and Bevan, 2011). TCR activation is coupled to intracellular signalling by means of CD3. CD3 is a nonpolymorphic surface-expressed hexamer made up of  $\gamma$ ,  $\delta$ ,  $\epsilon$  and  $\zeta$  subunits (**Figure 1.1C**). The CD3 $\gamma\epsilon$ -CD3 $\delta\epsilon$ -CD3 $\zeta\zeta$  hexamer non-covalently associates with the TCR constant domain forming an essential part of the signalling complex (Frank *et al.*, 1990; Straus and Weiss, 1993; Ghendler *et al.*, 1998; Dong *et al.*, 2019). The cytoplasmic tails of CD3 subunits contain immunoreceptor tyrosine-based activation motifs (ITAMs) (Reth, 1989; Weiss, 1993; Dong *et al.*, 2019). Upon ligation of a pMHC complex, conformational change is thought to expose CD3 ITAMs to targeting by cytosolic Src family protein tyrosine kinases (such as Lck) (Rudd *et al.*, 1988; Barber *et al.*, 1989; June *et al.*, 1990; Straus and Weiss, 1993). An alternative model of T cell activation, the kinetic segregation model, proposes that in the absence of a target cell, phosphorylation of ITAMs is minimised by the presence of bulky, cell surface-expressed phosphatases such as CD45 and CD148. The close cell-cell contact brought about by TCR-pMHC interaction excludes the large phosphatases from the vicinity of the contact zone, allowing the TCR ITAMs to be phosphorylated (Davis and van der Merwe, 1996, 2006; Van Der Merwe *et al.*, 2000; Choudhuri *et al.*, 2005, 2009). The conformational changes of CD3 and the kinetic segregation of phosphatases may not be mutually exclusive and both

facilitate phosphorylation of CD3 ITAMs which recruits the Syk kinase family member ZAP-70 which propagates downstream signalling (Chan *et al.*, 1992; Bu, Shaw and Chan, 1995). Thus, in this manner the TCR, which has no enzymatic function of its own, is able to initiate a cascade of signalling events that result in activation of the T cell.

Phosphorylation of CD3 ITAMs is assisted by the co-receptors, CD4 and CD8 (Barber *et al.*, 1989; Veillette *et al.*, 1989) because, intracellularly, they are able to bind to Lck (Rudd *et al.*, 1988; Barber *et al.*, 1989; Van Laethem *et al.*, 2007). Extracellularly, CD8 and CD4 assist TCR binding to two different types of MHC molecule; MHC class I and MHC class II respectively (Doyle and Strominger, 1987; Norment *et al.*, 1988) (although despite this generalisation, TCRs have been known to be capable of binding both MHC-I and MHC-II ligands (Yin *et al.*, 2011)). MHCs are discussed in detail in **Section 1.3**. When a TCR binds a pMHC the CD8/CD4 co-receptor is recruited along with its accompanying pool of sequestered Lck (Veillette *et al.*, 1988; Abraham *et al.*, 1991; Van Laethem *et al.*, 2007). In this way, the CD4/CD8-MHC interaction recruits Lck for the phosphorylation of CD3 $\zeta$  ITAMS (Purbhoo *et al.*, 2001). Thus, the CD8/CD4 co-receptor both facilitates T cell activation, by providing a concentrated pool of signalling molecules, and simultaneously biases TCR-signalling towards MHC-dependence (Turner *et al.*, 1990; Van Laethem *et al.*, 2007), this raises an interesting debate as to whether the TCR is inherently MHC-restricted, or whether the bias is imposed by the co-receptor (see **Section 1.4.2**). Interestingly, Lck is thought to associate more frequently with the co-receptors in memory T cells than naïve T cells. Bachmann *et al.*, found that more Lck co-immunoprecipitated with CD8 isolated from memory or effector T cells than CD8 isolated from naïve T cells. The resulting distributional difference between experienced and inexperienced cells may contribute to the more rapid response of the former (Bachmann *et al.*, 1999).

In addition to CD8, CD4, and CD3 there are a number of surface molecules that can also influence the success of the TCR-pMHC interaction. For example, CD80 and CD86 molecules on the surface of antigen presenting cells (APCs), such as dendritic cells, can interact with either CD28 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4; also known as CD152) on the surface of T cells and have a co-stimulatory or co-inhibitory effect respectively (Linsley, Brady, Grosmaire, *et al.*, 1991; Linsley, Brady, Urnes, *et al.*, 1991). An additional co-stimulatory interaction is the binding of CD2 on the T cell surface to CD58 on the APC surface (Selvaraj *et al.*, 1987; Pan *et al.*, 2019). In contrast, binding of PD-1 on the T cell surface to PD-L1 or PD-L2 on the surface of other cells can provide inhibitory signals (Ishida *et al.*, 1992; Freeman *et al.*, 2000; Latchman *et al.*, 2001; Brown *et al.*, 2003; Rodig *et al.*, 2003). The role

of co-stimulation in regulating T cell activation and preventing autoimmunity is discussed further in **Section 1.5.2**. Clearly, the success of an interaction between the TCR and its cognate pMHC can be exquisitely fine-tuned through the regulation of accompanying surface molecules.

Following activation, a CD8<sup>+</sup> T cell will respond by undergoing clonal expansion – it will proliferate in order to expand the pool of CD8<sup>+</sup> T cells that can recognise that specific foreign antigen (Butz and Bevan, 1998; Badovinac, Haring and Harty, 2007). In a positive feedback manner, activated T cells produce a range of proinflammatory cytokines and chemokines that propagate the immune response. For example, interleukin (IL)-2 which promotes cell-cycle progression (Mier and Gallo, 1980; Smith, 1988; Williams, Tyznik and Bevan, 2006) and differentiation (Bachmann *et al.*, 2007; Pipkin *et al.*, 2010), and macrophage inflammatory protein (MIP)-1 $\beta$  (also known as CCL4) a chemoattractant that encourages T cell migration to the site of response (Taub *et al.*, 1993; Bystry *et al.*, 2001; Kenway-Lynch *et al.*, 2014). Activated T cells can also produce interferon (IFN)- $\gamma$  (Wheelock, 1965; Boehm *et al.*, 1997) and tumour necrosis factor (TNF) (Kolb and Granger, 1968) which have many immune functions including stimulating the upregulation of MHC expression in surrounding cells (Boehm *et al.*, 1997), the suppression of viral replication (Mestan *et al.*, 1986; Bouley *et al.*, 1995; Finke *et al.*, 1995; van den Broek *et al.*, 1995; Seo and Webster, 2002), and enhancing the proliferation of CD8<sup>+</sup> T cells (Yokota, Geppert and Lipsky, 1988).

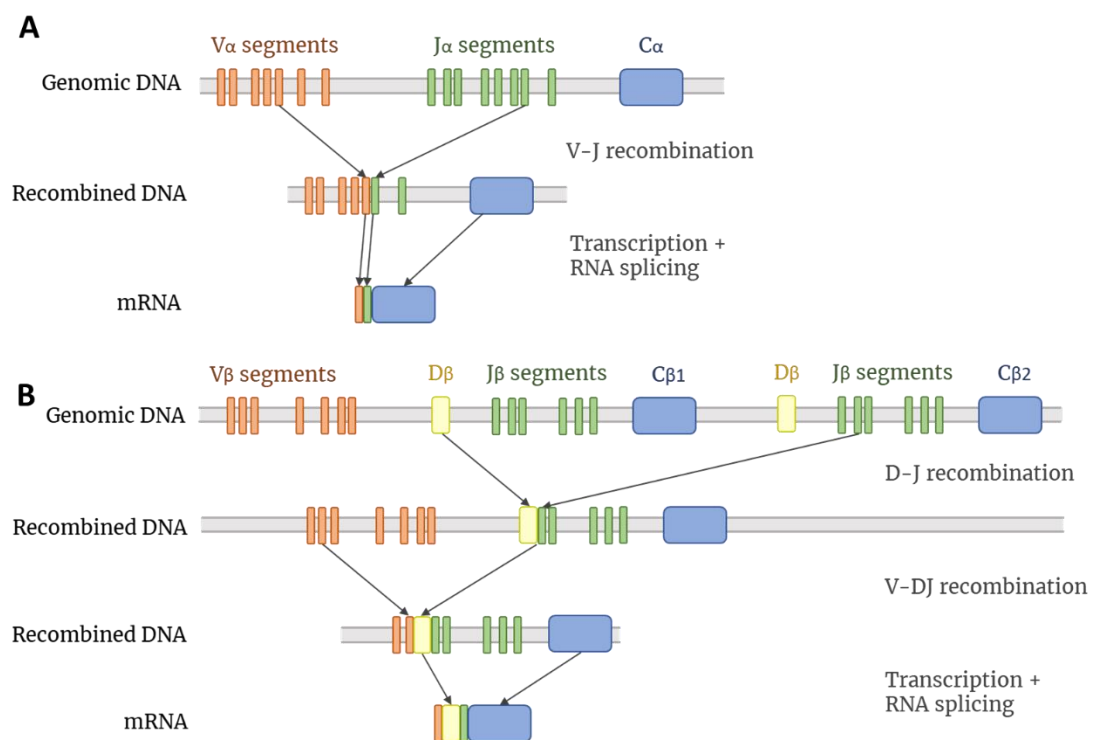
Activated CD8<sup>+</sup> T cells also have a more direct role in pathogen removal. They can induce apoptosis in target cells via two main pathways: the secretory perforin-granzyme pathway, and the Fas pathway (Podack, Hengartner and Lichtenheld, 1991; Henkart, 1994; Heusel *et al.*, 1994; Kägi *et al.*, 1994; Ebnet *et al.*, 1995). The former involves Ca<sup>2+</sup>-dependant degranulation, engagement of the TCR causes the contents of cytosolic granules to be released in an localised manner directly onto the target cell (Weiss *et al.*, 1984; Burkhardt *et al.*, 1993; Berke, 1995). Stored within these granules are perforin – a lytic protein that polymerises to form pores in the surface membrane of the target cell – and granzymes – serine proteases that enter the target cells through the newly created pores and damage cellular machinery to induce the intrinsic apoptotic pathway (Heusel *et al.*, 1994; Kägi *et al.*, 1994; Berke, 1995). The latter involves the upregulation of FasL on the surface of the activated CD8<sup>+</sup> T cell (Shresta *et al.*, 1998). The interaction of FasL with Fas (expressed on the surface of the target cell) induces the extrinsic apoptotic pathway in the target cell (Kägi *et al.*, 1995; Jensen *et al.*, 1998).



In summary, the CD8<sup>+</sup> T cell is capable of a vast array of antipathogenic activities, and central to it all is the engagement of its TCR.

### 1.2.3 – TCR gene rearrangement

The TCR repertoire is thought to consist of  $\sim 10^7$  unique receptors (Arstila *et al.*, 1999; Turner *et al.*, 2006). A unique gene for every unique TCR would require more genomic material than would fit in the cell nucleus. Instead, this sizeable diversity arises from the random combination of a finite number of gene segments - a process known as somatic gene rearrangement (Hozumi and Tonegawa, 1976). This enables a limited number of genes to give rise to a vast range of different TCRs (Griesser *et al.*, 1988; Alt *et al.*, 1992; Hodges *et al.*, 2003; Attaf *et al.*, 2015). The TCR gene segments lie far apart in the genomic configuration of deoxyribonucleic acid (DNA), but are brought together to form a continuous transcript by somatic recombination (Alt and Baltimore, 1982; Tonegawa, 1983; Hodges *et al.*, 2003).



**Figure 1.2: V(D)J recombination.** **A)** shows the recombination events required to produce a TCR  $\alpha$  chain. Recombination occurs between a V segment and a J segment, the intervening DNA is excised. The recombined DNA is transcribed into pre-mRNA. Subsequent RNA splicing places the C segment next to the VJ. **B)** shows the recombination events required to produce a TCR  $\beta$  chain. An additional recombination event, D-J, occurs first. Followed by V-DJ recombination. One of two C segments is juxtapositioned during RNA splicing.

The variable domain of the TCR  $\alpha$ -chain (encoded at the *tra* locus), consists of two recombined gene segments known as variable (V) and junctional (J) gene segments. V-J recombination brings together a randomly selected V segment and a randomly selected J segment (**Figure 1.2A**) (Market and Papavasiliou, 2003). The DNA is broken and reannealed to create the continuous exon that will encode the V domain of the TCR. The stretch of DNA is transcribed into pre-messenger ribonucleic acid (mRNA), and subsequent RNA splicing removes the genetic material between the VJ sequence and the single constant domain segment present at the *tra* locus, prior to translation into polypeptide (Alt *et al.*, 1992; Market and Papavasiliou, 2003). There are 70 V segments and 61 J segments at the *tra* locus, resulting in a large number of potential combinations and giving rise to TCR  $\alpha$ -chain diversity (Market and Papavasiliou, 2003).

The variable domain of the TCR  $\beta$ -chain, in contrast, is encoded by three gene segments. At the *trb* locus there is an additional recombination step in which one of two diversity (D) segments are selected (Griesser *et al.*, 1988; Alt *et al.*, 1992; Arstila *et al.*, 1999; Market and Papavasiliou, 2003), see **Figure 1.2B**. For the TCR  $\beta$ -chain there are 52 V segments, 13 J segments, and two possible constant segments (Market and Papavasiliou, 2003). Given the vast number of potential combinations, it is not difficult to envision how such diversity arises.

Somatic recombination is strictly regulated and carried out by a group of enzymes collectively called the recombinase. To ensure recombination occurs in the correct places each gene segment is flanked with recombination signal sequences (RSSs) (Alt and Baltimore, 1982; Alt *et al.*, 1992; Bassing, Swat and Alt, 2002; Hodges *et al.*, 2003). The RSSs are recognised by two key members of the recombinase, recombination activating gene (RAG)-1 and RAG-2 (Schatz, Oettinger and Baltimore, 1989; Oettinger *et al.*, 1990; Alt *et al.*, 1992). This recognition was visualised by a cryo-electron microscopic structure in 2015 (Ru *et al.*, 2015).

Recombination at the V-J or V-D-J junctions is imperfect, and nucleotides often get deleted during the process (Sakano *et al.*, 1979; Weigert *et al.*, 1980; Alt and Baltimore, 1982; Alt *et al.*, 1992). V-J or V-D-J junctions are also subject to the addition of palindromic (P)- or non-template (N)-nucleotides by another member of the recombinase – terminal deoxynucleotidyl transferase (TdT) (Alt and Baltimore, 1982; Alt *et al.*, 1992; Hodges *et al.*, 2003; Peralta-Zaragoza, Recillas-Targa and Madrid-Marina, 2004). The resulting heightened diversity at the junctional regions (Tonegawa, 1983; Alt *et al.*, 1992; Gilfillan *et al.*, 1993) gives rise to the ‘hypervariable’ CDR3 loops of both TCR chains (Jorgensen *et al.*, 1992; Hodges *et al.*, 2003). In contrast, the ‘variable’ CDR1 and CDR2 loops of both chains are

germline encoded and arise from variable gene segments (Jorgensen *et al.*, 1992; Hodges *et al.*, 2003).

The rearrangement of the genome is tightly controlled as dysregulation can result in transformation of lymphocytes (Mijušković *et al.*, 2015). V(D)J recombination has been extensively reviewed (Alt and Baltimore, 1982; Griesser *et al.*, 1988; Bassing, Swat and Alt, 2002; Market and Papavasiliou, 2003; Attaf *et al.*, 2015).

#### 1.2.4 – Maturation and thymic selection

T cell progenitor cells arise in the adult bone marrow, but the process of somatic gene rearrangement described above occurs in the thymus during T cell maturation. Maturing T cells undergo a process called selection. During selection recognition of self-pMHC complexes presented on thymic epithelial cells is simultaneously essential for and detrimental to thymocyte survival (Von Boehmer, Haas and Jerne, 1978; Zinkernagel, G. Callahan, *et al.*, 1978; Kappler, Roehm and Marrack, 1987; Boehmer, 1988).

Thymocytes are positively selected in the thymic cortex if they are able to recognise a self-pMHC, ligation with the pMHC saves the precursor cell from cell death (Von Boehmer, Haas and Jerne, 1978; Boehmer, 1988). Only those that can recognise self-antigen in the context of self-MHCs survive because those that are unable to recognise a self-peptide are likely to be equally inefficient at recognising foreign antigen, these thymocytes are said to “die by neglect” (Von Boehmer, Teh and Kisielow, 1989; Attaf *et al.*, 2015).

In the thymic medulla, a second phase of thymic selection – known as negative selection – destroys any potentially harmful T cells that recognise pMHC too strongly (Kappler, Roehm and Marrack, 1987; Von Boehmer, Teh and Kisielow, 1989). It is thought to be the strength, or dwell-time, of the TCR-pMHC interaction which ultimately decides cell fate (Kalergis *et al.*, 2001; Gascoigne, 2014; Stepanek *et al.*, 2014). A weak interaction is essential to prevent cell death and promote positive selection however too strong an interaction results in negative selection and apoptosis (Alam *et al.*, 1996; Williams *et al.*, 1999; Starr, Jameson and Hogquist, 2003).

The microenvironment in which the interaction occurs (Koble and Kyewski, 2009; Klein *et al.*, 2014) is also important in determining which thymocytes survive to populate the periphery (Ashton-Rickardt *et al.*, 1994; Sebzda *et al.*, 1994). Epithelial cells in the thymic cortex possess a special proteasome – known as the thymoproteasome – which produces peptides unique

to the thymus for positive selection (Takada, Kondo and Takahama, 2017). With regards to negative selection, the developing T cell repertoire will only be tolerated to antigen it is exposed to. The peripheral pMHCs on display may vary in different microenvironments due to differences in protein expression, post-translational modification, or level of surface presentation (Lanzavecchia, 1995; Salemi *et al.*, 1995; Simitsek *et al.*, 1995; Wood and Elliott, 1998). This may explain the lack of tolerance towards 'cryptic' self-antigens (Moudgil and Sercarz, 1994). Exposure of cryptic self-antigens, for example during an infection or inflammation in the local microenvironment (Lanzavecchia, 1995; Salemi *et al.*, 1995), can drive the development of autoimmunity (Lanzavecchia, 1995; Fairchild, Pope and Wraith, 1996), discussed in detail in **Section 1.5.2**.

In addition to eradicating harmful self-reactive T cells, thymic selection determines another key stage of thymocyte maturation. Prior to positive selection thymocytes express both CD4 and CD8, whether their final phenotype will be CD8<sup>+</sup> or CD4<sup>+</sup> depends on whether they encounter a peptide presented on MHC-I or MHC-II respectively (Sha *et al.*, 1988; Scott *et al.*, 1989; Von Boehmer, Teh and Kisielow, 1989).

## 1.3 – The major histocompatibility complex (MHC)

### 1.3.1 – Overview

The MHCs were first discovered in the mouse in 1936 (Gorer, 1936; Klein, 1986). They are a family of cell surface-expressed glycoproteins (Parham *et al.*, 1977; Bjorkman, M A Saper, *et al.*, 1987) that play a vital role in the immune response, via their activation of  $\alpha\beta$  T cells (Heber-Katz, Hansburg and Schwartz, 1983). Human MHCs are also known as the human leukocyte antigens (HLA). MHCs present peptide fragments, generated by the cleavage of proteins (Babbitt *et al.*, 1985; Guillet *et al.*, 1986; Townsend, Rothbard, *et al.*, 1986), thus allowing the  $\alpha\beta$  T cells to scrutinise the proteome at the cell surface (Davis and Bjorkman, 1988; Germain, 1994; Garcia, Teyton and Wilson, 1999).

The classical MHC molecules are divided into two broad families; MHC class I and MHC class II (Hood, Steinmetz and Malissen, 1983; Davis and Bjorkman, 1988). The former is expressed on almost all nucleated cells and presents peptides from endogenous sources to CD8<sup>+</sup> T cells (Bjorkman, M A Saper, *et al.*, 1987; David-Watine, Israël and Kourilsky, 1990; Ting and Baldwin, 1993; Van Den Elsen, 2011). In contrast, MHC class II expression is largely restricted to APCs and thymic epithelial cells (Glimcher and Kara, 1992; Van Den Elsen *et al.*, 2003). MHC class II molecules present peptides from exogenous sources to CD4<sup>+</sup> T cells, thus T cell

immunity can sample both the intracellular and the extracellular proteome (Swain, 1981; Spits *et al.*, 1982; Swain *et al.*, 1984; Van Den Elsen, 2011). Whilst other cell types do not constitutively express MHC class II, expression can be induced by the cytokines produced during an immune response such as IFN- $\gamma$  (Piskurich *et al.*, 1998; Holling *et al.*, 2002).

### 1.3.2 – The *HLA* loci

The MHCs are encoded by one of the most diverse set of alleles in the human genome (Campbell and Trowsdale, 1993; Beck *et al.*, 1999; Kumánovics, Takada and Lindahl, 2003; Horton *et al.*, 2004). The two aforementioned classical MHC classes are further divided into three subfamilies apiece. *HLA-A*, *HLA-B*, and *HLA-C* in the case of class I, and *HLA-DR*, *HLA-DQ*, and *HLA-DP* in the case of class II. Each subfamily itself is highly polymorphic (Campbell and Trowsdale, 1993; Beck *et al.*, 1999; Kumánovics, Takada and Lindahl, 2003; Mungall *et al.*, 2003; Horton *et al.*, 2004; Trowsdale and Knight, 2013). This enormous diversity is surmised to be an evolutionary safeguard to ensure that detection of any potential foreign pathogen is covered across the human population (Horton *et al.*, 2004). The vast majority of this diversity occurs in the peptide-binding groove of the MHC molecule (Bjorkman, M. A. Saper, *et al.*, 1987; Kumánovics, Takada and Lindahl, 2003; Trowsdale and Knight, 2013). It is the sequence of the peptide-binding groove that determines which peptide fragments can be loaded into the groove and presented to the T cells (Barber *et al.*, 1995; Gao *et al.*, 2001).

Despite the huge number of available *HLA* alleles each individual only expresses a small subset of MHC molecules (Stern and Wiley, 1994). As such, the complement of MHC molecules expressed by an individual determines the peptide fragments that that individual is able to present to their T cell repertoire. Even a single amino acid difference between two MHC molecules can alter the epitopes that can be presented (Stern *et al.*, 1994; Barber *et al.*, 1995). Thus, possession of certain *HLA* alleles can confer susceptibility or resistance to viral disease, based on the ability, or lack thereof, to present key pathogenic epitopes (Fabio *et al.*, 1992; Gao *et al.*, 2001; Geldmacher *et al.*, 2009; Kløverpris *et al.*, 2012; Kløverpris, Cole, *et al.*, 2015). Expression of certain *HLA* alleles has also been linked to susceptibility to autoimmune disease (Todd, Bell and McDevitt, 1987; Mignot *et al.*, 1997; Price *et al.*, 1999; Chabas *et al.*, 2003; Larsen and Alper, 2004; Trowsdale, 2005; Horton *et al.*, 2008), see **Section 1.5.2.**

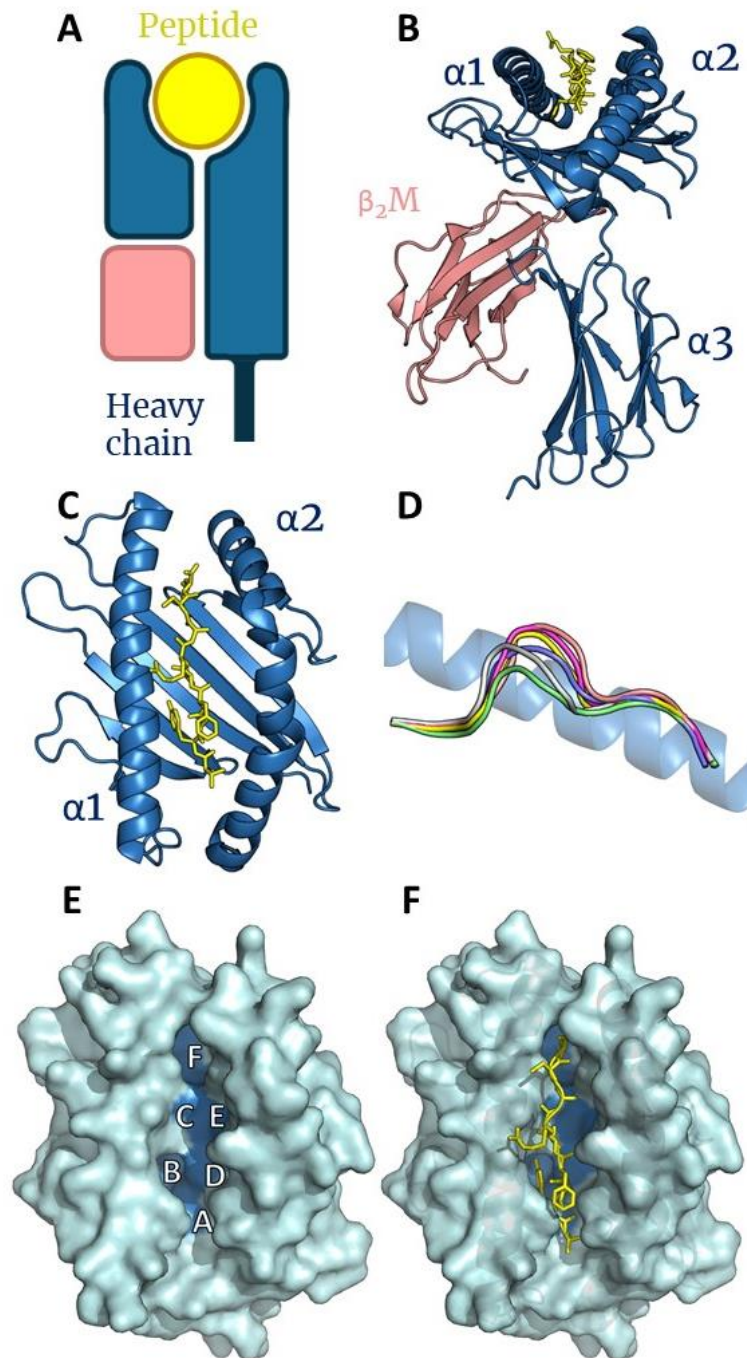
### 1.3.3 – pMHC structure

The first MHC structure (HLA-A\*0201, HLA-A2 hereafter) was solved in 1987 (Bjorkman, M A Saper, *et al.*, 1987). This was achieved by purifying the protein from a human Epstein-Barr virus (EBV)-transformed lymphoblastoid cell line, JY. As such, the groove contained a mixture of peptides (Bjorkman, M A Saper, *et al.*, 1987). By 1992 MHC molecules could be artificially refolded with a single peptide to produce clear crystal structures (Fremont *et al.*, 1992; Garboczi, Hung and Wiley, 1992; Wilson and Fremont, 1993).

MHC class I molecules are heterodimers consisting of a heavy  $\alpha$ -chain associated with a smaller  $\beta_2$ -microglobulin ( $\beta_2M$ ), **Figure 1.3A**. The  $\alpha$ -chain is a transmembrane glycoprotein folded into three domains, denoted  $\alpha_1$ ,  $\alpha_2$ , and  $\alpha_3$ , **Figure 1.3B** (Bjorkman, M A Saper, *et al.*, 1987).

Domains  $\alpha_1$  and  $\alpha_2$  form the peptide binding groove - two parallel  $\alpha$ -helices linking a seven-stranded  $\beta$ -sheet 'floor' (Bjorkman, M A Saper, *et al.*, 1987). The  $\alpha_2$  helix possesses a distinctive kink at residue 162 (Bjorkman, M A Saper, *et al.*, 1987). The binding groove is closed at either end and can generally accommodate peptides of 8-13 amino acids in length, **Figure 1.3C**. Peptides longer than eight amino acids are accommodated in a bulged fashion **Figure 1.3D** (Chicz *et al.*, 1992; Henderson *et al.*, 1992; Matsumura *et al.*, 1992; Chen *et al.*, 1994).

Within the peptide-binding groove are six pockets (known as pockets A-F), **Figure 1.3E-F** (Bjorkman, M A Saper, *et al.*, 1987; Bjorkman, M. A. Saper, *et al.*, 1987; Bjorkman and Parham, 1990). These pockets are responsible for most of the contacts with the peptide, and can accommodate its side chains (Bjorkman, M. A. Saper, *et al.*, 1987). These pockets also house most of the allelic variety of the MHC-I molecules and are chemically distinct, giving them varying binding preferences for different amino acids. The pockets are largely responsible for determining which peptides can be presented to TCRs (Bjorkman, M. A. Saper, *et al.*, 1987; Van Bleek and Nathenson, 1991). Altering the peptide sequence even slightly can affect how well it is able to be presented by a particular MHC molecule (Van Bleek and Nathenson, 1991).



**Figure 1.3: The structure of MHC-I.** **A)** shows a schematic representation of the MHC-I heavy chain (blue) associated with  $\beta_2M$  (pink) and presenting a peptide (yellow). **B)** The heavy chain is further divided into three globular domains donated  $\alpha 1$ ,  $\alpha 2$ , and  $\alpha 3$ . **C)**  $\alpha 1$  and  $\alpha 2$  form the binding groove in which the peptides sit. **D)** Peptides sit within this groove in a bulged fashion, anchored at their termini with their central residues protruding. **E+F)** Deep 'pockets' within the peptide binding groove floor form interactions with peptide side chains helping to anchor it in place. Images generated using PDB: 2BCK. Overlaid peptides in (D): ALWGPDPAAA (PDB 3UTQ) magenta, AQWGPDPAAA (PDB 5C0D) yellow, YLGGPDFPTI (PDB 5C0G) pink, ELAGIGILTV (PDB 1JF1) grey, SLLMWITQC (PDB 2BNR) blue, and AAGIGILTV (PDB 3QEQ) green.

The third globular domain, known as the  $\alpha 3$  domain, is an immunoglobulin-like domain containing the membrane-spanning portion of the molecule (Bjorkman, M A Saper, *et al.*, 1987). Together  $\alpha 1$ ,  $\alpha 2$ , and  $\alpha 3$  are known as the MHC-I heavy chain. The heavy chain non-covalently associates with  $\beta_2M$  to form a heterodimer (Bjorkman, M A Saper, *et al.*, 1987; Tysoe-Calnon, Grundy and Perkins, 1991). The  $\alpha 3$  domain also has an important role in the association with the CD8 co-receptor. A negatively charged loop formed by residues 222-228 within the  $\alpha 3$  domain was suggested as the binding site for CD8 (Salter *et al.*, 1990). This was later confirmed with atomic resolution crystal structures (Gao *et al.*, 1997; Wang, Natarajan and Margulies, 2009).

As mentioned briefly above, class I MHC molecules generally present short peptides (8-13mers) (Cerundolo *et al.*, 1991; Falk *et al.*, 1991; Jardetzky *et al.*, 1991; Madden *et al.*, 1991; Hunt *et al.*, 1992; Chen *et al.*, 1994; Rist *et al.*, 2013; Trolle *et al.*, 2016). This length-restriction is imposed by the binding groove of MHC-I which adopts a closed conformation, with the termini of the peptide typically forming clusters of hydrogen bonds with the B and F pockets of the MHC (Bjorkman, M A Saper, *et al.*, 1987; Saper, Bjorkman and Wiley, 1991; Garboczi, Hung and Wiley, 1992; Falk *et al.*, 1994; Fidelis Maier, 1994; Stern and Wiley, 1994; Garboczi *et al.*, 1996). The principle anchor residues for the peptide-MHC-I interaction are generally located at the termini of the peptides (often peptide positions 2 and the C-terminal residue) (Bjorkman, M A Saper, *et al.*, 1987; Garboczi, Hung and Wiley, 1992; Stern and Wiley, 1994; Garboczi *et al.*, 1996; Ding *et al.*, 1999; Hausmann *et al.*, 1999; Rudolph, Stanfield and Wilson, 2006). In addition to the clusters of hydrogen bonds that occur at primary anchor residues, other contacts can be made elsewhere between the MHC molecule and the peptide. These contacts can increase the overall binding affinity between peptide and MHC and subtly adjust the peptide position (Fremont *et al.*, 1992; Silver *et al.*, 1992; Jardetzky *et al.*, 1994; Stern and Wiley, 1994; Cole *et al.*, 2016; Cole, Fuller, *et al.*, 2017).

The centre of the peptide tends to arch away from the MHC molecule and thus interacts with it less (Fremont *et al.*, 1992; Guo *et al.*, 1992; Silver *et al.*, 1992; Madden, Garboczi and Wiley, 1993). This central peptide region is further away from the MHC-I molecules and is therefore more exposed for recognition by the TCR (Madden *et al.*, 1992; Silver *et al.*, 1992; Madden, Garboczi and Wiley, 1993). The peptide side chains that are not nestled within the MHC pockets can protrude upwards and interact with the TCR (Garboczi *et al.*, 1996; Garcia *et al.*, 1996; Ding *et al.*, 1999). Even the smallest sequence difference within the peptide can lead to a striking alteration in the resulting conformation (Madden, Garboczi and Wiley, 1993;



Borbulevych *et al.*, 2009). Longer peptides can bind MHC-I by protruding further out of the groove, known as super-bulged peptides (Guo *et al.*, 1992; Speir *et al.*, 2001; Tynan, Borg, *et al.*, 2005; Tynan, Burrows, *et al.*, 2005; Tynan *et al.*, 2007), or by extending out of the 'closed' groove in a manner akin to MHC-II peptide presentation (Motozono, Pearson, *et al.*, 2015; Pymm *et al.*, 2017).

MHC class II molecules are also heterodimers, consisting of two transmembrane glycoproteins –  $\alpha$  and  $\beta$ . Both chains fold into two domains –  $\alpha 1$  and  $\alpha 2$ , or  $\beta 1$  and  $\beta 2$  respectively – with the groove-like binding site formed by the  $\alpha 1$  and  $\beta 1$  domains (Brown *et al.*, 1993). In contrast to MHC class I molecules, the binding sites of class II molecules are 'open-ended' meaning that the termini of the peptide can protrude at either side and longer peptides (13-25 amino acids) can be accommodated (Chicz *et al.*, 1992; Brown *et al.*, 1993; Jardetzky *et al.*, 1994; Stern and Wiley, 1994; Stern *et al.*, 1994). The termini of MHC-II-bound peptides are able to play a role in the TCR interaction whereas in MHC-I presentation they tend to be buried (Sant'Angelo *et al.*, 2002; Zavala-Ruiz *et al.*, 2004; MacLachlan *et al.*, 2019). Unlike class I-restricted proteins that tend to have anchor residues at the peptide termini, class II restricted peptide anchor residues can be distributed throughout the peptide (Stern and Wiley, 1994; Stern *et al.*, 1994; Sant'Angelo *et al.*, 2002; MacLachlan *et al.*, 2019)

#### 1.3.4 – Peptide presentation by MHC

The peptides presented by MHC-I molecules are largely derived from cellular proteins that have been degraded by proteases or – following ubiquitination – by the proteasome (Townsend, Gotch and Davey, 1985; Townsend *et al.*, 1986), **Figure 1.4A**. In addition to degraded cellular proteins, another, more immediate source of presentable material is defective ribosomal products (DRiPs). DRiPs are N-terminal polypeptide fragments that are generated when the ribosomal translation process fails; they cannot be used by the cell and are rapidly delivered to the proteasome (Yewdell, Antón and Bennink, 1996; Berglund *et al.*, 2007; Yewdell, 2011). Presentation of DRiPs offers the advantage of very early detection of viral infection, soon after the start of viral protein translation (Yewdell, 2011).

Proteins, both self and viral in the case of an infected cell, can be broken down by the host proteasome. The proteasome became theoretically implicated in MHC-I antigen processing through a sequential summation of evidence, reviewed in 1992 by Goldberg and Rock (Goldberg and Rock, 1992). It was previously known that MHC class II presentation required the uptake of extracellular antigens and their degradation in acidic lysosomal compartments

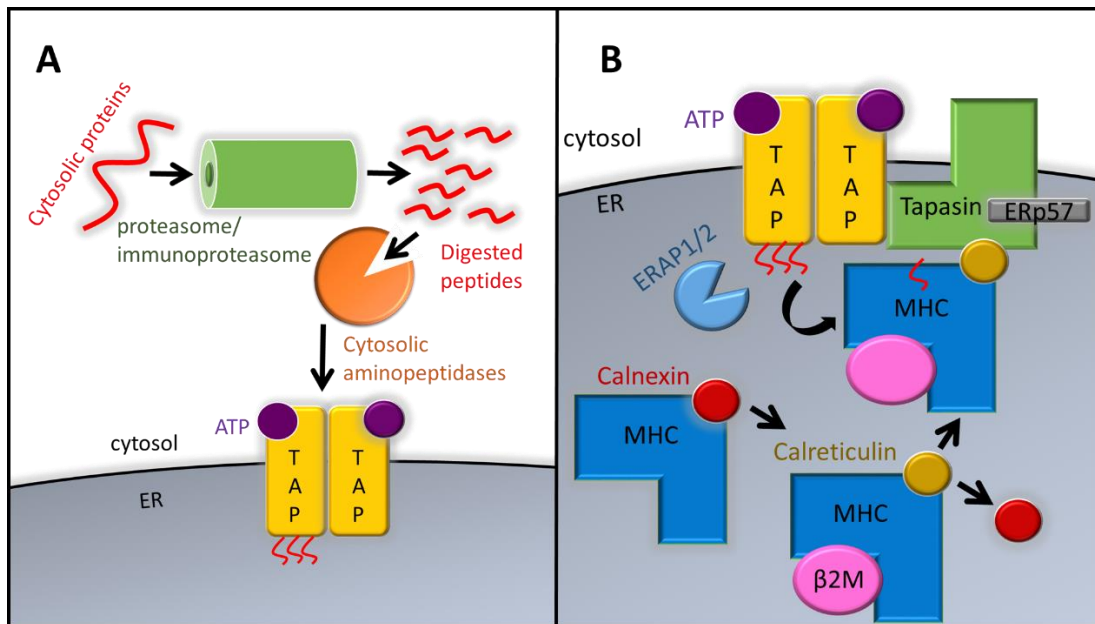
(Allen, Babbitt and Unanue, 1987; Puri and Factorovich, 1988). Yet MHC-I presentation did not seem to require acidophilic lysosomal proteases (Morrison *et al.*, 1986). In contrast, degradation of antigen for presentation by MHC-I was thought to occur in the cytosol because antigens that were injected into the cytosol were effectively processed (Townsend, Gotch and Davey, 1985; Townsend, Bastin, *et al.*, 1986; Moore, Carbone and Bevan, 1988; Yewdell, Bennink and Hosaka, 1988). An identified 'MHC-linked low molecular mass protein' was found to be similar in size and sequence to the 20S subunit of the proteasome (Brown, Driscoll and Monaco, 1991; Glynne *et al.*, 1991; Kelly *et al.*, 1991; Martinez and Monaco, 1991). It was also encoded by a gene located within the MHC locus (Brown, Driscoll and Monaco, 1991; Ortiz-Navarrete *et al.*, 1991), this pointed towards a role for the proteasome in MHC-I peptide presentation. In 1993 the role of the proteasome was demonstrated experimentally. Michalek *et al.* used a temperature sensitive ubiquitinase to demonstrate that MHC-I presentation utilises ubiquitinase-mediated proteasome degradation (Michalek *et al.*, 1993). This observation was supported by subsequent similar observations (Rock *et al.*, 1994; Hughes *et al.*, 1996). It is worth noting that the proteasome is not essential for the presentation of all MHC-I epitopes (Bai and Forman, 1997).

In addition to the standard 'housekeeping' proteasome, there is the immunoproteasome (expression of which can be upregulated by the IFN $\gamma$  produced in an antiviral response) (Brown, Driscoll and Monaco, 1991; Glynne *et al.*, 1991; Martinez and Monaco, 1991; Aki *et al.*, 1994; Griffin *et al.*, 1998; Kingsbury, Griffin and Colbert, 2000; Huber *et al.*, 2012). The immunoproteasome cleaves proteins in a more suitable manner for MHC-I ligation than the standard proteasome as it favours cleavage after hydrophobic amino acids, reflecting the preference of MHC-I for C terminal binding (Romero *et al.*, 1991; Falk *et al.*, 1994; Gaczynska *et al.*, 1994). Whilst the immunoproteasome cleaves peptides at more MHC-I-compatible positions, both proteasomes are important to the generation of MHC-I ligands and cells lacking an immunoproteasome can still process peptides for MHC-I-presentation (Yewdell *et al.*, 1994; Cerundolo *et al.*, 1995).

Peptides cleaved by the respective proteasomes may not be at optimal length for MHC-I presentation (8-13mers), consequently they can be cleaved further by cytosolic peptidases **Figure 1.4A** (Craiu *et al.*, 1997; Mo *et al.*, 1999; Reits *et al.*, 2004; Hulpke and Tampé, 2013). The relevance of this further cleavage to MHC-I presentation of antigen is particularly interesting and has generated some debate as to its usefulness. Some argue that the overall effect of the peptidases might be negative to the adaptive immune response as they may destroy more MHC-I ligands than they create (Chapiro *et al.*, 2006; York *et al.*, 2006; Marcilla,

Villasevil and López de Castro, 2008; Van Endert, 2011). On the other hand, this peptidase-dependent method of antigen generation may actually be able to compensate for an absence of proteasomal activity (Glas *et al.*, 1998). It is interesting to speculate that these apparently degenerate mechanisms for protein breakdown may function as evolutionary safeguards against pathogens.

Peptides are loaded onto MHC-I complexes in the endoplasmic reticulum (ER) by a collection of proteins known as the peptide loading complex (PLC), **Figure 1.4B** (Hulpke and Tampé, 2013). Following degradation peptide fragments are translocated into the ER by the transporter associated with antigen processing (TAP). TAP is a member of the antigen-binding cassette (ABC) transporter superfamily that resides in the ER membrane (Spies *et al.*, 1990; Kleijmeer *et al.*, 1992). TAP is a heterodimer composed of a TAP1 subunit and a TAP2 subunit (Spies *et al.*, 1992). It carries two nucleotide-binding domains that bind adenosine triphosphate (ATP) (Spies *et al.*, 1992), and two transmembrane domains that help anchor it in the ER membrane (Spies *et al.*, 1990). The gene encoding TAP (*peptide supply factor gene*) was mapped to the MHC loci in 1990 (Deverson *et al.*, 1990; Monaco, Cho and Attaya, 1990; Spies *et al.*, 1990; Trowsdale *et al.*, 2008). In 1991, experimental evidence showed that TAP expression is required for normal levels of surface MHC expression (Spies and DeMars, 1991). Further supporting evidence for the necessity of TAP was generated in 1992 using mutant cell lines deficient in the transporter; deficiencies resulted in unstable MHC-I and significantly reduced presentation of intracellular peptides (Kelly *et al.*, 1992). In 1994, immunoprecipitation experiments demonstrated that the TAP complex associates with the MHC heavy chain (Ortmann, Androlewicz and Cresswell, 1994; Suh *et al.*, 1994). In 1996, HLA-A2 was conclusively shown to interact with TAP. Disruption of this interaction by point mutation in HLA-A2 resulted in unstable HLA-A2- $\beta$ 2M dimers at the cell surface (Lewis *et al.*, 1996). In the presence of mutated TAP, MHC molecules were loaded with suboptimal peptides; without TAP binding to tether them to the ER they rapidly progress to the cell surface before peptide optimisation (mediated by molecular chaperones and discussed below) can occur (Lewis and Elliott, 1998).



**Figure 1.4: Peptide loading of MHC. A)** Cytosolic proteins or DRiPs are broken down by proteasomes into peptides which are further cleaved by cytosolic peptidases. Cleavage products are translocated into the ER by the ABC transporter family member, TAP. **B)** Within the ER free MHC heavy chain become associated with  $\beta_2M$  with the aid of protein chaperones. This complex is brought into close contact with translocated peptides. This juxtaposition is brought about via a PLC scaffold made up of TAP, tapasin, ERp57, and calreticulin. Prior to MHC loading peptides can be trimmed to optimal length by ERAP1/2.

TAP performs the characteristic cycling of ATP binding and hydrolysis which induces a conformational change in TAP that allows peptide fragments to be transported across the ER membrane (Kelly *et al.*, 1992; Androlewicz, Anderson and Cresswell, 1993; Shepherd *et al.*, 1993; Knittler *et al.*, 1999; Boyle *et al.*, 2015). TAP has a peptide-binding region (Nijenhuis and Hämmerling, 1996), which binds peptides in an ATP-independent manner (Van Endert *et al.*, 1994). Binding and hydrolysis of ATP then facilitates the translocation of the bound peptide (Neefjes, Momburg and Hämmerling, 1993; Van Endert *et al.*, 1994). Similar to MHC-I binding, TAP can bind promiscuously to peptide fragments due to the requirement of relatively few anchor residues. The remaining residues in the peptide are then open to diversity (Van Endert *et al.*, 1995; Uebel *et al.*, 1997; Herget *et al.*, 2011), although interestingly TAP is stereospecific – meaning that it is unable to bind peptides composed of D-amino acids (Grommé *et al.*, 1997; Uebel *et al.*, 1997). TAP can transport peptides of a range of different lengths because it is thought that the ends of the peptide are bound to

TAP, whilst the centre protrudes in an extended kink conformation – not interacting with TAP (Uebel *et al.*, 1995; Van Endert *et al.*, 1995; Koopmann *et al.*, 1996; Herget *et al.*, 2011).

Once in the ER, potential antigenic peptides can be cleaved further (Snyder, *et al.*, 1994; Elliott, *et al.*, 1995) by an ER aminopeptidase associated with antigen processing, ERAP1/2 (known as ERAAP in mice). This enzyme serves to trim peptides for insertion into the MHC-I binding pocket (Saric *et al.*, 2002; Serwold *et al.*, 2002; York *et al.*, 2002; Saveanu *et al.*, 2005; Hulpke and Tampé, 2013). Polymorphisms in this enzyme can predispose to autoimmune diseases through alterations in the peptide repertoire that is presented at the cell surface to T cells (Kemming *et al.*, 2019).

Also in the ER lumen are free MHC-I heavy chains, the folding of which is assisted and stabilised by an ER-resident chaperone protein called calnexin and the ER-resident thiol oxidoreductase ERp57 enzyme which catalyses the formation of di-sulphide bonds **Figure 1.4B** (Degen and Williams, 1991; Hochstenbach *et al.*, 1992; Hughes and Cresswell, 1998). These chaperones stabilise intermediate forms of the quaternary structure, and promote the association MHC-I heavy chain with  $\beta_2M$  (Degen and Williams, 1991; Ortmann, Androlewicz and Cresswell, 1994). Following association with  $\beta_2M$ , calnexin is exchanged for calreticulin, another ER-resident chaperone protein that associates with MHC-I/ $\beta_2M$ , it also associates with ERp57 and serves to facilitate recruitment of this intermediate complex to the aforementioned TAP via an additional member of the PLC, tapasin (Ortmann, Androlewicz and Cresswell, 1994; Sadasivan *et al.*, 1996).

Tapasin forms a heterodimer with ERp57 (Oliver *et al.*, 1999; Zhang, Baig and Williams, 2006; Santos *et al.*, 2007), the crystal structure of which was reported in 2009 (Dong *et al.*, 2009). Tapasin facilitates the physical proximity of the MHC-I/ $\beta_2m$  complex and TAP (Ortmann, Androlewicz and Cresswell, 1994; Sadasivan *et al.*, 1996; Ortmann *et al.*, 1997) by simultaneous binding of TAP and calreticulin (Ortmann, Androlewicz and Cresswell, 1994; Sadasivan *et al.*, 1996; Ortmann *et al.*, 1997; Koch *et al.*, 2004). In this way, the Tapasin-ERp57 complex tethers the TAP – which is transporting peptides – to the empty MHC-I molecule which will receive the peptides. Tapasin has binding sites on the  $\alpha_2$  and  $\alpha_3$  domains of the MHC class I heavy chains (Peace-Brewer *et al.*, 1996; Kulig *et al.*, 1998; Suh *et al.*, 1999; Varela-Rohena *et al.*, 2008) and on the N-terminal domains of the TAP subunits (Koch *et al.*, 2004). The presence of tapasin increases the quantity of peptides that can be presented by most MHC molecules (Barber *et al.*, 2001). However, in addition to its tethering role, tapasin has a role in optimisation – ensuring that high-affinity peptides are preferentially presented,

thus mediating both quantity and quality of peptide presentation (Barber *et al.*, 2001; Williams *et al.*, 2002; Howarth *et al.*, 2004; Chen and Bouvier, 2007; Wearsch and Cresswell, 2007; Praveen *et al.*, 2010). Tapasin is thought to bind to the MHC-I binding groove, enforcing an open conformation and causing lower-affinity peptides to dissociate. Higher affinity peptides can overcome this energy barrier and their binding induces maturing conformational changes in the MHC that induces its release from the PLC (Elliott *et al.*, 1991, 1992; Rigney *et al.*, 1998; Chen and Bouvier, 2007). Kinetically stable pMHC complexes can then be released from the PLC to make their way to the cell surface for scrutiny by TCRs (Chen and Bouvier, 2007; Wearsch and Cresswell, 2007; Hulpke and Tampé, 2013). Tapasin is assisted in its role by an additional, related, MHC-I-peptide editor, TAPBR (Boyle *et al.*, 2013; Hermann *et al.*, 2013, 2015).

#### 1.4 – The TCR-pMHC interaction

At the heart of the adaptive immune response is the interaction between the  $\alpha\beta$  TCR and the pMHC. This interaction was alluded to by a series of studies in the 1970s (discussed below). One such study demonstrated that an interaction between cytotoxic T cells and somatic cells infected with lymphocytic choriomeningitis only occurred if the two cell types share expression of at least one H-2 allele (a mouse MHC variant) (Zinkernagel and Doherty, 1974).

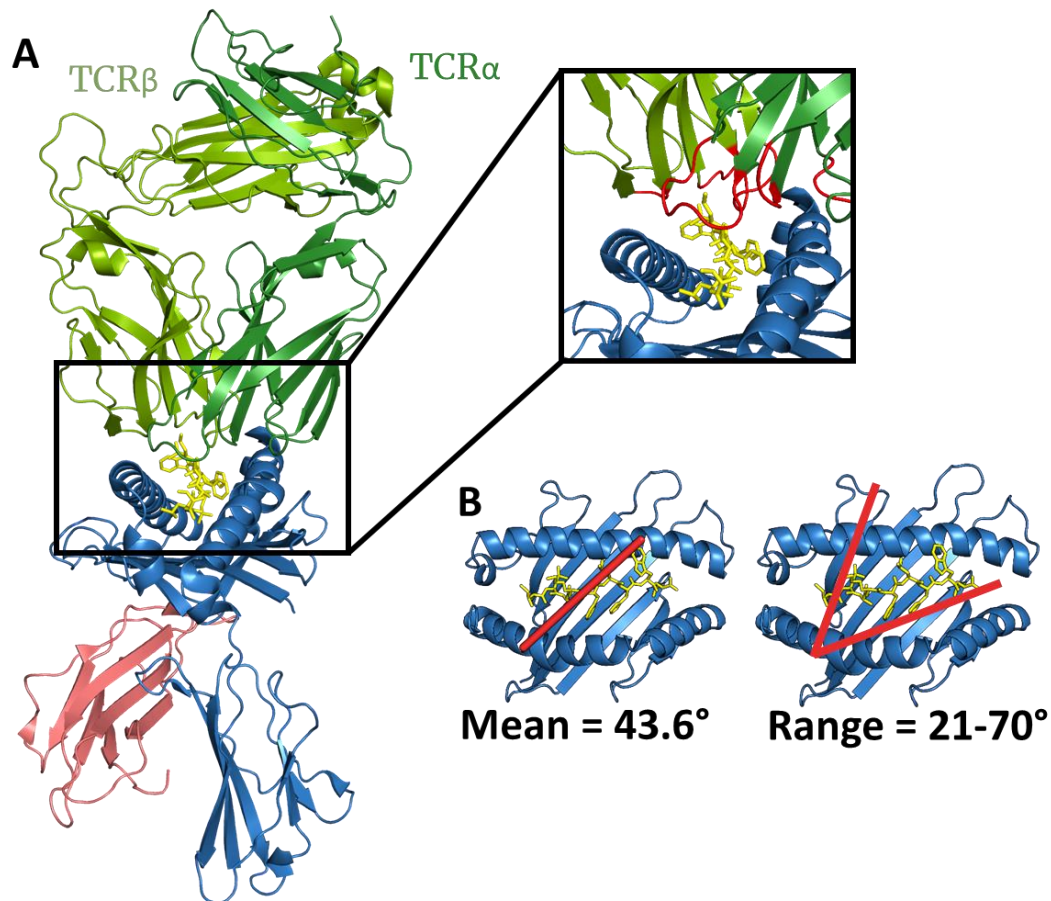
The TCR itself remained elusive for another decade (Hedrick *et al.*, 1984; Yanagi *et al.*, 1984). By the 1980s, monoclonal antibodies raised against T cell lines had identified disulphide linked heterodimers with immunoglobulin-like constant and variable domains (Acuto *et al.*, 1983; McIntyre and Allison, 1983). These antibodies were shown to be able to stimulate and inhibit T cell functions, suggesting they targeted the unknown surface receptor (Allison, McIntyre and Bloch, 1982; Infante *et al.*, 1982; Haskins *et al.*, 1983; Kaye *et al.*, 1983; Meuer *et al.*, 1983; Samelson, Germain and Schwartz, 1983). Hendrick *et al.* used  $^{32}\text{P}$ -labelled cDNA probes to isolate genes that were expressed in T but not B cells, encoded a membrane-bound polypeptide that would be amendable to somatic gene recombination (similar to what had been described for B cell receptors (Hozumi and Tonegawa, 1976; Tonegawa, 1983)); and would have variable and constant regions. Using this method they successfully isolated the TCR (Hedrick *et al.*, 1984).

The TCR-pMHC interaction serves to bring the T cell into proximity with its target cell, and facilitates T cell activation (Davis and Bjorkman, 1988; Garcia, Teyton and Wilson, 1999). In the case of the CD8<sup>+</sup> T cell, TCR interactions with pMHC-I can mediate killer cell functions

such as target cell lysis (see **Section 1.2.2**) (Davis and Bjorkman, 1988; Garcia, Teyton and Wilson, 1999). The interaction is also central to the process of maturation and selection that thymocytes undergo whilst developing in the thymus (see **Section 1.2.3**).

Multiple structural studies of the TCR-pMHC interaction have collectively revealed a relatively conserved binding mode, **Figure 1.5A** (Garboczi *et al.*, 1996; Garcia *et al.*, 1996, 1998; Ding *et al.*, 1998; Teng *et al.*, 1998). The variable domain of the TCR  $\alpha$ -chain docks over the  $\alpha 2$  helix of the MHC and the N-terminal region of the presented peptide. The variable domain of the TCR  $\beta$ -chain, in contrast, docks over the  $\alpha 1$  helix of the MHC and the C-terminal region of the peptide (Hong *et al.*, 1992; Sun *et al.*, 1995; Sant'Angelo *et al.*, 1996; Garcia, Teyton and Wilson, 1999; Rudolph, Stanfield and Wilson, 2006).

The 'contact zone' on the TCR is formed by its variable domains, the six hypervariable CDR loops form the antigen-binding site **Figure 1.5A inset** (see **Section 1.2.2**) (Garboczi *et al.*, 1996; Garcia *et al.*, 1996; Ding *et al.*, 1998). The germline-encoded CDR1 and CDR2 loops mainly contact the MHC surface (Garboczi *et al.*, 1996; Garcia *et al.*, 1996; Ding *et al.*, 1998), although CDR1 and CDR2 loops have been shown to contact both the MHC and the peptide (Ding *et al.*, 1998; Cole *et al.*, 2009; Cole, Van Den Berg, *et al.*, 2017). CDR3 loops principally form contacts with the presented peptide (Garboczi *et al.*, 1996; Garcia *et al.*, 1996; Ding *et al.*, 1998). As the peptide tends to sit in the MHC binding groove anchored at either end with the central region of the peptide bulging out of the groove (Garcia *et al.*, 1996; Ding *et al.*, 1998; Hausmann *et al.*, 1999; Speir *et al.*, 2001), most interactions occur between the TCR and central peptide residues with upward facing side chains (Degano *et al.*, 2000; Chen *et al.*, 2005; Tynan, Borg, *et al.*, 2005; Cole *et al.*, 2016; Cole, Fuller, *et al.*, 2017; Madura *et al.*, 2019), although many exceptions to this rule exist (Hahn *et al.*, 2005; Yili Li *et al.*, 2005; Ekeruche-Makinde *et al.*, 2012; Gras *et al.*, 2016; Harris *et al.*, 2016).



**Figure 1.5: The TCR-pMHC interaction.** **A)** shows the consensus binding mode between TCR and pMHC. The antigen binding site is formed by the six CDR loops, three CDR $\alpha$  and three CDR $\beta$  (inset, red). **B)** The  $\alpha\beta$  TCR adopts an approximately diagonal orientation when interacting with pMHC. Image generated using PDB 5MEN. Crossing angles calculated by Dr Bruce MacLachlan.

When the  $\alpha\beta$  TCR binds the pMHC class I complex it tends to adopt an approximately diagonal orientation relative to the peptide, **Figure 1.5B**, although there is much variability with little difference in the ability of the complex to promote T cell activation (Garboczi *et al.*, 1996; Ding *et al.*, 1998; Teng *et al.*, 1998; Rudolph and Wilson, 2002; Rudolph, Stanfield and Wilson, 2006).

The consensus manner of binding thus described led to the proposal of a two-step binding model in 2002 (Wu *et al.*, 2002). This model suggests that the TCR initially forms transient contacts with the surface of the MHC, holding the TCR in close proximity and thus allowing the 'scanning' of the presented peptide (Wu *et al.*, 2002). This model has been subject to much controversy. For example, in the case of super-bulged peptides where centrally bulging



peptides in the peptide-binding groove can act as an obstacle, impeding TCR access to the MHC (Tynan, Burrows, *et al.*, 2005). The consensus binding mode has been challenged further by Cole *et al.* who showed that affinity-enhanced TCRs whose enhanced affinity was mediated by an increase in TCR-MHC contacts still exhibited exquisite peptide-specificity (Cole *et al.*, 2014). These results were inconsistent with the two-step binding model (Wu *et al.*, 2002) leading the authors to propose an alternative model, known as the “synchronised docking” model, in which there is no temporal difference between the binding of TCR-MHC and that of TCR-peptide (Cole *et al.*, 2014).

The interaction between the TCR and pMHC can be highly plastic. To facilitate engagement of a pMHC ligand, a TCR can undergo substantial conformational changes – principally within its CDR loops (Garcia *et al.*, 1998; Reiser *et al.*, 2003; Colf *et al.*, 2007; Ayres *et al.*, 2016). It may be supposed that a requirement for conformational change might result in a less potent interaction due to the entropic cost of the movement. However, Cole *et al.* showed in 2017 that the naturally-occurring high-affinity HIV-specific 868 TCR undergoes extensive CDR rearrangement in order to engage its ligand (Cole, Fuller, *et al.*, 2017).

Flexibility can also occur on the side of the MHC (Hawse *et al.*, 2013; Van Hateren *et al.*, 2017; Hopkins *et al.*, 2020). The human T-lymphotropic virus (HTLV)-1 Tax peptide (LLFGYPVYV), and the *Saccharomyces cerevisiae* peptide Tel1p (MLWGYLQYV) are both presented by HLA-A2 to the human  $\alpha\beta$  TCR, A6 (Borbulevych *et al.*, 2009). When both peptides were crystallised in complex with HLA-A2 they showed superimposable structures. However, when these pMHC-I pairs were bound to the A6 TCR they showed a substantially different interface of contacts, facilitated by a conformational change of the HLA-A2  $\alpha$ -helices when in complex with Tel1p but not Tax (Borbulevych *et al.*, 2009). Many other examples of MHC flexibility have been described (Hülsmeier *et al.*, 2002; Pöhlmann *et al.*, 2004; Tynan, Borg, *et al.*, 2005; Borbulevych *et al.*, 2011; Kass, Buckle and Borg, 2014).

Peptide conformational changes can also occur within this interaction, such as the flattening of super-bulged peptides (Tynan *et al.*, 2007), and the ‘flexing’ of the peptide to occupy different binding groove pockets (Madura *et al.*, 2019)

The natural affinity of functional TCR-pMHC-I interactions tends to range between  $K_D \sim 0.1$ – $100 \mu\text{M}$  (Weber *et al.*, 1992; Corr *et al.*, 1994; Sykulev *et al.*, 1994; Alam *et al.*, 1996; Garcia *et al.*, 1997, 2001; Kersh *et al.*, 1998; Boniface *et al.*, 1999; Willcox *et al.*, 1999; Ding *et al.*, 1999; van der Merwe and Davis, 2003; Cole *et al.*, 2007; Bridgeman *et al.*, 2011). This is low relative to antibody-ligand affinities (Goldman *et al.*, 1997; Conte, Chothia and Janin, 1999).

The range of off-rates observed for the TCR-pMHC interaction is narrow ( $\sim 0.01\text{-}0.73\text{ s}^{-1}$ ), with a mean of  $0.24\text{ s}^{-1}$  (Bridgeman *et al.*, 2011). It has been suggested that the off-rate of the interaction must fall within a narrow 'window' so that the interaction is long enough to allow the induction of intracellular signal transduction but brief enough to allow each pMHC to be 'scanned' by, and trigger, multiple TCRs in a short time (Valitutti *et al.*, 1995; Cole *et al.*, 2007).

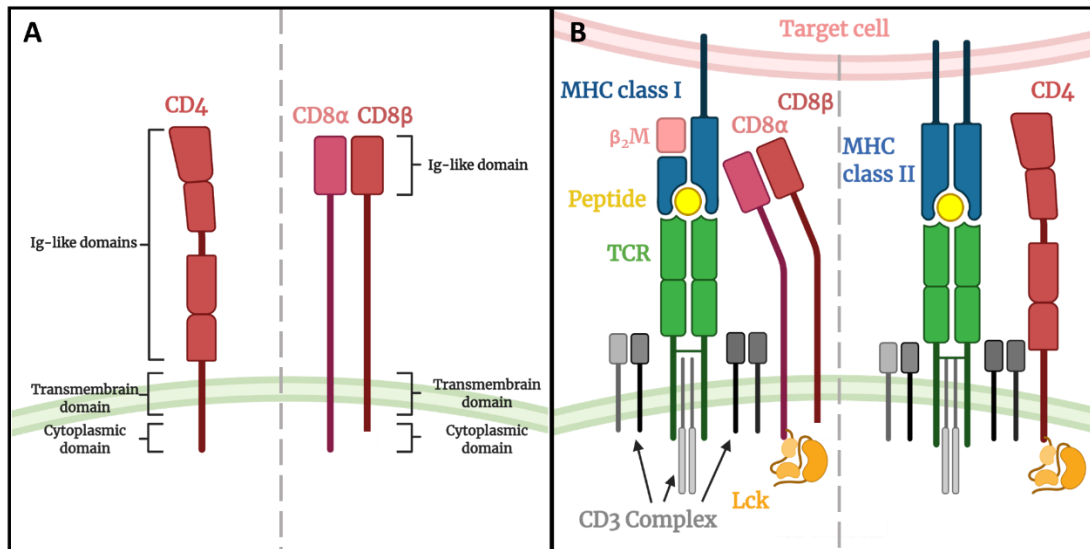
#### 1.4.1 – The co-receptors

Another important consideration for the TCR-pMHC interaction is the role of the co-receptors. As mentioned briefly above, CD4 and CD8 both phenotypically characterise the two major subsets of  $\alpha\beta$  T cells and dictate which class of MHC they interact with. CD4 specifically recognises MHC class II molecules, whilst CD8 specifically recognises MHC class I molecules (Wettstein *et al.*, 1978; Swain and Panfili, 1979; Swain, 1980; Engleman *et al.*, 1981).

An interesting series of observations prompted the suggestion of an interaction between MHC-I and CD8 long before it was confirmed crystallographically. Firstly, it was observed that mature T cells tended to express either CD8 or CD4 (Cantor and Boyse, 1977; Reinherz *et al.*, 1979, 1980). Amounting evidence in the 1970s and 80s demonstrated that MHC-I-expressing cells associated with CD8<sup>+</sup> T cells and MHC-II with CD4 (Swain, 1983). The interaction between co-receptor and respective MHC was then demonstrated by cell-adhesion experiments (Doyle and Strominger, 1987; Norment *et al.*, 1988). Antibodies against CD4 or CD8 could block T cell functions against MHC-II<sup>+</sup> or MHC-I<sup>+</sup> cells respectively (Engleman *et al.*, 1981; MacDonald, Thiernesse and Cerottini, 1981; Swain, 1981; Krensky *et al.*, 1982; Meuer, Schlossman and Reinherz, 1982; Wilde *et al.*, 1983; Swain *et al.*, 1984; Rosenstein *et al.*, 1989). It was further observed that gene transfer of CD8 could redirect a MHC-II-reactive T cell to MHC-I<sup>+</sup> target cells (Gabert *et al.*, 1987).

In 1988, Connolly *et al.* showed that a point mutation in the  $\alpha 3$  domain of the MHC molecule (E227K) could abrogate binding to CD8 and subsequent killing by cytotoxic lymphocytes (Connolly *et al.*, 1988). A negatively charged loop formed by residues 222-228 within the  $\alpha 3$  domain was suggested as the binding site for CD8 (Salter *et al.*, 1990). This theoretical binding location was further supported by the crystallisation of CD8, demonstrating that the proposed binding manner would be feasible (Leahy, Axel and Hendrickson, 1992). Finally, this successive series of events culminated in the 1997 crystallisation of a HLA-A2-CD8

complex that visually demonstrated MHC-I binding to the co-receptor (Gao *et al.*, 1997). CD4 has also been crystallised in complex with MHC-II (Yin, Wang and Mariuzza, 2012).



**Figure 1.6: The co-receptors. A)** Schematic representation of the CD4 and CD8 co-receptors. CD4 exists as a single polypeptide chain folded into four immunoglobulin (Ig)-like domains. CD8 exists as a heterodimer. Both co-receptors have transmembrane domains, and cytoplasmic tails that associate with the cytosolic Src family protein tyrosine kinase, Lck. **B)** This association allows the juxtapositioning of Lck and CD3 ITAMs when either co-receptor binds their respective MHC.

On conventional cytotoxic T cells, CD8 exists as a heterodimer made up of CD8α and CD8β chains, **Figure 1.6A** (Fung-Leung *et al.*, 1991; Moebius *et al.*, 1991; Casey Crooks and Littman, 1994; Nakayama *et al.*, 1994). As discussed above, CD8 binds primarily to the α3 domain of MHC-I (Gao *et al.*, 1997; Wang, Natarajan and Margulies, 2009). CD4 binds the α2 and β2 regions of MHC-II near to the membrane of the antigen presenting cell (Wang *et al.*, 2001, 2011). Thus, in both cases, the peptide-MHC complex can be simultaneously bound by both the TCR and its associated co-receptor, **Figure 1.6B**.

CD8 interacts with pMHC-I with relatively low affinity ( $K_D \sim 150 \mu M$ ) (Wyer *et al.*, 1999; Huang, Edwards, *et al.*, 2007; Li, Yin and Mariuzza, 2013), with respect to most TCR-pMHC interactions (Wooldridge, Clement, *et al.*, 2010; Wooldridge, Laugel, *et al.*, 2010). Evidence presented by Wooldridge *et al.* in 2010 suggested that this low affinity is actually an evolutionary advantage, and in fact essential for the maintenance of TCR specificity

(Wooldridge, Clement, *et al.*, 2010; Wooldridge, Laugel, *et al.*, 2010). Artificially increasing the strength of the CD8-MHC interaction resulted in non-specific binding of the TCR to pMHC and T cell activation, irrespective of the peptide presented. Thus, the low affinity of the CD8-MHC interaction is essential to maintain specificity of CD8<sup>+</sup> T cells (Wooldridge, Clement, *et al.*, 2010).

The co-receptors are thought to enhance the kinetics of the TCR-pMHC engagement (Veillette *et al.*, 1989; Holler and Kranz, 2003; Wooldridge *et al.*, 2005; Wooldridge, Clement, *et al.*, 2010; Wooldridge, Laugel, *et al.*, 2010). This is achieved in a number of ways. Despite its low affinity for MHC-I, CD8 can contribute to an enhanced overall avidity of the interaction (Holler and Kranz, 2003; Li, Yin and Mariuzza, 2013). Laugel *et al.* showed that TCRs with high affinities for their cognate pMHC could be labelled with pMHC tetramers despite mutations to remove CD8 from the interaction (Laugel *et al.*, 2007). However, in incidences where the TCR-pMHC affinity was low (>200 $\mu$ M), the presence of the co-receptor was essential to T cell activation (Laugel *et al.*, 2007).

By recruiting downstream signalling molecules to the localised site of the interaction (see **Section 1.2.2**), CD8 can lower the threshold needed for T cell activation by facilitating the propagation of the signalling cascade (Artyomov *et al.*, 2010; Jiang *et al.*, 2011). Blocking the binding of CD8 to the  $\alpha$ 3 domain of MHC-I can reduce some, but not all, T cell functions (Xu *et al.*, 2001). It has been postulated that the evolutionary benefit conferred by co-receptor-mediated recruitment of downstream signalling molecules might have influenced the binding conformation of the TCR-pMHC-I interaction. As discussed in **Section 1.2.2**, the cytoplasmic tail of the co-receptor sequesters Lck (Veillette *et al.*, 1989; Van Laethem *et al.*, 2013). Lck phosphorylates the ITAMs on the cytoplasmic tail of the TCR-associated CD3 (Barber *et al.*, 1989; Straus and Weiss, 1993; Purbhoo *et al.*, 2001; Artyomov *et al.*, 2010). Thus, interactions that align CD8 in proximity to CD3 (see **Figure 1.6B**) may be evolutionary favourable and contribute to the conserved docking mode observed in so many TCR-pMHC interactions (Garboczi *et al.*, 1996; Ding *et al.*, 1998; Rudolph and Wilson, 2002; Rudolph, Stanfield and Wilson, 2006; Van Laethem *et al.*, 2007; Li, Yin and Mariuzza, 2013).

#### 1.4.2 – MHC-restriction

The propensity of  $\alpha\beta$  TCRs to bind antigen in the context of MHC molecules is known as MHC-restriction (Zinkernagel and Doherty, 1974; Kappler and Marrack, 1976). MHC-restriction was first demonstrated by a series of studies in the 1970s. In 1973, Rosenthal and Shevach

highlighted the requirement of antigen presentation on histocompatible macrophages for T cell activation (Rosenthal and Shevach, 1973). Ern and Feldmann subsequently demonstrated that histocompatible, but not allogenic, macrophages could activate CD4<sup>+</sup> T cells (Erb and Feldmann, 1975b, 1975a, 1975c). Thymic grafting between mice of different strains was used to show that T cells develop MHC-restriction in the thymus during their maturation process (Fink and Bevan, 1978). The *K* and *D* regions were isolated as the specific regions within the H-2 complex that needed to match between target cell and T cell in order to facilitate T cell activation (Doherty, Blanden and Zinkernagel, 1976). A number of studies were published showing that the T cell response to virus occurred only with MHC-matched target cells (Doherty, Zinkernagel and Ramshaw, 1974; Shearer, 1974; Wainberg *et al.*, 1974; Zinkernagel and Doherty, 1974; Koszinowski and Ertl, 1975; Lewandowski, Gerhard and Palmer, 1976). The preference for self-MHC was further demonstrated in mice, where heterozygous AB stem cells were used to repopulate a non-lethally irradiated strain A mouse. T cell activity was mainly detected against cells expressing the MHC of strain A cells, little or no reactivity was detected against strain B cells (Bevan, 1977; Zinkernagel, G. Callahan, *et al.*, 1978; Zinkernagel, G. N. Callahan, *et al.*, 1978).

The **Dual Recognition Theory** was proposed by Katz, in order to explain MHC-restriction. It suggested that a T cell might express two receptors – one to recognise the self-MHC and one to recognise the foreign antigen (Katz and Armerding, 1976; Katz, Dixon and Kunkel, 1977; Fink and Bevan, 1978). This hypothesis was largely superseded by the **Altered-Self Theory**, proposed by Doherty *et al.* which suggested that T cells had only one receptor that could recognise the antigen-MHC as a complex (Doherty and Zinkernagel, 1976; Doherty, Blanden and Zinkernagel, 1976). The altered-self theory to explain MHC-restriction was ultimately confirmed with the publication of TCR-pMHC complex structures demonstrating how the  $\alpha\beta$  TCR can simultaneously engage self-MHC and foreign peptide (Garboczi *et al.*, 1996; Garcia *et al.*, 1996). How MHC restriction is imposed is generally debated by two key theories, the **Germ-line Encoded Theory** and the **Selection Theory** (La Gruta *et al.*, 2018).

The germ-line encoded theory of MHC restriction proposes that  $\alpha\beta$  TCRs have evolved a preference for MHC recognition (Jerne, 1971; La Gruta *et al.*, 2018). This theory is supported by the conserved binding mode observed for the interaction that was revealed by the first crystal structures in the 1990s (Garboczi *et al.*, 1996; Ding *et al.*, 1998). The germ-line encoded theory poses that MHC-restriction is driven by pairwise interactions between evolutionary conserved residues in both of the interacting molecules (Huseby *et al.*, 2005). These so-called 'interaction codons' are a product of side-by-side evolution of the MHC and

TCR, and structural evidence in their favour was published in 2007 by Feng *et al.* in a study involving four V $\beta$ 8.2 TCRs (Feng *et al.*, 2007). Feng *et al.* observed a number of structurally superimposable interactions amongst these complexes, and proposed that these were germline-encoded interaction codons (Feng *et al.*, 2007). A similar observation was made of 12 TCR-pMHC complexes in 2016 (Adams *et al.*, 2016). Interaction codons were also proposed to explain why certain TCR chains tend to interact with certain MHC alleles, explaining why TCRs tend to be restricted to a single MHC allele (Garcia *et al.*, 2009).

Subsequent examples of interaction codons bolstered support for the germ-line encoded theory of MHC restriction (Dai *et al.*, 2008; Marrack *et al.*, 2008; Scott-Browne *et al.*, 2009; Yin, Li and Mariuzza, 2012). In 2005, Tynan *et al.* observed that three MHC positions (65, 69, and 155) invariably made contact with the TCR in all TCR-pMHC-I structures that had been described at the time. They postulated that this might represent the minimum binding footprint required for the interaction and termed the three residues the “restriction triad” (Tynan, Burrows, *et al.*, 2005). Mutating the conserved contact regions was shown to significantly diminish the size of the naïve T cell pool (Scott-Browne *et al.*, 2009, 2011; Yin *et al.*, 2011). In further evidence for the germ-line encoded theory, other classes of TCR interact with other classes of MHC molecules. For example, mucosal-associated invariant T cells (MAITs) which recognise metabolites in the context of MHC class I-related (MR1), and CD1-restricted T cells which respond to lipid antigen (McMichael *et al.*, 1979; Treiner *et al.*, 2003; Gold *et al.*, 2010; Rossjohn *et al.*, 2012; Godfrey *et al.*, 2015; Van Rhijn *et al.*, 2015). The fact that other classes of TCR also interact with ligands in the context of MHC-like models is often cited evidence in support of the germ-line theory.

The contrasting argument, the selection theory, proposes that MHC-restriction is not an intrinsic feature of TCRs but rather a feature that is imposed upon them during the selection process in the thymus, see **Section 1.2.3** (La Gruta *et al.*, 2018). Support for this theory comes from studies showing that the interaction codons are not essential, and structural compensations can be made to accommodate mutations in these residues (Van Laethem, Tikhonova and Singer, 2012). In fact, Rossjohn’s group queried their own 2005 study (Tynan, Burrows, *et al.*, 2005) which first proposed the restriction triad by demonstrating that these residues could be mutated with minimal destructive effect on the interaction (Burrows *et al.*, 2010). Further support for the selection theory comes from evidence that only around 30% of the preselection TCR repertoire is MHC-restricted (Merkenschlager *et al.*, 1997; Zerrahn, Held and Raulet, 1997), suggesting that the selection process itself is responsible for these relatively few TCRs dominating the mature repertoire. Additionally several incidences of

unconventional  $\alpha\beta$  TCRs have been described in the literature (Beringer *et al.*, 2015; Gras *et al.*, 2016), including  $\alpha\beta$  TCRs that are not actually MHC-restricted (Barnd *et al.*, 1989; Jerome, Domenech and Finn, 1993; Magarian-Blander *et al.*, 1998; Hanada *et al.*, 2011).

The selection theory proposes that MHC-restriction occurs because the co-receptors, CD8 and CD4, sequester a key component of TCR signalling – Lck (Van Laethem, Tikhonova and Singer, 2012; Van Laethem *et al.*, 2013; Rangarajan and Mariuzza, 2014; La Gruta *et al.*, 2018). As briefly discussed in **Section 1.2.2**, Lck is a protein tyrosine kinase that initiates the signal transduction pathway in both immature thymocytes and mature T cells (Rudd *et al.*, 1988). Successful TCR signalling requires Lck, which tends to associate with the cytoplasmic tails of the co-receptors (Turner *et al.*, 1990; Miceli, Von Hoegen and Parnes, 1991; Van Laethem, Tikhonova and Singer, 2012; Van Laethem *et al.*, 2013; Rangarajan and Mariuzza, 2014; La Gruta *et al.*, 2018). Lck initiates T cell signal transduction by phosphorylating regions within the cytosolic tail of the TCR/CD3 complex, resulting in the recruitment of downstream signalling molecules such as ZAP-70 (Meuer *et al.*, 1983; Abraham *et al.*, 1991; Chan *et al.*, 1992; Van Laethem *et al.*, 2013).

When a TCR recognises a ligand in the context of an MHC molecule, the co-receptor also binds the MHC (Rosenstein *et al.*, 1989), as such extracellular MHC-co-receptor engagement brings intracellular co-receptor bound Lck into the vicinity of the ITAMs on the cytoplasmic tail of CD3 (see **Section 1.2.2**) (Artyomov *et al.*, 2010). This intracellular juxtaposition gives MHC co-receptor-dependent signalling an advantage over co-receptor-independent signalling, in which “free” Lck must be passively captured by an activated TCR before signal transduction can begin (Lee, Loh and Lacy, 1992; Van Laethem, Tikhonova and Singer, 2012; Van Laethem *et al.*, 2013; Rangarajan and Mariuzza, 2014; La Gruta *et al.*, 2018). Deletion of the co-receptors, allowed thymocytes to differentiate into mature  $\alpha\beta$  T cells carrying TCRs that could recognise non-MHC ligands (Van Laethem *et al.*, 2007, 2013; Van Laethem, Tikhonova and Singer, 2012). Furthermore, van Laethem *et al.* showed that if the two cysteine residues in the N-terminal region of the Lck protein (that are used to associate with CD8 and CD4) are mutated a MHC-independent  $\alpha\beta$  TCR repertoire is generated (Van Laethem *et al.*, 2013). The selection theory proposes that V(D)J recombination produces a broad range of  $\alpha\beta$  TCRs but that only those that are MHC-restricted survive thymic selection (Van Laethem *et al.*, 2007)

The germ-line encoded and the selection theories of MHC-restriction both offer up compelling evidence and it is likely to be a combination of the two that contribute to the

MHC-restriction so commonly seen amongst  $\alpha\beta$  TCRs. Regardless of how it is imposed, the characteristic MHC-restriction is undoubtedly a driving factor behind the specificity of TCRs in the peripheral population of T cells.

#### 1.4.3 – Specificity and crossreactivity within the TCR-pMHC interaction

In order to maintain host health, the immune system must comprise of enough TCRs to specifically recognise any potential pathogen that could be encountered. Incomplete immune coverage would quickly be exploited by opportunistic pathogens. Ideas concerning how this comprehensive immune coverage is achieved have evolved over time.

In 1955 Neils Kaj Jerne described the natural selection theory of antibody formation (Jerne, 1955). Jerne postulated that instead of manufacturing antibodies (and by extrapolation, TCRs) in response to a newly-encountered antigen (Talmage, 1957), a host already possessed the full cohort of immunoreceptors that would be required to combat any foreign antigen it might encounter (Jerne, 1955). The exposure of the immune system to an antigen would result in the selection of the immunoreceptor best fit to fight it, from the existing pool of antibodies (Jerne, 1955). In 1959, Frank Macfarlane Burnet proposed the clonal selection theory of acquired immunity (Burnet, 1959). Burnet suggested that that when an antigen was encountered the immune cell specific for that antigen would undergo clonal expansion (Burnet, 1959). Whilst cementing the concept of specificity in the acquired immune response, this theory propagated the assumption that an immune receptor would be highly specific, responding to a single antigen (Burnet, 1959, 1976).

The clonal selection theory satisfied the question of specificity and was generally accepted by the field. However, in 1998 Don Mason and others demonstrated that the number of theoretical pMHC targets that could be encountered far exceeds the number of unique TCRs that exist within the host's immune system (Mason, 1998; Arstila *et al.*, 1999; Sewell, 2012). There are  $10^{12}$  T cells in humans, carrying  $\sim 10^7$  unique TCRs. Between them these TCRs must recognise  $>10^{15}$  possible foreign antigen in order to provide comprehensive immune coverage and avoid potentially fatal immunological gaps (Sewell, 2012; Wooldridge, 2013). Mason argued that whilst somatic gene recombination could theoretically produce sufficient variability (Arstila *et al.*, 1999; Turner *et al.*, 2006; Bhati *et al.*, 2014), the number of individual T cells (each expressing a single highly specific TCR) required to meet the clonal selection requirements would be more than the host body could physically accommodate (Mason, 1998). Drawing on other evidence (Bhardwaj *et al.*, 1993; Reay, Kantor and Davis, 1994;



Wucherpfennig and Strominger, 1995; Kersh and Allen, 1996), Mason called for the abandonment of the belief that every single immunogenic peptide must have its own unique TCR. He instead proposed that TCRs could recognise multiple peptides, (Mason, 1998), and that crossreactivity must be an essential feature of T cell immunity.

#### 1.4.4 – How is crossreactivity achieved?

Many examples of crossreactivity in T cells have since been described in the literature. In 1995, a myelin basic protein (MBP)-specific T cell clone isolated from a multiple sclerosis patient was shown to recognise a number of other peptides (Wucherpfennig and Strominger, 1995). These included 7 viral- and 1 bacterial-derived peptide, indicating that molecular mimicry between T cell epitopes and self-antigen might underlie some incidences of autoimmunity, see **Section 1.5.2** (Wucherpfennig and Strominger, 1995). The following year Kersh and Allen showed that the 3.L2 T cell clone could respond to 12 altered peptide ligands (APLs) (Kersh and Allen, 1996).

One way that TCRs might achieve crossreactivity is by focused binding to peptide ‘hotspots’. This describes a situation in which the TCR only recognises a few residues of the peptide. The same TCR could theoretically recognise all other peptides which contained the essential motif, allowing for variation at other residues of the peptide outside of the hotspot. For example, the type I diabetes-associated 1E6 T cell clone can recognise over 1 million peptides (Wooldridge *et al.*, 2012) and does so largely by focusing on a central three-residue peptide motif, GPD (Cole *et al.*, 2016).

TCRs might also exhibit crossreactivity by being flexible in their binding mode. For example, the murine 2C TCR adopts different conformations when in complex with self (L<sup>d</sup>-QL9) and non-self (K<sup>b</sup>-dEV8) targets (Colf *et al.*, 2007). The two peptides included in this study were entirely distinct, differing at every residue. When interacting with QL9 the 2C TCR focused mainly on the C-terminal region. Whereas the interaction with dEV8 spanned the length of the peptide (Colf *et al.*, 2007). In another example of a TCR seeing two peptides with completely distinct sequences, the BM3.3 TCR recognised INFD<sup>d</sup>FNTI and RGYVYQGL peptides in the context of H-2K<sup>b</sup> via CDR3 $\alpha$  loop flexibility (Reiser *et al.*, 2003). Crossreactivity in the context of antipathogenicity and autoimmunity is discussed in further detail in **Sections 1.5** and **4.1**.

## 1.5 – Application of the TCR-pMHC interaction

The TCR-pMHC interaction has a fundamental role in the immune response, it can be highly effective against pathogens and neoplasms. The TCR or pMHC can also be manipulated and employed as a therapeutic. The generic T cell response, including mechanisms of cytotoxicity, is discussed in **Section 1.2.2**.

### 1.5.1 – The TCR-pMHC interaction in immunity

Viruses are obligate intracellular pathogens and the CD8<sup>+</sup> T cell response to viruses is well characterised. In fact, the murine lymphocytic choriomeningitis virus has provided a model system from which much of what is known about T cell responses has been derived (Marker and Volkert, 1973; Moskopodis *et al.*, 1987; Ashton-Rickardt *et al.*, 1994; van den Broek *et al.*, 1995; Butz and Bevan, 1998; Gallimore *et al.*, 1998; Williams, Tyznik and Bevan, 2006). The TCR-pMHC interaction in the context of a number of example viral responses is discussed below.

Influenza virus (IVs) are common human pathogens (Grant *et al.*, 2016). Influenza viruses undergo rapid antigenic drift allowing them to continually evade the immune system (Taubenberger and Kash, 2010). Despite this, CD8<sup>+</sup> T cells are demonstrably important in controlling IV infections (McMichael *et al.*, 1983; Sridhar *et al.*, 2013; Wang *et al.*, 2015). A large number of influenza-specific CD8<sup>+</sup> T cell epitopes have been described (Wu *et al.*, 2011; Grant *et al.*, 2013, 2014).

Given the CD8<sup>+</sup> T cell involvement in the response to IVs, efforts have been made to produce polypeptide vaccines that trigger a CD8<sup>+</sup> T cell response (Moriyama *et al.*, 2017; Skibinski *et al.*, 2018; Zheng *et al.*, 2019). In 2018 Miles *et al.* proposed that non-natural amino acids, that were resistance to gastric degradation, could be delivered by oral administration (Miles *et al.*, 2018). A T cell clone specific for a matrix protein 1 (M1) epitope, the HLA-A2–restricted GILGFVFTL<sub>58–66</sub> (GIL), was subjected to combinatorial peptide library (CPL) screening (see **Section 3.1.2**) with a D-amino acid library (Miles *et al.*, 2018). The resulting D-amino acid peptide (gppqwnpp; lower case letters used to indicate the D isomer in single letter amino acid code) bore little resemblance to the original GILGFVFTL peptide sequence, yet could stimulate GILGFVFTL-specific T cells clones and importantly was resistant to serum degradation for over 1 hour (Miles *et al.*, 2018). Oral administration of gppqwnpp was shown to protect mice against a lethal challenge with the influenza PR8 strain. This work

exemplifies how greater understanding of specific TCR-pMHC interactions in clinically relevant contexts has the potential to influence vaccine design.

In another example, strains of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) have caused two major outbreaks since the turn of the century, an epidemic in 2003 (Peiris *et al.*, 2003) and a global pandemic in 2019 (Dong and Bouey, 2020). Following the 2003 epidemic, SARS-CoV-specific CD8<sup>+</sup> T cell responses were detected in patients who had recovered from the infection (Huang, Cao, *et al.*, 2007; Yang *et al.*, 2007). A number of HLA-A2-restricted spike protein epitopes were identified (Wang *et al.*, 2004; Tsao *et al.*, 2006; Lv *et al.*, 2009). These include HLA-A2-ILPDPLKPT<sub>(S787-795)</sub> and HLA-A2-KLPDDFMGCV<sub>(S411-420)</sub> (Zhao *et al.*, 2010). These HLA-A2-restricted spike protein epitopes trigger specific CD8<sup>+</sup> T cells responses that can protect the host from infection (Zhao *et al.*, 2010). Since the beginning of the 2019 pandemic, wide ranging CD8<sup>+</sup> T cell responses to SARS-CoV have been detected in recovered patients (Kared *et al.*, 2021). Furthermore, vaccines developed against SARS-CoV have been shown to successfully trigger T cell responses (Ramasamy *et al.*, 2020).

Specific TCR-pMHC interactions have been described in many other viral infections including Epstein-Barr virus (EBV) (Steven *et al.*, 1997; Hislop *et al.*, 2002, 2005; Pudney *et al.*, 2005; Forrest *et al.*, 2018), varicella zoster virus (VZV; chicken pox) (Frey *et al.*, 2003; Chiu *et al.*, 2014), and herpes simplex virus-1 (HSV-1) (Ott *et al.*, 2017; Treat *et al.*, 2017; Russell *et al.*, 2018). Understanding the TCR-pMHC interactions involved in various human pathologies can be useful in directing the quest for therapeutic interventions. A viral pathogen of particular interest to this thesis is the human immunodeficiency virus (HIV) which is discussed in detail in **Appendix Section 7.1**.

### 1.5.2 – CD8<sup>+</sup> T cells in autoimmunity

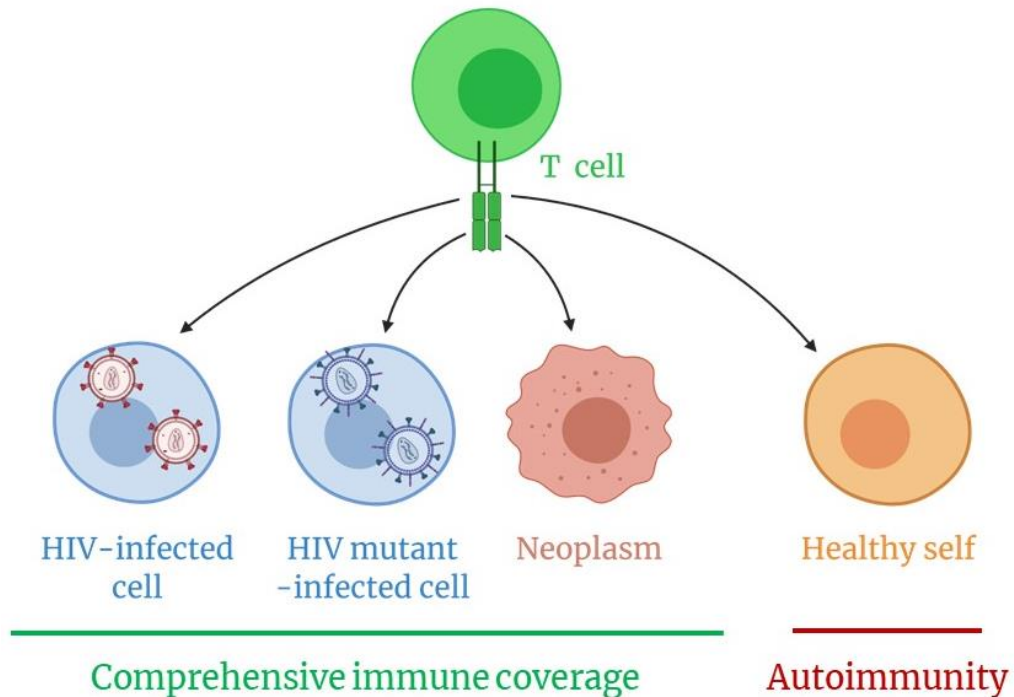
Viruses will evolve quickly to try to evade the immune system (viral escape mutations are discussed in the context of HIV in **Section 4.1.1**). However, not all viral escape mutations successfully avoid the immune system. As discussed in **Section 1.4.3**, this is because many TCRs are highly promiscuous. T cell crossreactivity (Mason, 1998; Sewell, 2012), can be of significant benefit as it can prevent immunological gaps, and allow the recognition of escape mutants or as-yet unencountered pathogens (Lee *et al.*, 2004; Varela-Rohena *et al.*, 2008; Wen *et al.*, 2017). However, this comprehensive immune coverage comes at a price.

Autoimmunity arises when crossreactive T cells unintentionally recognise self-antigen, see **Figure 1.7**.

Autoimmune diseases develop in approximately 5% of people (Goodnow, 2007). They are commonly classified based on the nature of their target antigen (Khan and Ghazanfar, 2018). They can be systemic – such as rheumatoid arthritis (RA) and multiple sclerosis (MS) – or organ-specific – such as type 1 diabetes (T1D), discussed in detail in **Section 3.1.1**, or Goodpasture’s syndrome (Khan and Ghazanfar, 2018).

Given the extensive variation generated by V(D)J gene recombination (see **Section 1.2.3**) (Tonegawa, 1983; Alt *et al.*, 1992; Lewis, 1994), the production of self-reactive TCRs is almost inevitable (Schwartz, 2012; Khan and Ghazanfar, 2018). To account for this, a number of regulatory mechanisms have evolved, including negative selection in the thymus, peripheral suppression through regulatory T cell subsets, and the requirement for co-stimulation for full T cell activation (Schwartz, 2012). Collectively these mechanisms achieve ‘self-tolerance,’ a notion first introduced in 1945 to describe how the immune system is able to ignore self (Owen, 1945).

In the 1950s, studies on transplant rejections in mice supported the idea that the immune system could tolerate self, whilst maintaining aggression against foreign antigens (Billingham, Brent and Medawar, 1954). In the context of Burnet’s clonal selection theory, proposed in the same decade, the logical assumption was that self-reactive clones must be eliminated by an undiscovered mechanism that functions to prevent autoimmunity (Burnet, 1959; Schwartz, 2012). Proof-of-concept for the clonal elimination theory occurred in 1987 when Kappler *et al.* showed that a specific TCR present in the immature thymocyte population would not emerge in the periphery if a high affinity ligand for that TCR was expressed in the thymus (Kappler, Roehm and Marrack, 1987). In 1994, staining for DNA strand breaks was used to demonstrate that negative selection occurs through apoptosis, and to localise the event to the thymic medulla (Surh and Sprent, 1994). The, now well-recognised, process of negative selection is discussed in **Section 1.2.3**.



**Figure 1.7: The advantages and disadvantages of crossreactivity.** T cell crossreactivity allows a limited number of specific TCRs to provide comprehensive immune coverage, protecting the host from any potential immune challenge that could be encountered – including viral escape mutants and neoplastic changes in host cells. The downside to crossreactivity is the risk of autoimmunity.

Despite this fairly robust system for removing self-reactive T cells, autoimmunity does occur. Autoreactive T cells can be found in the peripheral circulation of healthy individuals (Kitze *et al.*, 1988; Naquet *et al.*, 1988; Mason and Fowell, 1992; Fowell and Mason, 1993; King and Sarvetnick, 1997). It is also evident that if a self-antigen is not expressed in the thymus, a T cell cannot be tolerised to it, this includes both self-antigens that do not occur in the thymus and splice variants that may differ between tissues (Akkaraju *et al.*, 1997; Pugliese *et al.*, 1997; Klein *et al.*, 2000; Kamradt and Avrion Mitchison, 2001; Schwartz, 2012). Some antigens exist in immune privileged sites or are protected from the immune system by the blood-brain barrier, these can cause an immune reaction should they become aberrantly expressed elsewhere (Pachter, De Vries and Fabry, 2003; Benhar, London and Schwartz, 2012; Li, Wang and Han, 2012; Khan and Ghazanfar, 2018). These observations necessitate the existence of additional regulatory mechanisms to suppress autoreactive T cells that

escape thymic selection. Thymic selection is often referred to as central tolerance, to distinguish from autoreactive suppression that occurs in the periphery – known as peripheral tolerance (Schwartz, 2012).

Peripheral tolerance is executed by a subset of CD4-expressing T cells known as T regulatory cells (Tregs) (Schwartz, 2012). Cells with suppressive function were shown to populate the periphery in the 1990s (Powrie and Mason, 1990; Modigliani *et al.*, 1995; Sakaguchi *et al.*, 1995). Such cells intentionally recognise self-antigen and negatively influence any response in the vicinity of the ligand. This so-called ‘bystander suppression’ involves the secretion of inhibitory cytokines such as IL-4, IL-10, and TGF- $\beta$  (Schwartz, 2012).

If, despite peripheral suppression, a T cell does recognise an antigen in the periphery an additional layer of protection exists. A naïve T cell generally requires additional co-stimulation to achieve full activation, in the absence of these additional stimuli expressed by professional APCs presenting foreign antigen it will become senescent or undergo apoptosis (known as clonal deletion) (Lafferty and Woolnough, 1977; Ragazzo, 2001; Steinman, Hawiger and Nussenzweig, 2003; Schwartz, 2012). This ‘two signal’ model allowing discrimination between self and non-self has long been appreciated (Bretscher and Cohn, 1970; Lafferty, Misko and Cooley, 1974). In 1987, Jenkins and Schwartz demonstrated that chemically-fixed APCs presenting the appropriate antigen failed to activate T cells. These chemical-fixed presenting cells were metabolically inactive and thus unable to produce proinflammatory cytokines or upregulate their expression of co-stimulatory molecules (Jenkins and Schwartz, 1987). From these, and other, experiments it became clear that engagement of a suitable pMHC target is insufficient to activate naïve T cells without additional costimulatory signals (Bach *et al.*, 1977; Lafferty and Woolnough, 1977; Roska and Lipsky, 1985; June *et al.*, 1987; Mueller, Jenkins and Schwartz, 1989). The two signal model is discussed further, in the context of T1D, in **Section 3.1.2**.

One well-described co-stimulatory interaction is that of CD28 on T cell surfaces with CD80/CD86 on the surface of APCs (Gmünder and Lesslauer, 1984; Lesslauer *et al.*, 1986; Aruffo and Seed, 1987; Linsley, Clark and Ledbetter, 1990; Howard, Rochelle and Seldin, 1991; Nalvai *et al.*, 2000). CD28 is constitutively expressed on CD8<sup>+</sup> and CD4<sup>+</sup> T cells (Gross, St John and Allison, 1990; Lee *et al.*, 1990), but expression of CD80/CD86 on APCs such as dendritic cells depends upon their encountering foreign antigen (Freeman, Borriello, *et al.*, 1993; Freeman, Gribben, *et al.*, 1993; Inaba *et al.*, 1994). CD28 engagement contributes to the intracellular T cell signalling pathways and enhances the overall avidity of the interaction

between the two cells (Riha and Rudd, 2010). Its importance can be demonstrated by the immunocompromisation seen in CD28-deficient mice (Shahinian *et al.*, 1993). Additionally, enhancement of this regulatory pathway can amplify the response against traditionally weaker antigen such as tumour antigen (Chen *et al.*, 1992; Baskar *et al.*, 1993; Townsend and Allison, 1993; Allison, Hurwitz and Leach, 1995).

In a further regulatory effort, CTLA-4 is upregulated in response to activation (Alegre, Frauwirth and Thompson, 2001). CTLA-4 is a homolog of CD28 and thus also a ligand for the CD80/CD86 proteins, but provides a negative feedback loop (Khan and Ghazanfar, 2018). Blocking CTLA-4 by means of antibodies has an encouraging effect on the anti-tumour immune response (Walunas *et al.*, 1994; Leach, Krummel and Allison, 1996). Whereas triggering CTLA-4 signalling inhibits the T cell response (Walunas *et al.*, 1994; Krummel and Allison, 1995). It competes with CD28 for CD80/CD86 thus balancing the positive and negative signals (Alegre, Frauwirth and Thompson, 2001).

Another noteworthy regulatory signalling mechanism is programmed cell death protein (PD)-1. PD-1 is expressed on activated T cells (Ishida *et al.*, 1992; Agata *et al.*, 1996; Vibhakar *et al.*, 1997). It was first described in 1992 (Ishida *et al.*, 1992). Deficiencies in PD-1 led to mice displaying increased rates of multiple autoimmune disorders and a loss of peripheral tolerance (Nishimura *et al.*, 1999, 2001; Ansari *et al.*, 2003). Its ligands, PD-L1 and PD-L2 are expressed on APCs and activated lymphocytes (Carreno and Collins, 2002; Eppihimer *et al.*, 2002; Mazanet and Hughes, 2002; Sheppard *et al.*, 2004). Interaction of PD-1 with its ligands evokes negative feedback on T cell activation, including reducing proliferation and cytokine secretion (Freeman *et al.*, 2000; Latchman *et al.*, 2001; Carter *et al.*, 2002; Brown *et al.*, 2003). PD-1 activation results in a reduction in the phosphorylation of early T cell signalling molecule ZAP-70 – thus mediating its effects by dampening down signalling from engaged TCRs (Sheppard *et al.*, 2004).

Despite these regulatory mechanisms to prevent it, autoimmunity does occur. This indicates that these regulatory systems must be surmountable, resulting in the aberrant activation of lymphocytes (Khan and Ghazanfar, 2018). This can occur in a number of ways, including molecular mimicry and the emergence of cryptic self.

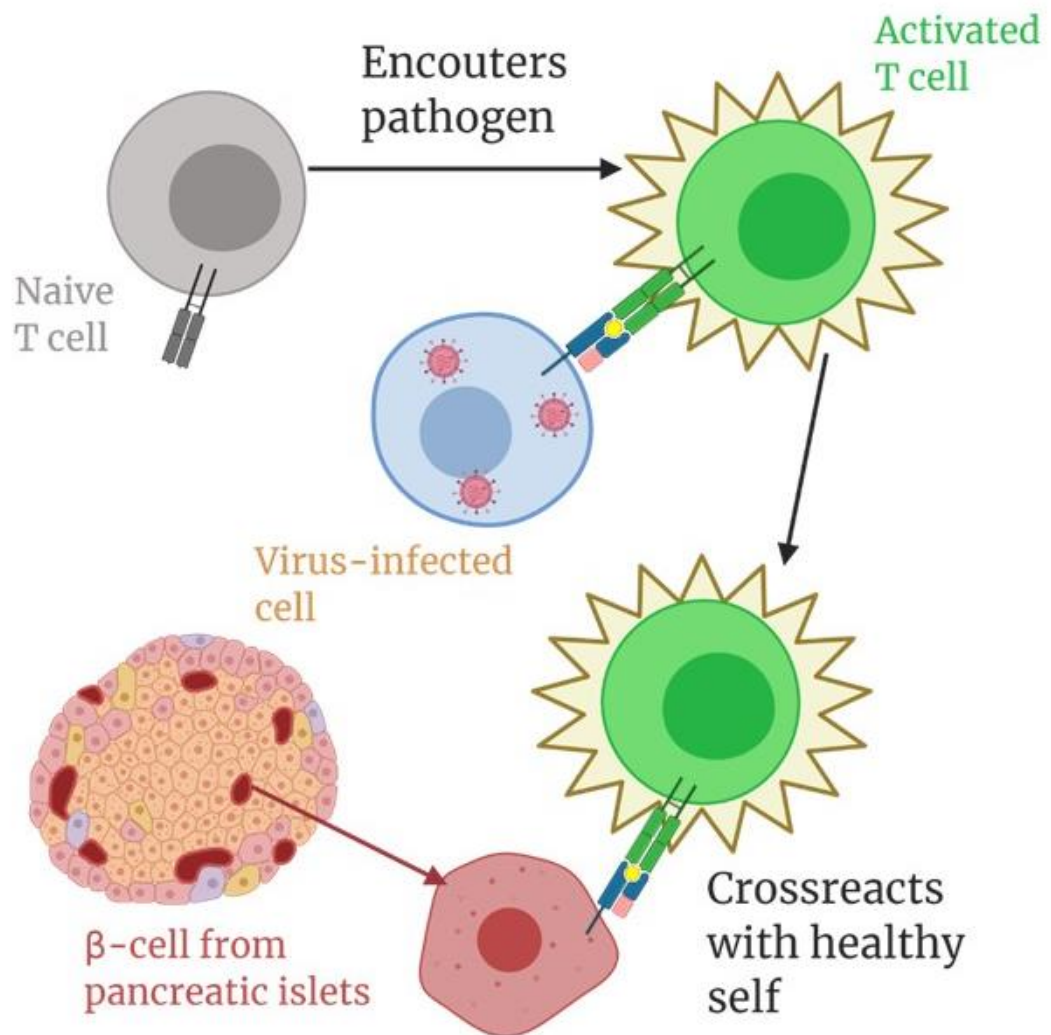
T cells with the potential for autoreactivity can ignore a self-target that is insufficiently potent to induce activation (Akkaraju *et al.*, 1997). The naïve T cell can be activated by a stronger – often pathogen derived – antigen that is a molecular mimic of the self-antigen and with

which it can cross-react, **Figure 1.8** (Raman *et al.*, 2016; Cole, Van Den Berg, *et al.*, 2017; Khan and Ghazanfar, 2018).

Many examples of molecular mimicry between pathogenic peptides and self-peptides have been described in the literature. An example is the ASVKVLLGRKSDSERG peptide epitope, derived from herpes simplex virus (HSV)-1. It was shown to be recognised by autoreactive T cells that could target a corneal antigen in mice. Mutated strains of the virus, lacking this epitope, were not recognised (Zhao *et al.*, 1998). In another example, group A streptococcal proteins were shown to be capable of triggering T cell responses against the cardiac protein myosin (Dale and Beachey, 1986).

Molecular mimicry has been cited as a trigger for multiple sclerosis (MS). Levin *et al.* observed that Epstein-Barr virus (EBV) infection increases the susceptibility to MS in young adults (Levin *et al.*, 2003). A case of molecular mimicry between an EBV peptide and a myelin-derived self-peptide has been demonstrated and shown to be able to trigger TCR-dependent demyelination (Lang *et al.*, 2002). In this manner, crossreactivity following exposure to particular viral infections could feasibly contribute to the development of MS (Olson *et al.*, 2001; Harkiolaki *et al.*, 2009; Münz *et al.*, 2009).





**Figure 1.8: Molecular mimicry in autoimmunity.** A schematic representation showing how exposure to a true pathogenic antigen can cause a T cell to become activated and crossreact with self-antigens to which it had previously been ignorant. For illustrative purposes this figure envisions a viral antigen acting as a molecular mimic for an antigen expressed on pancreatic  $\beta$  cells.

Other examples of molecular mimicry occur in cases of rheumatoid arthritis (RA). RA has been associated with alphavirus infections (Laine, Luukkainen and Toivanen, 2004; Rulli *et al.*, 2007; Toivanen, 2008). This has prompted bioinformatical investigation into potential cases for molecular mimicry (Venigalla, Premakumar and Janakiraman, 2020). Venigalla *et al.* analysed the proteomes of RA-associated alphaviruses to identify homology to human proteins – particularly those specifically associated with joint cartilage and RA (Venigalla, Premakumar and Janakiraman, 2020). From this analysis they identified epitopes with

predicted ability to bind RA-associated class II MHC molecules. Potential epitopes include the viral epitope YSGGRFTIPTGAGKPGDSGRPIFDN as a homolog to the GKPGDDG sequence found in human collagen type II (Venigalla, Premakumar and Janakiraman, 2020). T cell clones and antibodies directed against this protein had already been identified in RA patients (Londei *et al.*, 1989; Mullazehi *et al.*, 2012). The viral epitope SKDVYANTQLVLQRPAAGTVHVPYS is also a potential homolog to the QSPAAGTVQGRVP sequence from human cartilage intermediate layer protein 1 (Venigalla, Premakumar and Janakiraman, 2020). Antibodies against this protein have been previously described in the context of RA (Tsuruha *et al.*, 2001).

Molecular mimicry has also been described in the context of Goodpasture's syndrome, an autoimmune disorder in which the principle target is type IV collagen, found in the glomerular basement membranes (GBM) of the kidneys and in the alveoli of the lungs (Hudson *et al.*, 1989; Merkel *et al.*, 1996). A role for T cells in Goodpasture's syndrome was indicated by T cell infiltration in diseased kidneys, which correlated with disease severity (Bolton *et al.*, 1987; Hu *et al.*, 2016, 2017). The symptoms can be alleviated through administration of anti-T cell therapies such as inhibition of the CD28-CD80/CD86 interaction (Reynolds *et al.*, 2000, 2004), or via anti-CD4/CD8 antibodies (Reynolds *et al.*, 2002). T cell clones specific for the GBM have been detected (Merkel *et al.*, 1996; Derry *et al.*, 2008), and a number of T cell epitopes have been described (Merkel *et al.*, 1996; Wu *et al.*, 2003). In 2006, Arends *et al.* identified microbial peptides that resembled known T cell epitopes and could induce glomerular inflammation in rats suggesting a role for molecular mimicry in yet another autoimmune disease (Arends *et al.*, 2006).

During an immune response the cytokine-rich environment encourages endogenous cells to increase pMHC expression at the cell surface, exposing antigen that would not normally be presented (Lehmann *et al.*, 1992; Schwartz, 2012). This emergence of 'cryptic self' has the potential to propagate autoimmunity as T cells would not have been tolerised to unencountered antigen in the thymus (Lehmann *et al.*, 1992; Schwartz, 2012). These T cells are specific – not to pathogenic molecular mimics but to self-antigens that are not normally exposed to immune scrutiny (Moudgil and Sercarz, 1994; Itariu and Stulnig, 2014). T cells with specificities to non-dominant self-epitopes can escape negative selection (Schild *et al.*, 1990; Cibotti *et al.*, 1992; Slifka *et al.*, 2003), these T cells then reside in the periphery harmlessly until the, fairly unlikely but potential, emergence of their cryptic self-antigen (Gammon and Sercarz, 1989; Lipham *et al.*, 1991).

A final noteworthy mechanism for escaping the extensive self-tolerance regulations is through the expression of dual TCRs. This describes a cell that has multiple V $\alpha$  or V $\beta$  chains rearranged and expressed. Differing pairings can provide the cell with differing specificities (Heath and Miller, 1993; Elliott and Altmann, 1995; Padovan *et al.*, 1995). T cells expressing two fully rearranged TCR $\beta$  chains were first isolated from mice in 1988 (Matis, Ezquerra and Coligan, 1988; Triebel *et al.*, 1988). Dual  $\alpha$ -chain expression was demonstrated shortly afterwards (Furutani *et al.*, 1989; Kuida *et al.*, 1991). In 1993, the existence of dual TCRs in humans was demonstrated (Padovan *et al.*, 1993). It is thought that ~10% of  $\alpha\beta$  TCRs co-express two  $\alpha$ -chains (Heath *et al.*, 1995; Niederberger *et al.*, 2003; Schuldt and Binstadt, 2019), whilst ~1% of  $\alpha\beta$  TCRs co-express two  $\beta$ -chains (Balomenos *et al.*, 1995; Davodeau *et al.*, 1995; Sieh and Chen, 2001; Brady, Steinel and Bassing, 2010).

In the context of autoimmunity, an interesting example of dual TCR expression was described in 2010 by Qingyong *et al.* They described a case of MS in mice that could be induced by infection with vaccinia virus (Ji, Perchellet and Goverman, 2010). This causality could not be explained by locating crossreactive molecular mimics between viral peptides and peptides within the MBP sequence (Ji, Perchellet and Goverman, 2010). Instead a co-expressed, alternative V $\beta$  chain was identified within virus-specific T cells, allowing for two unique TCR pairs. One pair was specific for MBP and the other for vaccinia virus. Activation of the clone by exposure to vaccinia virus initiated cytotoxicity against MBP via its co-expressed TCR (Ji, Perchellet and Goverman, 2010).

Mechanisms for the breakdown of self-tolerance emphasise the importance of investigating the specific TCR-pMHC interactions involved in autoimmune settings. An autoimmune disorder of particular interest to this thesis is type 1 diabetes mellitus (T1D). It is discussed in detail in **Section 3.1**.

## 1.6 – Aims and objectives

Functionally, the TCR-pMHC interaction encompasses an exceptional degree of plasticity and crossreactivity that enables comprehensive immune coverage to protect the host from any potential, often as yet unencountered, immune threat (see **Section 1.5.1**). Yet this crossreactive nature of the TCR-pMHC interaction can result in aberrant recognition of self in autoimmune diseases (see **Section 1.5.2**).

Ultimately, further investigation into the plasticity of the TCR-pMHC interaction will enhance understanding of the nature of T cell antigen recognition. Here, three main aspects of the TCR-pMHC interaction are investigated:

- 1) Can understanding the inherent crossreactivity of a particular TCR-pMHC interaction aid the identification of specific pathogenic triggers for an autoimmune disease?
- 2) How would amino acid substitutions in the CDR3 loops of a TCR influence the repertoire of peptides it can interact with?
- 3) Finally, on the other side of the interaction, how is the molecular flexibility of the MHC influenced by the peptide cargo it carries?

To address these questions, I aimed to:

- Investigate whether the T1D-associated InsB4 T cell clone could crossreact with peptides from pathogenic proteomes to address the hypothesis that pathogenic molecular mimics might act as triggers of T1D.
- Use the extensively described interaction between the 868 TCR and its cognate HIV-1 Gag-derived peptide SLYNTVATL in the context of HLA-A2 to investigate how single point mutations in the amino acid sequence of a CDR3 loops might alter the peptide repertoire of the TCR.
- Probe the flexible nature of the pMHC using a T1D-relevant epitope (HLA-A2-ALWGPDPAAA) and a series of APLs designed around this epitope to investigate whether the red edge excitation shift (REES) phenomenon might be able to provide information regarding the peptide-dependent flexibility of the pMHC complex and add to the tool-kit of experimental techniques that can be used to assess pMHC flexibility.

## 2 – Materials and methods

---

### 2.1 – Protein production

#### 2.1.1 – Buffers and media used in protein production

<i>Buffer</i>	<i>Composition</i>
Lysogeny broth (LB) agar plates	10 g/L tryptone (VWR Chemicals), 5 g/L yeast extract (VWR Chemicals), 10 g/L NaCl (Fisher Scientific), 15 g/L bacteriological agar (Oxoid). Supplemented following autoclaving with 50 µg/mL carbenicillin (Fisher Bioreagents).
TYP media	16 g/L tryptone (VWR Chemicals), 16 g/L yeast extract (VWR Chemicals), 5 g/L NaCl (Fisher Scientific), 3.3 g/L potassium phosphate dibasic (Sigma Aldrich). Supplemented following autoclaving with 50 µg/mL carbenicillin (Fisher Bioreagents).
Lysis buffer	10 mM TRIS pH 8.1, 10 mM MgCl <sub>2</sub> (Sigma), 150 mM NaCl (Fisher Scientific), 10% glycerol (Fisher Chemical).
Triton wash	0.5 % Triton X (Sigma), 50 mM TRIS pH 8.1, 100 mM NaCl (Fisher Scientific), 2 mM ethylenediaminetetraacetic acid EDTA (Sigma Aldrich).
Resuspension buffer	50 mM TRIS pH 8.1, 100 mM NaCl (Fisher Scientific), 2 mM (EDTA) (Sigma Aldrich).
Guanidine buffer	6 M guanidine (Fisher Bioreagents), 50 mM TRIS pH 8.1, 100 mM NaCl (Fisher Scientific), 2 mM EDTA (Sigma Aldrich).
pMHC refold buffer	50 mM TRIS pH 8.1, 2 mM EDTA (Sigma Aldrich), 400 mM L-arginine (Sigma Aldrich), pH adjusted to 8 with HCl (Sigma Aldrich). 0.74 g/L cysteamine (Sigma) and 0.83 g/L cystamine (Aldrich) added prior to use.
TCR refold buffer	50 mM TRIS pH 8.1, 2 mM EDTA (Sigma Aldrich), 2.5 M urea (Sigma), pH adjusted to 8 with HCl (Sigma Aldrich). 0.74 g/L cysteamine (Sigma) and 0.83 g/L cystamine (Aldrich) added prior to use.
2M TRIS pH 8.1	242.24 g/L TRIS-base (Sigma), pH adjusted to 8.1 with HCl (Sigma Aldrich), filtered 0.45 µM.
0.5M EDTA pH 8	146.12 g/L EDTA (Sigma Aldrich), pH adjusted to 8 with NaOH (Fisher Chemical).

---

Dialysis buffer	20 L dH <sub>2</sub> O 10 mM TRIS, pH 8.1.
Ion exchange buffer 'A'	10 mM TRIS, filtered 0.45 mM, pH 8.1.
Ion exchange buffer 'B'	10 mM TRIS, 1 M NaCl (Fisher Scientific), filtered 0.45 mM, pH 8.1.
Biomix A	0.5 M bicine buffer, pH 8.3
Biomix B	100 mM ATP, 100 mM magnesium acetate, 500 µM d-Biotin.
Phosphate buffered saline (PBS)	Oxoid, tablets composed of: 8 g/L sodium chloride, 0.2 g/L potassium chloride, 1.15 g/L di-sodium hydrogen phosphate, 0.2 g/L potassium dihydrogen phosphate.
HEPES buffer	150 mM NaCl (Fisher Scientific), 50 mM HEPES (Fisher Bioreagents).
Crystal buffer	10 mM TRIS pH 8.1, 10 mM NaCl (Fisher Scientific).
Sodium dodecyl sulphate (SDS) reducing sample buffer	Lithium dodecyl sulphate at pH 8.4 (Novex, Thermo Fisher), 20 mM dithiothreitol (DTT) (Fisher Chemical).
SDS non-reducing sample buffer	Lithium dodecyl sulphate at pH 8.4 (Novex, Thermo Fisher).
Psi Broth	5 g/L yeast extract (VWR Chemicals), 20 g/L tryptone (VWR Chemicals), 5 g/L magnesium sulphate (Fisher Chemical). pH to 7.6 with potassium hydroxide (AnalR) before autoclaving.
Transformation buffer I	30 mM potassium acetate (0.588 g) (Sigma), 100 mM rubidium chloride (2.42 g) (Sigma), 10 mM calcium chloride (0.294 g) (Sigma Aldrich), 50 mM magnesium chloride (2.0 g) (Fisher Chemical), 15 % glycerol (w/v) (30 mL) (Fisher Chemical). pH to 5.8 with acetic acid (Fisher Scientific) before filtering 0.45 µm and storing at 4°C. Measurements given for making 200 mL.
Transformation buffer II	10 mM MOPS (0.21 g) (Sigma), 75 mM calcium chloride (1.1 g) (Sigma Aldrich), 15 % glycerol (w/v) (15 mL) (Fisher Chemical). pH to 6.5 with sodium hydroxide (Fisher Chemical) before filtering 0.45 µm and storing at 4°C. Measurements given for making 100 mL.

**Table 2.1:** A summary of the buffers and media used during protein production throughout this thesis.

### 2.1.2 – Vectors and protein sequences

The carbenicillin-resistant pGMT7 vector was used for expression of proteins of interest in bacterial cells, **Figure 2.1**. pGMT7 is an extensively used bacterial expression vector that utilises the T7 RNA polymerase promoter (Rosenberg *et al.*, 1987; William Studier *et al.*, 1990; Banham and Smith, 1993; Reid *et al.*, 1996). The constructs shown below were cloned into pGMT7 using BamH1 and EcoR1 restriction sites. In addition to conferring resistance to carbenicillin, pGMT7 allows inducible expression of the construct via isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) and the lac operon (Hughes *et al.*, 1991). This expression system was used to produce soluble HLA-A2 and human  $\beta$ 2M. In some cases, construct sequences were modified slightly from wildtype sequences to optimise *in vitro* experiments (Boulter *et al.*, 2003). For tetramerisation studies an N-terminal 15 amino acid biotinylation sequence was added to HLA-A2 (GLNDIFEAQKIEWHE) (AviTag™, Avidity).

#### HLA-A2wt:

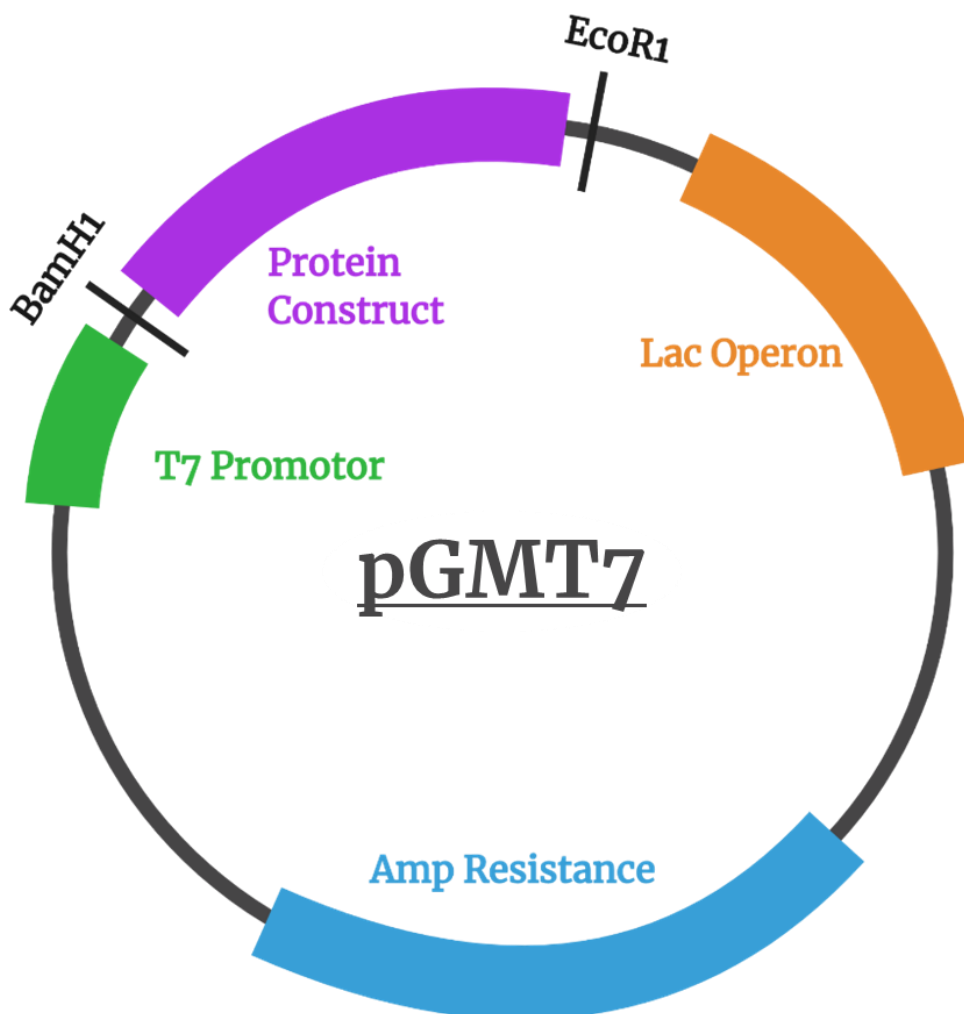
MGSHSMRYFFTSVSRPGRGEPFRFIAVGYVDDTQFVRFDSDAASQRMetEPRAPWIEQEGPEY  
WDGETRKKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGS DWRFLRGYHQYAYDGK  
DYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRLRYLENGKETLQRTD  
APKTHMTHHAVSDHEATLRCWALS FYPAEITLTWQRDGEDQTQDTELVE TRPAGDGT FQKWA  
AVVVPSGQEQRYTCHVQHEGLPKPLTLRWEF

#### HLA-A2tag:

MGSHSMRYFFTSVSRPGRGEPFRFIAVGYVDDTQFVRFDSDAASQRMetEPRAPWIEQEGPEY  
WDGETRKKVKAHSQTHRVDLGTLRGYYNQSEAGSHTVQRMYGCDVGS DWRFLRGYHQYAYDGK  
DYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRLRYLENGKETLQRTD  
APKTHMTHHAVSDHEATLRCWALS FYPAEITLTWQRDGEDQTQDTELVE TRPAGDGT FQKWA  
AVVVPSGQEQRYTCHVQHEGLPKPLTLRWEFGLNDIFEAQKIEWHE

#### $\beta$ 2M:

MIQRTPKIQVYSRHPAENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWS  
FYLLYYTEFTPTKDEYACRVNHVTL SQPKIVKWDRDM



**Figure 2.1: Schematic of the pGMT7 plasmid used for protein production.** The desired protein construct is cloned into the pGMT7 using the BamHI and EcoRI restriction sites. Successful transformation of bacterial cells is detected via the conferred ampicillin resistance. The presence of the lac operon allowed inducible protein production.

### 2.1.3 – Making chemically competent cells

A vial of the previous (or original) batch of competent cells (either BL21 DE3, TOP10, or XL-10 Gold) was used to inoculate an agar plate supplemented with the relevant antibiotic (chloramphenicol, streptomycin, or chloramphenicol respectively), plates were grown at 37°C overnight. The following day a single colony was used to inoculate 50 mL Psi broth supplemented with the relevant antibiotic, this was grown overnight in an orbital shaking



incubator (37°C, 100 revolutions per minute; RPM) (Thermo Fisher). 1 mL of the resulting culture was used to inoculate a fresh 100 mL of Psi broth supplemented with the relevant antibiotic. This was then grown in an orbital shaking incubator (37°C, 100 RPM) (Thermo Fisher) until it reached an optical density at 600 nm (OD<sub>600nm</sub>) of 0.45. Cells were pelleted by spinning in a pre-cooled centrifuge 6371 x g (6000 RPM) for 10 minutes (Beckman Coulter Avanti J-E centrifuge). Cells were then washed in 40 mL of Transformation Buffer I and incubated on ice for 15 minutes. Cells were pelleted by spinning in a pre-cooled centrifuge 6371 x g (6000 RPM) for 10 minutes (Beckman Coulter Avanti J-E centrifuge). Cells were then resuspended in 4 mL Transformation Buffer II, aliquoted into sterile microcentrifuge tubes, and snap frozen. Performed by Anna Fuller, Aaron Wall, or Théo Morin.

#### 2.1.4 – Transformation of competent cells

For transformation, 20 µL aliquots of bacterial cells were first thawed on ice for 5 minutes. 1 µL (containing 50-100 ng of DNA) of vector DNA was added to the 20 µL bacterial cells followed by a further 5-minute incubation on ice. To encourage uptake of vector DNA, bacterial cells were then heat-shocked by a 2-minute incubation at 42°C. Following heat-shock, cells were allowed to recover on ice for 5 minutes before the full volume was plated onto LB agar plates supplemented with 50 µg/mL carbenicillin. Plates were incubated at 37°C overnight to allow growth of colonies.

BL21 DE3 chemically competent *Escherichia coli* cells were used to produce MHC chains in the form of inclusion bodies for artificial refolding. TOP10 chemically competent *E. coli* cells were used to amplify DNA. XL-10 Gold ultracompetent cells were used to amplify the larger pELNS plasmid for viral work, discussed in **Section 2.3**.

#### 2.1.5 – Amplifying DNA via mini preparation

Competent, TOP10, cells were transformed with the relevant DNA as described in **Section 2.1.4**. Single colonies were selected to inoculate 5 mL of TYP media and grown overnight in an orbital shaking incubator (37°C, 220 RPM) (Thermo Fisher). The following day, bacterial cells were harvested by centrifuging the cultures at 16,000 x g for 5 minutes (Eppendorf Centrifuge 5424). DNA extraction was then carried out using a PureLink Quick Plasmid Miniprep kit (Thermo Fisher) according to the manufacturer's instructions. The resulting pellets were resuspended in 250 µL of Resuspension Buffer (R3) supplemented with 20 mg/ml RNase. Cells were then lysed in 250 µL Lysis Buffer (L7), incubating at room

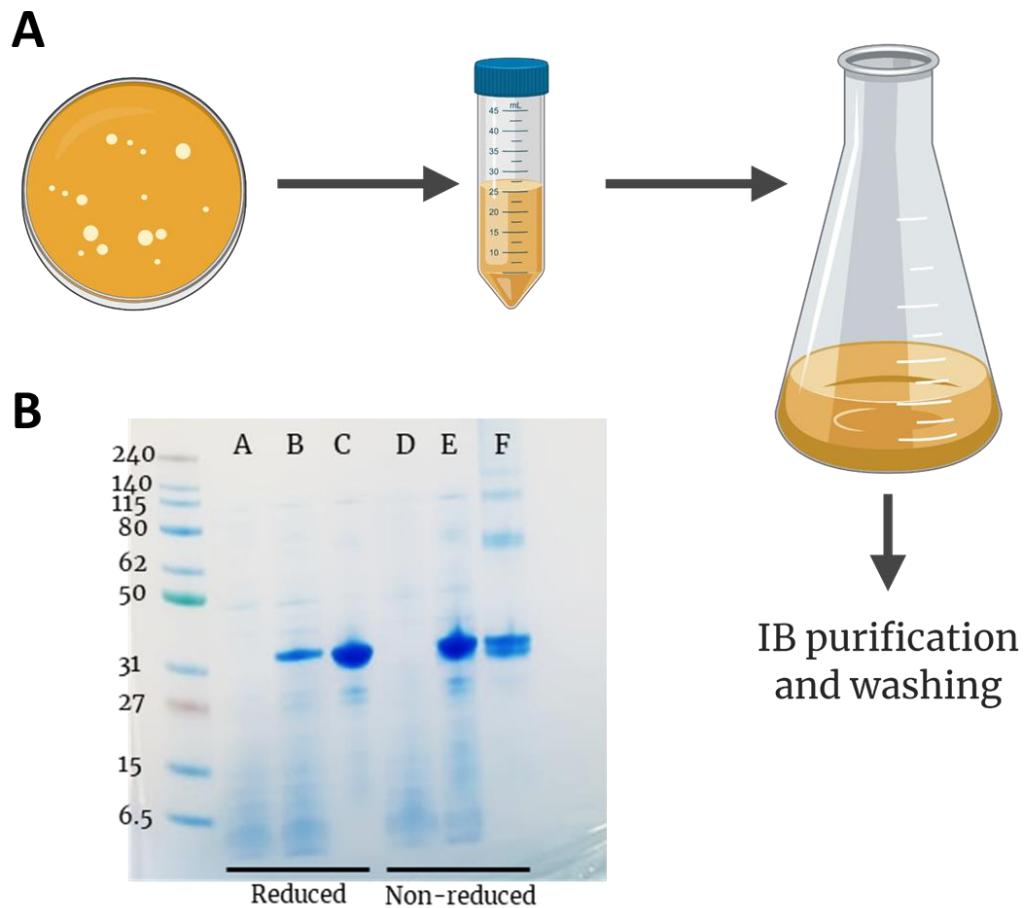
temperature for 5 minutes to allow for lysis. 350  $\mu$ L of Precipitation Buffer (N4) was used to precipitate protein and genomic DNA from the resulting lysate, leaving circular plasmid DNA in solution. Lysates were then centrifuged at 16,000 x g for 10 minutes (Eppendorf Centrifuge 5424) to pellet the precipitant. Supernatants were loaded on to HiPure spin columns (Thermo Fisher) assembled with wash tubes to collect flow through. Samples were centrifuged at 12,000 x g for 1 minute (Eppendorf Centrifuge 5424) to bind DNA to the column. Columns were then washed to remove impurities, first with 500  $\mu$ L of Wash Buffer (W10) supplemented with 60 %v/v ethanol, then with 700  $\mu$ L of Wash Buffer (W9) supplemented with 70 %v/v ethanol. Columns were centrifuged at 12,000 x g for 1 minute (Eppendorf Centrifuge 5424) once more to remove residual Wash Buffer and ethanol. To recover DNA, columns were incubated in 30  $\mu$ L of nuclease-free water for 5 minutes at room temperature before being centrifuged at 12,000g for 2 minutes (Eppendorf Centrifuge 5424). Concentrations were measured using a spectrophotometer (Geneflow). For DNA, optical density was measured at 260 nm and concentration was calculated by the instrument using Beer's Law:  $A = \epsilon \cdot l \cdot C$ . The extinction coefficient of the protein was then used to determine final concentration. Samples were sequenced using Eurofins Genomics' Mix2Seq service, for protein preparation all constructs were within the pGMT7 vector so could be sequenced with the T7 forward primer (TAATACGACTCACTATAGGG). For longer constructs, the SP6 primer can also be used for sequencing (ATTTAGGTGACACTATAG).

#### 2.1.6 – Expression of constructs and purification of inclusion bodies

Following overnight growth on agar at 37°C (as described above), single colonies were selected (**Figure 2.2A**) and grown in 10 mL TYP media supplemented with 50  $\mu$ g/mL carbenicillin (37°C, 220 RPM) until an  $OD_{600nm}$  of 0.5 was reached (**Figure 2.2A**). The following day these starter cultures were expanded in 1 L of TYP media supplemented with 50  $\mu$ g/mL carbenicillin until an  $OD_{600nm}$  of 0.5 was reached (**Figure 2.2A**). Based on previous experiments by my laboratory this OD is considered to be indicative of the peak of the bacterial exponential growth phase, and so is the optimal point at which to induce expression of the protein of interest before the cells begin to enter the death phase. A 500  $\mu$ L 'pre-induction' sample of culture was taken to later be run on an SDS gel to verify protein expression (**Figure 2.2B**). Protein expression was induced using 500  $\mu$ L of 1 M IPTG (to give a working concentration 0.5 mM). 3 hours post-induction a 500 mL sample of culture was taken to later be run on an SDS gel to verify protein expression (**Figure 2.2B**), bacterial cells were then harvested by centrifuging in an Eppendorf 5810R centrifuge for 20 minutes at

2755 x g (3700 RPM). The resulting cell pellets were resuspended in 40 mL of lysis buffer for overnight freeze-thaw to encourage cell lysis. To further aid cell lysis, thawed cells were sonicated using a Bandelin Sonoplus sonicator for 20 minutes at 50 % power, using a 2 x 10 % cycle. Dirtier lysates were subject to a second round of freeze-thaw and sonication. The cell lysate was treated with 200  $\mu$ L of 20 mg/mL DNase enzyme (working concentration 0.1 mg/mL) and incubated for 1 hour at 37°C in an orbital shaking incubator (220 RPM) (Thermo Fisher). For purification, the inclusion bodies were washed three times, twice in 200 mL of a triton wash buffer, and finally in 200 mL of resuspension buffer. Between each wash, the supernatant was discarded, and the pellet was resuspended in the next buffer by mechanical homogenisation (VWR, VDI 25). A final 500  $\mu$ L sample of inclusion body dissolved in resuspension buffer was taken to be run on an SDS gel to verify protein purification (**Figure 2.2B**). Inclusion bodies were pelleted by centrifuging at 17,696 X g (10,000 RPM) for 20 minutes (Beckman Coulter Avanti J-E centrifuge). Finally, the pelleted inclusion bodies were dissolved in 10 mL guanidine buffer. To remove any insoluble debris inclusion bodies were centrifuged in an Eppendorf 5810R centrifuge for 30 minutes at 2755 x g (3700 RPM). Inclusion body concentration was measured using a spectrophotometer (Geneflow) at 280 nm, as described in **Section 2.1.5**. The extinction coefficient was then used to determine final concentration. On average 50 mg of purified inclusion bodies was yielded per 1 L of TYP culture.

To verify protein expression and purification, an SDS gel was used to compare pre- and post-IPTG induction samples to a 'post-wash' sample of inclusion bodies in resuspension buffer, this gel was used to verify purity of the inclusion bodies (**Figure 2.2B**). 4  $\mu$ L of SDS reducing sample buffer or SDS non-reducing sample buffer was added to 20  $\mu$ L of sample and incubated at 95°C for 10 minutes. To remove bubbles, samples were centrifuged at 16,000 x g for 30 seconds. 20  $\mu$ L of each sample was loaded onto a gel (BOLT 4-12 % Bis-Tris Plus, Invitrogen), with the pre-stained protein ladder (Elite pre-stained protein ladder, 6.5-270 kDa, Generon). Gels were run at 165 V for 35 minutes in 1X BOLT MES SDS-PAGE Running Buffer (Invitrogen). To visualise bands gels were stained with 25 mL Quick Coomassie Stain (Generon) before being destained with water.



**Figure 2.2: Protein production via *E. coli* expression systems. A)** Bacterial cells that have received the pGMT7 vector containing the protein of interest are able to grow on carbenicillin agar plates. Single colonies are selected for expansion in liquid culture before protein production is induced using IPTG. **B)** Protein production and subsequent washing is verified using SDS-PAGE. Rows A, B, and C represent pre-induction, post-induction, and resuspension buffer samples respectively, in reducing conditions. Rows D, E, and F contain the same under non-reducing conditions. A band at ~43 kDa indicates production of HLA-A2 heavy chain.

### 2.1.7 – Refolding soluble protein from inclusion bodies

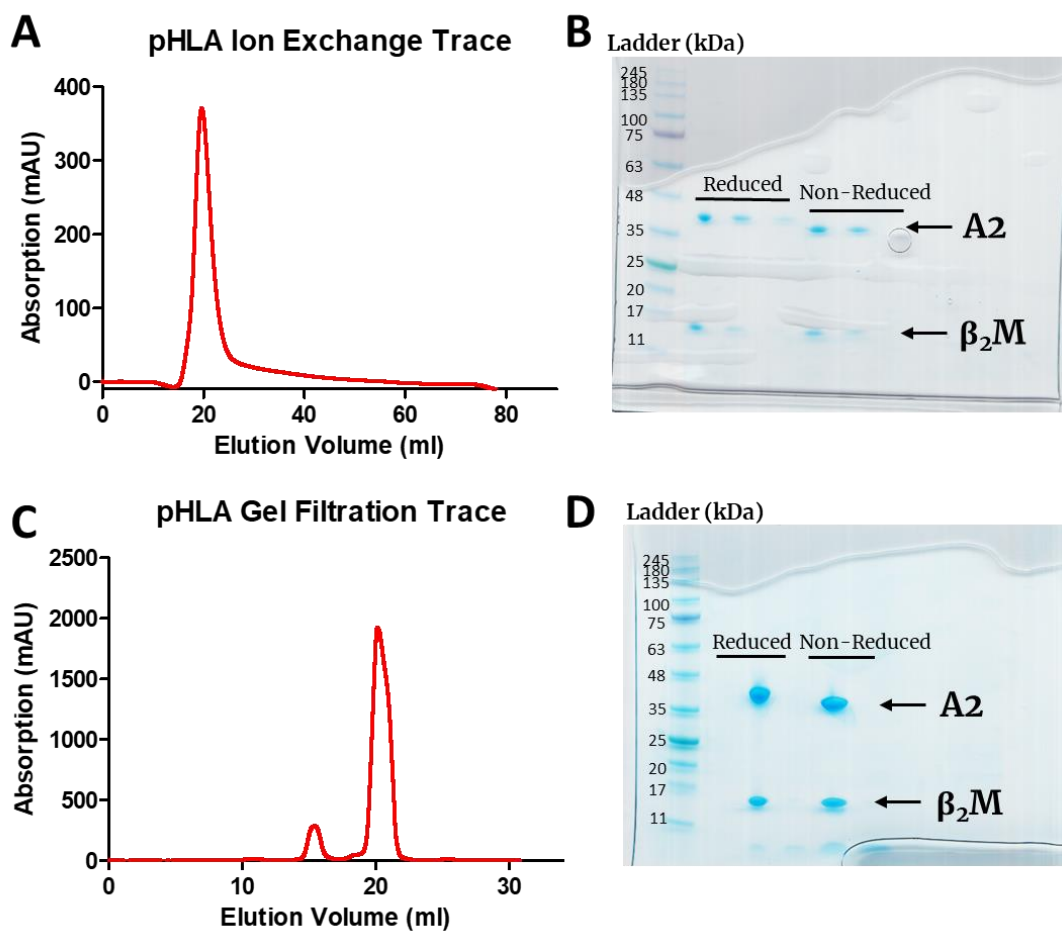
pMHC refold buffer were made up according to recipes in **Table 2.1**, filtered 1.2  $\mu\text{M}$  (Fisher Scientific) and 0.45  $\mu\text{M}$  (Sartorius) and pre-cooled to 4°C. For pMHC refolds, 30 mg HLA-A2  $\alpha$  chain inclusion bodies, 30 mg human  $\beta 2\text{M}$  inclusion bodies, and 4 mg of a relevant synthetic peptide (Peptide Synthetics), per 1 L refold buffer, was incubated with 10 mM DTT (Fisher Chemical) at 37°C for 30 minutes. The inclusion body mixture was then diluted into the cold refold buffer and stirred at 4°C for 6-8 hours or overnight. Refold mixtures were then dialysed twice in 20 L of 10 mM TRIS pH 8.1 to ensure the conductivity of the refold was <1 mS/cm. Dialysed refold mixtures were then re-filtered (as above) prior to purification (**Section 2.1.8**).

### 2.1.8 – Purification of soluble protein by fast protein liquid chromatography (FPLC)

All FPLC procedures were carried out using an ÄKTA Pure (GE Healthcare) and Unicorn 6.3 software (GE Healthcare). A Poros 50 HQ anion exchange column (Life Technologies) was washed with 100 mL of ion exchange buffer 'B' (see **Table 2.1**) before being equilibrated using 100 mL of ion exchange buffer 'A'. Unless otherwise stated, a flow rate of 20 mL/minute and a pressure alarm of 5 MPa was used throughout. A protein sample was then loaded onto the column. The sample was eluted, using increasing concentrations of ion exchange buffer 'B' (**Figure 2.3A**), into 5 mL FPLC tubes (Greiner Bio-One) held in a fraction collector (F9-R, GE Healthcare). Fractions were then analysed by SDS-PAGE (as described in **Section 2.1.6**) for presence of pure protein of interest (**Figure 2.3B**). Fractions verified to contain protein of interest were concentrated to ~1 mL using a Amicon Ultra 4 mL 10 kDa centrifugal filter (Merck) by centrifuging at 2755 x g (3700 RPM, Eppendorf 5810R centrifuge) (for approximately 20 minutes). If pMHC samples required biotinylation, samples were instead concentrated to 700  $\mu\text{L}$  using the same method. Following this, 100  $\mu\text{L}$  each of Biomix A, Biomix B, and 500  $\mu\text{M}$  d-Biotin (Avidity) were added to the sample along with 2  $\mu\text{L}$  of BirA biotin-protein ligase at 3 mg/mL (Avidity). The solution was left at room temperature overnight to allow for biotinylation of the HLA heavy chain.

A Superdex<sup>TM</sup> 200 Increase 10/300 GL size-exclusion column (VWR) was used to purify sample by size (removing aggregates and excess biotin) and for buffer exchange, the column was pre-equilibrated in the appropriate buffer (PBS, unless otherwise stated). The aforementioned ~1 mL sample was transferred into a 1 mL syringe (BD Plastipak), avoiding air bubbles, and manually loaded onto the column. As above, sample was eluted into 5 mL

FPLC tubes (**Figure 2.3C**). Fractions were analysed by SDS-PAGE (**Section 2.1.6**) for presence of pure protein of interest (**Figure 2.3D**), verified fractions were concentrated to ~1 mL using a Amicon Ultra 4 mL 10 kDa centrifugal filter (Merck) by centrifuging at 2755 x g (3700 RPM, Eppendorf 5810R centrifuge) (for approximately 20 minutes). Sample concentrations were measured using a spectrophotometer (Geneflow) at 280 nm, as described above. Samples were used in ongoing experiments, or frozen at -20°C for storage.



**Figure 2.3: Purification of soluble protein.** Protein purification occurs in two stages. **A)** shows a typical ion exchange chromatography trace. **B)** Fractions corresponding to peaks are run on SDS-PAGE under both reducing and non-reducing conditions to verify presence of desired protein. **C)** Fractions are concentrated and subject to size exclusion chromatography to remove any residual impurities. **D)** Fractions corresponding to peaks are once again verified by SDS-PAGE.

## 2.2 – Biochemical assays

### 2.2.1 – Dynamic light scattering to exclude aggregated samples

Dynamic light scattering (Zetasizer) was used to confirm that freeze-thawed proteins had not aggregated or unfolded. 50  $\mu$ L sample was incubated at 4°C before measurements were taken. Peaks between 1-10 nm represented refolded proteins. Samples displaying peaks of larger values were discarded as aggregated protein.

### 2.2.2 – Red edge excitation measurements of HLA-A2 complexes

Fluorescence experiments were performed as previously described (Catici *et al.*, 2016). REES measurements were performed using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA) which was connected to a circulating water bath in order to maintain a temperature of 15°C ( $\pm$ 1°C). Samples were equilibrated at this temperature for 5 minutes. Tryptophan emission was measured between 325 nm and 500 nm with excitation and emissions slit widths of 3.5 nm. The excitation wavelength was initially set at 292 nm and subsequently increased at intervals of 1 nm for a total of 19 scans (in triplicate), thus the highest excitation wavelength used was 310 nm. The PBS buffer spectra was subtracted from the sample spectra at each experimental condition. The centre of spectral mass was calculated as follows:

$$CSM = \frac{\sum(f_i \times \lambda_{Em})}{\sum(f_i)}$$

where  $f_i$  is the measured fluorescence intensity and  $\lambda_{Em}$  is the emission wavelength.

### 2.2.3 – Buffer exchange using a PD-10 desalting column

Samples prepared in Cardiff were in a PBS buffer (produced as described in **Section 2.1**). For pressure/temperature dependent fluorimetry measurements carried out in Bath samples were exchanged into a HEPES buffer (50 mM HEPES, 150 mM NaCl, pH 7.4) using a PD-10 desalting column containing Sephadex™ G-25 medium (GE Healthcare), according to the manufacturer's instructions. The column storage solution was discarded and the column equilibrated in the desired HEPES buffer – to achieve this, a column volume of HEPES buffer was allowed to flow through the column by gravity pull four times. The sample was then added to the column and allowed to enter the packages bed. The volume was topped up to 2.5 mL with HEPES buffer, the flow through was discarded. The sample was then eluted by adding an additional 3.5 mL HEPES buffer. Eluate was collected in a series of test tubes which

were analysed by a spectrophotometer (measuring at 280 nm) for the presence of protein. Relevant eluate tubes were combined and the concentration measured, as described above.

#### 2.2.4 – Pressure/temperature dependent fluorimetry

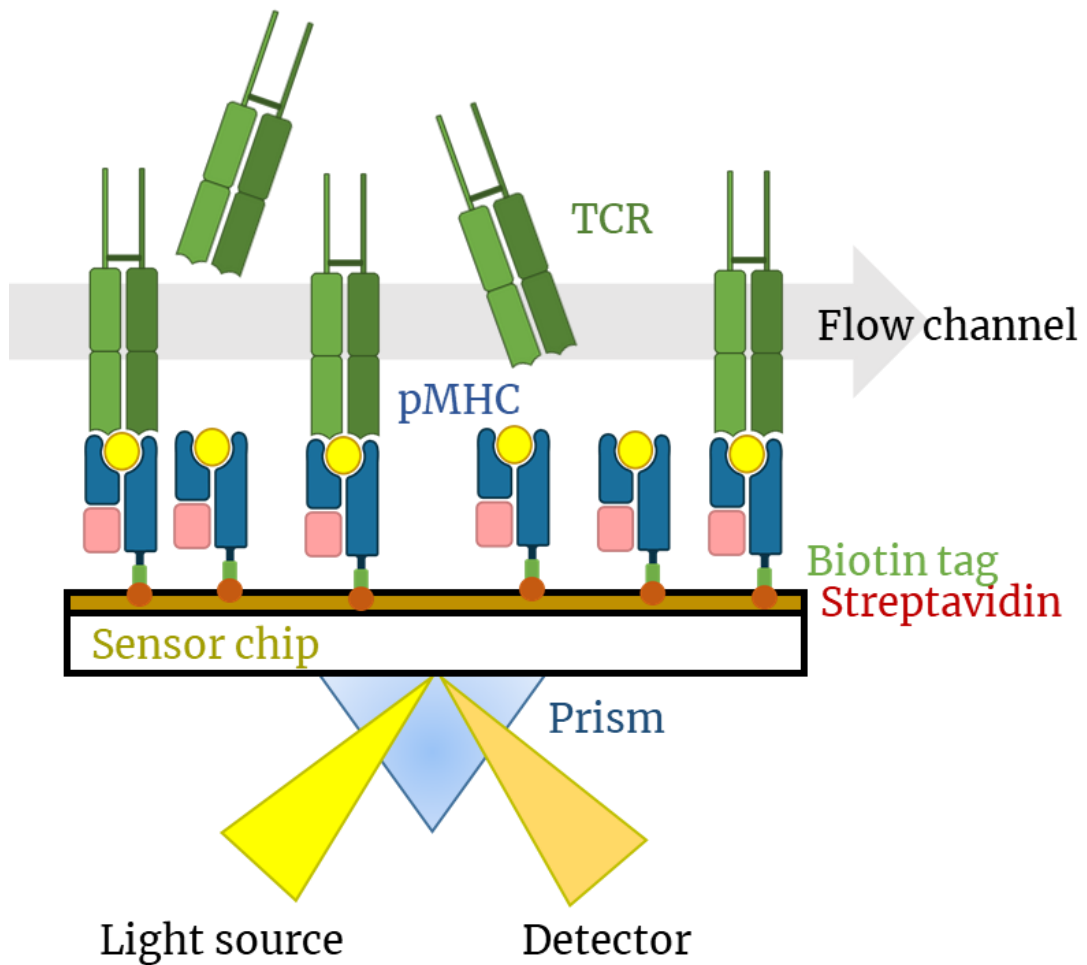
Pressure/temperature measurements were performed using an ISS high-pressure cell (ISS, Champaign, IL, USA) fitted with a custom fibre optic wires connecting to the fluorimeter and the water bath. pMHC complexes were excited at 295 nm, tryptophan emission was measured between 325-500 nm. Emission and excitation slits were set to 15 nm to minimise the signal to noise ratio (due to optimal set up of the pressure cell). Initial measurements were made at 10°C and increased in 5°C increments, up to 30°C. The pressure dependence at each temperature was measured at 50, 400, 800, 1200, and 1600 bar. Measurements were taken in triplicate. Following each full pressure/temperature range, repeat scans were taken at lower pressure/temperature conditions to ensure extreme the pressure/temperature conditions had not denatured the protein. For all measurements the appropriate buffer controls (HEPES) were subtracted prior to data processing.

#### 2.2.5 – Surface plasmon resonance (SPR)

SPR experiments were carried out by Aaron Wall using a BIAcore T200™ and CM5 sensor chips (GE Healthcare) as described previously (Cole *et al.*, 2008; MacLachlan *et al.*, 2017; Whalley *et al.*, 2020). A typical SPR immunoassay is shown schematically in **Figure 2.4**. Soluble pMHC molecules were bound to a CM5 Sensor Chip (GE Healthcare). A negative control was bound to flow cell 1. Flow cells 2-4 were occupied by either test samples or a positive control (if available). Approximately 500 response units (RUs) of each pMHC was immobilised on the chip. Equilibrium binding analysis experiments were then performed at 25°C by injecting TCR at varying concentrations over the chip. A flow rate of 30 µL/min was used. The  $K_D$  values of each TCR-pMHC interaction were calculated by assuming 1:1 binding and Michaelis-Menten kinetics. Data was analysed using GraphPad Prism and fitted to a global fit algorithm:

$$AB = B * AB_{MAX} / (K_D + B)$$





**Figure 2.4: Schematic of surface plasmon resonance.** Biotinylated pMHC monomers are immobilised on a CM5 sensor chip (gold) due to a high affinity interaction between biotin, streptavidin, and a dextran matrix that coats the surface of the chip. The chip is coupled to a prism that refracts light from a light source, this is then measured by an optical detector. Known concentrations of soluble TCR are injected over the chip at a controlled flow rate. Successful interaction between TCR and pMHC alters the refractive properties of the sensor chip, this change is measured by the optical detector. Variations in light intensity from baseline is used to monitor interaction between the proteins. This measurement is carried out at a range of TCR concentrations to allow plotting of a response curve.

## 2.3 – Molecular cloning

### 2.3.1 – Buffers and media used in molecular cloning and lentiviral production

<i>Buffer</i>	<i>Composition</i>
LB media	10 g/L tryptone (VWR Chemicals), 5 g/L yeast extract (VWR Chemicals), 10 g/L NaCl (Fisher Scientific). Supplemented following autoclaving with 50 µg/mL carbenicillin (Fisher Bioreagents).
TAE buffer	242 g TRIZMA-BASE (Sigma), 57.1 mL acetic acid (Fisher Scientific), 100 mL 0.5 M EDTA pH 8, made up to 1 L with ddH <sub>2</sub> O.
0.1 X TRIS-EDTA (TE) buffer	1 mM TRIS, 0.1 mM EDTA in ddH <sub>2</sub> O, pH 8.0. Sterilised with 0.22 µM filter (Merck).
CaCl <sub>2</sub>	2.5 M CaCl <sub>2</sub> = 9.18 g CaCl <sub>2</sub> .2H <sub>2</sub> O (Sigma), in 25 mL H <sub>2</sub> O. Sterilised with 0.22 µM filter (Merck).
2xHEPES-buffered saline (HeBS)	0.28 M NaCl (Fisher Scientific), 0.05 M HEPES (Fisher Scientific), 1.5 mM anhydrous Na <sub>2</sub> HPO <sub>4</sub> (Fisher Bioreagents) in ddH <sub>2</sub> O. pH to 7.0 using NaOH (Fisher Chemical). Sterilised with 0.22 µM filter (Merck).
Buffered water	125 µL, 1 M HEPES (Fisher Scientific), in 50 mL ddH <sub>2</sub> O. Sterilised with 0.22 µM filter (Merck).
MACS buffer	5 g/L Bovine serum albumin (Sigma), 2 mM EDTA. Sterilised with 0.22 µM filter (Merck).

**Table 2.2:** The buffers and media used for molecular cloning and lentiviral production.

### 2.3.2 – 868 construct and design of mutants

Constructs containing 868  $\alpha$ - and  $\beta$ -chains was ordered from GeneArt, Thermo Fisher. The TCR chains were separated by a 2A self-cleaving sequence (Kim *et al.*, 2011; Liu *et al.*, 2017), and flanked by XbaI and XhoI restriction sites at the start of the  $\alpha$ -chain and end of the  $\beta$ -chain respectively, see **Figure 2.5**. This was in keeping with their intended viral vector, pELNS, which contains a marker gene, Rat CD2 (rCD2), flanked by restriction sites XhoI and Sall, and is separated from the TCR chains by a second 2A sequence. The 2A sequences ensure equal expression of both TCR chains and the marker gene (Kim *et al.*, 2011; Liu *et al.*, 2017). In addition, the 2A sequence between the TCR chains contains a SmaI restriction site in order to allow chains to be taken in and out if required.

868 mutants were designed based on the complex structure and table of contacts published by Cole *et al.* in 2017 (Cole, Fuller, *et al.*, 2017) PDB 5NME. Amino acid substitutions are discussed in detail in **Section 4.2.1**. In short, key contact residues were substituted to amino acids of different chemical properties. For example, in the case of TCR22 ( $\beta$ D5S), the negatively charged aspartic acid was exchanged for the polar, uncharged serine. TCR constructs were codon optimised for human expression and ordered from GeneArt, Thermo Fisher.

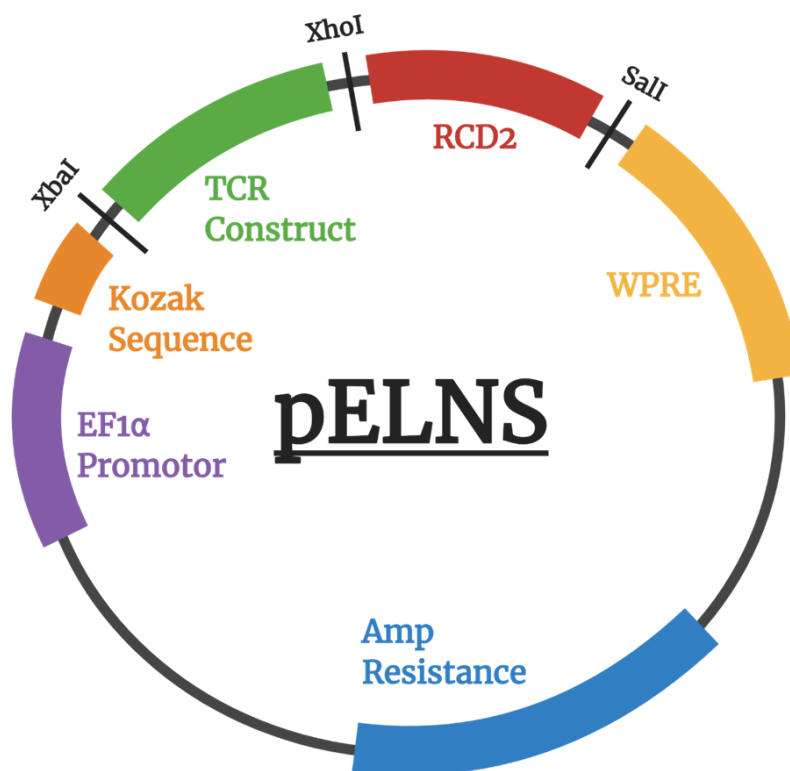


**Figure 2.5: TCR construct design for lentiviral work.** TCR  $\alpha$  and  $\beta$  chains were flanked with XbaI and XhoI restriction sites to allow cloning into pELNS transfer vector. Chains were separated by 2A self-cleaving peptides to aid equal expression. A SmaI restriction site sits between the TCR chains to allow editing of the TCR pair should the need arise. Once in the pELNS vector the TCR construct is immediately followed by marker gene rCD2. The two are separated by a 2A cleavage site to aid equal expression.

### 2.3.3 – Cloning constructs into the pELNS lentiviral vector

As discussed above, the 3<sup>rd</sup> generation lentiviral vector, pELNS contains the rCD2 marker gene, see **Figure 2.6**. CD2 is a T cell surface antigen involved in cell adhesion, it interacts with CD48 and CD58 (LFA-3) (Sandrin *et al.*, 1993; van der Merwe *et al.*, 1994; Davis and van der Merwe, 1996; Dustin *et al.*, 1997). In addition to the marker gene, pELNS contains an elongation factor (EF)-1 $\alpha$  promoter that controls expression of the inserted gene, a Kozak sequence, a woodchuck hepatitis post-transcriptional regulatory element (WPRE), and an ampicillin resistance gene. The EF-1 $\alpha$  promoter allows for constitutive expression in a number of different eukaryotic cell types, and has been shown to induce more efficient transduction and improved long-term transgene expression than the cytomegalovirus CMV promoter (X. Wang *et al.*, 2017). It is thought that human EF-1 $\alpha$  is more resistant to silencing

than viral-origin promoters (Gill *et al.*, 2001; Teschendorf *et al.*, 2002; X. Wang *et al.*, 2017). The Kozak sequence is a eukaryotic protein translation initiation site upstream of most protein encoding genes (Kozak, 1984, 1987), its presence in DNA vectors can significantly upregulate expression of the transgene (Ólafsdóttir *et al.*, 2008). The WPRE was isolated in 1998 through shared homology with the hepatitis B virus post-transcriptional regulatory element (Huang and Liang, 1993; Donello, Loeb and Hope, 1998). Both regulatory elements serve to increase expression of associated genes at the post-transcriptional level, by facilitating trafficking of RNA transcripts from the nucleus to the cytoplasm (Huang and Liang, 1993; Donello, Loeb and Hope, 1998). Inclusion of the WPRE sequence in viral vectors has been shown to enhance expression of the transgene (Schwenter, Déglon and Aebischer, 2003; Klein *et al.*, 2006). The ampicillin resistance is principally employed during the amplification of the DNA via maxiprep (Section 2.3.5).



**Figure 2.6: pELNS vector for lentiviral work.** The TCR construct is cloned into the pELNS vector using XbaI and XhoI restriction sites. It is preceded by an EF1 $\alpha$  promoter and a Kozak sequence to boost expression. It is immediately followed by marker gene, rCD2. rCD2 is flanked by XhoI and SalI restriction sites to facilitate exchange of the marker gene should the need arise. The pELNS vector also contains a WPRE to further enhance expression of the transgene and an ampicillin resistance gene to allow easy recognition of successful cell uptake during amplification of the vector in bacterial cells.

The wildtype 868 construct and all mutants were ordered from the GeneArt service by Thermo Fisher. Constructs arrived in either pMK or pMA transport vectors, which confer resistance to kanamycin or ampicillin respectively. All constructs were immediately amplified via mini prep, using XL-10 Gold cells and agar plates supplemented with the relevant antibiotic (**Section 2.1.5**). The DNA was then digested in order to remove the construct from the transport vector; 1-5 µg of DNA was mixed with 1 µL XbaI (Thermo Scientific), 1 µL XhoI (Thermo Scientific), and 2 µL 10X Fast Digest buffer (Thermo Scientific). The solution was made up to 20 µL with nuclease-free water (Ambion). 5-10 µg of the recipient vector, pELNS, was digested in the same manner. The digestion mixtures were centrifuged briefly and incubated at 37°C overnight. The following morning the digestion mixtures were loaded onto a 1% agarose gel (Sigma), along with the Hyperladder 1 kb (Bioline), and run at 90 V for 45 minutes. This gel was produced by dissolving 0.7 g agarose (Sigma) in 70 mL TAE buffer, when the gel was sufficiently cooled 2.5 µL Midori Green DNA dye (Nippon Genetics Europe) was added and it was poured into the mould. A 1 kb DNA Hyperladder (Bioline) was also included. The gel was visualised using an UV illuminator (Nippon Genetics Europe).

The relevant bands were cut out of the gel using a scalpel, and the gel fragments were digested using a Promega Wizard SV Gel and PCR Clean-up System according to the manufacturer's instructions. Gel slices were weighed and 10 µL of Membrane Binding Solution was added per 10 mg. This was incubated at 65°C, vortexing every few minutes until the gel had completely dissolved. This solution was then loaded onto a SV Minicolumn/Collection Tube set up and incubated at room temperature for 1 minute to allow DNA to bind to the column. The tube was centrifuged 16,000 x g for 1 minute to remove impurities (Eppendorf Centrifuge 5424). The column was washed twice with Membrane Wash Solution (supplemented with ethanol), and the empty column was centrifuged once more to remove any residual ethanol. The DNA was eluted in 20 µL nuclease-free water (Ambion), and the concentration measured using a spectrophotometer (Geneflow) as described in **Section 2.1.5**. The molar concentration was calculated using the Promega biomath tool.

Pure digested construct DNA was then ligated into the digested pELNS vector. The digested insert and vector were mixed at a ratio of 150 fmol:30 fmol, with the insert in molecular excess. 2 µL 10x T4 ligase buffer (Thermo Scientific), and 1 µL T4 ligase (Thermo Scientific) was added to the ligation mixture and the total volume made up to 20 µL with nuclease-free water (Ambion). The ligation solution was mixed gently and incubated at room temperature

for 2 hours. A negative control in which the insert DNA was replaced with an equal volume of nuclease-free water (Ambion) was also set up.

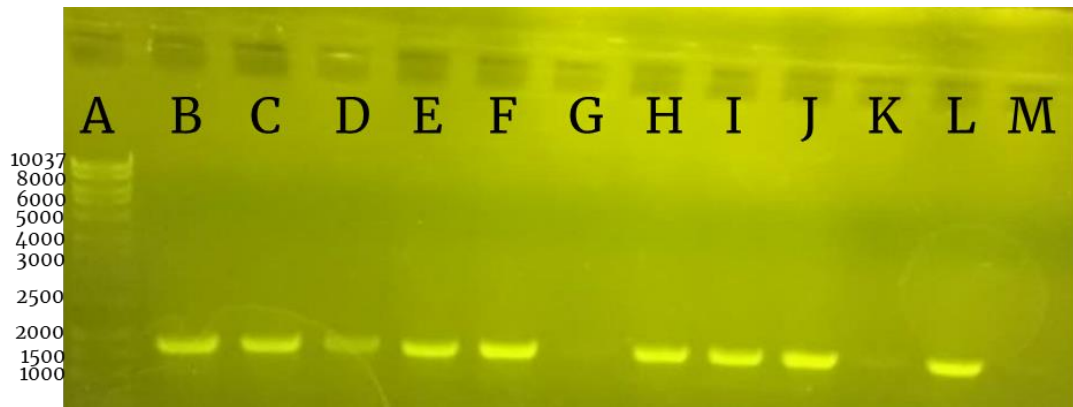
5 µL ligation mixture was then used to transform XL-10 Gold cells, undigested empty pELNS acted as a positive control and the water-containing ligation mixture as a negative control. XL-10 Gold cells were allowed to defrost on ice for 5 minutes before the DNA was added. They were then incubated for a further 30 minutes on ice before a 30 second heat shock at 42°C to encourage uptake of DNA. In order to amplify cell numbers 500 µL of pre-warmed SOC media (Clontech) was added and tubes were placed in an orbital shaking incubator for 1 hour (37°C, 220 RPM) (Thermo Fisher). This culture was used to inoculate agar plates supplemented with 50 µg/mL carbenicillin, they were grown overnight at 37°C.

The following morning, individual colonies were selected for colony polymerase chain reaction (PCR) to confirm the presence of the desired insert. For lentiviral work, constructs were cloned into the pELNS vector and the pELNS F1 and pELNS R1 primers (Eurofins Genomics) (see **Table 2.3**) were used for colony PCR. For each colony a PCR mixture was prepared, containing: 12.5 µL DreamTaq MasterMix (Thermo Scientific), 1 µL pELNS F1, 1 µL pELNS R1, 10.5 µL nuclease-free water (Ambion). Sterile P2 tips were used to inoculate each mix. The tubes were briefly centrifuged to remove air bubbles and then placed in a thermal cycler for the following conditions:

94°C	10 minutes	} x 20 cycles
94°C	20 seconds	
57°C	20 seconds	
72°C	1 minute	
72°C	7 minutes	

Each PCR mixture was then run on a 1% agarose gel, prepared as described above. The gel was visualised using an UV illuminator (Nippon Genetics Europe). Lanes with bands of the appropriate size (~1836 base pairs) indicate positive ligation reaction, see **Figure 2.7**.

Plasmids were extracted from positive colonies using a PureLink Quick Plasmid Miniprep kit (Thermo Fisher), as described above (**Section 2.1.5**). Concentrations were measured using a spectrophotometer (Geneflow). Samples were sequenced using Eurofins Geonmics' TubeSeq service with the relevant primers (see **Table 2.3**).



**Figure 2.7: Representative colony PCR.** The transgene is 1836 base pairs in size. A colony PCR uses primers to amplify a region within the transfer vector and provides a very clear demonstration of which colonies have successfully taken up the vector. In this example gel, the construct has successfully been incorporated into all colonies apart from colonies G, K, and M. Well A contains a Hyperladder 1 kb (Bioline).

#### 2.3.4 – Sequencing constructs for validation

Isolated plasmids were sequenced to confirm presence of the inserted construct using Eurofins Genomics. Following mini prep, 15  $\mu\text{L}$  of DNA at 100  $\text{ng}/\mu\text{L}$  was sent along with 2  $\mu\text{L}$  pELNS F2 primer (**Table 2.3**) at 10  $\text{pmol}/\mu\text{L}$  using the Mix2Seq service (Eurofins genomics). Sequences were checked using the NCBI Nucleotide Blast tool. Following maxi prep (**Section 2.3.5**), 15  $\mu\text{L}$  of DNA at 100  $\text{ng}/\mu\text{L}$  was sent to Eurofins genomics along with 2  $\mu\text{L}$  each of pELNS F2, Alpha Constant, Beta Fw, and Rat Reverse primers at 10  $\text{pmol}/\mu\text{L}$  (**Table 2.3**) using the TubeSeq service. Overlapping sequence results were checked manually using SnapGene Viewer to confirm the absence of any mutation aside from the intended change.

<i>Primer Name</i>	<i>Primer Sequence</i>	<i>Use</i>
pELNS F1	GAGTTTGGATCTTGGTTCATTC	Sequencing constructs in pELNS, and PCR.
pELNS F2	CTTCCATTTTCAGGTGTCGTG	Sequencing constructs in pELNS
pELNS R1	GCATTAAAGCAGCGTATCCAC	Sequencing constructs in pELNS, and PCR
pELNS R2	CCAGAGGTTGATTGTCGAC	Sequencing constructs in pELNS
CMV Fw	CGCAAATGGGCGGTAGGCGTG	Sequencing pMD2.G and pMDLg
M13 Fw	GTAAAACGACGGCCAG	Sequencing pRSV-Rev
pSF Fw	TACCATCCACTCGACACACC	Sequencing constructs in pSF
pSF Rev	AAGCAGCGTATCCACATAGC	Sequencing constructs in pSF
Early Alpha C	GACTCCAAGTCCAGCGACAA	Sequencing 868TCR
Alpha Constant	AACCTGCTGATGACACTGAG	Sequencing 868TCR
Beta Fw	GTTGGGCTCTGCTGTGTCTC	Sequencing 868TCR
Rat Rev	AACTTGCACCGCATATGCAT	Sequencing 868TCR

**Table 2.3:** A summary of the primers used throughout this thesis. Primers sourced from Eurofins Genomics.

### 2.3.5 – Amplifying DNA via maxi preparation

Competent, XL-10 Gold, cells were transformed with the relevant DNA as described in **Section 2.1.4**. Single colonies were selected from the resulting LB agar plates to inoculate 250 mL of LB media, these were grown overnight in an orbital shaking incubator (37°C, 220 RPM) (Thermo Fisher). The following morning, bacterial cells in these cultures were harvested by centrifuging at 2755 x g (3700 RPM, Eppendorf 5810R centrifuge) for 30 minutes, DNA extraction was then carried out using a PureLink HiPure Plasmid Filter Maxiprep kit (Thermo Fisher) according to the manufacturer’s instructions. 10 mL of Resuspension Buffer (R3) supplemented with RNase A was used to dissolve the pellet, before an equal volume of warmed Lysis Buffer (L7) was used to lyse cells. Samples were incubated at room temperature for 5 minutes to allow for lysis. 10 mL of Precipitation Buffer (N3) was added to the lysates to precipitate protein and genomic DNA, leaving circular plasmid DNA in solution. Samples were loaded onto pre-equilibrated PureLink HiPure Maxi Columns (Thermo Fisher) and allowed to filter by gravity flow. The column was washed using 50 mL



of Wash Buffer (W8) before 15 mL of Elution Buffer (E4) was used to recover the purified plasmid DNA. 10.5 mL of propan-2-ol (Fisher Chemical) was added, and the eluate was centrifuged at 16,000 x g for 30 minutes (Beckman Coulter Avanti J-E) at 4°C to precipitate the DNA. Finally, the DNA was washed using 5 mL 70% ethanol (Fisher Chemical), before being re-pelleted by centrifuging at 16,000 x g for 5 minutes (Beckman Coulter Avanti J-E). Air-dried pellets were resuspended in 500 µL of nuclease-free water (Ambion). As above, concentrations were measured using a spectrophotometer (Geneflow). Samples were sequenced using Eurofins Genomics' TubeSeq service with the relevant primers, **Table 2.3**.

## 2.4 – Lentiviral production and cell transduction

### 2.4.1 – Production of lentiviruses in HEK 293T cells

3<sup>rd</sup> generation lentiviruses were produced using HEK 293T cells plated in T175 flasks (Grenier Bio-one) at approximately 60-80% confluency. HEK 293T D10 media (**Table 2.4**) was refreshed 6 hours before transduction. The transfer plasmid used was pELNS (kindly provided by Dr. James Riley, University of Pennsylvania) carrying 868 constructs and marker gene rCD2, separated by self-cleaving 2A peptide sequences (**Section 2.3.2**). 15 µg of transfer plasmid was co-transduced with 7 µg of envelope plasmid pMD2.G (Addgene plasmid #12259) and 18 µg of both packaging plasmids pMDLg/pRRE and pRSV-Rev (Addgene plasmids #12251 and #12253 respectively). Plasmids were co-transduced into HEK 293T cells using calcium phosphate precipitation. To achieve this DNA was first diluted in a solution of 1.1 mL 0.1 X TE Buffer and 580 µl buffered water (**Table 2.2**). 188 µL of 2.5 M CaCl<sub>2</sub> was added to the diluted DNA before 1.9 mL 2 X HEPES Buffered Saline (**Table 2.2**) was added to the solution in a dropwise manner. During this addition the solution was aerated via a vortex (Vortex Genie 2, Scientific Industries) to prevent sudden changes to pH and buffer salt concentrations. This ensured formation of a fine CaCl<sub>2</sub>-DNA precipitate and minimise DNA clumping which would hamper cell uptake. Precipitation occurred during a 20-minute incubation at room temperature, before the solution was transferred to HEK 293T cells via Pasteur pipette (Starlab). HEK 293T D10 media was refreshed 16 hours following transduction. Media containing lentiviral product was harvested at 48 hours and 72 hours post-transduction, and filtered through 0.45 µM filters (Sigma) to remove cells and debris. Lentiviral product was stored at 4°C for up to 1 week, during which time transduction efficiency was tested in the Jurkat cell line (**Section 2.4.2**). Following this confirmation, lentivirus supernatants were concentrated and either used immediately for transduction of primary T cells or frozen at -80°C for long term storage (**Section 2.4.3**).

#### 2.4.2 – Transduction of immortal cell lines

Antigen presenting cells (such as Jurkat or MOLT-3 cells) were plated in 24 well plates (Grenier) at a density of 100,000 cells/well. 1 mL of lentiviral supernatant (prior to concentration) was added to each test well. Cells were then spininfected by centrifuging at 500 x g for 2 hours (Heraeus Megafuge 1.0R). Lentiviral supernatant was removed the following morning and replaced with R10 media.

Lentiviral efficiency was evaluated by transducing the T cell leukaemia Jurkat cell line (clone E6.1). Jurkat cells were cultured for 1 week following transduction before lentiviral uptake was assessed by surface marker staining and flow cytometry (**Section 2.6.2**).

#### 2.4.3 – Concentration of lentiviral supernatant

Lentivirus supernatants were concentrated by centrifugation at 140,000 x g (28,000 RPM) for 2 hours (Beckman Coulter Optima™ ultracentrifuge, SW28 rotor). The lentiviral pellet was resuspended in 300 µL 200 international units (IU) T cell media and either used immediately for transduction of CD8<sup>+</sup> T cells, or frozen at -80°C for storage.

#### 2.4.4 – Transducing primary CD8 T cells

In preparation for lentiviral transduction, CD8<sup>+</sup> T cells were isolated from peripheral blood mononuclear cells (PBMCs) as described in **Section 2.5.4**. CD8<sup>+</sup> T cells were plated in 48 well plates (Grenier) at a density of 500,000 cells/well in 200 IU media. Cells were activated overnight to increase susceptibility to viral uptake (Bilal, Vacaflares and Houtman, 2015). This was achieved by mixing T cells with CD3/CD28 activator beads (Dynabeads, Life Technologies) at a ratio of 3:1 (beads:cells), thus a density of 1.5 million beads/well. The total volume of each well was 1 mL 200 IU media. T cells were incubated at 37°C 5% CO<sub>2</sub> overnight (BB 15 CO<sub>2</sub> incubator, Thermo Scientific).

The following morning 700 µL of media was removed from each well and 100 µL of concentrated lentivirus added. 0.25 µL of polybrean (Santa Cruz Biotechnology) at a concentration of 10 mg/mL was added to each well to give a working concentration of 5 µg/mL. The total volume in each well was made up to 500 µL with 200 IU media, and cells were incubated overnight at 37°C (BB 15 CO<sub>2</sub> incubator, Thermo Scientific).

The following morning 500 µL of T cell transduction media was added to each well. Subsequently T cells were fed three times a week (Monday, Wednesday, and Friday) by

replacing half of the media. T cells were fed with T cell transduction media for at least two weeks following transduction, before moving on to 200 IU media.

Following the initial one-week period CD8<sup>+</sup> T cells were assessed for lentiviral uptake by rCD2 surface marker staining and flow cytometry (**Section 2.6.2**). If staining indicated that uptake of the transgenes had been successful, then transduced CD8<sup>+</sup> T cells were separated from untransduced CD8<sup>+</sup> T cells by means of rCD2 surface marker purification (**Section 2.4.5**) and expanded (**Section 2.5.5**).

#### 2.4.5 – Purification based on surface marker expression

Transduced CD8<sup>+</sup> T cells were isolated from their untransduced peers by means of surface expression of the marker gene, rCD2, using the miniMACS separation protocol (Miltenyi Biotec) according to the manufacturer's instructions. T cells were harvested, washed with 15 mL of cold MACS buffer, and pelleted by centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R). Pelleted cells were resuspended in 50 µL of cold MACS buffer and 2 µL of anti-rCD2-PE (clone OX-34, Biolegend). Cells were incubated on ice for 20 minutes. MACS buffer, the centrifuge, and cells were kept cold at all times to prevent beads from being internalised by cells.

Excess antibody was removed by washing with 15 mL of cold MACS buffer. Following centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R) cells were resuspended in 80 µL of cold MACS buffer and 20 µL of anti-PE microbeads (Miltenyi Biotec) per 10<sup>7</sup> cells and incubated at 4°C for 15 minutes.

Excess beads were removed by washing with 15 mL of cold MACS buffer. Following centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R) cells were resuspended in 500 µL of cold MACS buffer. This suspension was passed through a 30 µM filter (Celltrics, Wolf Labs) to remove aggregated cells before being loaded onto pre-equilibrated miniMACS separation columns (Miltenyi Biotec). The column was washed with 500 µL of cold MACS buffer three times to remove rCD2<sup>-</sup> cell populations. The column was then removed from the magnetic holder and the rCD2<sup>+</sup> population eluted in 500 µL of 200 IU media using the plunger supplied with the column. rCD2<sup>+</sup> T cells were counted and plated at the appropriate density in 24 well plates (Grenier).

This protocol can be used to isolate subpopulations expressing other surface markers by replacing the rCD2-PE antibody with a PE-conjugated antibody targeting the marker of

interest. In contrast, marker-negative populations can be collected by preserving the flow through.

## 2.5 – Cell culture

### 2.5.1 – Buffers and media used in cell culture work throughout this thesis

<i>Buffer/media</i>	<i>Composition</i>
D10	Dulbecco's Modified Eagle Medium (DMEM) – high glucose (Sigma Life Science), 10% heat inactivated foetal bovine serum (FBS) (Gibco Life Technologies), 100 U/ml penicillin (Sigma-Aldrich) 100 µg/mL streptomycin (Sigma-Aldrich), 2 mM L-glutamine (Sigma-Aldrich).
D-PBS-EDTA	Dulbecco's PBS (D-PBS) (Sigma-Aldrich) supplemented with 2 mM EDTA.
R10	RPMI 1640 (Sigma-Aldrich), 10% FBS, 100 U/ml penicillin (Sigma-Aldrich) 100 µg/mL streptomycin (Sigma-Aldrich), 2 mM L-glutamine (Sigma-Aldrich).
R0	RPMI 1640 (Sigma-Aldrich), 100 U/ml penicillin (Sigma-Aldrich) 100 µg/mL streptomycin (Sigma-Aldrich), 2 mM L-glutamine (Sigma-Aldrich).
R5	RPMI 1640 (Sigma-Aldrich), 5% FBS, 100 U/ml penicillin (Sigma-Aldrich) 100 µg/mL streptomycin (Sigma-Aldrich), 2 mM L-glutamine (Sigma-Aldrich).
200IU T cell culture media	R10 supplemented with 25 ng/ml interleukin (IL)-15 (Miltenyi Biotech), 200 IU/mL IL-2 (Proleukin), 1 x non-essential amino acids solution (Sigma-Aldrich), 1 mM sodium pyruvate (Sigma-Aldrich), 10 mM HEPES buffer (Sigma-Aldrich).
20IU T cell expansion media	R10 supplemented with 25 ng/ml interleukin (IL)-15 (Miltenyi Biotech), 20 IU/mL IL-2 (Proleukin), 1 x non-essential amino acids solution (Sigma-Aldrich), 1 mM sodium pyruvate (Sigma-Aldrich), 10 mM HEPES buffer (Sigma-Aldrich).
Transduction media	200 IU T cell culture media supplemented with 20% FBS (final concentration).
Red blood cell (RBC) lysis buffer	155 mM Ammonium chloride (NH <sub>4</sub> Cl) (Acros Organics), 10 mM potassium bicarbonate (KHCO <sub>3</sub> ) (Sigma-Aldrich), 0.1 mM EDTA at pH 8.0, solution made up to 500 mL with ddH <sub>2</sub> O, pH to 7.2-7.4 with HCl (Sigma-Aldrich).

Freezing buffer	90 % FBS (Gibco Life Technologies), 10 % dimethylsulfoxide (DMSO) (Sigma-Aldrich).
MACS buffer	0.5 % Bovine serum albumin (Sigma-Aldrich), 2 mM EDTA, in D-PBS (Sigma-Aldrich).
Fixing buffer	4 % paraformaldehyde (Fisher Chemicals) in PBS.
Fluorescence-activated cell sorting (FACS) buffer	2 % FBS in PBS.
Wash buffer	0.05 % Tween-20 (Merck) in PBS.
Reagent diluent	1 % Bovine serum albumin (Sigma-Aldrich) in PBS.

**Table 2.4:** Buffers and media used for cell culture throughout this thesis.

### 2.5.2 – Culturing of adherent cells lines

Adherent cell lines (such as HEK 293T cells and mm909.24 cells) were sourced from the American Type Cell Collection and cultured as recommended in their respective medias (**Table 2.4**) in incubators maintaining 37°C and 5% CO<sub>2</sub> humidity (BB 15 CO<sub>2</sub> incubator, Thermo Scientific). Whilst being maintained in culture, cell was routinely refreshed Mondays and Fridays. In general, adherent cells were split once a confluency of 80% was exceeded using a non-enzymatic detachment method. Old cell culture media was removed and the cells were washed in 2 mM EDTA in Dulbecco's PBS (Sigma-Aldrich) to remove any remaining media and detached cells. Fresh EDTA-D-PBS was added to the flask in sufficient volume to coat the surface. Cells were incubated at 37°C until all cells had detached, approximately 10 minutes. Following this, the EDTA in the D-PBS was neutralised by adding an equal volume of cell culture media to the flask. Detached cells were then pelleted by centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R) in a falcon tube. The pellet was resuspended in fresh cell culture media, and seeded back at a density suitable for the immediate experimental requirements. If not needed in the near future, adherent cells were generally split 1:20.

### 2.5.3 – Culturing of suspension cell lines

The suspension cell lines used throughout this thesis are summarised in **Table 2.5**. Suspension cells were grown in R10 media (**Table 2.4**) in incubators maintaining 37°C and 5% CO<sub>2</sub> humidity (BB 15 CO<sub>2</sub> incubator, Thermo Scientific). Cells were sourced from the American

Type Cell Collection and cultured as recommended. Whilst being maintained in culture, suspension cells were split Mondays and Fridays routinely or when media colour indicated a change in pH due to a build-up of waste products. Cells were split by resuspending fully and then removing half of the media to be replaced by fresh media. If cell lines were being expanded for experimental purposes fresh media was added incrementally each day, and cells were split over multiple flasks to maintain appropriate culture volumes.

<b>Cell line name</b>	<b>Media</b>	<b>Use</b>	<b>Cell type</b>
Jurkat	R10	Testing lentiviral products.	Immortalised T cells, suspension.
T2	R10	Antigen presenting target cells.	Lymphoblastic cell line, suspension.
MOLT-3	R10	Transduction of lentiviral constructs.	Immortalised T cells, suspension.
HEK 293T	D10	Production of lentiviral particles.	Human embryonic kidney cells, adherent.
C1R	R10	Transduction of lentiviral constructs.	B lymphoblastic cell line, suspension.
MOLT-3	R10	Transduction of lentiviral constructs.	T lymphoblastic cell line, suspension.
K562	R10	Transduction of lentiviral constructs.	Myelogenous leukemia line, suspension.
MM909.24	R10	Transduction of lentiviral constructs.	Melanoma cell line, adherent.

**Table 2.5:** A summary of cell lines used in this thesis.

2.5.4 – Isolation of CD8<sup>+</sup> primary T cells from whole blood  
 EDTA-treated buffy coats from three healthy donors were acquired regularly from the Welsh Blood Service (WBS) in accordance with the Human Tissue Act and local ethical approval. PBMCs were isolated using a Histopaque™ (Stemcell Technologies) density gradient approach. Blood was delivered by the WBS in the afternoon, diluted 1:2 with R10 and rolled overnight at room temperature in 50 mL falcons. The following morning 13 mL Histopaque™

was added to the bottom compartment of 50 mL SepMate Tubes (Stemcell Technologies). 25 mL of diluted blood was added slowly to the SepMate Tube to minimise mixing, and tubes were topped up to 50 mL with R0 to further dilute blood (approximately 10-12 mL). SepMate tubes were centrifuged at 1200 x g for 10 minutes (Heraeus Megafuge 1.0R). The top layer of primarily lymphocytes and some RBCs was transferred to a 50 mL falcon and diluted further with R0. Cells were pelleted by centrifuging at 800 x g for 10 minutes (Heraeus Megafuge 1.0R). All pellets from the same donor were combined and dissolved in the same 25 mL of RBC lysis buffer, see **Table 2.4**. Tubes were then incubated at 37°C for 10 minutes in a water bath. Following this, the tubes were topped up to 50 mL with R0 and centrifuged 400 x g for 5 minutes (Heraeus Megafuge 1.0R). This step was repeated with a fresh 25 mL of RBC lysis buffer if, following centrifugation, the pelleted cells were still visibly red. RBC-clear pellets were resuspended in 25 mL of R10 for counting.

If primary CD8<sup>+</sup> T cells were not required, isolated PBMCs were non-fatally irradiated to be used for T cell expansions (**Section 2.5.6**). If primary CD8<sup>+</sup> T cells were required for lentiviral transduction, they were isolated at this point using the miniMACS separation protocol (Miltenyi Biotec) according to the manufacturer's instructions. For this protocol MACS buffer, the centrifuge, and cells were kept cold at all times to prevent CD8 microbeads (Miltenyi Biotec) from being internalised by cells.

Lymphocytes were washed with 15 mL of cold MACS buffer, and pelleted by centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R). Pelleted cells were resuspended in 80 µL of cold MACS buffer and 20 µL of CD8 microbeads (Miltenyi Biotec) per 10<sup>7</sup> cells and incubated at 4°C for 15 minutes, the number of expected CD8<sup>+</sup> cells was estimated based on the frequencies of CD8<sup>+</sup> cells recovered by previous attempts.

Excess beads were removed by washing with 15 mL of cold MACS buffer. Following centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R) cells were resuspended in 500 µL of cold MACS buffer. This suspension was passed through a 30 µM filter (Celltrics, Wolf Labs) to remove aggregated cells before being loaded onto pre-equilibrated miniMACS separation columns (Miltenyi Biotec). The column was washed with 500 µL of cold MACS buffer three times to remove CD8<sup>-</sup> cell populations. The column was then removed from the magnetic holder and the CD8<sup>+</sup> population eluted in 500 µL of 200 IU media using the plunger supplied with the column. CD8<sup>+</sup> cells were counted and plated at the appropriate density in 24 well plates (Greiner). At this point cells were ready for the lentiviral transduction procedure described in **Section 2.4.4**.

### 2.5.5 – T cell purification based on multimer-binding specificity

CD8<sup>+</sup> T cells that are able to bind to certain pMHC molecules can be isolated in a similar manner to that described in **Section 2.4.5** using streptavidin-conjugated tetramers made as described in **Section 2.6.3**. They are isolated using the miniMACS separation protocol (Miltenyi Biotec) according to the manufacturer's instructions. T cells were harvested, washed with 15 mL of cold MACS buffer, and pelleted by centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R). Pelleted cells were resuspended in 50 µL of cold MACS buffer and 50 µL of 100 nM Protein Kinase Inhibitor (Dasatinib, Axon Medchem), cells were incubated at 37°C for 10 minutes. Subsequently 5 µg of tetramer was added and cells were incubated on ice for 30 minutes. MACS buffer, the centrifuge, and cells were kept cold at all times to prevent beads from being internalised by cells.

Excess tetramer was removed by washing with 15 mL of cold MACS buffer. Following centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R) cells were resuspended in 80 µL of cold MACS buffer and 20 µL of anti-PE microbeads (Miltenyi Biotec) per 10<sup>7</sup> cells and incubated at 4°C for 15 minutes.

Excess beads were removed by washing with 15 mL of cold MACS buffer. Following centrifuging at 400 x g for 5 minutes (Heraeus Megafuge 1.0R) cells were resuspended in 500 µL of cold MACS buffer. This suspension was passed through a 30 µM filter (Celltrics, Wolf Labs) to remove aggregated cells before being loaded onto pre-equilibrated miniMACS separation columns (Miltenyi Biotec). The column was washed with 500 µL of cold MACS buffer three times to remove tetramer<sup>-</sup> cell populations. The column was then removed from the magnetic holder and the tetramer<sup>+</sup> population eluted in 500 µL of 200 IU media using the plunger supplied with the column. Tetramer<sup>+</sup> cells were counted and plated at the appropriate density in 24 well plates (Grenier).

Alternatively, towards the end of my studies the laboratory acquired a SONY MA900 Multi-Application cell sorter which can be used to sort tetramer<sup>+</sup> populations. T cells were stained with 5 µg of PE-tetramer, as described above, prior to analysis. Tetramer<sup>+</sup> populations were cultured in 200 IU T cell media supplemented with Amphotericin B/Fungizone (Merck) following sorting. All FACS-based cell-sorting performed by Dr Cristina Ruis Rafael.

### 2.5.6 – Expansion of CD8<sup>+</sup> primary T cells and subsequent culturing

CD8<sup>+</sup> T cells were routinely expanded in per T25 flasks (Greiner) at 37°C, 5 % CO<sub>2</sub>. Up to 1 x 10<sup>6</sup> CD8<sup>+</sup> T cells were co-cultured with 15 x 10<sup>6</sup> cells from an allogenic, non-lethally irradiated



mix of PBMCs from three different WBS donors (known as a feeder mix). Feeder mix cells were isolated as described above (**Section 2.5.4**) and irradiated at 3000-3100 cGy. Each flask was made up to a total volume of 15 mL with 20 IU media and 1 µg/mL phytohemagglutinin (PHA, PAN Biotech), see **Table 2.4**.

Expansions were incubated at 37°C, 5 % CO<sub>2</sub>, tilted at an angle to allow cells to congregate and benefit from cell-cell proximity, for five days. On the 5<sup>th</sup> day, half of the media (7.5 mL) was exchanged for a fresh 7.5 mL of 20 IU. Seven days into the expansion cells were harvested, counted, and plated at the appropriate density (3-4 million per well) in 24 well plates (Greiner), in 2 mL of 200 IU media per well see **Table 2.4**.

T cells were subsequently cultured by changing half the media three times a week; Monday, Wednesday, and Friday. Following day 14 of the expansion cells were considered fit for downstream functionality assays. If necessary, cells were frozen within four weeks of expansion (**Section 2.5.7**).

#### 2.5.7 – Cell counting

For counting, cells were harvested, washed, and resuspended in R10 – the volume of which was dependent on the estimated number of cells. If cells were too densely packed to obtain accurate counts they were diluted further. 10 µL of resuspended cells was then mixed with 10 µL of 0.4 % trypan blue solution (Sigma Aldrich) (dilution factor, 2). Cells were added to a haemocytometer and counted based on the assumption that live cells would not take up trypan blue and so would appear bright, whereas dead cells would appear blue under the microscope. The following calculation was used:

$$\text{Average cells per grid} \times \text{dilution factor} \times 10^4 = \text{cells/mL}$$

#### 2.5.8 – Freezing cells for storage in liquid nitrogen

For freezing, cells were counted (**Section 2.5.6**) and centrifuged at 400 x g for 5 minutes to remove cell culture media (Heraeus Megafuge 1.0R). Cells were then resuspended in freezing buffer (**Table 2.4**) and placed, in 1 mL aliquots, in internal thread cryovials (Nunc). Cells were initially frozen at -80°C in isopropanol-filled controlled-rate freezing pots (Mr Frosty, Nalgene). Isopropanol was refreshed every five uses, as per the manufacturer's recommendation. After at least 24 hours at -80°C frozen cells were transferred to liquid nitrogen for long-term storage.

For thawing, cells were removed from liquid nitrogen and rapidly defrosted by incubation in a 37°C water bath. As soon as they had thawed, cells were transferred into 10 mL of warmed R10 to dilute freezing buffer. Cells were then centrifuged at 400 x g for 5 minutes to remove freezing buffer (Heraeus Megafuge 1.0R). Cells were resuspended in their required media, counted, and plated or placed in flasks according to their preference.

## 2.6 – Cell-based functional assays

### 2.6.1 – Antibodies used for flow cytometry throughout this thesis

Antibody specificity	Conjugated fluorochrome	Clone	Source
rCD2	FITC PE	OX-34	Biolegend
CD8	FITC APC	REA734	Miltenyi Biotec
CD8	PE PE-Vio770 APC-Vio770	BW135/80	Miltenyi Biotec
CD8	APC-H7 Per-CP	SK1	BD Biosciences Biolegend
CD4	PE APC-Vio770	VIT4	Miltenyi Biotec
CD4	FITC	REA623	Miltenyi Biotec
CD4	APC	M-T466	Miltenyi Biotec
CD3	PerCP PE	BW264/56	Miltenyi Biotec
CD19	Pacific Blue	HIB19	Biolegend
HLA-A2	APC FITC PE	BB7.2	Miltenyi Biotec
TNF	PE-Vio770	cA2	Miltenyi Biotec
TNF	PE-Cy7 PerCP/Cyanine5.5 APC	mAb11	BD Biosciences
CD107a	PE FITC	H4A3	BD Biosciences
Anti-PE	Unconjugated	PE001	Biolegend
TCR Vbeta5a	Unconjugated	1C1	Thermo Fisher
Anti-Mouse Ig	PE	Polyclonal	BD Biosciences

**Table 2.6:** The antibodies used for flow cytometry throughout this thesis.

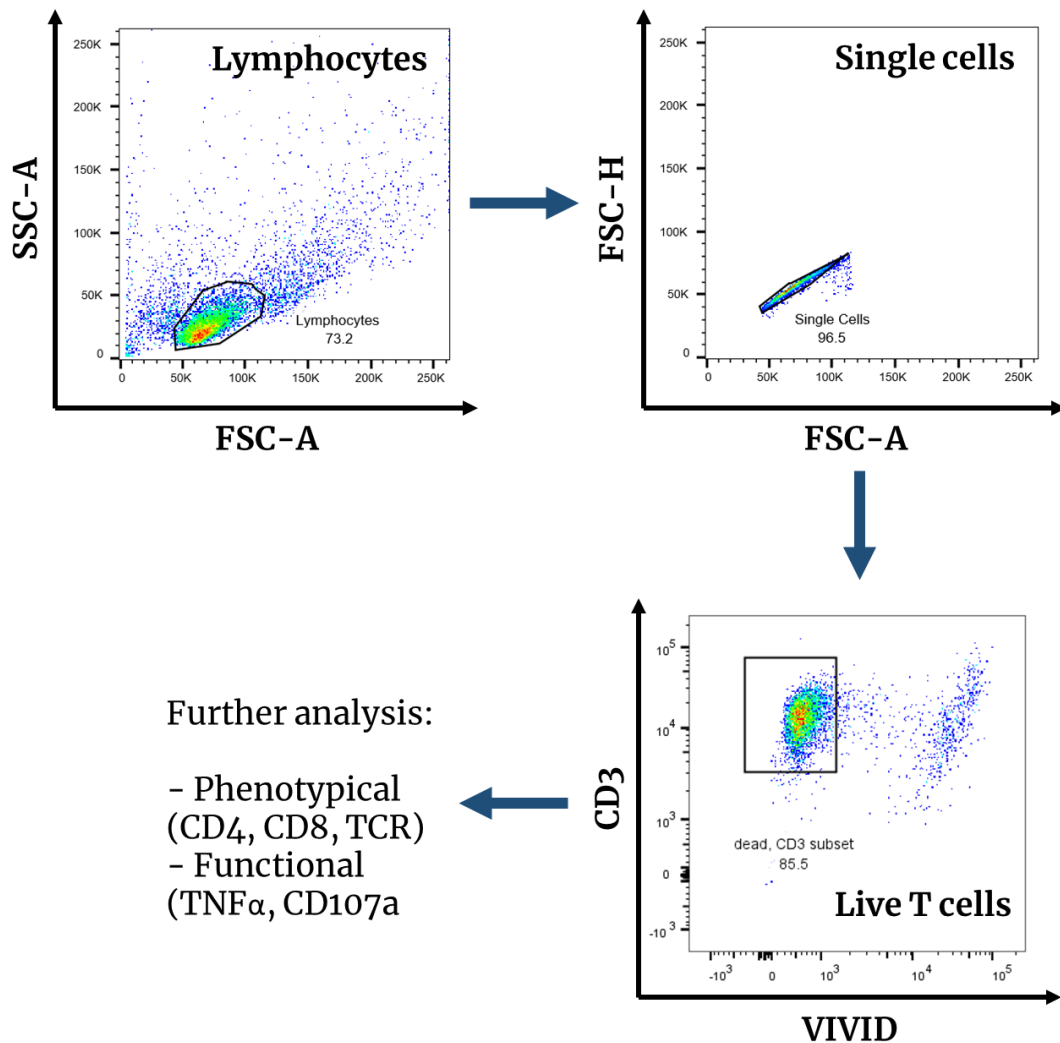
All antibodies were raised in mice against human proteins except for rCD2 (mouse anti-rat), and anti-mouse Ig (Goat anti-mouse). The latter was used in conjugation with the anti-human TCR Vbeta5a. PE = phycoerythrin; PerCP = peridinin chlorophyll protein; FITC = fluorescein isothiocyanate; APC = allophycocyanin.

### 2.6.2 – Flow cytometry: staining for surface markers

Approximately 50,000 cells were used per staining condition. Cells were harvested, transferred to FACS tubes (Elkay Labs), and washed twice with 3 mL of autoclave-sterilised PBS (made as described in **Table 2.1**) to remove any remaining components of cell media. Tubes were centrifuged at 800 x g for 2 minutes (Heraeus Megafuge 1.0R) to pellet cells before PBS was removed and cells were stained with an amine-reactive dye, LIVE/DEAD™ Fixable Violet Dead Stain (Thermo Fisher), referred to henceforth as vivid. Tubes were vortexed briefly to mix and incubated at room temperature in the dark for 5 minutes. Primary fluorochrome-conjugated antibodies were then added to stain the required surface antigen (**Table 2.6**). Each tube was vortexed briefly to mix and incubated with the surface antibody for 20 minutes on ice in the dark. Excess antibody and vivid was removed with 2x 3 mL PBS washes. If not immediately analysed, cells were fixed by adding 50 µL of paraformaldehyde (PFA) to each tube. Tubes were incubated for 20 minutes on ice in the dark before excess PFA was removed with 2x 3 mL PBS washes.

Compensation was achieved using anti-mouse Ig Compensation Beads (BD Biosciences). Beads were vortexed, diluted in PBS, and aliquoted 25 µL per tube. Using a single fluorochrome per tube, 2 µL of relevant antibody was added to the beads. Staining was analysed using a FACS Canto II machine (BD Biosciences) and data subsequently analysed using FlowJo (Tree Star Inc, Ashland OR).

A typical gating strategy is shown in **Figure 2.8**. In general, forward and side scatter was used to isolate lymphocytes. Doublets and debris were removed by gating on single cells. Dead cells were then removed based on staining with vivid, before further analysis of surface marker expression. The placing of gates was aided by biologically relevant negative controls (also known as fluorescence minus one; FMO) (for example untransduced CD8<sup>+</sup> T cells when assessing viral uptake).



**Figure 2.8: Typical gating strategy for analysis of T cells.** First lymphocytes are isolated using their forward and side scatter properties. Then the area to height ratio is used to gate on single cells, excluding doublets and debris. Finally, vivid uptake is used as a marker to exclude dead cells, and CD3 is used to indicate T cells.

### 2.6.3 – Flow cytometry: pMHC multimer staining

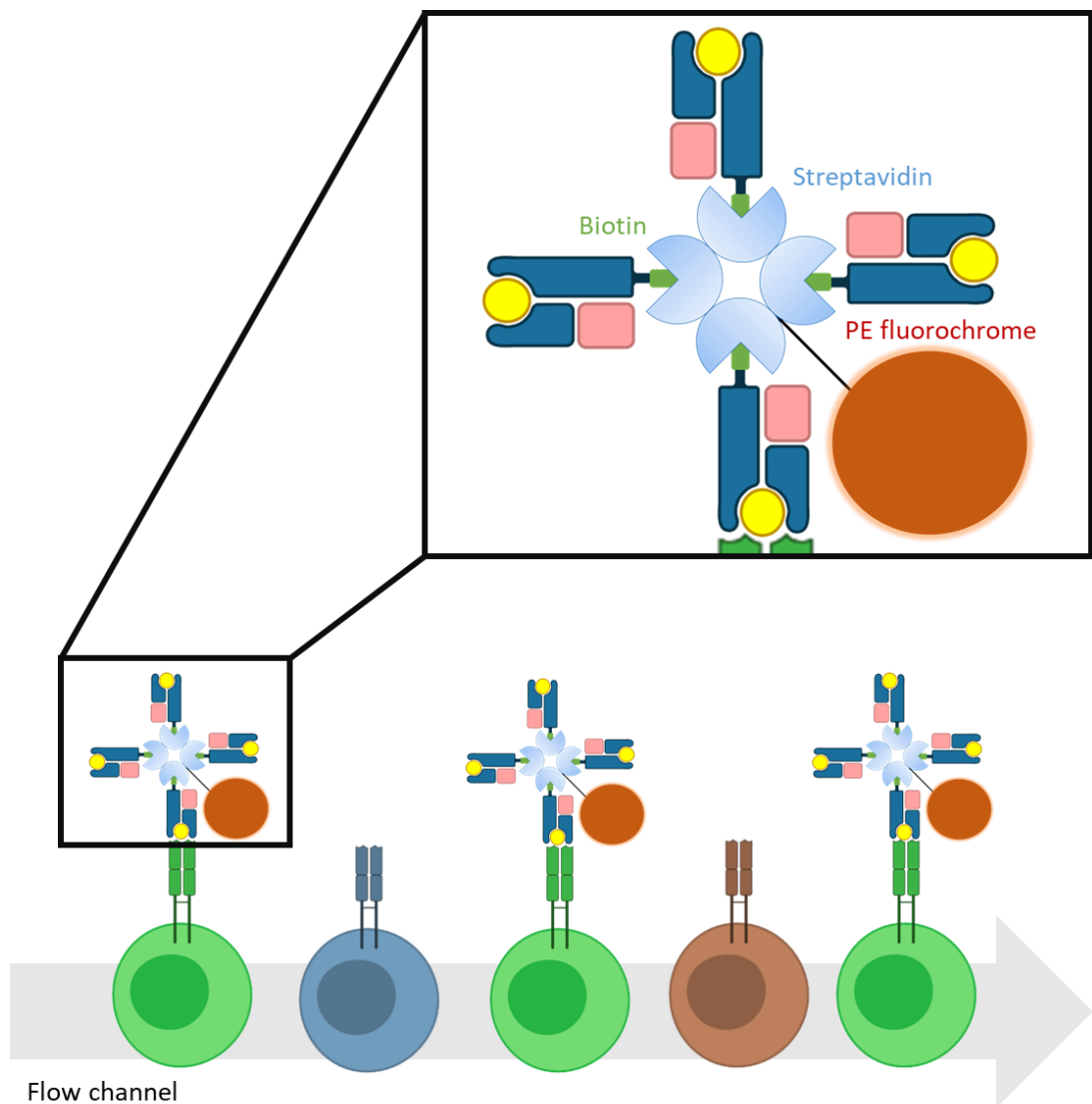
Biotinylated pMHC monomers were refolded as described in **Section 2.1**. Tetramerisation was achieved by incubating pMHC monomers with PE-conjugated streptavidin at a ratio of 1:4 was used with streptavidin in molar excess (Invitrogen, Thermo Fisher) on ice. Streptavidin was added to pMHC monomer incrementally, five additions each separated by 20 minute incubations to encourage fulfilment of all streptavidin valences. Dexamers were assembled by incubation of pMHC monomers with dextran-streptavidin-PE, at a molar ratio

of 3:1, for 30 minutes in the dark at room temperature. Tetramers were made to a final concentration of 0.1 µg/µL using PBS.

Tetramer staining was performed as previously described (Wooldridge *et al.*, 2009; Dolton *et al.*, 2015; Tungatt *et al.*, 2015). Approximately 50,000 cells were used per staining condition. Cells were harvested, transferred to FACS tubes (Elkay Labs), and washed twice with 3 mL of FACS buffer (**Table 2.4**) to remove any remaining components of cell media. Tubes were centrifuged at 800 x g for 2 minutes (Heraeus Megafuge 1.0R) to pellet cells before FACS buffer was removed. 50 µL of 100 nM Protein kinase inhibitor (Dasatinib, Axon Medchem) was added to each tube and tubes were incubated at 37°C for at least 10 minutes. Protein kinase inhibitors enhance tetramer staining by reducing TCR downregulation and internalisation of bound pMHC tetramers (Lissina *et al.*, 2009).

0.5 µg of tetramer (with respect to the pMHC component) was then added to each tube, tubes were vortexed briefly and incubated on ice for 30 minutes. Cells were washed twice in PBS to remove excess tetramer before a fluorochrome-unconjugated anti-PE antibody was added. This addition serves to stabilise the TCR-pMHC interaction at the cell surface, thus enhancing staining (Tungatt *et al.*, 2015). Cells were incubated with the unconjugated antibody on ice for 20 minutes before washing in PBS to remove excess.

Finally, cells were stained with vivid and surface marker antibodies as described in **Section 2.6.2**. If not immediately analysed, cells were fixed by adding 50 µL of PFA to each tube. Tubes were incubated for 20 minutes on ice in the dark before excess PFA was removed with 2x 3 mL PBS washes. Staining was analysed using a FACS Canto II machine (BD Biosciences), see **Figure 2.9**, and data subsequently presented using FlowJo (Tree Star Inc, Ashland OR). Negative controls included staining with an irrelevant tetramer, and the use of no tetramer (only surface marker antibodies), these controls were used to aid the gating strategy.



**Figure 2.9: Schematic explanation of tetramer staining.** The multivalent nature of streptavidin is used to associate four pMHC molecules with a fluorochrome, in this thesis the fluorochrome used is invariably PE but other conjugated streptavidins are available. TCR-pMHC interaction then tags the fluorochrome to the surface of the T cell. This schematic represents the basis of tetramer staining and does not include ‘tricks’ to improve staining, these are described by Tungatt *et al.* 2015. For simplicity, pMHC tetramers are shown making a single binding event.

#### 2.6.4 – Enzyme-linked immunosorbent assays (ELISA) of T cell function

Enzyme-linked immunosorbent assays (ELISA) were used to determine T cell responsiveness to the various stimuli such as peptides or cell lines transduced with potential target proteins via lentivirus. This assay uses DuoSet human ELISA kits (R + D Systems) to measure production of MIP-1 $\beta$  or TNF in response to stimuli as a proxy for responsiveness.

Assays were carried out as per the manufacturer's instructions. T cells were harvested, washed in R0, and rested in R5 overnight before the assay. The following morning, T cells were plated in a 96 well plate (Greiner) at a density of 30,000 cells/well. They were co-incubated with 60,000 antigen presenting cells (generally HLA-A2<sup>+</sup> T2 cells) and 10  $\mu$ L of peptide dilution or 60,000 target cells. Plates were kept at 37°C and 5 % CO<sub>2</sub> humidity (BB 15 CO<sub>2</sub> incubator, Thermo Scientific) overnight.

Meanwhile, half-area flat-bottomed 96 well plates (Greiner) were coated in 50  $\mu$ L of anti-human MIP-1 $\beta$  or TNF antibodies (R + D Systems) at a concentration of 1.0  $\mu$ g/mL diluted in PBS. These plates were sealed and incubated overnight at room temperature.

The following morning, these assay plates were washed three times with 190  $\mu$ L wash buffer (**Table 2.4**) using a plate washer (Wellwash Versa, Thermo Fisher). To prevent non-specific binding 150  $\mu$ L reagent diluent (**Table 2.4**) was used to block assay plates, incubating at room temperature for at least 1 hour.

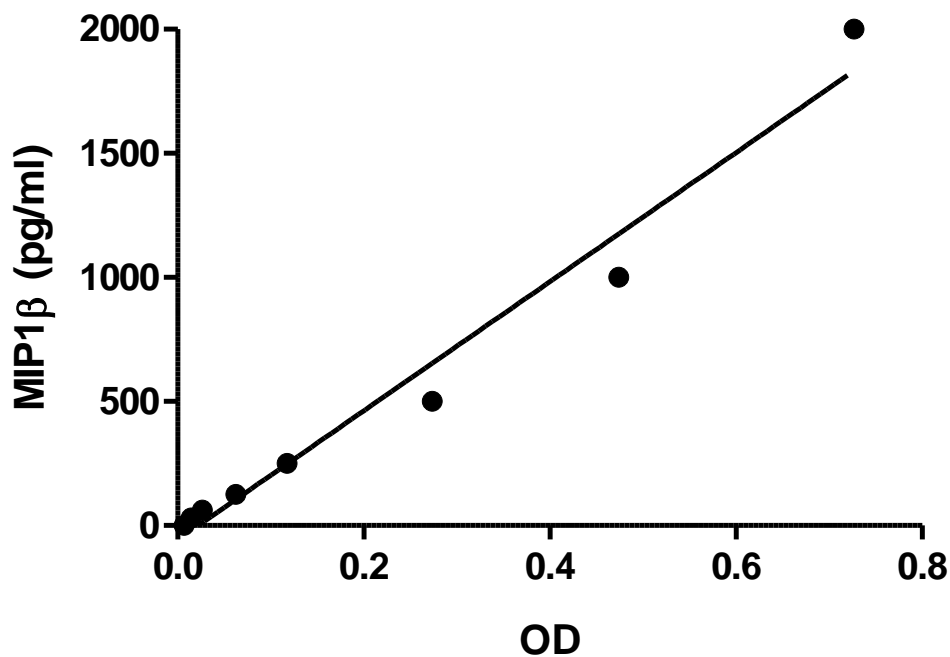
The co-incubation plates containing the T cells and targets were centrifuged 400 x g for 5 minutes (Heraeus Megafuge 1.0R) to pellet cells to prevent interference with the assay. 50  $\mu$ L of supernatant was then taken from each well into a fresh 96 well plate (Greiner) and diluted by adding 70  $\mu$ L R5 (**Table 2.4**). Following the aforementioned 1-hour blocking period, assay plates were washed three times with 190  $\mu$ L wash buffer using a plate washer (Wellwash Versa, Thermo Fisher). 50  $\mu$ L of diluted supernatant was added to assay plates and allowed to bind to the immobilised antibody by incubation at room temperature for 1 hour and 15 minutes. Recombinant human standards for the assayed cytokine (either MIP-1 $\beta$  or TNF) were also added to the assay plate. A serial dilution of standards (starting at 2000 pg/mL) was created by diluting in reagent diluent. This serial dilution would facilitate generation of a standard curve from which the concentration of MIP-1 $\beta$  or TNF in experimental wells could be calculated. An example standard curve is shown in **Figure 2.10**. Remaining supernatant was frozen at -20°C for storage and repeats if necessary.

Unbound supernatant was removed by washing three times with 190  $\mu$ L wash buffer using a plate washer (Wellwash Versa, Thermo Fisher). 50  $\mu$ L of the appropriate biotinylated



detection antibody (R + D Systems) diluted to 50 ng/mL was then added to the plate. The plate was incubated at room temperature for 1 hour and 15 minutes. Excess antibody removed by washing three times with 190  $\mu$ L wash buffer using a plate washer (Wellwash Versa, Thermo Fisher).

Next, the plate was incubated with 50  $\mu$ L of streptavidin-conjugated horseradish peroxidase (R + D Systems) per well for 20 minutes in the dark. Following this incubation, the plate was washed three times with 180  $\mu$ L wash buffer using a plate washer (Wellwash Versa, Thermo Fisher). Colour reagents A + B (R + D Systems) which were then mixed at a ratio of 1:1, 50  $\mu$ L of this mixture was immediately added to the plate in the dark to minimise bleaching. Typically, plates took 5-10 minutes to develop. The reaction was halted by the addition of 25  $\mu$ L 1 M sulphuric acid (Stop Solution, R + D Systems). The  $OD_{450nm}$  of each well was then immediately recorded using a Bio-Rad® iMark microplate reader and a correction set to 570nm.



**Figure 2.10: Standard curve for a MIP-1 $\beta$  ELISA.** The OD of known concentrations of MIP-1 $\beta$  is measured and used to generate a standard curve. The linear regression of the resulting data ( $y=m*x$ ) is then used to indicate MIP-1 $\beta$  concentrations from sample wells.

### 2.6.5 – Combinatorial peptide library (CPL) screens

CPL screens were performed using an ELISA format (**Section 2.6.4**) 30,000 rested T cells were co-incubated with 60,000 T2 antigen presenting cells and 10  $\mu$ L of a peptide mix at a final concentration of 100  $\mu$ g/mL from a 9-mer combinatorial peptide library (CPL) screen manufactured by Pepscan Presto Ltd. Peptide and T2 cells had previously been pulsed by incubation at 37°C and 5 % CO<sub>2</sub> humidity (BB 15 CO<sub>2</sub> incubator, Thermo Scientific) for 2 hours. MIP-1 $\beta$  production in response to each peptide mix was then measured using ELISA as a proxy for T cell responsiveness as described in **Section 2.6.4**.

A 9-mer CPL consists of individual peptide mixes in which, for each peptide position, a single L-amino acid is fixed. All other positions vary from any of the remaining 19 natural L-amino acids (cysteine is excluded from the non-fixed pool due to its propensity to form disulphide bonds causing aggregates within the peptide mix) (Wooldridge *et al.*, 2012; Ekeruche-Makinde *et al.*, 2013; Szomolay *et al.*, 2016). In a 9-mer CPL there are 180 peptide mixes (9 positions x 20 possible proteogenic L-amino acids) (Ekeruche-Makinde *et al.*, 2013). Within each mix there are  $1.7 \times 10^{10}$  peptide ( $19^9$ ). The total number of peptides in the full assay is  $4.8 \times 10^{11}$  ( $(9+19) \times 19^8$ ) (Ekeruche-Makinde *et al.*, 2013).

### 2.6.6 – TNF processing inhibitor-0 (TAPI-0) assays

A TAPI-0 assay assesses T cell functionality based on their ability to produce TNF in response to antigen (Haney *et al.*, 2011). The method was first described in 2011 and is thought to be particularly useful for low sensitivity TCRs, or those in which background noise is high – such as polyclonal T cell populations with transduced TCRs (Haney *et al.*, 2011). An additional benefit in the context of this thesis, is that analysis is achieved by flow cytometry allowing gating on the rCD2<sup>+</sup> population. The assay is based on the knowledge that TNF is originally produced in a membrane-bound form (Björnberg *et al.*, 1994; Haney *et al.*, 2011). It is subsequently cleaved by TNF converting enzyme (TACE) and released as a soluble protein. Inhibiting TACE traps TNF at the cell surface where it can be stained (Björnberg *et al.*, 1994; Haney *et al.*, 2011). Presence of CD107a at the cell surface can also be used as a marker of T cell activation (Betts *et al.*, 2003; Rubio *et al.*, 2003; Haney *et al.*, 2011).

An excess of the required number of CD8<sup>+</sup> T cells were rested overnight in R5 media. The following day target cells were plated at a density of 60,000 cells/well in a 96 U-well plate (Greiner) in a total volume of 50  $\mu$ L. Rested CD8<sup>+</sup> T cells were then harvested and added to the plate at a density of 30,000 cells/well in a total volume of either 40  $\mu$ L (or 39  $\mu$ L if 1  $\mu$ L of

peptide was to be included in the experiment). Negative controls lacking target cells or peptide (depending on the nature of the assay) were also included, along with a positive control in which T cells were activated by phytohaemagglutinin (PHA) (PAN Biotech).

A TAPI-0 mix consisting of 0.5 µL of TAPI-0 (Chem Cruz) (giving a working concentration of 30 µM), 1.5 µL anti-TNF-PE-Vio770 (clone cA2), 1.5 µL anti-CD107a-FITC (clone H4A3), and 6.5 µL R5 per well was made. 10 µL of this mixture was then added to each well.

Cells were incubated in the dark at 37°C and 5 % CO<sub>2</sub> humidity (BB 15 CO<sub>2</sub> incubator, Thermo Scientific) for a minimum of 4 hours to allow T cells to respond to antigen. Following this cells were washed in PBS, and stained with vivid and surface marker antibodies as described in **Section 2.6.2**. Staining was analysed using a FACS Canto II machine (BD Biosciences) and data subsequently presented using FlowJo (Tree Star Inc, Ashland OR).

#### 2.6.7 – Chromium-51 (<sup>51</sup>Cr)-release cytotoxicity assay

Assay performed by Dr Garry Dolton. Cytotoxicity of CD8<sup>+</sup> T cells can be measured by <sup>51</sup>Cr-release assay. In this assay target cells are harvested, washed, and labelled with 30 µCi of <sup>51</sup>Cr (sodium chromate, Perkin Elmer) per 1 x 10<sup>6</sup> cells. Labelling occurs over 1 hour at 37°C. Cells are subsequently washed with D-PBS to remove excess <sup>51</sup>Cr and resuspended in R10. These cells are then incubated at 37°C to allow for <sup>51</sup>Cr leaching from cells. Cells are then washed, resuspended in R10, and plated at 2000 cells/well (usually in duplicate) in 96 U-well plates. Target cells were also incubated in 5% Triton X-100 to determine *maximum* <sup>51</sup>Cr release, and in R10 to determine *spontaneous* <sup>51</sup>Cr release. Effector CD8<sup>+</sup> T cells, suspended in R10, were added to wells to achieve the desired effector:target ratio in a total volume of 150 µL. Co-incubation over 4 hours occurred at 37°C 5% CO<sub>2</sub>. Radioactivity of harvested supernatants was measured using a 1450-Microbeta™ counter (Perkin Elmer). Indirect T cell cytotoxicity against target cells (% specific lysis) was calculated thus:

$$\% \text{ Specific lysis} = \frac{\text{Experimental } ^{51}\text{Cr release} - \text{Spontaneous } ^{51}\text{Cr release}}{\text{Maximum } ^{51}\text{Cr release} - \text{Spontaneous } ^{51}\text{Cr release}} \times 100$$

## 3 – Insulin-specific T cells isolated from patients with type 1 diabetes strongly recognise common viral epitopes

---

### 3.1 – Introduction

I originally applied to undertake a PhD in the Sewell laboratory with Dr David Cole as I was very interested in the presentation of peptide antigens to  $\alpha\beta$  TCRs by MHC molecules. When I started my project, the laboratory had recently partially characterised an insulin B chain-specific CD8<sup>+</sup> T cell clone that was isolated from the blood of a patient with type 1 diabetes (T1D).

#### 3.1.1 – Type 1 diabetes

T1D, also known as insulin-dependent diabetes mellitus, commonly – but not exclusively – manifests in childhood (Katsarou *et al.*, 2017). The disease is a chronic metabolic disorder caused by defects in insulin secretion (Karamanou, 2016). The absence of insulin causes overproduction of glucose in the liver via glycogenolysis and gluconeogenesis, and decreased uptake of glucose by peripheral tissue, manifesting symptomatically as hyperglycaemia, polyuria, excessive thirst, and weight loss (Gepts, 1965; Eisenbarth, 1986; Atkinson, Eisenbarth and Michels, 2014). Metabolic ketosis caused by excessive fat breakdown occurs due to the need for alternative fuel source (Eiselein, Schwartz and Rutledge, 2004). Untreated, symptoms lead to coma and death (Eiselein, Schwartz and Rutledge, 2004). T1D is clinically managed by lifelong insulin administration to regulate blood glucose levels (Bluestone, Herold and Eisenbarth, 2010). Despite this effective treatment, complications arise over the lifetime of the patient as a result of vascular changes caused by long term exposure to elevated blood glucose levels (Eiselein, Schwartz and Rutledge, 2004). These include an increased risk of both cardiac and vasculature morbidities, renal disease, visual impairment and neuropathy. Life expectancy of T1D patients is reduced by 25% compared to healthy contemporaries (Eiselein, Schwartz and Rutledge, 2004), and the disease is expected to become the 7<sup>th</sup> leading cause of death by 2030 (Mathers and Loncar, 2006; Karamanou, 2016).

Descriptions of patients suffering from excessive thirst and polyuria date back to ancient times when an imbalance of bodily humours was believed to be the cause of illness. The sugar content of diabetic urine was first noted in the 5<sup>th</sup> century BC (Nduati *et al.*, 2011). In 1889, Minkowski and von Mering removed the pancreases of four dogs and observed that

they subsequently developed polyuria, with urine containing a high sugar content (Mering and Minkowski, 1890). Reimplanting pancreatic tissue subcutaneously prevented hyperglycemia. This landmark study demonstrated that the pancreas was responsible for glucose homeostasis, and at fault in T1D (Mering and Minkowski, 1890). This role was subsequently refined to the islets of Langerhans within the pancreas (Obstet, 1920).

Building on the observations of Minkowski and von Mering, Banting, Best, and colleagues isolated insulin from dog pancreases in 1922 (Banting *et al.*, 1922). They found that, when suitably extracted, 'pancreatic extract' had the ability to reduce blood glucose and the glucose present in urine when administered to diabetic dogs. Banting *et al.* further demonstrated that the pancreatic extract of another species – fetal calves – would achieve the same effect (paving the way for the future treatment of human diabetics with pig insulin), and that extracts of other organs such as liver and spleen did not (Banting *et al.*, 1922). They named their pancreatic extract insulin and used it to treat a 14 year old human boy, who had previously been adhering to the best available treatment of the day – 'eat as little as possible' (Karamanou *et al.*, 2014). Daily injections produced immediate improvement that regressed if injections ceased (Banting *et al.*, 1922). This was a life-changing advancement for diabetics. However, lifelong insulin-dependency, and the resulting economic cost (Björk, 2001; Ettaro *et al.*, 2004), means that the quest for a cure to T1D could not stop at this 'miracle' treatment.

#### 3.1.1.1 – T1D is a CD8<sup>+</sup> T cell mediated disease

Further investigations into the underlying pathology of T1D led to an appreciation for the strong associations of the disease with particular MHC alleles. In 1987, Todd *et al.* described how a polymorphism at position 57 of the HLA-DQ $\beta$  chain could influence susceptibility to T1D (Todd, Bell and McDevitt, 1987). DQ $\beta$  alleles with alanine, serine, or valine at this position were associated with T1D, whereas alleles with aspartic acid at this position were not (Todd, Bell and McDevitt, 1987). A genome wide association search involving over 2000 diabetic patients identified certain *HLA-I* genes associated with T1D susceptibility (Nejentsev *et al.*, 2007). HLA-B\*39, HLA-B\*18, and HLA-A\*24 were associated with T1D susceptibility whilst HLA-B\*27, HLA-A\*01, HLA-A\*11, and HLA-A\*31 were found to be protective (Nejentsev *et al.*, 2007). The susceptibility alleles confer a relative risk comparable to, or greater than, the more classically associated T1D genes, such as *INS* and *PTPN22*, with some alleles being associated with younger disease onset (Nejentsev *et al.*, 2007). HLA-A2, one of the most abundant Caucasian alleles, is also notably associated with T1D (Noble *et al.*, 2002; Howson *et al.*, 2009; Noble and Valdes, 2011). In 2002, Marron *et al.* demonstrated marked

acceleration of disease development in non-obese diabetic (NOD) mice following transgenic introduction of the *HLA-A\*02:01* gene (Marron *et al.*, 2002). Subsequently, in 2005, DiLorenzo *et al.* showed that abrogation of MHC class I expression in animal models could confer disease protection (DiLorenzo and Serreze, 2005). These studies indicate that MHC class I-mediated events contribute to the aetiology of T1D.

MHC-association is a hallmark of many autoimmune disorders, (Huey *et al.*, 1993; Karelle *et al.*, 2003; Yeo *et al.*, 2007; Chemin *et al.*, 2016) so observations of MHC-association in T1D provided strong evidence of an autoimmune component to the disease. Further proof came with the demonstration of inflammation and leukocyte infiltration within pancreatic islets. Post-mortem studies of patients close to disease onset showed CD8<sup>+</sup> T cells present in the islet mononuclear cell infiltrate (Gepts, 1965; Bottazzo *et al.*, 1985). CD8<sup>+</sup> T cells were quantified as the most abundant leukocyte in the infiltrate, with macrophages, CD4<sup>+</sup> T helper cells, and Tregs also present (Willcox *et al.*, 2009). Interestingly, only a small fraction of islets exhibit leukocyte infiltration at any one time (Willcox *et al.*, 2009) and islets appear to be destroyed progressively over time corresponding to an advancing decline in insulin production (Foulis, Farquharson and Meager, 1987). Once islets become insulin-deficient the immune infiltrate dramatically declines suggesting that viable  $\beta$  cells are required to maintain the presence of immune cells (Willcox *et al.*, 2009).

There is abundant evidence in support of a role for CD8<sup>+</sup> T cells in T1D. Adoptive transfer of CD8<sup>+</sup> T cells in animal models was shown to induce disease state (DiLorenzo and Serreze, 2005). The CD8<sup>+</sup> T cells present in diabetic islets of mice were shown to be islet-specific, autoreactive T cells (Trudeau *et al.*, 2003; Wong *et al.*, 2007; Énée *et al.*, 2008). This was followed by similar demonstration in human diabetic islets (Coppieters *et al.*, 2012). Many diabetic autoantigens have been described, and autoreactive T cell clones capable of  $\beta$  cell destruction isolated (Liu *et al.*, 1999; Li *et al.*, 2010; Delong *et al.*, 2011; Nikoopour *et al.*, 2011; Unger *et al.*, 2012; Chujo *et al.*, 2013). For example, in 1995 Panina-Bordignon *et al.* demonstrated specific T cell cytotoxicity against a glutamic acid decarboxylase 65 (GAD<sub>65</sub>)-derived peptide (GAD<sub>114-123</sub>). They noted that the peptide was presented by HLA-A2 and cytotoxicity was only detected in T cells derived from HLA-A2<sup>+</sup> T1D patients (Panina-Bordignon *et al.*, 1995). In another example, CD8<sup>+</sup> T cells specific for zinc transporter 8 (ZnT8) were isolated from diabetic children (Énée *et al.*, 2012). ZnT8 is expressed in  $\beta$  cells and involved in the regulation of insulin storage and secretion (Kawasaki, 2012). The proinsulin (PPI)-derived epitope LWMRLLPLL (residues 3-11) is presented on the T1D-associated HLA-A24 molecule and is recognised by CD8<sup>+</sup> T cell clones found in diabetic

patients (Kronenberg *et al.*, 2012). An autoantigen that holds importance to this chapter is the HLVEALYLV peptide. This peptide is derived from the insulin B chain (residues 10-18) and is also presented on HLA-A2. HLVEALYLV was first described as a T1D-relevant epitope in 2005 (Pinkse *et al.*, 2005). It is recognised by a T cell clone called InsB4 that was isolated from a T1D patient in 2016 (Theaker *et al.*, 2016). Another T1D epitope used as a model system in this thesis is the HLA-A2-restricted PPI-derived peptide, ALWGPDPAAA (ALW)<sub>(15-24)</sub>. PPI<sub>15-24</sub> is a key diabetic epitope (Velthuis *et al.*, 2010) encoded within the signal peptide of PPI. ALWGPDPAAA was isolated by elution of peptides from *INS*-expressing HLA-A2<sup>+</sup> cells and is recognised by a patient-derived CD8<sup>+</sup> T cell clone, 1E6 (Skowera *et al.*, 2008). Tellingly, human pancreatic  $\beta$  cells only become targets for 1E6 when maintained in glucose-containing media where they manufacture insulin from stored PPI (Skowera *et al.*, 2008). Inflammatory environments promote MHC class I overexpression in the islets (Foulis, Farquharson and Meager, 1987) which could contribute to disease propagation through epitope spreading and the emergence of cryptic antigens.

The constellation of evidence described above strongly implicates CD8<sup>+</sup> T cells in the cause of T1D. One model of how autoimmune diseases are triggered (see **Section 1.5.2**) is that pathogen-specific T cells inadvertently recognise self-antigens because they are crossreactive.

### 3.1.2 – T cells are, by necessity, highly crossreactive

CD8<sup>+</sup> T cells, including those that implicated in various autoimmune diseases, are crossreactive (as described in **Section 1.4**). The notion of T cell crossreactivity has supplanted the previously accepted model of how lymphocytes contribute to adaptive immunity known as the Clonal Selection Theory (CST), which was proposed prior to 1960 and later refined (Burnet, 1959, 1976). The CST propagated the idea that each lymphocyte is highly specific for a single antigenic ligand. This paradigm faced mounting evidence to the contrary in the 1990s as understanding of the nature of the ligands recognised by antigen receptors, especially the  $\alpha\beta$  TCR, increased (Bhardwaj *et al.*, 1993; Wucherpfennig and Strominger, 1995; Mason, 1998; Misko *et al.*, 1999). Counterevidence questioning the CST included structural proof of a single TCR recognising multiple peptide ligands (Hagerty and Allen, 1995; Reiser *et al.*, 2003). In fact, a single TCR can even tolerate polymorphisms in the MHC component of its target (Hennecke and Wiley, 2002; Luz *et al.*, 2002). Further evidence in favour of crossreactive T cells stems from the fact that a single pMHC molecule can select for

a relatively wide ranging T cell repertoire (Ignatowicz, Kappler and Marrack, 1996), and a T cell that has been activated by one peptide can then respond to others (Ignatowicz *et al.*, 1997; Galloway *et al.*, 2019).

Don Mason's seminal opinion piece, published in 1998, documented the theoretical evidence for why crossreactivity is an essential characteristic of the TCR (Mason, 1998). Mason suggested that in order to achieve full immune coverage a single TCR would likely recognise between  $10^6$  and  $10^7$  different pMHC targets (Mason, 1998). The CST was further counteracted by calculations by Arstila *et al.* in 1999, describing how the  $10^{12}$  T cells present in the human body likely carry one of just 25 million ( $2.5 \times 10^7$ ) distinct TCRs (Arstila *et al.*, 1999). The 20 proteogenic L-amino acids can be combined as 9-mers in  $5.1 \times 10^{11}$  different ways with at least 1% of these combinations amenable to be presented by a given MHC molecule at the cell surface (Mason, 1998; Wooldridge, 2013). Thus, even the most conservative calculations, those that assume that there is only one MHC per individual and that it only presents 9-mer peptides, require that each TCR see hundreds of different peptides to provide full recognition coverage. Therefore, even lacking the abundant experimental evidence of T cell crossreactivity, it would be a logical necessity to provide comprehensive immune coverage to the host (Mason, 1998; Sewell, 2012).

### 3.1.2.1 – Benefits of T cell crossreactivity

T cell crossreactivity can facilitate recognition of viral escape mutants, post-translationally modified peptides, or neoepitopes allowing the immune system to remain in control of infections or malignancy despite a high mutational load (Elliott, Bodmer and Townsend, 1996; Glithero *et al.*, 1999). For example, the HIV-1-specific 868 TCR can recognise a number of common SLYNTVATL escape mutants (Cole, Fuller, *et al.*, 2017). A tumour-responsive T cell clone, MEL5, recognises epitopes from the Melan-A/MART-1 protein in melanoma. MEL5 is able to bind two overlapping epitopes expressed on melanoma cells in the context of HLA-A2 (Borbulevych *et al.*, 2007; Madura *et al.*, 2019). Interestingly, Wen *et al.* described an example of cross-species crossreactivity (also known as heterologous immunity) by showing that Dengue-responsive CD8<sup>+</sup> T cells could confer protection against Zika virus (Wen *et al.*, 2017). In another example, Chiu *et al.* identified an immunodominant varicella-zoster virus (VZV) epitope and subsequently found homologs in two other  $\alpha$ -herpesviruses (herpes simplex virus (HSV)-1, and HSV-2) and a  $\gamma$ -herpesvirus, Epstein-Barr Virus (EBV) (Chiu *et al.*, 2014). They found that PBMCs from VZV-seropositive individuals could respond to the 3



homolog epitopes regardless of the individuals prior exposure to each virus, suggesting cross-species crossreactivity (Chiu *et al.*, 2014).

T cell crossreactivity is believed to assist the immune system spatially and temporally. An infected cell can only be scanned by so many T cells in a given time scale, and it is important that foreign peptides are detected rapidly to help control the spread of the infection. Each T cell being capable of recognising many (even more than a million (Wooldridge *et al.*, 2012)) peptides can speed up detection time as any foreign peptide will likely be recognised by many different T cells (Sewell, 2012).

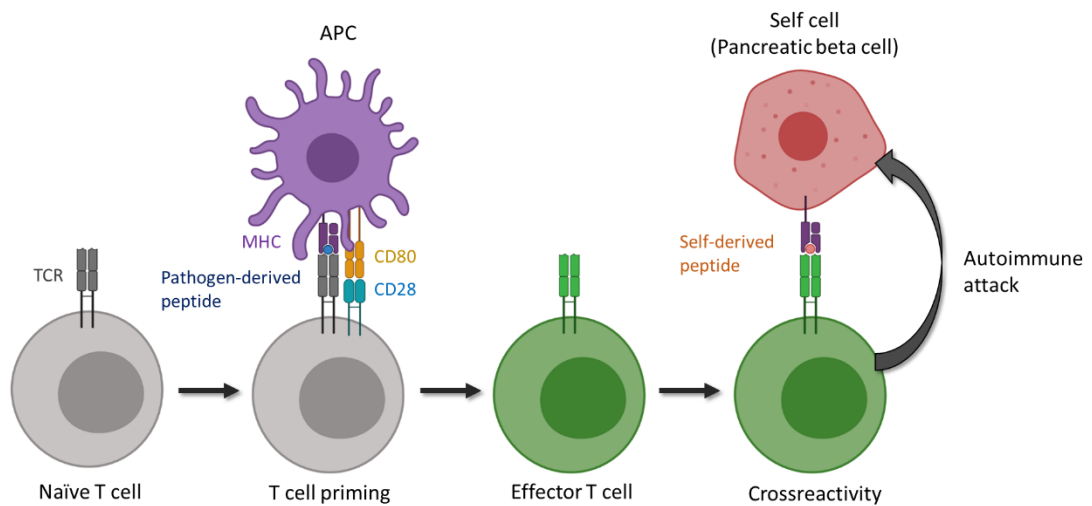
T cell crossreactivity can also be exploited therapeutically as peptide mimics can be used as vaccines to prime endogenous T cell populations. This can include use of more potent 'super agonists' as vaccines. Another PhD student in my research group recently used CPL screening to generate a super agonist peptide for a melanoma-reactive T cell clone isolated from the tumour-infiltrating lymphocytes (TIL) infusion product used to induce complete remission in a Stage IV malignant melanoma patient (Galloway *et al.*, 2019). The ST8.24 T cell clone used in this study was expanded in patient blood following cancer remission (Galloway *et al.*, 2019). T cell priming from HLA-A2<sup>+</sup> PBMC with a super agonist peptide for the ST8.24 T cell clone generated more robust killing of melanoma cells than those primed with the natural antigen (Galloway *et al.*, 2019). In another example, the crossreactive nature of T cells has allowed for an artificial D-amino acid peptide to be used as a vaccine to prime T cells to respond to an influenza epitope (Miles *et al.*, 2018). D-amino acid peptides have the advantage of being much longer lived in serum and resistant to degradation by gastric enzymes thereby making oral delivery possible (Miles *et al.*, 2018). Thus, well-understood crossreactivity can be harnessed to provide effective and intelligently designed therapeutics.

#### 3.1.2.2 – Drawbacks of T cell crossreactivity

Unfortunately, T cell crossreactivity can be the root cause of a number of negative clinical and biological outcomes. T cell crossreactivity can cause potentially fatal side effects when T cells or TCRs are used as a therapeutic. For example, an affinity-enhanced TCR targeting the multi-cancer associated MAGE-A3 antigen presented on HLA-A\*01 led to patient deaths due to severe myocardial damage (Linette *et al.*, 2013). Extensive investigation led to the identification of crossreactivity involving an unrelated cardiac protein, titin (Linette *et al.*, 2013). The titin epitope responsible was soon identified and shown to also be presented by HLA-A\*01 (Cameron *et al.*, 2013). Crystal structures showed that the two pMHCs were

molecular mimics (Raman *et al.*, 2016). Other examples of unintentional therapeutic crossreactivity have been reported (Johnson *et al.*, 2009; Parkhurst *et al.*, 2011), demonstrating the need for a more complete understanding of TCR peptide preferences to enable prediction of such crossreactivities. Experimental procedures such as those described in this chapter can be used to gain better understanding of TCR peptide preferences.

Aberrant T cell crossreactivity is also believed to initiate autoimmunity (see **Section 1.5.2**). Naïve T cells require strong TCR-pMHC interactions to activate them, so are not normally activated by self-ligands which tend to be weak (van der Merwe and Davis, 2003; Bridgeman *et al.*, 2011). Furthermore, naïve T cells generally require a ‘second signal’ as an in-built safety mechanism (Jenkins and Schwartz, 1987). The role of CD28 and the co-inhibitory receptors in preventing autoimmunity is discussed in detail in **Section 1.5.2**. Naïve T cells are, therefore, activated by professional APCs that present pathogenic ligands in the context of other co-stimulatory signals, this allows a pathogenic epitope to break tolerance (Shahinian *et al.*, 1993; Riha and Rudd, 2010). Effector-memory T cells, in contrast, can be re-activated by less potent ligands than those required to active naïve T cells and can be activated in the absence of a ‘second signal’ (Sewell, 2012). Thus, pathogenic T cell epitopes, to which the immune system should, and will, respond to can trigger autoimmunity through the activation of T cells with promiscuous TCRs, see **Figure 3.1** (Ruff *et al.*, 2019). These pathogenic epitopes may be molecular mimics of self-epitopes that would otherwise have gone unnoticed by the T cells with the capacity to crossreact to them (Ruff *et al.*, 2019). 1E6, a CD8<sup>+</sup> T cell clone implicated in T1D, recognises over a million peptides (Wooldridge *et al.*, 2012). Some of these peptides could be found in proteome databases for human pathogens (Wooldridge *et al.*, 2012; Cole *et al.*, 2016). One peptide epitope in particular, RQFGPDWIVA from the *C. asparagiforme* proteome, was recognised with 4-fold stronger affinity than the index T1D-associated epitope (Wooldridge *et al.*, 2012; Cole *et al.*, 2016). This raises the possibility that the autoreactive T cell in question, 1E6, could have initially been activated by a much more potent ligand of pathogenic origin and, as a result of its promiscuity, have subsequently activated in response to the insulin epitope.



**Figure 3.1: Pathogen-triggered autoimmunity.** In addition to strong TCR-pMHC interactions with professional APCs, naïve T cells require a ‘second signal’ such as the CD28/CD80 co-stimulatory interaction. Effector T cells are much easier to re-activate, requiring less potent TCR-pMHC interactions. They can therefore respond to self-peptides and initiate autoimmune disease.

### 3.1.3 – Measuring T cell crossreactivity

The ability to measure and define T cell crossreactivity is important both to the discovery of new TCR-pMHC interactions, and in the safety considerations of their therapeutic application (Cameron *et al.*, 2013). Understanding the ‘rules’ that govern the plasticity of a TCR-pMHC interaction, including those that dictate the promiscuity of a TCR, may help to identify autoimmune triggers (Yi Li *et al.*, 2005; Bulek *et al.*, 2012; Wooldridge *et al.*, 2012; Cole *et al.*, 2016), facilitate design of more potent TCRs as therapeutics (Molloy, Sewell and Jakobsen, 2005; Harper *et al.*, 2018; Sanderson *et al.*, 2020), and help prevent off-target toxicity in T cell therapeutics (Cameron *et al.*, 2013; Linette *et al.*, 2013). As a result, a number of methodologies for assessing T cell crossreactivity have been developed.

T cell crossreactivity can be demonstrated via the crystallisation of complex interactions, showing a single TCR in complex with MHC molecules presenting distinct peptides (Ding *et al.*, 1999; Reiser *et al.*, 2003; Yin *et al.*, 2011). Positive interactions can also be shown using SPR (Harkiolaki *et al.*, 2009; Cole, Van Den Berg, *et al.*, 2017). T cell assays can be used to measure response to distinct peptide ligands in many different ways including cytokine production (Whalley *et al.*, 2020), specific lysis (Miles *et al.*, 2018), CD69-upregulation as

measured by flow cytometry (Ishizuka *et al.*, 2009), and  $^3\text{H}$  incorporation or carboxyfluorescein succinimidyl ester (CFSE) loss as proxies for T cell proliferation (Kersh *et al.*, 1998; Galloway *et al.*, 2019). These methods are useful, on the small-scale, to confirm suspected crossreactivities. They do not, however discover or demonstrate the extent of T cell crossreactivity.

In an attempt to discover crossreactivities, Ishizuka *et al.* assessed nearly 30,000 TCR-pMHC interactions using a large library of synthetic peptides (Ishizuka *et al.*, 2009). They evaluated the specificities of 15 T cell clones by co-incubating pools of the peptides, the T cell clones, and an APC line. They then assessed reactivity using CD69-upregulation,  $^3\text{H}$  incorporation, and IFN $\gamma$  enzyme-linked immunospot (ELISpot). Despite this labour-intensive effort, they were only able to identify a single example of crossreactivity (Ishizuka *et al.*, 2009). 30,000 peptides represents just a fraction of the available MHC-binding peptide universe (Sewell, 2012; Wooldridge, 2013) so this result is unsurprising, however it does aptly demonstrate the need for very high throughput methodology for demonstrating and assessing T cell crossreactivity.

T cell specificity for a range of pMHC ligands can be demonstrated by staining with fluorescently labelled multimers and subsequent flow cytometry analysis (Wooldridge *et al.*, 2009; Dolton *et al.*, 2015). However, this tends to be limited by the available panel of fluorophores (Lee *et al.*, 2020). To overcome this limitation, pMHC multimers can be tagged with DNA barcodes (Bentzen *et al.*, 2016). The barcodes act as individual identifiers that allow the peptide agonists to be determined following staining and cell sorting. This method gives a more extensive insight into the crossreactive repertoire of a particular T cell clone. Bentzen *et al.* were able to screen over 1000 different peptides using the technique (Bentzen *et al.*, 2016). Measuring the full extent of T cell crossreactivity, however, requires greater breadth than even DNA barcoded pMHC can offer. Furthermore, technologies for assessing T cell crossreactivity involving any sort of pMHC multimers are expensive and are therefore still limited to very small fraction of the possible peptide universe. True assessment of T cell crossreactivity requires that a TCR is screened against all possible agonists – something made possible by combinatorial peptide library (CPL) screens (Hemmer *et al.*, 1997; Hiemstra *et al.*, 1997; Wooldridge *et al.*, 2012).

### 3.1.3.1 – CPL screens for measurement of T cell crossreactivity

CPL screen technologies were pioneered in the early 1990s by Dr Clemencia Pinilla and colleagues (Houghten *et al.*, 1991; Pinilla *et al.*, 1992). Originally developed to probe the ligand preferences of monoclonal antibodies, CPL screens consist of mixtures of free peptides in solution. Within each mixture a single amino acid is fixed at a single position, with all other positions allowed to vary amongst the 19 proteogenic L-amino acids, **Figure 3.2** (cysteine is generally excluded from the non-fixed amino acid pools due to its propensity to cause cross-linking via the formation of disulphide bonds) (Houghten *et al.*, 1991; Pinilla *et al.*, 1992; Hemmer *et al.*, 1998; Ninovasquez, 2004). A significant advantage to the use of CPL screens for ligand identification is the lack of a need for any prior knowledge of the likely ligand candidates (Houghten *et al.*, 1991). Preceding methods involved time-consuming synthesis and screening of individual peptides (Merrifield, 1963; Geysen, Meloen and Barteling, 1984), which were generally designed around the known index peptide (Houghten, 1985) and thus did not account for the ability of TCRs to see peptides of little or no similarity to the known ligand (Reiser *et al.*, 2003). For such methods, screening of 1000 peptides was a substantial achievement (Fodor *et al.*, 1991). In contrast, Houghten *et al.* had screened T cell recognition of a library of 34 million 6-mer peptides as early as 1991 (Houghten *et al.*, 1991).

Since their advent, CPL screens have been used to study the ligand preferences of many TCRs (Hemmer *et al.*, 1998; Wooldridge *et al.*, 2012; Szomolay *et al.*, 2016). In 2012 Wooldridge *et al.* used CPL screening to demonstrate that the aforementioned 1E6 T cell clone (Skowera *et al.*, 2008; Bulek *et al.*, 2012), can recognise more than one million peptides with a potency equal to or better than the 'index' PPI-derived sequence (Wooldridge *et al.*, 2012). CPL screens tend to be length-matched to the index peptide, if known, as the crossreactive behaviour of CD8<sup>+</sup> T cells with regards to peptide sequence does not generally extend to peptides of different length. Ekeruche-Makinde *et al.* showed that, in fact, MHC class I-restricted TCRs tend to have strong peptide-length preferences, and rarely respond to peptides of non-preferred length (Ekeruche-Makinde *et al.*, 2013). Ekeruche-Makinde *et al.* subjected 4 well-characterised TCRs to 6 CPL screens each (8-mers to 13-mers). They found that each TCR showed the highest reactivity to the CPL that matched the length of its index peptide. For example, ILA1 showed highest reactivity in the 9-mer CPL and little-to-no reactivity in the other screens. Whilst MEL5 and 1E6 showed highest reactivity in the 10-mer screen (Ekeruche-Makinde *et al.*, 2013). MHC class I molecules do not tend to show the same tight restriction regarding the length of peptides they can present (a single MHC class I molecule can generally present peptides of 8-13 amino acids in length) (Burrows *et al.*, 2008;

Bell *et al.*, 2009) and so it is likely that this length-preference is imposed by the TCR component of the interaction.

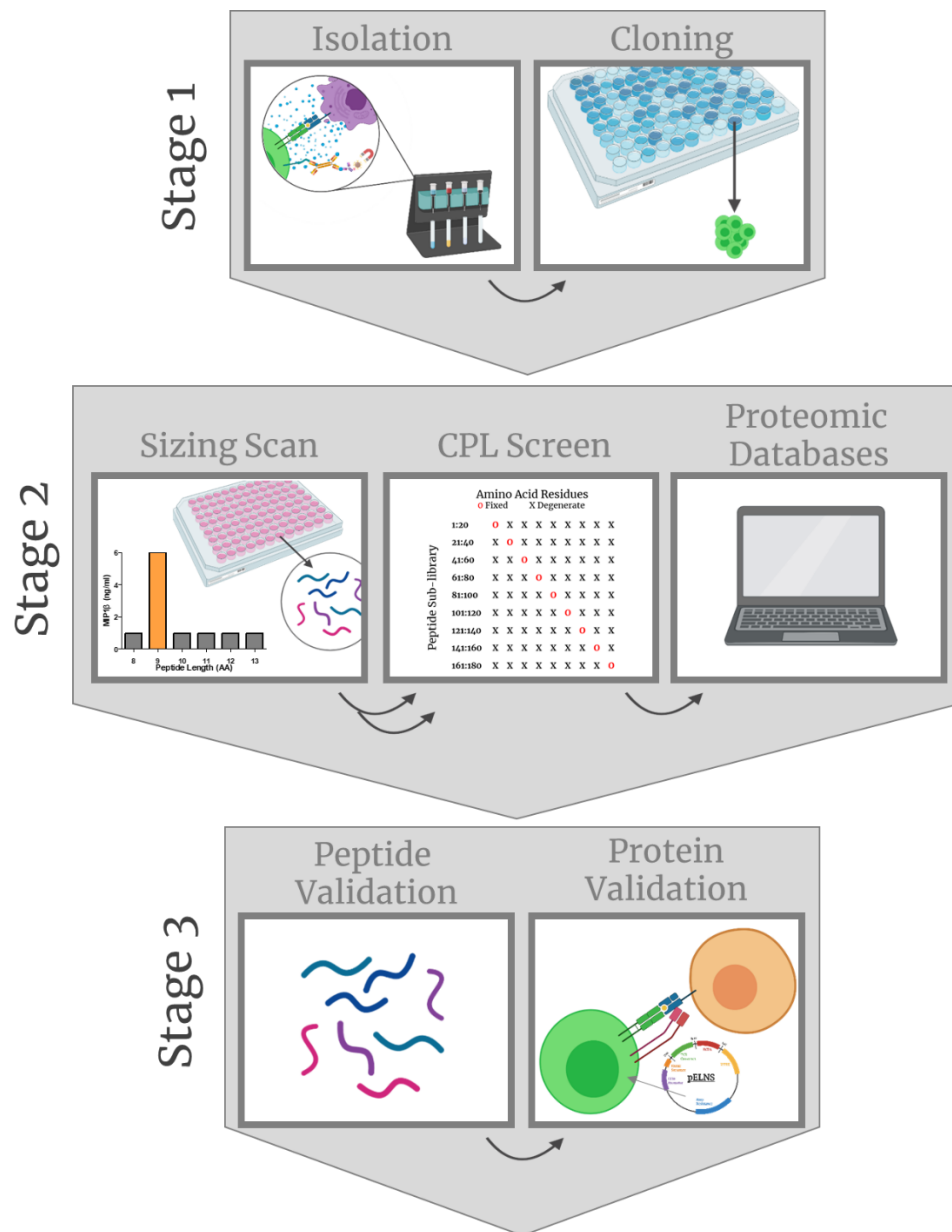
Amino Acid Residues									
	O Fixed	X Degenerate	X Degenerate	X Degenerate	X Degenerate	X Degenerate	X Degenerate	X Degenerate	X Degenerate
Peptide sublibrary 1-20	O	X	X	X	X	X	X	X	X
Peptide sublibrary 21-40	X	O	X	X	X	X	X	X	X
Peptide sublibrary 41-60	X	X	O	X	X	X	X	X	X
Peptide sublibrary 61-80	X	X	X	O	X	X	X	X	X
Peptide sublibrary 81-100	X	X	X	X	O	X	X	X	X
Peptide sublibrary 101-120	X	X	X	X	X	O	X	X	X
Peptide sublibrary 121-140	X	X	X	X	X	X	O	X	X
Peptide sublibrary 141-160	X	X	X	X	X	X	X	O	X
Peptide sublibrary 161-180	X	X	X	X	X	X	X	X	O

**Figure 3.2: Typical 9-mer CPL format.** The diagram represents the 180 of peptide mixtures that comprise a 9-mer CPL scan. Each peptide mixture contains one of the 20 proteogenic amino acids fixed at a one position (represented by O), with all other positions allowed to vary amongst the 19 proteogenic L-amino acid excluding cysteine (represented as X). Cysteine is generally excluded from X due to its propensity to cause cross-linking via the formation of disulphide bonds.

### 3.1.3.2 – CPL screens can identify novel peptide epitopes

In 2016, Szomolay *et al.* developed a publicly accessible webtool to facilitate ‘CPL-driven database searching’ (Szomolay *et al.*, 2016). This method uses raw CPL screen data to search proteome databases and identify likely ligands of the T cell clone in question (Szomolay *et al.*, 2016). Unlike previous CPL-based ligand identification, this webtool does not just identify likely ligands but ranks them in order of recognition likelihood. An overview of the TCR ligand discovery process is given in **Figure 3.3**. The first stage of the process involves isolating specific CD8<sup>+</sup> T cells from PBMC and cloning by dilution to obtain a single T cell (Theaker *et al.*, 2016). If the T cell cannot be cloned it can be manufactured by TCR replacement (Legut *et al.*, 2018). Once a monoclonal population has been obtained it is subject to a sizing scan to determine the peptide length preference (Ekeruche-Makinde *et al.*, 2013). A length-matched CPL screen is then conducted, the output of which is used to search proteomic databases (Szomolay *et al.*, 2016). Finally predicted peptide ligands require validation and

epitope confirmation, as demonstrated in this chapter. CPL-driven database searching can assess the entire peptide universe and thus has the potential to identify ligands that are stronger than the known index peptide, such as a pathogenic trigger for an autoreactive T cell (Wooldridge *et al.*, 2012; Szomolay *et al.*, 2016; Whalley *et al.*, 2020).



**Figure 3.3: An overview of the TCR ligand discovery process. Stage 1:** T cells are first isolated from PBMC based on their specificity. These are then diluted to less than 1 cell per well and plated to obtain a T cell clone. **Stage 2:** The clone is subject to sizing scan to uncover its peptide length preferences. Then an appropriately length-matched CPL is carried out. CPL data is used to search proteomic databases for likely ligands. **Stage 3:** These peptide ligands then require validation by expressing the parent protein in APC to confirm epitope processing and presentation.

### 3.1.4 – Aims

I hypothesised that a previously-characterised T1D-relevant T cell clone might be crossreactive and would potentially recognise peptides from pathogenic proteomes. To investigate this, I used CPL-driven database searching to explore the crossreactive profile of this T cell clone and then used the resulting profile to search for potential pathogenic peptides that act as strong agonists. This approach has potential to identify the ‘initial trigger’ that stimulates T cell activation, breaks self-tolerance, and promotes autoimmune development. Specifically, I aimed to:

- Investigate the crossreactive nature of the InsB4 T cell clone through CPL-driven database screening of pathogenic databases.
- Validate any notable peptides as legitimate HLA-A2-presented epitopes.
- Explore the potential for crossreactivity between the cognate antigen and any identified pathogenic epitopes in T1D patient blood.



## 3.2 – Results

### 3.2.1 – Work leading up to the project

InsB4 is a T1D-associated T cell clone that was isolated from a diabetic patient in 2016 by Dr Garry Dolton (Theaker *et al.*, 2016). InsB4 is specific for a peptide from the insulin B chain (residues 10-18), HLVEALYLV. Expression of the insulin B chain is restricted to pancreatic islet  $\beta$  cells, making it a suitably specific target for autoreactive CD8<sup>+</sup> T cells in the context of T1D (Harding and Ron, 2002; Pinkse *et al.*, 2005). HLVEALYLV is a naturally processed HLA-A2 restricted human T cell epitope that was first described in 2005 (Pinkse *et al.*, 2005), it is therefore a highly relevant model system in which to investigate T cell crossreactivity. Following characterisation of the epitope and demonstration of strong HLA-A2 binding, Pinkse *et al.* used tetramer staining to demonstrate the presence of HLA-A2-HLVEALYLV-specific CD8<sup>+</sup> T cells in both healthy donors and diabetics. They observed that in healthy donors the immune system ‘remains ignorant,’ (Pinkse *et al.*, 2005) potentially until exposure to a pathogenic trigger.

This last piece of evidence, that HLA-A2-HLVEALYLV-reactive CD8<sup>+</sup> T cell clones found in healthy humans remain ignorant of their insulin-derived ligand is intriguing. Why is it that T cell clones could be activated and contributing to autoimmunity in one individual but remain naïve in another? To investigate this, we turned to the ample evidence for pathogenic triggers of autoimmunity (discussed in detail in **Section 1.5.2**).

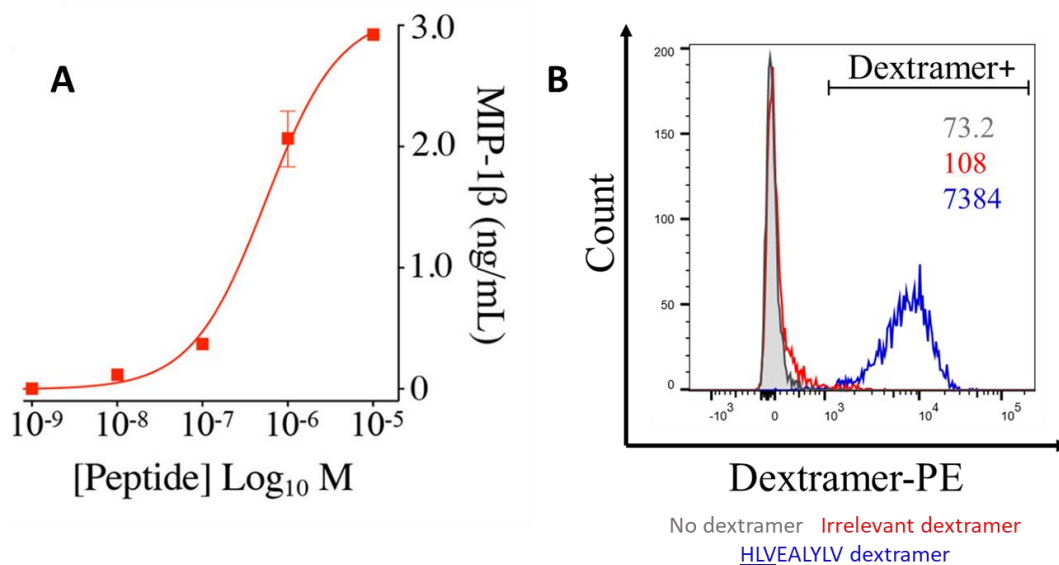
#### 3.2.1.1 – The HLA-A2-restricted HLVEALYLV epitope is presented at the surface of pancreatic $\beta$ cells.

My laboratory confirmed that the InsB4 T cell clone recognised the HLA-A2-restricted HLVEALYLV peptide (B chain residues 10-18). **Figure 3.4A** shows the dose-dependent response of the clone to this peptide, as measured by MIP-1 $\beta$  ELISA. The InsB4 T cell also stains well with HLA-A2-HLVEALYLV PE-conjugated pMHC dextramers using an optimised staining procedure (Wooldridge *et al.*, 2009; Dolton *et al.*, 2015, 2018). When cells were gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes, mean fluorescence intensity (MFI) values of 73.2, 108, and 7384 were given for staining with no dextramer, staining with HLA-A2-GILGFVFTL derived from the influenza virus matrix protein (irrelevant dextramer), and staining with HLA-A2-HLVEALYLV dextramer respectively, **Figure 3.4B**.

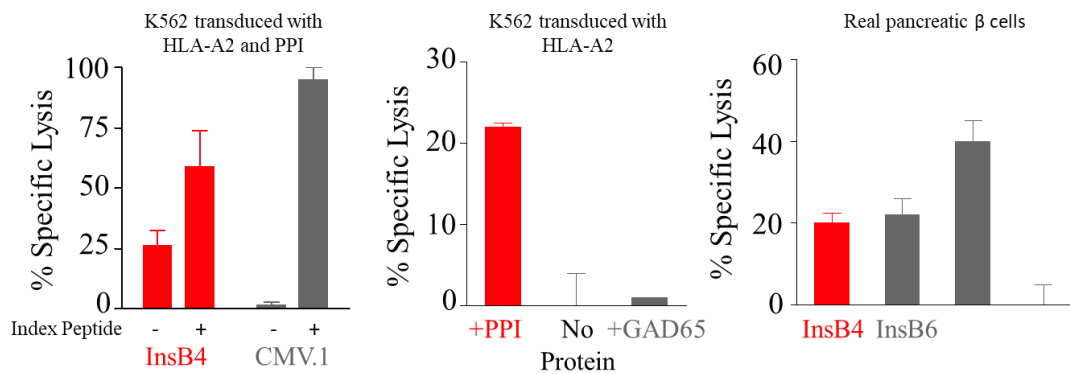
HLVEALYLV was then shown to be a *bona fide* epitope that can be appropriately processed and expressed at the surface of cells expressing PPI. K562 cells were transduced with HLA-A2 and PPI (thereby acting as surrogate  $\beta$  cells). These surrogate  $\beta$  cells were recognised and

killed by InsB4 but were not killed by an irrelevant CMV-specific T cell clone, **Figure 3.5A**. As a positive control, both clones were shown to respond when  $10^{-5}$  M of their cognate peptide (pp65 residues 495–503 for the irrelevant clone) was added, **Figure 3.5A**. This specific killing by InsB4 was dependent on the expression of PPI as K562 cells expressing only HLA-A2, or those expressing HLA-A2 and another transduced protein (GAD65), were not killed, **Figure 3.5B**. These data suggest that the HLVEALYLV is correctly processed from the PPI protein and expressed at the cell surface for scrutiny by T cells.

My laboratory subsequently demonstrated specific killing of real human pancreatic  $\beta$  cells by InsB4 T cells, **Figure 3.5C**. T1D-specific clones InsB6 (sister clone) and 1E6 (Bulek *et al.*, 2012) were used as positive controls. CMV-specific clone, CMV.1, was used as a negative control.



**Figure 3.4: The InsB4 T cell clone recognises an insulin-derived epitope. A)** MIP-1 $\beta$  response of the HLA-A2-restricted clone InsB4 to increasing concentrations of the insulin B chain HLVEALYLV peptide. 30,000 InsB4 T cells were cultured in the presence of 60,000 T2 cells as APC and varying concentrations ( $10^{-9}$  M to  $10^{-5}$  M) of HLVEALYLV overnight before the supernatant was harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis. Performed in duplicate, error bars depict standard error of the mean (SEM). Assay performed by Garry Dolton. **B)** 50,000 InsB4 T cells were stained with 0.5  $\mu$ g HLVEALYLV dextramer (relative to the pMHC component) with a PE-conjugated backbone. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. MFI values shown for no dextramer control (grey shaded), irrelevant dextramer (red), and HLVEALYLV dextramer (blue) are the mean of 10,000 events. Irrelevant pMHC used in this experiment is HLA-A2-GILGFVFTL, an M1-derived immunodominant influenza peptide.

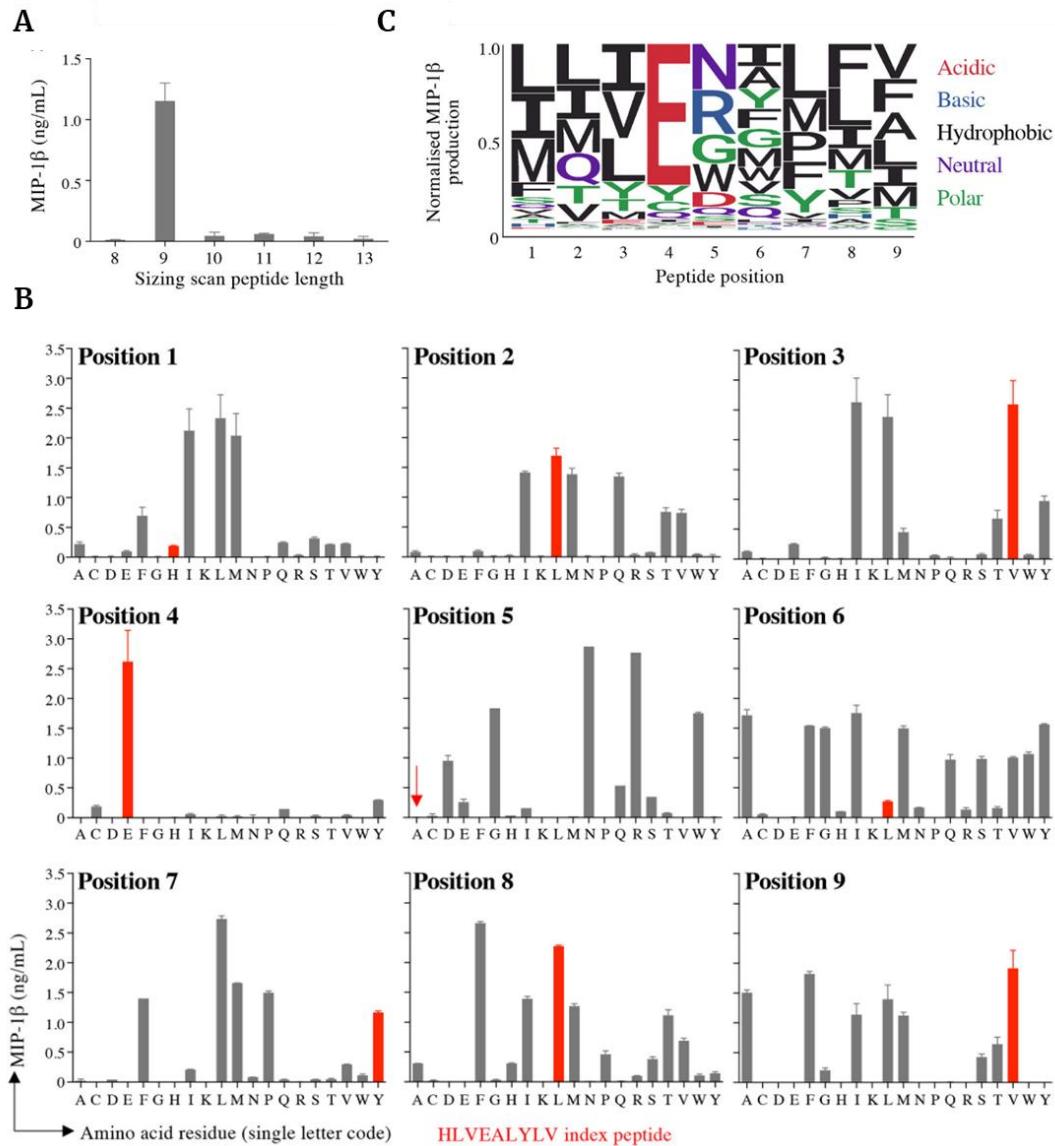


**Figure 3.5: InsB4 recognises and kills pancreatic  $\beta$  cells. A)** K562 cells transduced with HLA-A2 and preproinsulin are used as a proxy for pancreatic  $\beta$  cells. InsB4 clone kills surrogate  $\beta$  cells, as measured by chromium release cytotoxicity assay at a T cell to target cell ratio of 3:1 over 5 h. CMV.1 clone used as an irrelevant control.  $10^{-5}$  M of index peptide (pp65 residues 495–503 for the CMV.1 clone) was added exogenously for each clone to provide a positive control. **B)** InsB4 clone only specifically kills K562 cells transduced with both HLA-A2 and PPI. K562 cells transduced with HLA-A2 alone (no protein) or HLA-A2 with an irrelevant protein (GAD65) were not recognised. Chromium release cytotoxicity assay at a T cell to target cell ratio of 3:1 over 5 h. **C)** InsB4 kills real human pancreatic  $\beta$  cells, as measured by chromium release cytotoxicity assay at a T cell to target cell ratio of 3:1 over 5 h. T1D-specific clones InsB6 (sister clone) and 1E6 used as positive control. CMV-specific clone CMV.1 used as a negative control. All assays performed in duplicate, error bars depict SEM. Assays performed by Garry Dolton.

### 3.2.1.2 – CPL scanning identifies the peptide repertoire of the InsB4 T cell clone

The index peptide for InsB4, HLVEALYLV, is a 9-mer. The preference of InsB4 for 9-mer peptides was confirmed by sizing scan. This method uses random mixtures of peptides 8, 9, 10, 11, 12, and 13 amino acids in length and measures MIP-1 $\beta$  response to each pool by ELISA. InsB4 showed an extremely strong preference for 9-mer peptides, **Figure 3.6A**.

Based on these data, a 9-mer CPL screen was performed on the InsB4 clone to investigate amino acid preferences at each peptide position. Response to each of the 180 peptide mixtures in the CPL, as measured by MIP-1 $\beta$  production, is shown in **Figure 3.6B**. At many peptide positions, particularly positions 1, 5, 6, and 7, the index amino acids (indicated by the red bar) were not the preferred amino acid – suggesting that a stronger peptide agonist might be readily discoverable. These data are summarised as a logo plot in **Figure 3.6C**. It is noteworthy that the glutamic acid residue found at position 4 in the index peptide is so clearly preferred at this position. This suggests that the 4E residue of the peptide might be a key TCR contact residue for the interaction.

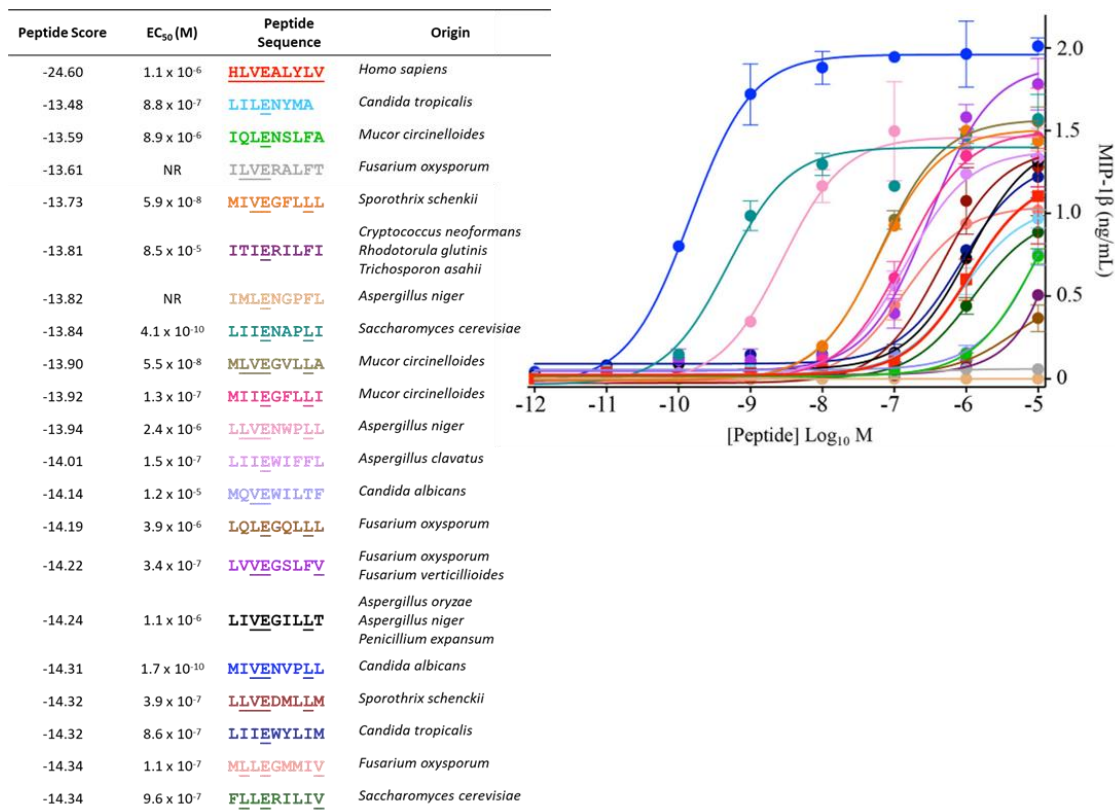


**Figure 3.6: Characterising the peptide preferences of InsB4.** **A)** Sizing scan of the InsB4 clone, based on MIP-1β production. 30,000 InsB4 T cells were cultured in the presence of 60,000 T2 cells as APC and peptides of varying lengths overnight before the supernatant was harvested for measurement of MIP-1β presence by ELISA analysis. Performed in duplicate, error bars depict SEM. Assay performed by Garry Dolton. **B)** CPL screen of the InsB4 T cell clone. 30,000 InsB4 T cells were co-cultured with 60,000 T2 cells as APC and 100 μg/mL peptide mix from a 9-mer CPL screen. Supernatants were harvested and ELISA analysis performed to measure MIP-1β presence as a proxy for T cell response. Red bars indicate the sequence of the index HLVEALYLV peptide. Performed in duplicate, error bars depict SEM. Assay performed by Garry Dolton. **C)** Logo plot representing amino acid preferences of InsB4 at each peptide position.

### 3.2.1.3 – Fungal-derived peptide agonists of the InsB4 T cell clone

CPL data, along with a publicly assessable webtool (Szomolay *et al.*, 2016; Whalley *et al.*, 2020), were then used to screen pathogenic proteome databases. This screening method, developed by Szomolay *et al.* allowed ranked predictions of peptide ligands for the InsB4 TCR.

The top 20 scoring fungal peptides ranked by a likelihood score, are shown in **Figure 3.7**. I assessed the recognition of these peptides in crude form (~40% purity) by InsB4 T cells alongside that of the insulin-derived HLVEALYLV peptide by MIP-1 $\beta$  ELISA, **Figure 3.7**. Half maximal effective concentration (EC<sub>50</sub>) values, as calculated by GraphPad Prism software, confirmed that many of the predicted fungal peptides were more potent ligands than HLVEALYLV.

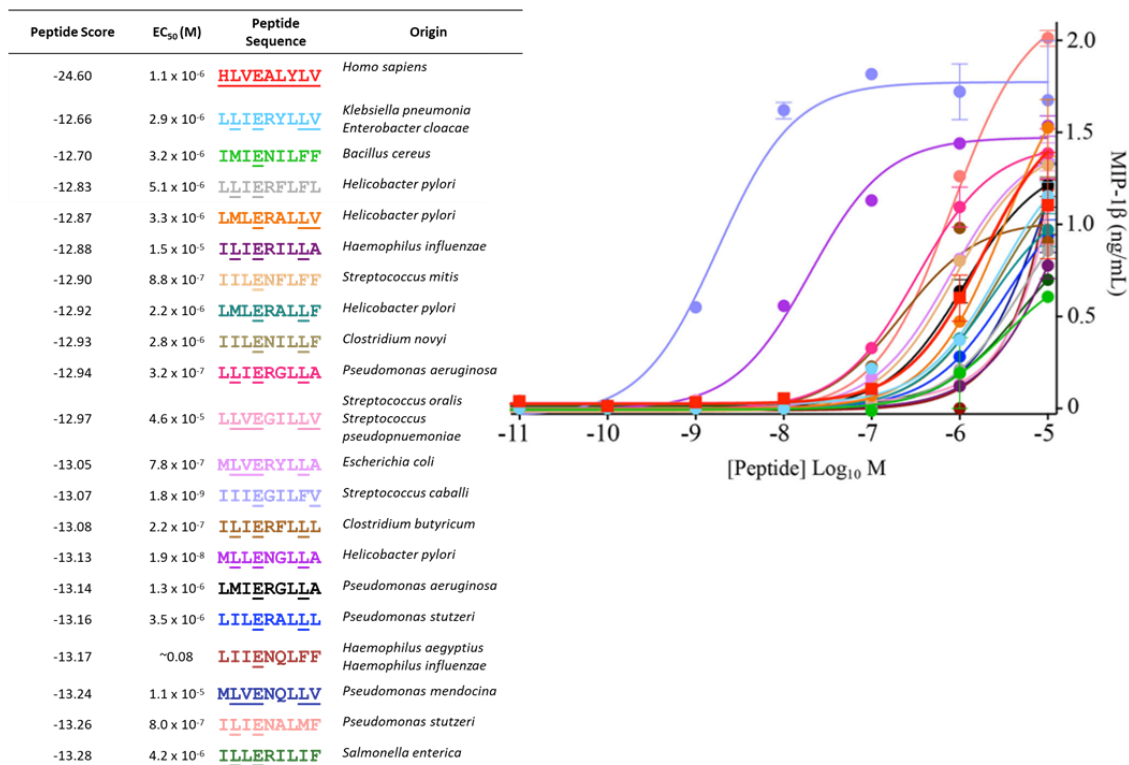


**Figure 3.7: InsB4 recognises fungal peptides with greater sensitivity than the index peptide.** The top 20 ranked peptides from the fungal proteomic database predicted to be InsB4 ligands generated as described by Szomolay *et al.* (Szomolay *et al.*, 2016). Greater 'score' indicates prediction for a more potent ligand. Peptides were tested for their ability to trigger MIP-1 $\beta$  production. 30,000 InsB4 T cells were cultured in the presence of 60,000 T2 cells as APC and varying concentrations ( $10^{-12}$  M to  $10^{-5}$  M) of each peptide overnight before the supernatants were harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis. The EC<sub>50</sub> for each peptide is shown in the accompanying table. NR = no response. Measurements performed in duplicate, error bars depict SEM.

#### 3.2.1.4 – Bacterial-derived peptide agonists of the InsB4 T cell clone

CPL data was also used to screen the pathogenic bacterial database collated by Szomolay *et al.* (Szomolay *et al.*, 2016). The top 20 scoring bacterial peptides ranked by a likelihood score, are shown in **Figure 3.8**. I assessed the ability of these peptides in crude form (~40% purity) to activate InsB4 T cells, alongside that of the insulin-derived HLVEALYLV peptide, by MIP-1 $\beta$  ELISA, **Figure 3.8**. EC<sub>50</sub>, as calculated by GraphPad Prism software, suggested that many of the predicted bacterial peptides were more potent ligands than HLVEALYLV.

At the time of my joining the lab, the above data were being assembled into a paper that has recently been published (Whalley *et al.*, 2020). The paper describes a novel graphics processing unit (GPU)-based algorithm that can be used to rapidly screen databases, such as the peptide databases assembled by my colleague Dr Barbara Szomolay (Szomolay *et al.*, 2016). This algorithm was used to identify agonist ligands for the InsB4 T cells that might be processed and presented from fungal and bacterial pathogens (Whalley *et al.*, 2020). As a new member of the group, my initial training in T cell assays involved performing the ELISAs that were used to verify the predicted peptides, as shown above, as such I was listed as a co-author on the Whalley *et al.* study which described agonist peptide ligands derived from bacterial and fungal proteomes (Whalley *et al.*, 2020)

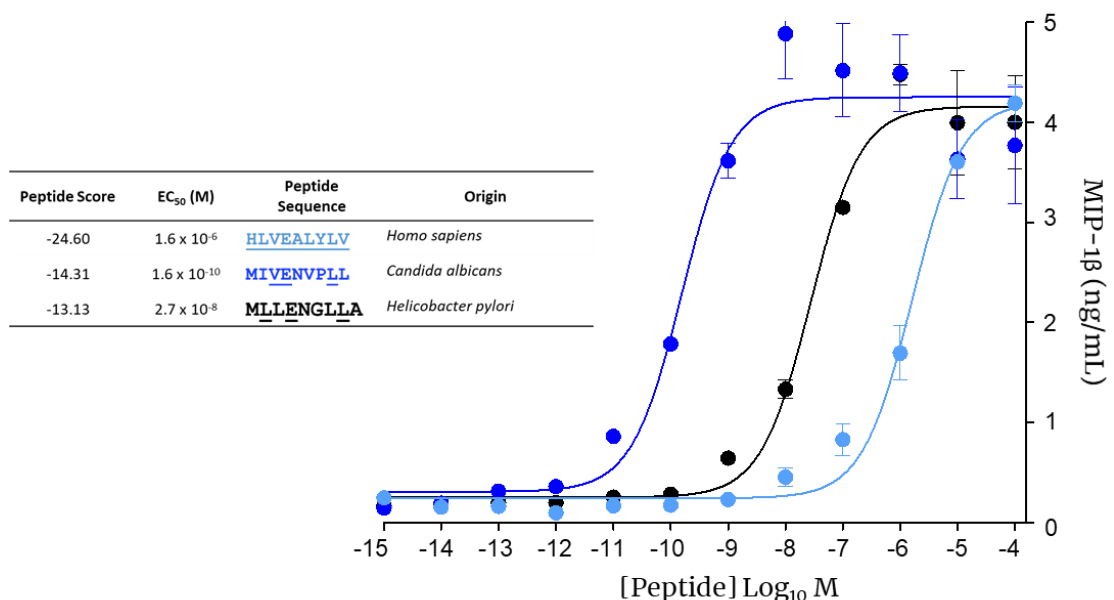


**Figure 3.8: InsB4 recognises bacterial peptides with greater sensitivity than the index peptide.** The top 20 ranked peptides from the bacterial proteomic database predicted to be InsB4 ligands generated as described by Szomolay *et al.* (Szomolay *et al.*, 2016). Greater 'score' indicates prediction for a more potent ligand. Peptides were tested for their ability to trigger MIP-1 $\beta$  production. 30,000 InsB4 T cells were cultured in the presence of 60,000 T2 cells as APC and varying concentrations ( $10^{-11}$  M to  $10^{-5}$  M) of each peptide overnight before the supernatant was harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis. The EC<sub>50</sub> for each peptide is shown in the accompanying table. ~ = ambiguous EC<sub>50</sub> based on GraphPad Prism software analysis. Measurements performed in duplicate, error bars depict SEM.



### 3.2.2 – Fungal and bacterial peptides act as functional ligands for the InsB4 T cell clone

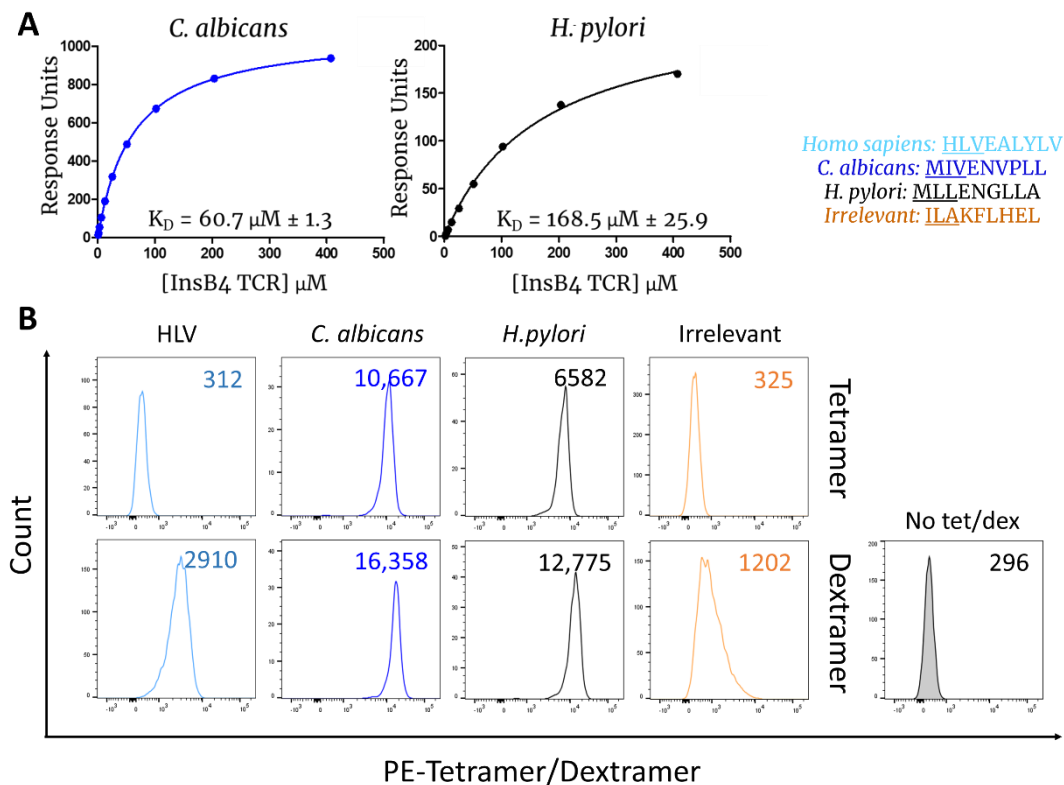
I next aimed to validate the top scoring fungal and bacterial peptides. The top fungal peptide (the *Candida albicans* peptide (MIVENWPLL)) and the top bacterial peptide (the *Helicobacter pylori* peptide (MLLENGLLA)) were ordered as pure preparations (>95% purity; Peptide Synthetics) and I re-assessed the MIP-1 $\beta$  response of InsB4 by ELISA (peptide concentrations ranging from  $10^{-15}$  to  $10^{-4}$  M), **Figure 3.9**. The EC<sub>50</sub> value for each titration was generated using GraphPad Prism software analysis. Each pathogenic peptide proved to be a more potent agonist for InsB4 than the T1D-associated index peptide, HLVEALYLV.



**Figure 3.9: Pure peptide titrations for the top pathogenic peptides.** The top bacterial and fungal peptides based on crude peptide titration EC<sub>50</sub> values were ordered as pure preparations. Peptides were tested for their ability to trigger MIP-1 $\beta$  production. 30,000 InsB4 T cells were cultured in the presence of 60,000 T2 cells as APC and varying concentrations ( $10^{-15}$  M to  $10^{-4}$  M) of each peptide overnight before the supernatant was harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis. The EC<sub>50</sub> for each peptide is shown in the accompanying table, based on GraphPad Prism software analysis. Measurements performed in duplicate, error bars depict SEM.

Binding of the InsB4 TCR to the two pathogenic agonists was then confirmed by SPR. Each of the pathogenic peptides had an affinity for InsB4 that was measurable by SPR, whereas the index HLVEALYLV ligand bound too weakly to be measured. HLA-A2-MIVENVPLL (*C. albicans*) and HLA-A2-MLEENGLLA (*H. pylori*) had affinities ( $K_D$ ) for InsB4 of 60.7  $\mu\text{M}$  and 168.5  $\mu\text{M}$  respectively (**Figure 3.10A**). These data confirm the interaction between InsB4 and each of the pathogenic epitopes in the context of HLA-A2.

I next demonstrated that the InsB4 T cell clone successfully bound by PE-conjugated HLA-A2 multimers made with the two pathogenic peptides. MFI values (displayed on the respective histograms) support the notion that InsB4 binds more strongly to the pathogenic peptides than to the index HLVEALYLV peptide (light blue) which only stains as a dextramer, **Figure 3.10B**.



**Figure 3.10: InsB4 TCR binding of pathogen-derived agonist peptides is confirmed by SPR and pMHC multimer staining. A)** SPR responses of the InsB4 TCR against the top fungal and top bacterial peptides as soluble HLA-A2 complexes. Responses against an irrelevant peptide HLA-A2-NLSAIGIFST were used to remove background response. 10 serial dilutions of the InsB4 were used, with a maximum concentration of 407.0  $\mu\text{M}$ . Index A2-HLVEALYLV binding to InsB4 was too weak to measure. Assay performed by Aaron Wall. **B)** 50,000 InsB4 T cells were stained with 0.5  $\mu\text{g}$  of tetramer or dextramer (relative to the pMHC component), with a PE-conjugated backbone. Cells previously gated on live  $\text{CD3}^+\text{CD8}^+$  single lymphocytes. The human telomerase reverse transcriptase-derived ILAKFLEHEL was used as a negative control. Numbers on the histograms correspond to MFI values, mean of 10,000 events.

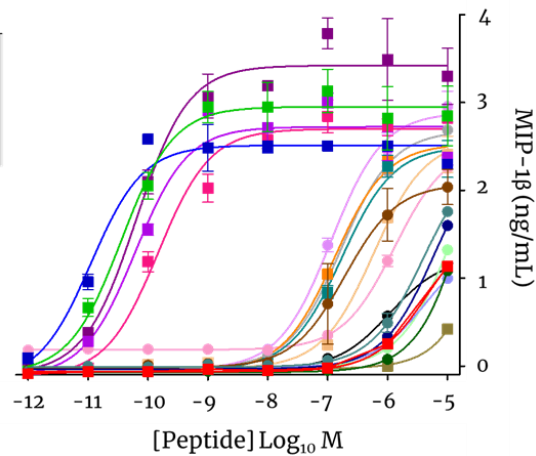
### 3.2.3 – Peptide sequences derived from human viruses act as potent agonists of the InsB4 T cell

After validating the bacterial and fungal crude peptide ligands that were described in the Whalley *et al.* paper (Whalley *et al.*, 2020), the majority of my own work with the InsB4 T cell clone revolved around examining viral-derived agonist peptides. Some of these ligands are known viral epitopes (discussed in **Section 3.3.4**) which acted as extremely potent agonists for the InsB4 T cell, making them strong candidates for being the triggering event that might have initiated T1D.

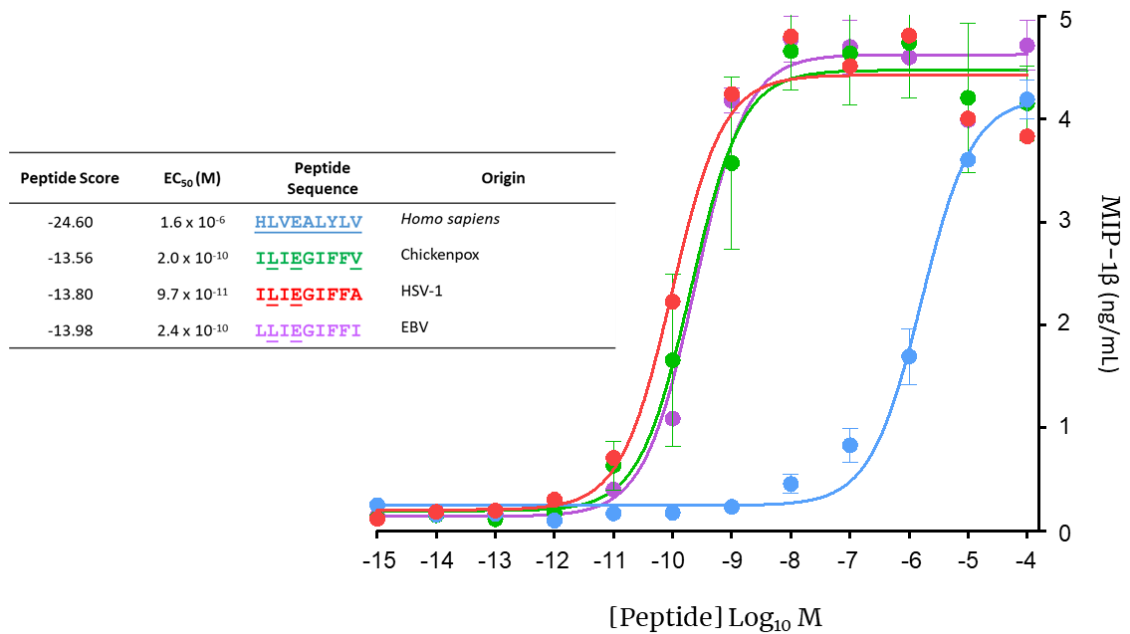
I began by using the CPL data and the Szomolay webtool (Szomolay *et al.*, 2016), to screen for potential ligands within the pathogenic viral proteome. The top scoring peptides from the viral database were synthesised (Peptide Synthetics) in crude form (~40% purity) and I assessed their ability to evoke a response from InsB4 T cells, **Figure 3.11**. InsB4 was incubated with titrations of each peptide (concentrations ranging from  $10^{-12}$  to  $10^{-5}$  M) and T2 cells overnight, the resulting MIP-1 $\beta$  production was measured by ELISA. All viral peptides gave measurable responses despite many having little sequence homology with the HLVEALYLV peptide. In fact, many proved to be far more potent ligands for InsB4 than the index HLVEALYLV peptide itself and were substantially more potent than the fungal and bacterial peptides that had previously been identified (**Figure 3.7** and **3.8**). These data, once again, show that InsB4 is capable of responding to more potent peptides than the T1D-associated epitope, and suggest that a pathogen-derived agonist may exist. Given that the top viral peptides were so much more potent than even the bacterial and fungal peptides, these data support the hypothesis that the initial trigger that activated InsB4 T cells *in vivo* could be virus-derived.

The three most potent viral epitopes for activating InsB4 T cells (LLIEGIFV; varicella-zoster virus (referred to as chickenpox virus henceforth), LLIEGIFA; HSV-1, and LLIEGIFI, EBV) are highlighted by grey shading in **Figure 3.11**. These peptides were ordered as pure preparations (>95 % purity; Peptide Synthetics) and re-assessed for activation of the InsB4 T cell clone by MIP-1 $\beta$  ELISA over concentrations ranging from  $10^{-15}$  to  $10^{-4}$  M, **Figure 3.12**. The EC<sub>50</sub> value for each peptide was generated using GraphPad Prism software analysis. Each viral peptide proved to be a substantially more potent agonist for InsB4 than the T1D-associated index peptide, HLVEALYLV. Indeed, all three viral peptides were 100,000-fold more potent at activating the InsB4 T cell clone than the insulin-derived sequence known to be presented at the surface of HLA-A2<sup>+</sup>  $\beta$  cells.

Peptide Score	EC <sub>50</sub> (M)	Peptide Sequence	Origin
-24.60	4.6 × 10 <sup>-6</sup>	<u>H</u> LVEALYL <u>V</u>	Homo sapiens
-13.56	1.1 × 10 <sup>-11</sup>	<u>I</u> LIEGIF <u>F</u> V	Varicella-Zoster virus
-13.80	3.6 × 10 <sup>-11</sup>	<u>I</u> LIEGIF <u>F</u> FA	HSV-1
-13.98	6.2 × 10 <sup>-12</sup>	<u>L</u> LIEGIF <u>F</u> I	Epstein-Barr virus
-14.35	1.5 × 10 <sup>-10</sup>	<u>I</u> LIEGV <u>F</u> FA	HSV-2
-14.3	6.4 × 10 <sup>-11</sup>	<u>L</u> IVEGI <u>F</u> I	Kaposi's sarcoma-associated herpesvirus
-14.86	5.8 × 10 <sup>-7</sup>	<u>I</u> TIENAL <u>V</u> A	Variola virus
-15.20	1.7 × 10 <sup>-7</sup>	<u>I</u> LIE <u>N</u> FF <u>T</u> T	TMEV-like cardiovirus
-15.34	~0.03	<u>L</u> QTERAL <u>V</u> V	HSV-2
-16.02	1.3 × 10 <sup>-7</sup>	<u>L</u> VIENS <u>P</u> L <u>S</u>	Human parainfluenza virus 1
-16.26	1.3 × 10 <sup>-6</sup>	<u>I</u> LQGI <u>L</u> L <u>F</u>	Cytomegalovirus
-16.66	1.1 × 10 <sup>-7</sup>	<u>L</u> QIED <u>W</u> L <u>L</u>	HSV-1
-16.67	4.1 × 10 <sup>-6</sup>	<u>I</u> LTE <u>G</u> GP <u>V</u> L	HSV-6
-16.71	1.2 × 10 <sup>-5</sup>	<u>M</u> QIEN <u>N</u> L <u>L</u> S	HSV-6
-17.00	1.6 × 10 <sup>-7</sup>	<u>L</u> LIER <u>F</u> V <u>S</u> L	Human coronavirus
-17.16	1.1 × 10 <sup>-6</sup>	<u>Q</u> LIE <u>Q</u> SL <u>F</u> L	Metapneumovirus
-17.18	6.3 × 10 <sup>-6</sup>	<u>I</u> L <u>L</u> ER <u>V</u> SL	Human coronavirus
-17.30	3.1 × 10 <sup>-5</sup>	<u>I</u> V <u>Y</u> ER <u>Q</u> PP <u>F</u>	Torque teno midi virus
-16.74	1.9 × 10 <sup>-7</sup>	<u>F</u> VI <u>Y</u> R <u>F</u> LV	Variola virus
-17.21	3.9 × 10 <sup>-6</sup>	<u>L</u> MIER <u>F</u> V <u>S</u> L	SARS coronavirus



**Figure 3.11: InsB4 recognises viral peptides with greater sensitivity than the index peptide.** The top 19 ranked peptides from the viral proteomic database predicted to be InsB4 ligands, generated as described by Szomolay *et al.* (Szomolay *et al.*, 2016). Greater 'score' indicates prediction for a more potent ligand. Peptides were tested for their ability to trigger MIP-1 $\beta$  production. 30,000 InsB4 T cells were cultured in the presence of 60,000 T2 cells as APC and varying concentrations ( $10^{-12}$  M to  $10^{-5}$  M) of each peptide overnight before the supernatant was harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis. The EC<sub>50</sub> for each peptide is shown in the accompanying table. ~ = ambiguous EC<sub>50</sub> based on GraphPad Prism software analysis. Measurements performed in duplicate, error bars depict SEM. Grey shading indicates the viral super agonists discussed henceforth.

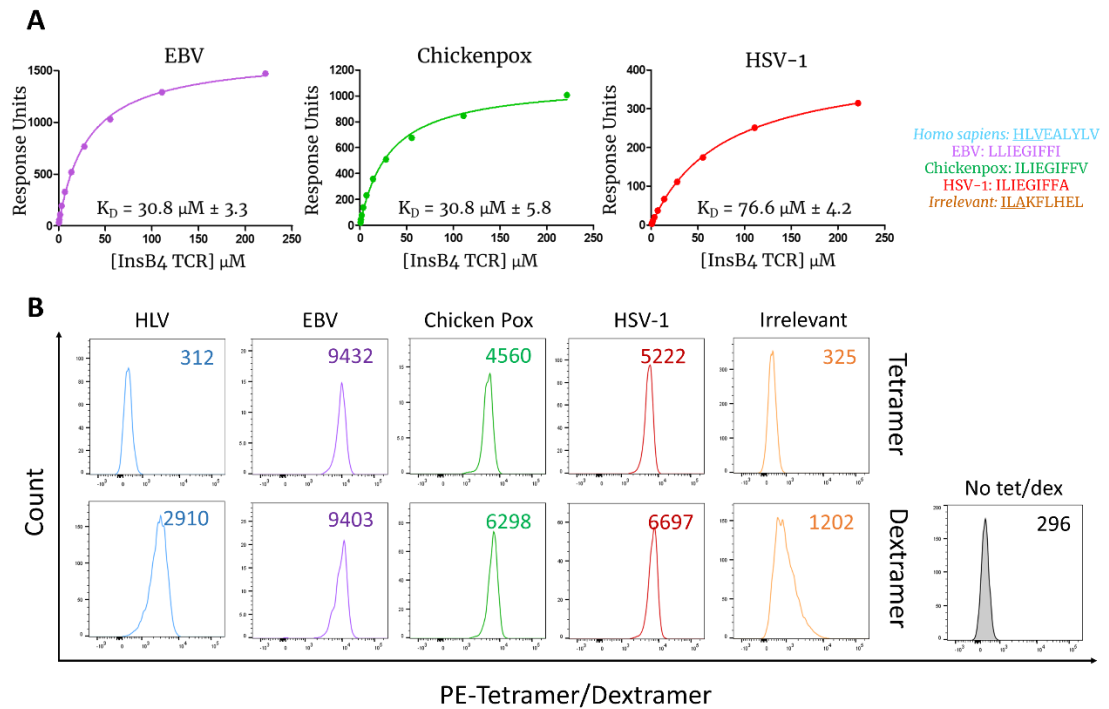


**Figure 3.12: Viral-derived peptides act as strong agonists for the insulin-specific InsB4 T cell clone.** The top viral peptides based on crude peptide titration EC<sub>50</sub> values were ordered as pure preparations (>95 % purity). Peptides were tested for their ability to trigger MIP-1β production. 30,000 InsB4 T cells were cultured in the presence of 60,000 T2 cells as APC and varying concentrations (10<sup>-15</sup> M to 10<sup>-4</sup> M) of each peptide overnight before the supernatant was harvested for measurement of MIP-1β presence by ELISA analysis. The EC<sub>50</sub> for each peptide is shown in the accompanying table, based on GraphPad Prism software analysis. Measurements performed in duplicate, error bars depict SEM.

### 3.2.3.1 – The InsB4 TCR binds to viral epitopes strongly

Binding of the InsB4 TCR to the three viral agonists was confirmed by SPR. Each peptide had an affinity for InsB4 that was measurable by SPR, whereas the index HLVEALYLV ligand bound too weakly to be measured. HLA-A2-LLIEGIFFI, (EBV), HLA-A2-ILIEGIFV (chickenpox virus), and HLA-A2-ILIEGIFFA (HSV-1) had affinities ( $K_D$ ) for InsB4 TCR of 30.8  $\mu$ M, 30.8  $\mu$ M, and 76.6  $\mu$ M, respectively (**Figure 3.13A**). These data confirm the interaction between InsB4 and each of the viral epitopes in the context of HLA-A2.

The InsB4 T cell clone also successfully stained with PE-conjugated HLA-A2 multimers made with the three viral peptides. As seen with the bacterial and fungal peptides, MFI values (displayed on the respective histograms) suggest that InsB4 binds more strongly to the viral peptides than to the index HLVEALYLV peptide (light blue) which only stains as a dextramer, **Figure 3.13B**.



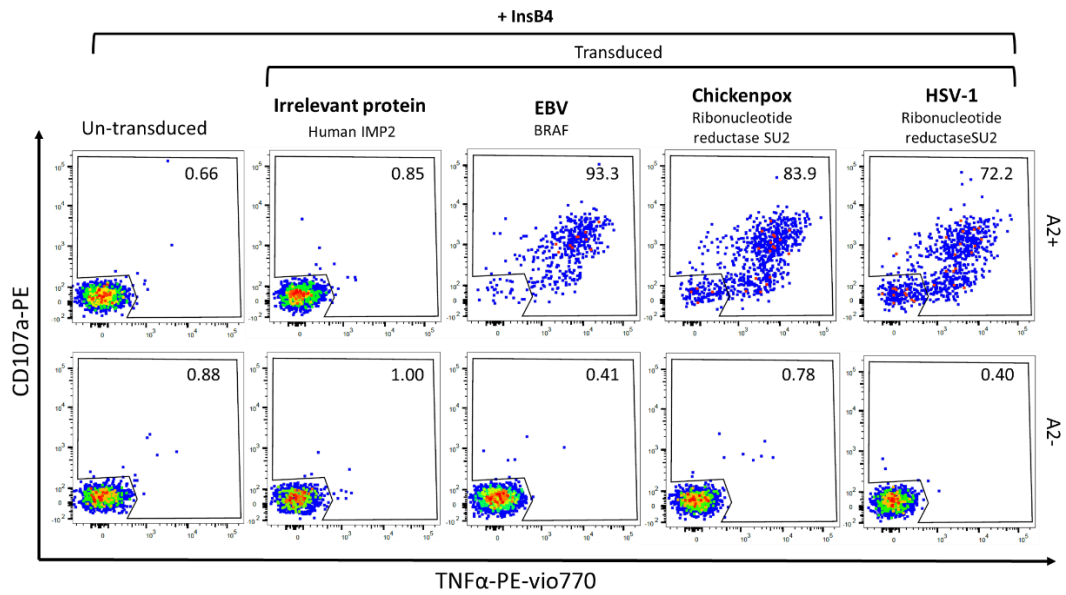
**Figure 3.13: InsB4 TCR binding of pathogenic agonists is confirmed by SPR and multimer staining. A)** SPR responses of the InsB4 TCR against the viral peptides as soluble HLA-A2 complexes. Responses against an irrelevant peptide HLA-A2-NLSAIGIFST were used to remove background response. 10 serial dilutions of the InsB4 were used, with a maximum concentration of 221  $\mu\text{M}$ . Index A2-HLVEALYLV binding to InsB4 was too weak to measure. Assay performed by Aaron Wall. **B)** 50,000 InsB4 T cells were stained with 0.5  $\mu\text{g}$  of tetramer or dextramer (relative to the pMHC component), with a PE-conjugated backbone. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. The human telomerase reverse transcriptase-derived ILAKFLHEL was used as a negative control. Numbers on the histograms correspond to MFI values, mean of 10,000 events.

### 3.2.3.2 – Viral epitopes are correctly processed and presented

Following confirmation of the physical interaction between the viral-derived peptides and the InsB4 TCR, I next validated each of the three viral peptides as legitimately processed and presented epitopes. ILIEGIFV (chickenpox virus), ILIEGIFFA (HSV-1), and LLIEGIFFI, (EBV) are derived from viral proteins ribonucleotide reductase (chickenpox virus), ribonucleotide reductase (HSV-1) and BARP-1 (EBV) respectively, see **Table 1**. These proteins were cloned into a lentiviral vector, pELNS, (**Section 2.3.3**) and transduced into MOLT-3 cells (**Section 2.4.2**), in order to assess whether the peptide epitopes contained within these proteins would be successfully processed and presented by HLA-A2 at the cell surface. The proteins were transduced into both the wildtype HLA-A2<sup>-</sup> MOLT-3 line, and a MOLT-3 line that had previously been stably transduced with HLA-A2. Transduced MOLT-3 cells were cultured for 1 week following transduction before InsB4 reactivity was measured via TAPI-0 assay (**Section 2.6.6**).

InsB4 T cells produced both TNF and CD107a in response to HLA-A2<sup>+</sup> targets expressing all three viral proteins, suggesting that all the epitopes were successfully processed and presented from the full protein sequence, **Figure 3.14**. The response was HLA-A2-dependent as InsB4 cells did not respond to HLA-A2<sup>-</sup> MOLT-3 cells that had been transduced with any of the viral proteins. Flow cytometry analysis showed that 93.3, 83.9, and 72.2 % of the InsB4 T cells in the assay responded to HLA-A2<sup>+</sup> MOLT-3 targets expressing the target antigen from EBV, chickenpox virus, and HSV-1 respectively. These data confirm that InsB4 T cells recognise HLA-A2<sup>+</sup> MOLT-3 target cells that have been transduced with the ribonucleotide reductase genes from chickenpox virus and HSV-1 or BARP-1 from EBV.

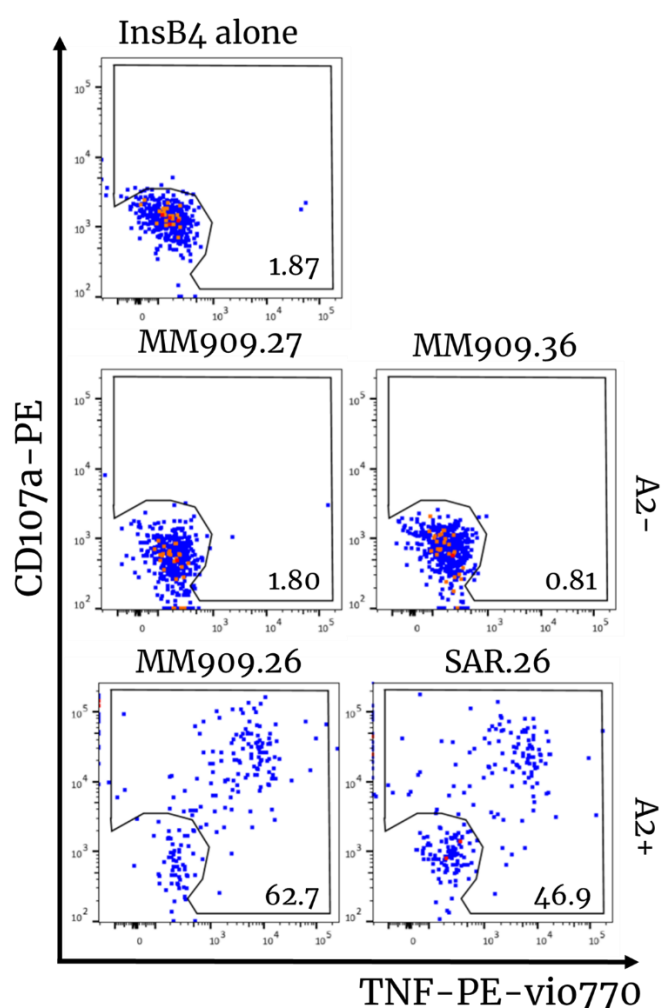




**Figure 3.14: Putative viral epitopes are efficiently processed and presented to InsB4 T cells in the context of HLA-A2.** The three viral epitopes are correctly processed and presented by MOLT-3 cells allowing response from InsB4, as measured by TAPI-0 assay. MOLT-3 cells were transduced with lentiviral constructs encoding the three parental proteins from which the viral epitopes are derived. 30,000 InsB4 cells were co-cultured with 60,000 MOLT-3 cells for 4 hours before cells were harvested and assessed for surface expression of TNF and CD107a. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Numbers on dot plots correspond to the percentage of cells in the gated population, mean of 10,000 events.

### 3.2.4 – InsB4 recognises EBV-infected HLA-A2<sup>+</sup> B cells (LCL lines)

EBV is routinely used to transform lymphoblastic cell lines (LCLs). I therefore wondered if InsB4 would recognise EBV-transformed LCLs. **Figure 3.15** shows that the InsB4 clone responds to EBV-transformed LCL lines in an HLA-A2-dependent manner. TNF<sup>+</sup> CD107a<sup>+</sup> response to the two HLA-A2<sup>-</sup> LCL lines, MM909.27 and MM909.36, was 1.80 % and 0.81 % respectively. Whereas 62.7 % and 46.9 % of the T cells responded to the two HLA-A2<sup>+</sup> LCL lines MM909.26 and SAR.26 respectively. This result confirms that the InsB4 T cell can respond strongly to EBV-infected cells and suggests that herpesviruses like EBV may have been involved in the initial activation of this T cell in the T1D patient from which it was derived.

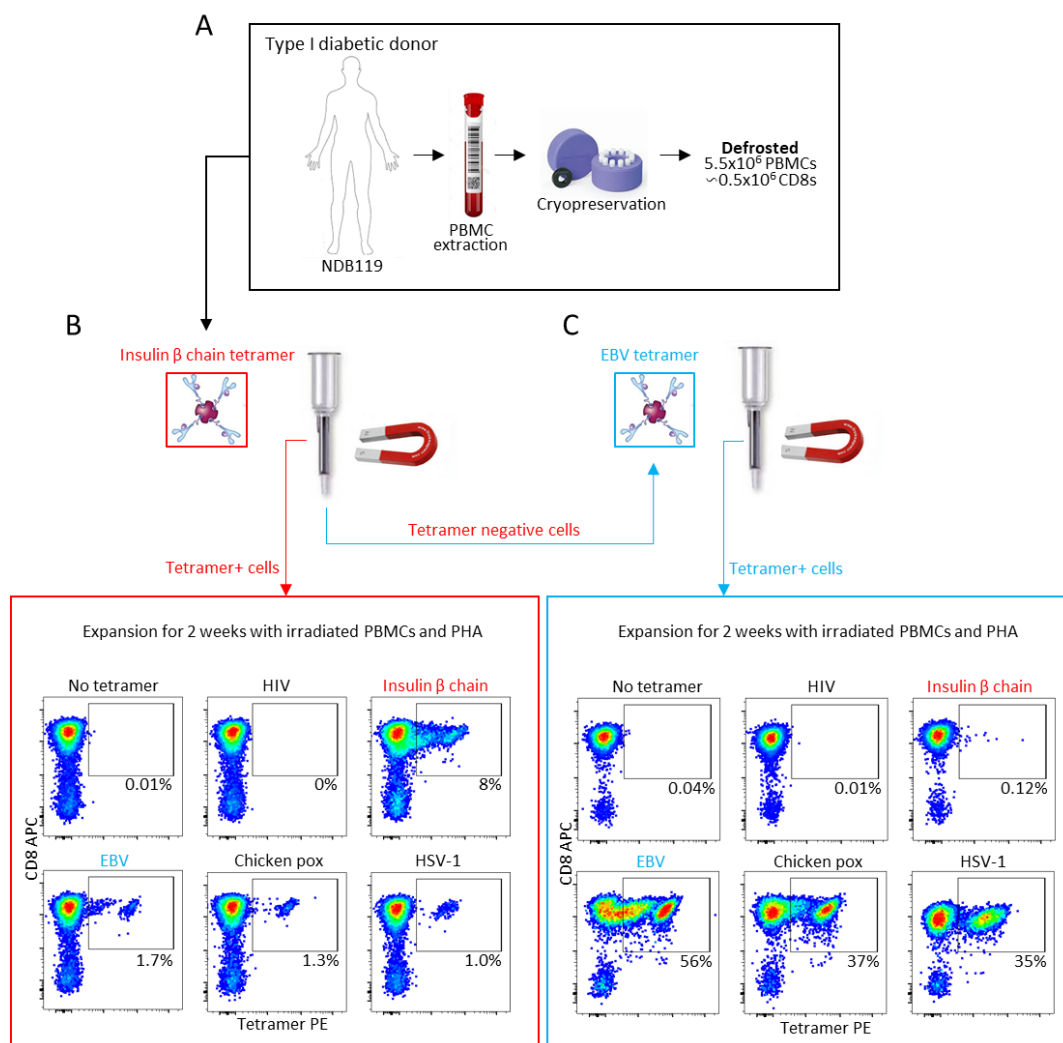


**Figure 3.15: InsB4 responds to EBV-transformed lymphoblastic cell lines (LCLs) in an HLA-A2-dependent manner.** 30,000 InsB4 T cells were cultured in the presence of 60,000 HLA-A2<sup>+</sup> or HLA-A2<sup>-</sup> LCLs for 4 hours before cells were harvested and assessed for surface expression of TNF and CD107a. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Numbers on dot plots correspond to the percentage of cells in the gated population, mean of 10,000 events.

3.2.5 – Insulin-specific T cells in an HLA-A2<sup>+</sup> T1D patient recognise EBV  
Thus far, I have demonstrated that the insulin-specific InsB4 T cell clone, isolated from a patient with T1D could respond strongly to epitopes derived from common human viruses. I next aimed to determine whether this crossreactivity might occur in other patients with T1D. Fortunately, I had a PBMC sample taken from a T1D patient, NDB119, taken just prior to the March COVID-19 lockdown.

Extracted lymphocytes from patient NDB119 were subjected to magnetic separation with HLA-A2-HLVEALYLV tetramer, as described in **Section 2.5.5**. HLA-A2-HLVEALYLV tetramer<sup>+</sup> cells were expanded (**Section 2.5.6**) prior to tetramer staining and analysis by FACS as shown in **Figure 3.16B**. 8 % of the resulting cell population could bind the HLA-A2-HLVEALYLV tetramer and, notably, 1.7 %, 1.3 %, and 1.0 % of the CD8<sup>+</sup> T cells isolated with the HLA-A2-HLVEALYLV tetramer could be bound by HLA-A2-LLIEGIFFI (EBV), HLA-A2-ILIEGIFV (chickenpox virus), and HLA-A2-ILIEGIFFA (HSV-1) tetramers respectively. These data suggest that some insulin-specific T cells from patient NDB119 might bind the three viral epitopes that acted as strong agonists for the InsB4 T cell clone that had been isolated from different patient with T1D. Confirmation of crossreactivity will require TCR sequencing of the tetramer<sup>+</sup> cells to show that identical TCRs are present in each respective tetramer<sup>+</sup> population. Unfortunately, I was unable to sequence the TCRs from these T cell populations, but they have been stored and I hope to examine them fully in the future.

In order to extract maximum data from the NDB119 PBMC, I also stained them with the HLA-A2-LLIEGIFFI (EBV) tetramer *after* first removing the insulin-specific T cell population. After expansion of these cells over 50% of the resultant T cell line stained with HLA-A2 EBV tetramer. Over 30% of the cells from the expanded NDB119 line also stained with HSV-1 and chickenpox virus tetramers suggesting that many, but not all, EBV-specific T cells can respond to peptide sequences from other herpesviruses. This EBV-specific T cell line that had been grown after prior removal of insulin-specific T cells did not stain well with the HLA-A2-HLVEALYLV (insulin) tetramer. Taken together, this experiment shows that a further T1D patient has EBV-specific T cells that may crossreact with an insulin epitope. Future experiments could aim to sequence the TCRs from the tetramer<sup>+</sup> T cells to confirm this potential crossreactivity.



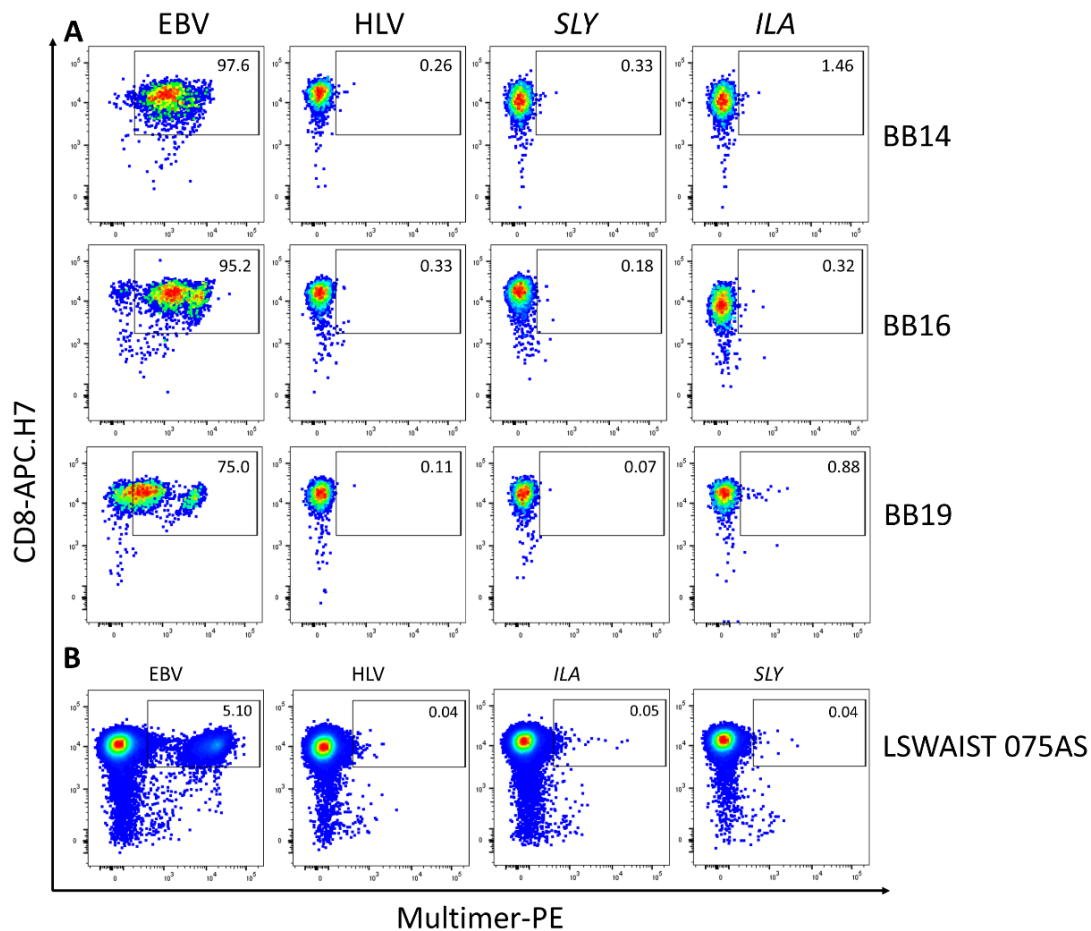
**Figure 3.16: Insulin-specific cells in an HLA-A2<sup>+</sup> T1D donor can recognise three viral epitopes.** **A)** A schematic overview of the procedure used in this work. **B)** HLA-A2-HLVEALYLV<sup>+</sup> cells from the NDB119 donor were isolated using magnetic separation and expanded. Following expansion 50,000 T cells were stained with 0.5  $\mu$ g of various tetramer (relative to the pMHC component), with a PE-conjugated backbone. Numbers on dot plots represent percentage of tetramer-positive cells in the 10,000 events recorded. **C)** HLA-A2-HLVEALYLV<sup>-</sup> cells from the NDB119 donor were subject to HLA-A2-LLIEGIFFI tetramer binding and subsequent magnetic separation. HLA-A2-LLIEGIFFI<sup>+</sup> cells were then expanded. Following expansion 50,000 T cells were stained with 0.5  $\mu$ g (relative to pMHC component) of various tetramers, with PE-conjugated backbones. Numbers on dot plots represent percentage of tetramer-positive cells in the 10,000 events recorded. HLA-A2-SLYNTVATL tetramers used as a negative control.

Following my return to work after the summer 2020 lockdown I was unable to access to any more freshly donated samples from patients with T1D. I was, however, fortunate to benefit from three frozen PBMC samples from healthy donors, and a frozen T1D PBMC sample (LSWAIST 075AS) kindly donated by Professor Susan Wong. I also reasoned that if the EBV/insulin crossreactivity could be responsible for triggering disease, T cells specific for the EBV-derived peptide LLIEGIFFI derived from healthy donors would not cross-recognise the insulin-derived epitope HLVEALYLV.

FACS-based cell sorting, performed by Dr Cristina Ruis Rafael, was used to isolate CD8<sup>+</sup> T cells specific for the HLA-A2-LLIEGIFFI (EBV) tetramer from these PBMC samples. Following expansion of these HLA-A2-LLIEGIFFI<sup>+</sup> cells I showed that the resulting cell populations from all three healthy donors (BB14, BB16, and BB19) did not stain with HLA-A2-HLVEALYLV tetramer **Figure 3.17A**. Thus, I was unable to detect any insulin crossreactivity within HLA-A2-LLIEGIFFI-specific T cell populations from three healthy donors.

I next examined a frozen T1D PBMC sample, kindly donated by Professor Susan Wong, LSWAIST 075AS. Only 5.1 % of the EBV tetramer sorted T cells stained with the HLA-A2-LLIEGIFFI tetramer (compared to >75% with all the healthy donor lines) (**Figure 3.17B**). This T1D line did not stain with the HLA-A2-HLVEALYLV tetramer, even when a larger number of events were collected.

Overall, I have examined the peptide recognition landscape of an insulin-specific T cell clone isolated from a patient with T1D (Theaker *et al.*, 2016) and proved that this T cell can strongly respond to genuinely processed epitopes from three herpesviruses that commonly infect children and/or adolescents. This same crossreactivity was then observed in the fresh PBMC from a further donor (**Figure 3.16**). EBV/insulin crossreactivity was not observed in three healthy donors, nor was it observed in a further HLA-A2<sup>+</sup> T1D patient (**Figure 3.17**). It is interesting that I found EBV/insulin crossreactivity in two T1D donors but not a third as these data highlight the heterogeneity of T1D. Several  $\beta$  cell-specific epitopes have potential to be involved in triggering disease and it is likely that the initial causal trigger differs between T1D patients.



**Figure 3.17: EBV/Insulin B chain crossreactivity is not present in healthy donors. A)** Frozen PBMC samples from healthy donors were subject to HLA-A2-LLIEGIFFI tetramer-based cell sorting and subsequent expansion. Following this 50,000 T cells were stained with 0.5  $\mu$ g of various tetramer (relative to the pMHC component), with a PE-conjugated backbone. Numbers on dot plots represent percentage of tetramer-positive cells in the 10,000 events recorded. **B)** A frozen PBMC sample from a T1D patient was subject to HLA-A2-LLIEGIFFI tetramer-based cell sorting and subsequent expansion. Following this 50,000 T cells were stained with 0.5  $\mu$ g of various tetramer (relative to the pMHC component), with a PE-conjugated backbone. Numbers on dot plots represent percentage of tetramer-positive cells in the 100,000 events recorded.

### 3.3 – Discussion

#### 3.3.1 – The InsB4 T cell kills human pancreatic $\beta$ cells

In this chapter, I aimed to investigate the crossreactive nature of a previously-identified T1D-associated T cell clone following the experimental procedure depicted in **Figure 3.3** (Pinkse *et al.*, 2005; Theaker *et al.*, 2016). First, my laboratory confirmed that the InsB4 T cell clone isolated from a patient with T1D (Theaker *et al.*, 2016) recognised the HLVEALYLV insulin-derived peptide in the context of HLA-A2. The clone produced MIP-1 $\beta$  in response to the peptide presented by HLA-A2<sup>+</sup> APCs, cytokine release was dose-dependent (**Figure 3.4A**). The InsB4 T cell clone also stained well with HLA-A2-HLVEALYLV PE-conjugated dextramers (**Figure 3.4B**). HLA-A2<sup>+</sup> K562 cells were transduced with a PPI expression cassette to create artificial – or surrogate – pancreatic  $\beta$  cells. PPI-transduced K562 cells were lysed by InsB4 but not an irrelevant CMV-specific control T cell clone (**Figure 3.5A**). InsB4 T cells lysed K562 target cells transduced with PPI; they did not lyse untransduced cells or those transduced to express with an irrelevant protein (**Figure 3.5B**). Encouraged by the killing of surrogate  $\beta$  cells, we next demonstrated the ability of InsB4 to kill real human  $\beta$  cells (**Figure 3.5C**). Thus, the InsB4 T cell clone responds to the *bona fide* insulin B chain-derived, HLA-A2-restricted epitope, HLVEALYLV. Importantly, this T1D-patient derived T cell clone is capable of killing human pancreatic  $\beta$  cells and could thus contribute to the pathological loss of  $\beta$  cells characteristic of T1D.

#### 3.3.2 – Molecular mimicry may trigger autoimmune disease

The HLA-A2-HLVEALYLV epitope was first described in 2005 when Pinkse *et al.* demonstrated the presence of HLA-A2-HLVEALYLV-specific CD8<sup>+</sup> T cells in both T1D patients and healthy donors (Pinkse *et al.*, 2005). Clearly, therefore, in some situations CD8<sup>+</sup> T cells that have the capacity to recognise self-antigens remain inert and do not kill pancreatic  $\beta$  cells. In 2019, Cullina *et al.* reported that CD8<sup>+</sup> T cells specific for a T1D-associated HLA-A2-presented epitope (ZnT8<sub>186-194</sub>) circulated at similar frequencies in healthy controls and T1D patients, but were enriched in the pancreases of T1D patients (Culina *et al.*, 2018). The ability of these autoreactive T cells to remain inactive in healthy donors suggests the presence of autoreactive T cells and the auto-epitope alone is insufficient to trigger the response to  $\beta$  cells.

In 1985, Fujinami and Oldstone demonstrated that myelin basic protein (MBP) shared six consecutive amino acids with hepatitis B virus polymerase (HBVP) and that rabbits given injections of 8-mer and 10-mer HBVP peptides encompassing this region developed histology

reminiscent of experimental allergic encephalomyelitis (Fujinami and Oldstone, 1985). Subsequent studies showed that the activation and clonal expansion of autoreactive lymphocytes was a critical step in the pathogenesis of autoimmune disease. Activated T cells but not resting naïve T cells could transfer autoimmunity in experimental models (Zamvil and Steinman, 1990). Microbially-derived peptides were shown to have sufficient homology with self-peptides to be able to activate autoimmune T cells (Wucherpfennig and Strominger, 1995; Zhao *et al.*, 1998; Lang *et al.*, 2002); a mechanism now referred to as molecular mimicry. The concept of molecular mimicry suggests that harmless naïve T cells that possess a TCR capable of binding to a self-antigen can be activated by pathogen-derived peptides with similar sequence (as depicted in **Figure 3.1**).

### 3.3.3 – InsB4 can be activated by pathogen-derived peptides

We set out to identify a potential ‘triggering’ epitope for InsB4. After confirming the peptide length preference (Ekeruche-Makinde *et al.*, 2013) of InsB4 (9-mers, see **Figure 3.6A**), CPL screening was performed to investigate the peptide recognition repertoire of the clone (**Figure 3.6B**). These data (summarised in **Figure 3.6C**) demonstrated that at several peptide positions the index amino acids, HLVEALYLV, are not the preferred amino acids. Positions 1, 5, 6, and 7 in particular showed strong preference for non-index amino acids. This observation supports the possibility that alternative peptides could act as ligands for InsB4. Ligands which I then endeavored to find using CPL-driven database searching (Szomolay *et al.*, 2016). I was thus able to identify fungal, bacterial, and viral peptides that proved to be more potent antigens than the index HLVEALYLV peptide (**Figures 3.7**, **3.8**, and **3.11** respectively). This observation supports the hypothesis that InsB4 is initially activated by more potent pathogenic ligands displayed on ‘professional’ APCs that can generate the ‘second signal’ required to activate naïve T cells as depicted in **Figure 3.1**. In the absence of such molecular mimicry, autoreactive T cells can remain naïve in healthy donors (Pinkse *et al.*, 2005). The pathogenic peptides described throughout this chapter are shown in **Table 3.1**. Viral peptides derived from viruses that commonly infect children and adolescents acted as very strong agonists of the InsB4 T cell. These peptides were of particular interest given that one recent study linked EBV to T1D (Harley *et al.*, 2018). Furthermore, the three virus-derived peptides I identified here by unbiased CPL screening have previously been associated with each other. Chiu *et al.* endeavoured to identify HLA-A2-restricted immunodominant epitopes for the CD8<sup>+</sup> T cell response to chickenpox virus. In doing so they not only isolated the ILIEGIFV peptide (derived from chickenpox virus ribonucleotide reductase) but found



homologs in other herpesviruses (ILIEGIFFA from HSV-1 ribonucleotide reductase and LLIEGIFFI from EBV BARF-1) and demonstrated the presence of CD8<sup>+</sup> T cell specificity to all three epitopes in PBMC of healthy subjects who had previously developed chickenpox virus, regardless of their EBV- or HSV-1-infection status, (Chiu *et al.*, 2014). These data, particularly that from patients naïve to EBV or HSV-1, is suggestive of crossreactivity to these three epitopes.

Peptide	Origin	Parental Protein
<u>HL</u> VEALYLV	<i>Homo sapiens</i>	Insulin B chain
IL <u>IE</u> GIF <u>FF</u> V	Chickenpox	Ribonucleotide reductase
IL <u>IE</u> GIF <u>FF</u> A	HSV-1	Ribonucleotide reductase
LL <u>IE</u> GIF <u>FF</u> I	EBV	BARF-1
MIV <u>EN</u> V <u>PL</u> L	<i>Candida albicans</i>	Putative protein
ML <u>LE</u> NG <u>LL</u> A	<i>Helicobacter pylori</i>	Putative protein

**Table 3.1: The pathogenic peptides used throughout Chapter 3.** Underlining indicates amino acid is identical to the index insulin-derived peptide sequence.

### 3.3.4 – Viral epitopes strongly activate InsB4 T cells

Using data from 27,000 newly diagnosed T1D patients, and 107,000 healthy controls Lai *et al.* demonstrated that T1D patients have a 1.17-fold increase risk of developing shingles. Shingles occurs when the dormant varicella-zoster virus (chickenpox virus) reactivates in later life (Lai, Lin and Liao, 2019). This propensity could arise because the virus is more likely to have the opportunity to reactivate in an environment of immune dysfunction, or because T1D patients have an increased likelihood of harboring the virus compared to healthy controls. A number of other studies describe similar associations between T1D and shingles (Heymann *et al.*, 2008; Okamoto *et al.*, 2009; Suaya *et al.*, 2014; Muñoz-Quiles *et al.*, 2017; Queenan *et al.*, 2018).

These intriguing associations, along with the fact that the viral peptides were the most potent of the predicted pathogenic ligands, led me to mainly focus my attention primarily on the viral agonists identified by the CPL screen. The top three viral peptides chosen were considerably stronger agonists than any predicted peptides from the much larger fungal and bacterial proteomics databases. The InsB4 T cell was shown to stain well with HLA-A2 tetramers of all five pathogenic peptides studied (ILIEGIFV; chickenpox virus, ILIEGIFA; HSV-1, LLIEGIFI, EBV; MIVENVPLL, *C. albicans*; and MLLENGLLA, *H. pylori*, see **Table 1**). Each putative pathogen-derived epitope stained InsB4 T cells more efficiently than the index HLA-A2-HLVEALYLV insulin sequence as a dextramer (**Figures 3.10B** and **3.13B**). Furthermore, as soluble molecules, each viral agonist was able to bind InsB4 with measurable affinity (**Figures 3.10A** and **3.13A**). The binding affinity between InsB4 and HLA-A2-HLVEALYLV was consistently too weak to be measured by SPR, further supporting the hypothesis that a more potent ligand might initially trigger the T cell clone allowing it to subsequently crossreact with weaker self-ligands. In combination, the data discussed thus far demonstrate that the putative pathogenic agonists both bind to and induce a cytokine response from InsB4, they appear to act as much more powerful agonists than the index insulin-derived sequence used to kill human  $\beta$  cells, HLVEALYLV.

The molecular mimicry hypothesis only remains valid if the identified pathogenic peptides are genuine epitopes that are correctly processed and presented at the cell surface in the context of HLA-A2, and are thus available for scrutiny by T cells like InsB4. To validate these epitopes, I transduced MOLT-3 cells with the parental proteins for each of the three viral peptides, see **Table 1**, and demonstrate that InsB4 only responds to MOLT-3 cells transduced with both HLA-A2 and one of the relevant viral proteins (**Figure 3.14**). These results strongly suggest that the peptides identified by CPL-driven database screening are real epitopes, capable of activating InsB4 and potentially triggering downstream autoimmunity through the crossreactive recognition of HLA-A2-HLVEALYLV. Formal confirmation that the pathogenic epitopes are displayed at the surface of infected cells will require peptide elution and mass spectrometry.

EBV-transformed lymphoblastic cell lines (LCLs) are used routinely in the work of my group. Given that InsB4 produced a cytokine response to BARF-1-transduced HLA-A2<sup>+</sup> MOLT-3 cells I wondered whether InsB4 would recognise HLA-A2<sup>+</sup> LCLs. I demonstrated that InsB4 produces both TNF and CD107a in response to LCLs in an HLA-A2-dependant manor (**Figure 3.15**). Thus, InsB4 can respond to EBV-infected cells.

### 3.3.5 – T1D/insulin crossreactivity in T1D patients

I began this project because my laboratory had recently partially characterised an insulin-specific patient-derived T cell clone, InsB4. I thought that InsB4 would provide an opportunity for me to investigate the plasticity of a clinically-relevant TCR-pMHC interaction. I wanted to probe the crossreactive nature of InsB4 as I am particularly interested in the theory of pathogenic autoimmune triggers. I demonstrated that InsB4 could crossreact with three viral epitopes and that it responded to these viral epitopes with substantially greater potency than the insulin epitope, HLVEALYLV (**Figures 3.12** and **3.9**).

InsB4 was isolated from a T1D patient (Theaker *et al.*, 2016), and my demonstration of viral/autoimmune crossreactivity led me to wonder if similar crossreactivity could be found in other T1D patients. I thus showed that a HLVEALYLV-specific T cell line isolated from diabetic patient, NDB119, could be stained with HLA-A2-LLIEGIFFI tetramers (**Figure 3.16**). Confirmation of crossreactivity will require sequencing of the TCRs in the tetramer positive population but these data (**Figure 3.16**) hint at viral/autoimmune crossreactivity similar to that observed for InsB4. I subsequently demonstrated the absence of EBV/insulin crossreactivity in three healthy donors (**Figure 3.17A**). It is most interesting to me that EBV/insulin crossreactivity was absent from a further, frozen T1D donor sample, LSWAIST 075AS (**Figure 3.17B**). The underlying causes of T1D, of which I believe pathogenic molecular mimics play a substantial role, are likely to be extremely heterogeneous with many pathogenic epitopes triggering many different autoimmune T cells. In this chapter I propose three viral triggers for T1D but in many patients, LSWAIST 075AS included, disease could be initiated by crossreactivity with pathogenic epitopes hitherto undiscovered. This fascinating potential for a heterogeneous range of triggers for autoimmune diseases like T1D highlights the importance of studying the plasticity of the TCR-pMHC interaction.

Greater understanding of the underlying mechanisms of T1D, particularly the TCR-pMHC interactions involved in disease initiation and propagation, may lead to improved treatment options that somewhat alleviate the life-long dependence on injected insulin experienced by T1D patients. My laboratory is currently working with Dmitriy Chudakov, founder of the TCR database VDJdb (Bagaev *et al.*, 2020) to understand the nature of diabetogenic TCRs. The ultimate aim is to be able to predict disease before the onset of symptoms (which generally only occur once >80 % of  $\beta$  cells mass is lost). Loss of  $\beta$  cells is thought to occur over a period of ~3 years prior to the onset of symptoms. Insulin management can somewhat protect any remaining  $\beta$  cells from T cell destruction so early diagnosis is crucial to prognosis in terms of

extending the well-described honeymoon period that follows diagnosis (Abdul-Rasoul, Habib and Al-Khouly, 2006; Fonolleda *et al.*, 2017; Zhong *et al.*, 2020).

Knowing the initial pathogen triggers of T1D could lead to the development of a preventative vaccine (Metzler and Wraith, 1993, 1999; Liu *et al.*, 1998; Burkhart *et al.*, 1999; Streeter *et al.*, 2015). However, it is important to recognise, that there are likely to be many potential triggers (in this chapter alone I propose five pathogenic peptides as potential triggers), and many TCR-pMHC interactions involved. The interactions described in this chapter focus on an insulin B chain-derived peptide, yet there are many other well-described self-antigens derived from proteins such as GAD65 (Lohmann *et al.*, 1994), and ZnT8 (Wenzlau *et al.*, 2007). Furthermore, it is important to recognise that T1D is likely a collection of similar disease states with highly heterogeneous causes, with molecularly mimicry such as that described in this chapter making up only one.

### 3.3.6 – Summary

In this chapter, I have shown that the T1D-associated InsB4 clone strongly crossreacts with pathogenic ligands from the viral, bacterial, and fungal proteomes. I have demonstrated that the identified viral peptides are genuine epitopes that are processed correctly and presented at the cell surface in the context of HLA-A2. I have also shown that InsB4 is activated by HLA-A2<sup>+</sup> EBV-transformed cell lines.

I next searched for the same EBV/insulin crossreactivity in other donors. I generated EBV-specific T cell lines from three healthy donors and two further patients with T1D. Insulin-specific T cells could be clearly detected within the EBV-specific T cell line from one T1D patient, NDB119. This crossreactivity was not detected in EBV-specific T cell lines from the other T1D patient (LSWAIST 075AS), nor was it present in three healthy controls suggesting that these T cell lines did not contain EBV/insulin crossreactive TCRs. The absence of EBV/insulin crossreactivity in the LSWAIST 075AS T1D patient highlights the likely highly heterogeneous nature of T1D initiation. There are likely many ways to trigger T1D via the T1D risk allele HLA-A2.

Future work should aim at confirming that individual TCRs in the NDB119 line respond to both EBV and insulin epitopes by sequencing the responsible TCRs or generating T cell clones. Overall, my work suggests that some insulin-reactive T cells in patients with T1D may have initially been triggered and expanded to provide immunity to EBV, chickenpox virus, or HSV-1.

## 4 – Exploring how mutation of key peptide contact residues in CDR3 impact TCR peptide specificity

---

### 4.1 – Introduction

#### 4.1.1 – The 868 TCR

In **Chapter 3** I demonstrated that autoreactive T cells can be strongly activated by crossreactive pathogenic peptide epitopes. This highlighted the importance of understanding how such crossreactivities occur and highlighted the benefit that would be gained from being able to predict crossreactivity from known characteristics of TCRs, such as their sequence.

As shown in **Chapter 3**, CPL screens can be used to find preferred agonists for a given TCR. I reasoned that this ability might allow me to explore how changing key peptide contact residues within a TCR altered its preferred peptide agonists. I hypothesised that altering the amino acids in the CDR3 loops of a well-characterised naturally-occurring TCR would alter its peptide recognition repertoire. In order to make an initial assessment of how amino acid substitutions at key peptide contacts might impact peptide specificity I needed to select a TCR where there was an atomic resolution structure of the TCR in complex with a cognate antigen. The HLA-A2-restricted 868 TCR was selected as this TCR was known to have an unusually high natural affinity for its cognate antigen and other members of my laboratory had recently solved the structure of this TCR with the natural, HIV-derived epitope and several common escape variants (Cole, Fuller, *et al.*, 2017).

The 868 TCR recognises an immunodominant HLA-A2-restricted epitope from HIV-1 (Sewell *et al.*, 1997). It originates from an HLA-A2<sup>+</sup> HIV-1-infected male, patient 9300868 (Sewell *et al.*, 1997; Wilson *et al.*, 1998). T cells from this patient responded well to the immunodominant HLA-A2-restricted p17 Gag epitope (SLYNTVATL, residues 77-85 of HIV p17 Gag) (Sewell *et al.*, 1997). Using pMHC tetramers and V $\beta$ -specific antibodies, the cytotoxic lymphocytes cultured from this patient were shown to undergo clonal, tetramer-specific expansions in response to SLYNTVATL (Wilson *et al.*, 1998). The TRBV 5-6  $\beta$ CASSDTVSYEQY TCR  $\beta$  chain was shown to be present in the majority of single cells sorted from the patient PBMC using an HLA-A2-SLYNTVATL tetramer (Wilson *et al.*, 1998). Repeat attempts to generate a T cell clone from this line were unsuccessful and the 868 TCR was instead isolated by phage display of TCR sequences derived from a polyclonal HLA-A2-SLYNTVATL-specific T cell line grown from patient 9300868 (Varela-Rohena *et al.*, 2008). The TCR was shown to

possess a TRAV 12-2  $\alpha$  chain with the CDR3 sequence, CAVRTNSGYALNF (Varela-Rohena *et al.*, 2008; Cole, Fuller, *et al.*, 2017). All HLA-A2-SLYNTVATL-tetramer<sup>+</sup> T cells in the 868 SLYNTVATL-specific T cell line stained with antibodies specific for TRAV 12-2 and TRBV 5-6 variable domains (Varela-Rohena *et al.*, 2008). The 868 TCR binds to its cognate HLA-A2-SLYNTVATL antigen with an extremely high affinity for a naturally occurring TCR ( $K_D = 210$  nM at 37°C) (Cole, Fuller, *et al.*, 2017).

The HIV-1 Gag protein from which SLYNTVATL is derived is a large viral precursor protein that is cleaved by viral protease enzymes into proteins required for viral replication. These include p24, the capsid protein, and p17 – or the matrix protein (MA) from which the SLYNTVATL epitope arises (Freed *et al.*, 1994; Cannon *et al.*, 1997). The p17 protein is encoded towards the amino-terminal of the Gag protein (Freed *et al.*, 1994). It is thought to play a number of key roles during viral replication including localising the nascent viral particles to just under the lipid bilayer of the host cell where assembly generally takes place, and aiding the incorporation of Env glycoproteins into the portion of the host bilayer destined to become the new viral bilayer (Freed *et al.*, 1994; Cannon *et al.*, 1997).

The immunodominant response to SLYNTVATL is uncommon during the acute stage of HIV-1 infection and instead dominates during the long chronic infection phase (Goulder, Altfeld, *et al.*, 2001). Responses to HLA-A2-SLYNTVATL occurs in over 70% of chronically infected HLA-A2<sup>+</sup> patients, suggesting the epitope is favourably processed and presented at the cell surface of infected cells (Goulder, Sewell, *et al.*, 1997; Brander *et al.*, 1998; Goulder, Altfeld, *et al.*, 2001). Accordingly, patient 9300868 was first diagnosed as HIV-1<sup>+</sup> in 1993 but the SLYNTVATL-responsive T cells were isolated from a PBMC sample taken in 1996 when the patient would likely be in the chronic stage of the infection (Sewell *et al.*, 1997).

Over the course of HIV-1 infection, a number of naturally occurring escape mutations arise within the SLYNTVATL epitope in HLA-A2<sup>+</sup> individuals (Sewell *et al.*, 1997). The evolution of these viral escape mutants has been well described (Brander *et al.*, 1998; Jamieson *et al.*, 2003; Iversen *et al.*, 2006; Martinez-Hackert *et al.*, 2006; Christie *et al.*, 2009). A 2006 study showed that 58% of HLA-A2<sup>+</sup> patients in the chronic phase of infection had at least one example of an SLYNTVATL escape mutation (Iversen *et al.*, 2006). Mutations frequently occur at positions 3, 6, and 8 of the 9-mer epitope, yet rarely occur at positions 2, 9, or 4 (Sewell *et al.*, 1997; Iversen *et al.*, 2006; Tenzer *et al.*, 2009; Cole, Fuller, *et al.*, 2017). The mutations occur accumulatively over the course of the chronic infection (Iversen *et al.*, 2006), with the triple mutant, SL<sub>F</sub>NTI<sub>A</sub>VL, having been dubbed the ‘ultimate escape mutant’ (Iversen *et al.*,

2006; Cole, Fuller, *et al.*, 2017). In the absence of a T cell selective pressure (e.g. when the virus is transmitted to a HLA-A2<sup>+</sup> individual) SLYNTVATL-mutated HIV-1 rapidly reverts to the wildtype sequence, suggesting the mutants result in a reduction in viral fitness (Iversen *et al.*, 2006; Tenzer *et al.*, 2009; Cole, Fuller, *et al.*, 2017).

Viral mutations occur in order to escape recognition by host T cells (Sewell *et al.*, 1997). In a 2006 study, 8% of patients did not mount responses to any mutant variant of SLYNTVATL tested at all (Iversen *et al.*, 2006). As well as escaping recognition, mutations can have antagonistic effects (Sewell *et al.*, 1997), in which they bind the TCR yet do not fully trigger downstream signalling – this occupancy but failure to activate the TCR prevents the TCR from encountering other variants that could potentially induce full activation (Purbhoo *et al.*, 1998).

A common viral escape strategy is mutation of the residues that anchor the peptide to MHC so that the peptide is no longer presented at the surface of the infected cell for T cell inspection (Morikawa *et al.*, 1998; Kelleher *et al.*, 2001; Altfeld *et al.*, 2005; Varela-Rohena *et al.*, 2008). The primary anchor residues for HLA-A2 are the p2 and C-terminal residues of the peptide. MHC anchor mutations rarely arise in SLYNTVATL (Sette *et al.*, 1994; Lee *et al.*, 2004; Iversen *et al.*, 2006) as the amino acids at peptide positions 2 and 9 are crucial to the correct formation of the p17 protein, meaning that mutation would have a detrimental impact on viral fitness (Freed *et al.*, 1994; Cannon *et al.*, 1997; Morikawa *et al.*, 1998; Ono, Orenstein and Freed, 2000; Martinez-Hackert *et al.*, 2006). It was therefore hypothesised that the common viral escape strategy at positions 3, 6, and 8 must have an impact on TCR binding (e.g. these positions must form key contacts with host TCRs) (Iversen *et al.*, 2006).

Cole *et al.* found that, in fact, these residues were not the key contact residues. Structural analysis of the 868 TCR in complex with HLA-A2-SLYNTVATL and its escape variants demonstrated that most TCR contacts instead occur with the 4N peptide residue (Cole, Fuller, *et al.*, 2017). This residue was noted in 1997 to rarely be mutated in SLYNTVATL escape variants (Sewell *et al.*, 1997). Cole *et al.* demonstrated that immune escape is achieved by destabilising the pMHC structures on the surface of the cell over a long-term period (24 hours), a mechanism that had been missed in previous standard assays over a shorter duration (<7 hours) (Cole, Fuller, *et al.*, 2017). This unexpected mechanism of immune escape highlights the danger of making assumptions about how TCRs bind in the absence of an atomic resolution structure and highlights the importance of perusing greater understanding of the molecular rules that govern the TCR-pMHC interaction.

The 868 TCR is restricted to HLA-A2, the most common *HLA* allele in the western population (Nunes *et al.*, 2014; Gourraud *et al.*, 2015) and ~75% of infected, HLA-A2\* individuals mount a response against HLA-A2-SLYNTVATL (Goulder, Sewell, *et al.*, 1997; Brander *et al.*, 1998; Goulder, Altfeld, *et al.*, 2001; Iversen *et al.*, 2006; Varela-Rohena *et al.*, 2008). The interaction has been extensively studied and the crystal structure of the 868-A2-SLYNTVATL interaction, as well as complex structures of interactions with escape mutants have been solved (Cole, Fuller, *et al.*, 2017). The TCR has already been subject to some degree of mutational studies. Varela-Rohena *et al.* introduced amino acid substitutions into the CDR3 loops of the 868 TCR and used a phage display system to identify variants with both enhanced affinity for the wildtype epitope and broader recognition of escape variants (Varela-Rohena *et al.*, 2008). 868-A2-SLYNTVATL therefore makes an excellent model system with which to expand upon this work and investigate the influence of CDR3 sequence on peptide preference.

#### 4.1.2 – Probing TCR ‘rules of engagement’

The sequences of TCR CDR loops are largely responsible for peptide recognition. Collectively, the six CDR loops form the antigen binding site; with the CDR3s of each chain being responsible for most – but not all – of the peptide contacts (Garboczi *et al.*, 1996; Garcia *et al.*, 1996; Ding *et al.*, 1998; Hausmann *et al.*, 1999; Cole *et al.*, 2009). Subtle differences in the CDR loops of a TCR can alter its antigen specificity (Yang *et al.*, 2011; Chen *et al.*, 2017). For example, in 2013, Motozono *et al.* used a collection of patient-derived T clones specific for the immunodominant HIV-1 Nef epitope, VY8 (VPLRPMTY) presented by HLA-B\*35:01 to show that, despite their shared recognition of this epitope, their individual peptide recognition ‘footprints’ varied considerably (Motozono *et al.*, 2013). The ability to recognise HIV-1 escape mutants of this epitope varied from clone to clone (Motozono *et al.*, 2013). In fact, in a similar experimental set up to that used in this chapter, the same group later used CPL screens to profile the crossreactive nature of T cells specific for the VY8 epitope (Motozono, Bridgeman, *et al.*, 2015). Motozono *et al.* showed that single amino acid differences in the CDR3s could alter the crossreactive footprints of the T cell clones (Motozono, Bridgeman, *et al.*, 2015).

In 2014, Smith *et al.* mutated CDR loop residues to successfully alter the specificity of the A6 TCR (Smith *et al.*, 2014). The A6 TCR was chosen as, like the 868 TCR, it is structurally and biochemically well characterised (Garboczi *et al.*, 1996; Ding *et al.*, 1999; Borbulevych *et al.*, 2009). The A6 TCR recognises the HTLV-1-derived Tax peptide (LLF~~L~~GYPVYV) (Smith *et al.*,



2014). Smith *et al.* aimed to re-direct the A6 TCR to recognise the unrelated cancer-associated MART-1 peptide (ELAGIGILTV) (Kawakami *et al.*, 1994). These The LLFGYPVYV and ELAGIGILTV peptides differ at every residue save the HLA-A2 anchor residues (P2 and the C-terminus of the peptide). In an example of using structural data to guide rational mutagenesis, they used molecular modelling of the published A6 structure to predict which CDR loop residues would most likely accommodate the MART-1 peptide. With just five amino acid substitutions they redirected the A6 TCR to see the desired peptide, completely 'switching' the specificity of the TCR (Smith *et al.*, 2014). The complex structure of the mutant A6 (known as RD1) with the MART-1 epitope was subsequently solved in 2016 (Harris *et al.*, 2016). It showed that the small amino acid substitutions that were introduced resulted in a significant alteration in binding mode, affecting interactions throughout the interface (Harris *et al.*, 2016). Despite the altered binding mode, the re-directed TCR maintained the ability to trigger T cell activation in an antigen-dependent manner (Smith *et al.*, 2014; Harris *et al.*, 2016).

The crossreactive profile of the RD1 TCR was recently compared to a MART-1-specific TCR clone that had been isolated directly from a polyclonal population of melanoma-reactive T cells and subject to standard, random mutation-based, affinity maturation, known as T1 (Smith *et al.*, 2013; Sharma *et al.*, 2019). Both TCRs saw MART-1 comparably, but RD1 was less crossreactive than the randomly affinity-enhanced TCR which recognised a number of self-peptides. This study suggests that rationally designed TCRs can be functionally comparable but carry less potential for off-target crossreactivity than TCRs engineered by random mutagenesis methods (Sharma *et al.*, 2019).

#### 4.1.3 – Aims

TCR CDR loop sequences are paramount to function and peptide specificity. Mutation of just a few residues can enhance, alter, or – as demonstrated by Zhou *et al.* – completely abrogate function (Zhou *et al.*, 2017). In this chapter, I investigated the impact of CDR3 amino acid substitutions on the function of the 868 TCR. In a manner akin to that described by Babad *et al.* in 2015, I used lentiviral vectors to 'reprogram' primary human CD8<sup>+</sup> T cells with 868 TCR or a mutant version of this TCR (Babad *et al.*, 2015). Babad *et al.* demonstrated that viral vectors containing TCR structures separated by 2A peptides, and produced using HEK 293T cells can efficiently confer antigen-specificity to primary polyclonal CD8<sup>+</sup> T cells as measured by antigen-dependent cytokine release and cytotoxic activity (Babad *et al.*, 2015).

Using transduced CD8<sup>+</sup> T cells, I subjected the 868 TCR, and mutants thereof, to CPL-driven database searching as detailed in **Section 3.1** first described by Szomolay *et al.* (Szomolay *et al.*, 2016) and later refined by Whalley *et al.* (Whalley *et al.*, 2020). Raw data from CPL screens were used to search proteomic databases and generate a ranked list of peptides predicted to be suitable ligands for a given TCR. This approach can also be used to rank potential TCR agonists from the entire peptide universe so as to predict the optimal epitopes for a given TCR. In order to begin exploring whether useful information regarding the relationship between sequence and function could be gained by this approach, I compared the CPL data generated with mutated 868 TCRs with that of the native parent TCR.

The specific aims of this project were:

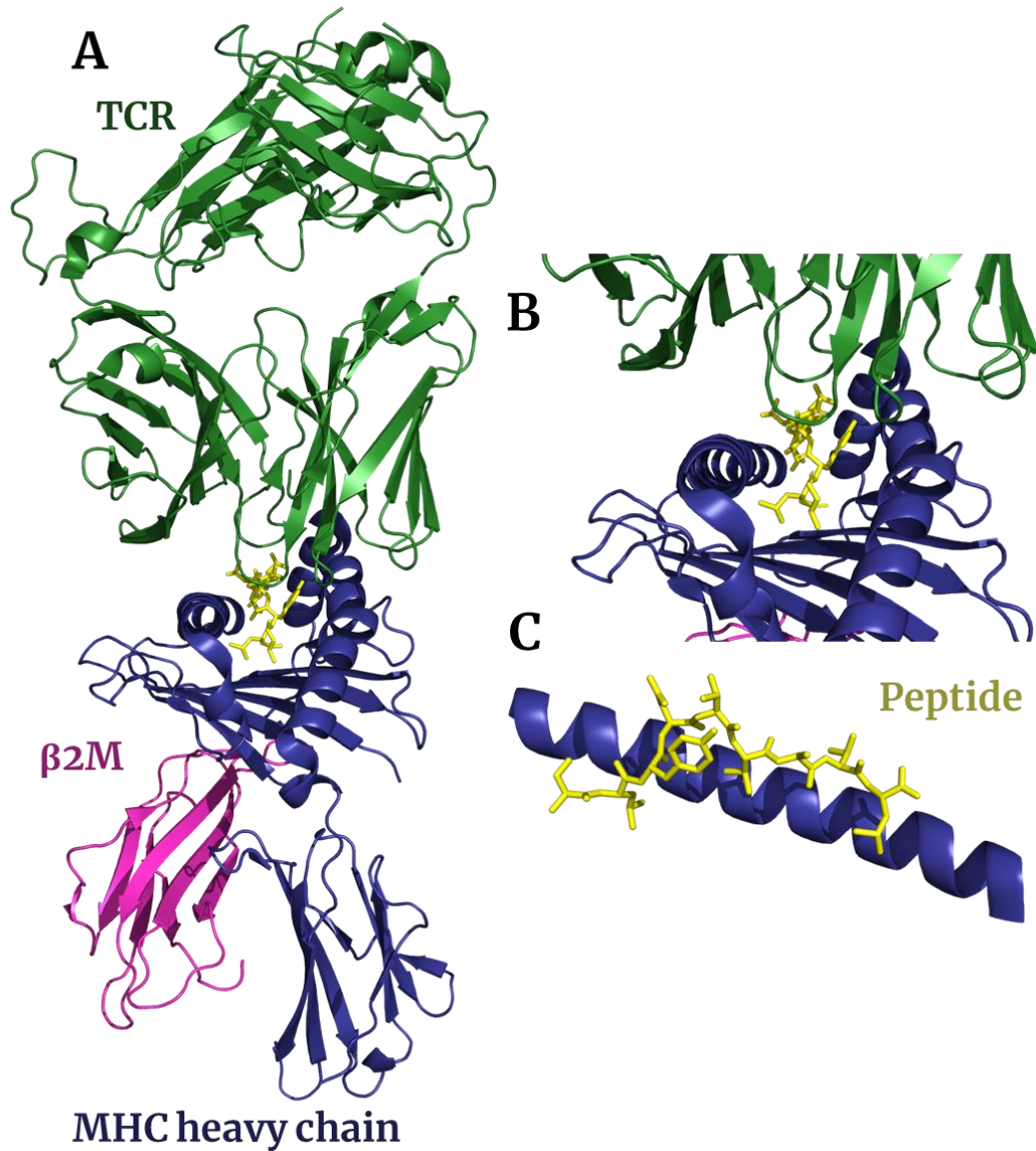
- Use a lentiviral transduction vector, pELNS, to stably transduce the 868 TCR into primary CD8<sup>+</sup> T cells.
- Investigate the crossreactive nature of the 868 TCR using these T cells and peptide length-matched CPL screens.
- Design lentiviral constructs containing mutated versions of the 868 TCR and use primary CD8<sup>+</sup> T cells transduced with these constructs to assess the impact of CDR3 amino acid substitutions on peptide recognition.

## 4.2 – Results

### 4.2.1 – Designing the mutant 868 TCRs

Mutant constructs of the 868 TCR were designed based on structural data of the 868-A2-SLYNTVATL interaction (**Figure 4.1A**) (Cole, Fuller, *et al.*, 2017). **Figures 4.1B** and **4.1C** demonstrate how the SLYNTVATL peptide is presented in the groove of HLA-A2 for inspection by the 868 TCR. This structure, along with the accompanying table of contacts (Cole, Fuller, *et al.*, 2017), reproduced in **Table 4.1**, was used to identify key contact residues responsible for the SLYNTVATL-specificity. As my aim was to alter the specificity of the TCR, key contact residues were substituted. It was assumed that substitutions to amino acids that possessed similar chemical properties (for example, glycine to alanine) might be well tolerated and therefore produce little change in peptide specificity so amino acid substitutions that radically changed the chemical properties or size of the residue were selected, for example replacing a polar amino acid with a hydrophobic amino acid, **Figure 4.2**. In total, 24 mutant versions of the 868 TCR were designed and synthesised (**Table 4.2**). Each mutant required cloning into the pELNS vector before analysis could commence, the progress of each mutant is indicated by grey shading in **Table 4.2**. Screening each TCR took a considerable amount of time, so I was only able to examine a few mutant TCRs during my PhD studies as my laboratory studies were interrupted by the March 2019 COVID-19 lockdown.

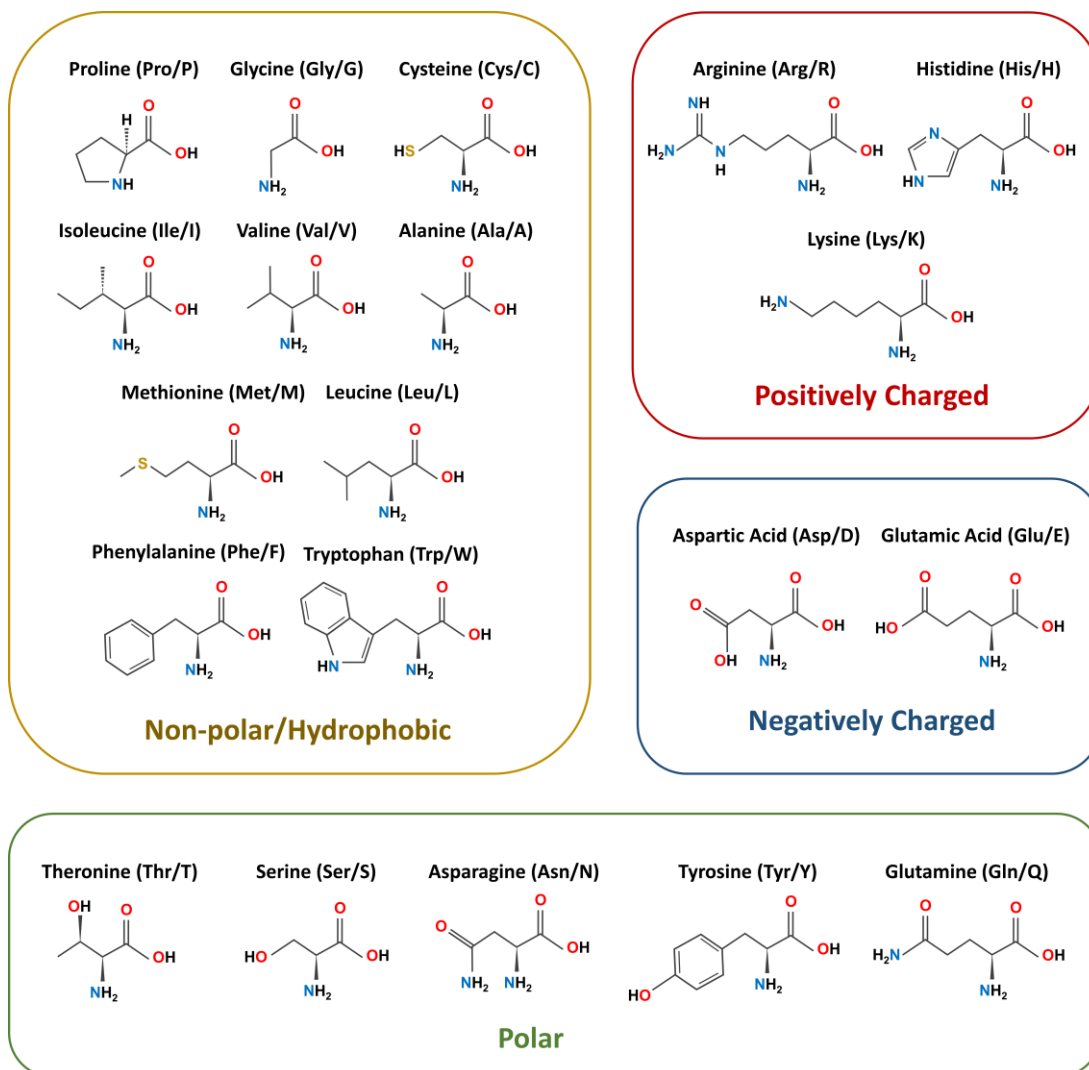
The first four mutant 868 TCRs that are discussed in this chapter, along with 868 itself, are shown in **Figure 4.3A**, each has a substitution or substitutions in the CDR3 $\beta$  loop. The TCRs, containing  $\beta$ T96F,  $\beta$ V97T,  $\beta$ D95S, and  $\beta$ D95I T96R V97S substitutions, are denoted TCR 3, TCR 6, TCR 22, and TCR 24 respectively, **Table 4.2**. The amino acid substitutions in each TCR are rationalised below.



**Figure 4.1: The interaction between 868 and HLA-A2-SLYNTVATL.** **A)** The 868-A2-SLYNTVATL interaction as solved by Cole *et al.* in 2017, PDB: 5NMD. **B)** shows a close up of the interface, demonstrating how 868 CDR loops interact with the SLYNTVATL peptide. **C)** the SLYNTVATL peptide is presented in a standard manner for MHC class I peptides, anchored at either end with a protruding centre.

CDR loop	TCR residue	Peptide residue	MHC residue	vdW $\leq 4$ Å	Electrostatic bonds $\leq 3.4$ Å
CDR3 $\alpha$	Arg92	Asn4		1	
	Arg92	Thr5		4	
	Thr93	Asn4		1	1 HB
	Asn94		Lys66	3	1HB
	Asn94	Leu2		1	1HB
	Asn94	Tyr3		2	
	Asn94	Asn4		4	2HB
	Ser95		Gly62	1	
	Ser95		Arg65	2	1HB
	Gly96		Arg65	1	
	Gly96	Asn4		2	1HB
	Tyr97		Lys66	3	
	Tyr97		Lys69	1	
	Tyr97	Asn4		7	1HB
	CDR3 $\beta$	Thr96	Ala7		2
Thr96		Thr8		2	1 HB
Val97			Lys146	1	
Val97			Trp147	1	
Val97			Ala150	1	
Val97			Val152	2	
Val97		Ala7		2	
Val97		Thr8		1	
Ser98			Ala150	1	
Tyr99		Thr5		15	
Tyr99		Val6		6	2HB

**Table 4.1: Contact table for the 868-A2-SLYNTVATL interaction.** Reproduced from (Cole, Fuller, *et al.*, 2017).



**Figure 4.2: The classification of amino acids based on chemical characteristics.** In this work amino acid substitutions were generally made to an amino acid of differing chemical property in the hope of altering the peptide preference of the 868 TCR.

TCR mutation	ID	CDR3 sequences	Cloned into pELNS	Expressed in CD8 <sup>+</sup> T cells	CPL	Specificity testing
868 wt	(TCR 2)	α: CAVRTNSGYALNF β: CASSDTVSYEQY	✓	✓	✓	✓
βT96F	TCR 3	α: CAVRTNSGYALNF β: CASSDE <u>V</u> SYEQY	✓	✓	✓	✓
βV97R	TCR 4	α: CAVRTNSGYALNF β: CASSDT <u>R</u> SYEQY	✓	✓		
βV97D	TCR 5	α: CAVRTNSGYALNF β: CASSDT <u>D</u> SYEQY				
βV97T	TCR 6	α: CAVRTNSGYALNF β: CASSDT <u>T</u> SYEQY	✓	✓	✓	✓
βY102W	TCR 7	α: CAVRTNSGYALNF β: CASSDTVSYEQ <u>W</u>				
αN94W	TCR 8	α: CAVRT <u>W</u> SGYALNF β: CASSDTVSYEQY				
αR92E	TCR 9	α: CAV <u>E</u> TNSGYALNF β: CASSDTVSYEQY	✓	✓	✓	Partially complete
αR92L	TCR 10	α: CAV <u>L</u> TNSGYALNF β: CASSDTVSYEQY	✓			
αT93V	TCR 11	α: CAVR <u>V</u> NSGYALNF β: CASSDTVSYEQY	✓	✓	✓	Partially complete
αS95V	TCR 12	α: CAVRTN <u>V</u> GYALNF β: CASSDTVSYEQY				
αG96S	TCR 13	α: CAVRTNS <u>S</u> YALNF β: CASSDTVSYEQY	✓			
αG96D	TCR 14	α: CAVRTNS <u>D</u> YALNF β: CASSDTVSYEQY	✓	✓		
αG96K	TCR 15	α: CAVRTNS <u>K</u> YALNF β: CASSDTVSYEQY	✓			
αY97I	TCR 16	α: CAVRTNSG <u>I</u> ALNF β: CASSDTVSYEQY				
αV91C	TCR 17	α: CAC <u>R</u> TNSGYALNF β: CASSDTVSYEQY	✓			
αV91W	TCR 18	α: CA <u>W</u> RTNSGYALNF β: CASSDTVSYEQY	✓			
αA98R	TCR 19	α: CAVRTNSGY <u>R</u> LNLF β: CASSDTVSYEQY	✓			
αA98E	TCR 20	α: CAVRTNSGY <u>E</u> LNLF β: CASSDTVSYEQY	✓			
βD95I	TCR 21	α: CAVRTNSGYALNF β: CASS <u>I</u> TVSYEQY	✓	✓		
βD95S	TCR 22	α: CAVRTNSGYALNF β: CASS <u>S</u> TVSYEQY	✓	✓	✓	✓
βD95I T96R	TCR 23	α: CAVRTNSGYALNF β: CASS <u>I</u> RVSYEQY	✓	✓		
βD95I T96R V97S	TCR 24	α: CAVRTNSGYALNF β: CASS <u>I</u> RSYEQY	✓	✓	✓	✓
βD95S T96R	TCR 25	α: CAVRTNSGYALNF β: CASS <u>S</u> RVSYEQY				
βD95S T96R V97S	TCR 26	α: CAVRTNSGYALNF β: CASS <u>S</u> RSYEQY	✓	✓		

**Table 4.2: The TCRs used in Chapter 4.** Each TCR was given an identification number to prevent mix ups. The CDR3 loop mutation is indicated by underlining. Grey shading indicates the progress with each TCR.

#### 4.2.1.1 – TCR 3, $\beta$ T96F

TCR 3 contains a mutation in the 96<sup>th</sup> residue of the 868  $\beta$  chain,  $\beta$ T96. In the wildtype TCR the threonine at this position makes two peptide contacts with SLYNTVATL residues, alanine (P7) and threonine (P8). The contact between  $\beta$ T96 and the threonine at P8 of the peptide is a hydrogen bond (defined according to the Cole *et al.* table of contacts as having a bond distance of  $\leq 3.4$  Å).  $\beta$ T96, a relatively small polar amino acid, was mutated to phenylalanine, a bulky hydrophobic amino acid with a large aromatic ring. The modelled  $\beta$ T96F mutation is shown in **Figure 4.3B**.

#### 4.2.1.2 – TCR 6, $\beta$ V97T

TCR 6 contains a mutation in the residue adjacent to that mutated in TCR 3. Residue 97 in the 868  $\beta$  chain is a valine,  $\beta$ V97. It makes two peptide contacts with the same two SLYNTVATL residues as  $\beta$ T96, alanine (P7) and threonine (P8).  $\beta$ V97 makes 4 additional contacts with the HLA-A2 molecule, **Table 4.1**.  $\beta$ V97, a hydrophobic amino acid, was mutated to threonine, a polar (hydrophilic) amino acid. The modelled  $\beta$ V97T mutation is shown in **Figure 4.3C**.

#### 4.2.1.3 – TCR 22, $\beta$ D95S

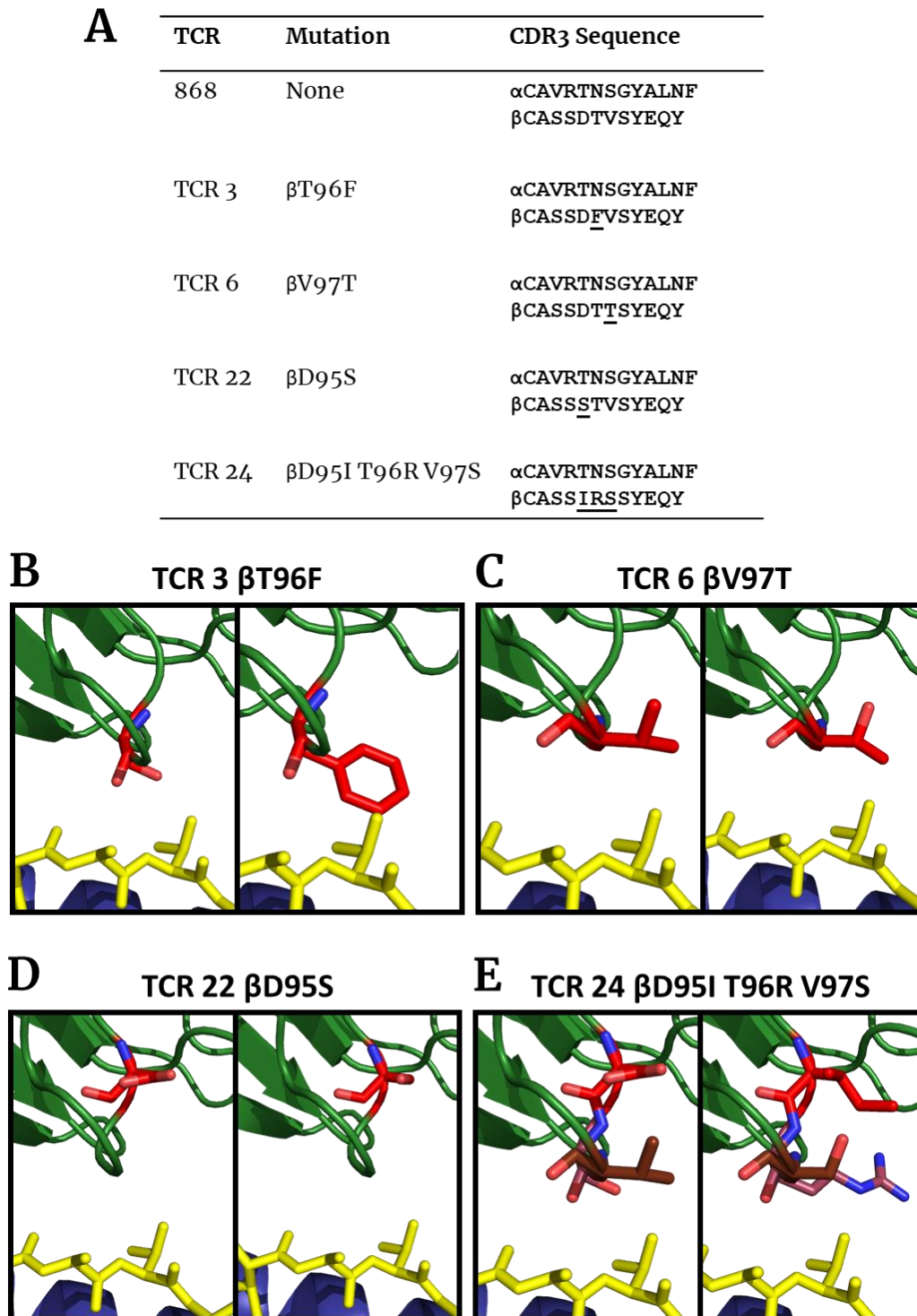
TCR 22 contains a mutation at the 95<sup>th</sup> residue in the 868  $\beta$  chain. According to the contact table published by Cole *et al.* this residue does not form any contacts with the HLA-A2-SLYNTVATL target at all (Cole, Fuller, *et al.*, 2017). This residue was selected for mutation to evaluate the impact of potential knock on effects that a non-contact residue might have on the recognition footprint of the TCR. In the wildtype sequence the 95<sup>th</sup> residue is a negatively charged aspartic acid residue  $\beta$ D95, for this study it was mutated to a polar, uncharged, serine  $\beta$ D95S (**Figure 4.3D**).

#### 4.2.1.4 – TCR 24, $\beta$ D95I T96R V97S

Finally, TCR 24 contains three amino acid substitutions at a stretch of three consecutive 868  $\beta$  chain residues, 95, 96, and 97. The wildtype sequence DTV was mutated to IRS ( $\beta$ D95I T96R V97S), **Figure 4.3E**. The  $\beta$ D95I mutation involves the replacement of a negatively charged amino acid for a hydrophobic amino acid. The  $\beta$ T96R mutation involves the replacement of a polar, uncharged amino acid with a positively charged amino acid, and the  $\beta$ V97S mutation involves the replacement of a hydrophobic amino acid with a polar amino acid. The model



of this triple mutation suggests a substantial structural rearrangement, **Figure 4.3E**. The amino acid substitutions chosen for TCR 24 were based on the published structure of a HLA-A2-restricted, influenza-specific TCR, JM22 (PDB:5HHO) (Valkenburg *et al.*, 2016). The JM22 TCR recognises an epitope from the M1 influenza protein (GILGFVFTL, residues 58-66). The TCR consists of TRAV27 and TRBV19 chains (Valkenburg *et al.*, 2016), in contrast to the TRAV12-2 and TRBV5-6 chains of the 868 TCR (Varela-Rohena *et al.*, 2008). Despite this difference the CDR3 $\beta$  sequences differ by just three residues (CASSDTVSYEQYF; 868 and CASSIRSSYEQYF; JM22) (PDB:5HHO) (Valkenburg *et al.*, 2016; Cole, Fuller, *et al.*, 2017). Thus, the three amino acid substitutions in TCR 24 construct are sufficient to make the CDR3 $\beta$  sequence of 868 identical to that of JM22, whilst other factors – such as variable genes and the CDR3 $\alpha$  sequence remain native to the 868 TCR. The similarity of the JM22 TCR was noted by Dr Thomas Whalley. We wondered whether these three amino acid substitutions alone would be sufficient to redirect the 868 TCR to recognising the M1 peptide. It is noteworthy that the SLYNTVATL and M1 peptides contain a VxTL motif at the C-terminus, whilst differing at all other residues (Valkenburg *et al.*, 2016; Cole, Fuller, *et al.*, 2017). According to the 868-A2-SLYNTVATL structure published by Cole *et al.*, three of the six key contact points between 868 CDR3 $\beta$  and the SLYNTVATL peptide are within the VxTL motif (Cole, Fuller, *et al.*, 2017). The  $\beta$ D95I T96R V97S triple mutation was, however, insufficient to redirect TCR 24 to the GILGFVFTL peptide (see **Supplementary Figure 7.1**), indicating that the other CDR loops play an important role in recognition of the influenza epitope by the JM22 TCR.



**Figure 4.3: The 868 mutants used in this chapter.** A) shows the CDR3 sequences of each of the first 5 TCR constructs used in this chapter, including the wildtype 868 sequence. All amino acid substitutions were in the CDR3 $\beta$  sequence and are underlined. B) Model prediction of the  $\beta$ T96F substitution in TCR 3. C) Model prediction of the  $\beta$ V97T substitution in TCR 6. D) Model prediction of the  $\beta$ D95S substitution in TCR 22. E) Model prediction of the  $\beta$ D95I T96R V97S substitution in TCR 24.

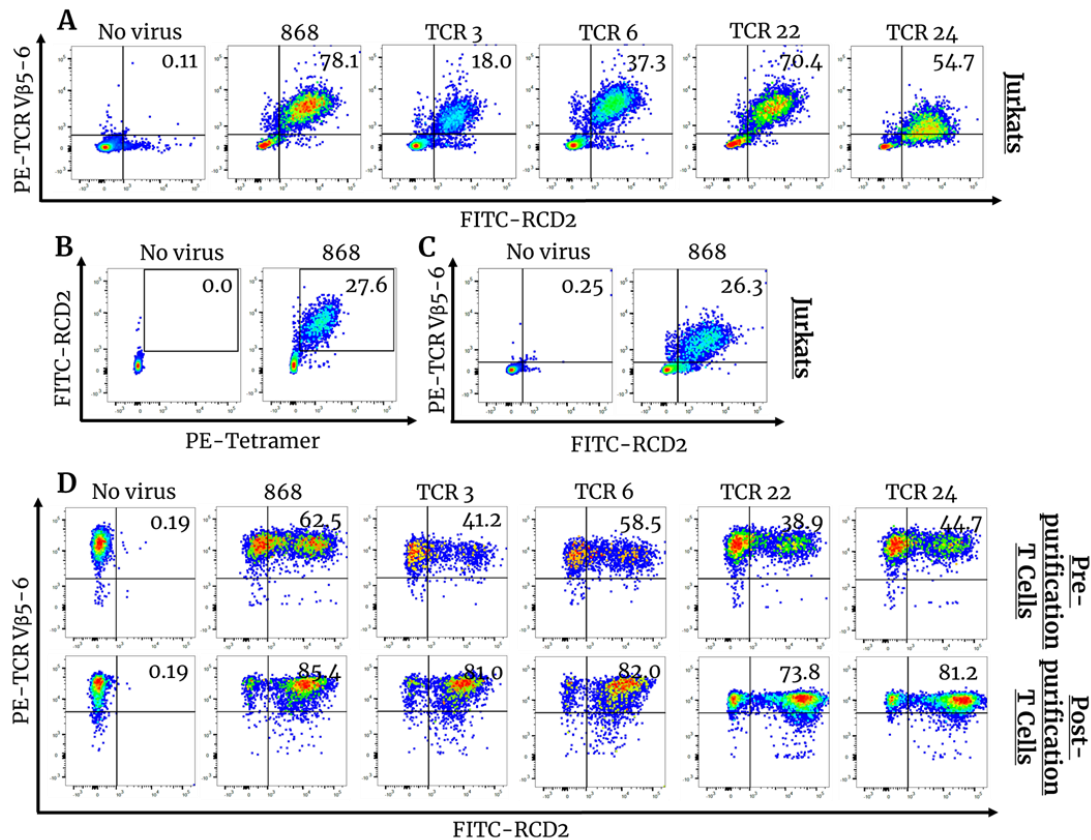
#### 4.2.2 – Validation of 868 mutants

As described in **Section 2.3.2**, the mutated TCR constructs were separated by 2A self-cleaving peptides to facilitate equal levels of expression (**Figure 2.5**). A rCD2 marker gene was also included to enable easy monitoring of cellular transduction by antibody staining. To ensure that my mutant TCRs were adequately expressed at the cell surface I first validated expression via lentiviral transduction in the T cell leukaemia line, Jurkat (clone E6.1). These readily available, immortalised CD4<sup>+</sup> T cells were chosen for initial testing due to their ease of growth in culture compared to primary CD8<sup>+</sup> T cells. This initial round of validation also served to prevent wastage of donated blood-derived primary CD8<sup>+</sup> T cells on potentially unviable TCR constructs.

As described in **Section 2.4.2**, viral vectors were used to deliver the TCR and marker genes via spinfection. **Figure 4.4A** shows the surface expression rCD2 and TRBV 5-6 in Jurkat cells transduced with the 868 TCR and the four mutant TCRs described above. All 5 TCRs were successfully expressed and trafficked to the cell surface, with varying degrees of transduction efficiency ranging from 18% to 78.1% transduction (TCR 3 and 868 respectively), **Figure 4.4A**. Polybrean is frequently used to increase uptake of viral vectors. Polybrean was not used during initial testing in Jurkat cells as attempts to transduce with polybrean led to large amounts of Jurkat cell death and exceedingly slow recovery of cell numbers for staining. It was reasoned that, at this stage, validation of the expression of each construct required a yes/no answer rather than high transduction efficacy.

An additional validation step involved staining 868-transduced Jurkat cells with HLA-A2-SLYNTVATL tetramers to determine whether the transduced TCR was functional. 27.6% of rCD2<sup>+</sup> 868-transduced Jurkat cells stained with the tetramer (**Figure 4.4B**), demonstrating specificity of the TCR – even in the absence of the CD8 co-receptor. The same 868-transduced Jurkat cells were then maintained in culture for over 1 month to evaluate the longevity of the transduction. The TCR construct would need to remain expressed in primary T cells for at least 1 month to allow time for purification, expansion, and functional testing. Surface expression was reassessed at the 1-month mark. The construct was still expressed at the surface, but a proportion of the Jurkat cells had lost expression of the transduced TCR (a fall from 78.1% to 26.3% of rCD2<sup>+</sup>TRBV<sup>+</sup> Jurkat cells, **Figure 4.4C**, highlighting the need for regular enrichment of construct-positive cells, particularly before any functional testing (see **Section 2.4.5**).

Following validation of my mutant constructs in Jurkat cells, the lentiviral constructs were transduced into primary CD8<sup>+</sup> T cells (see **Section 2.4.4**). Each construct was successfully expressed and trafficked to the cell surface with transduction efficiency ranging from 38.9% to 62.5%, **Figure 4.4D**. Following purification based on the rCD2 surface marker (see **Section 2.4.5**) surface expression increased to range between 73.8% to 85.4%, **Figure 4.4D**.



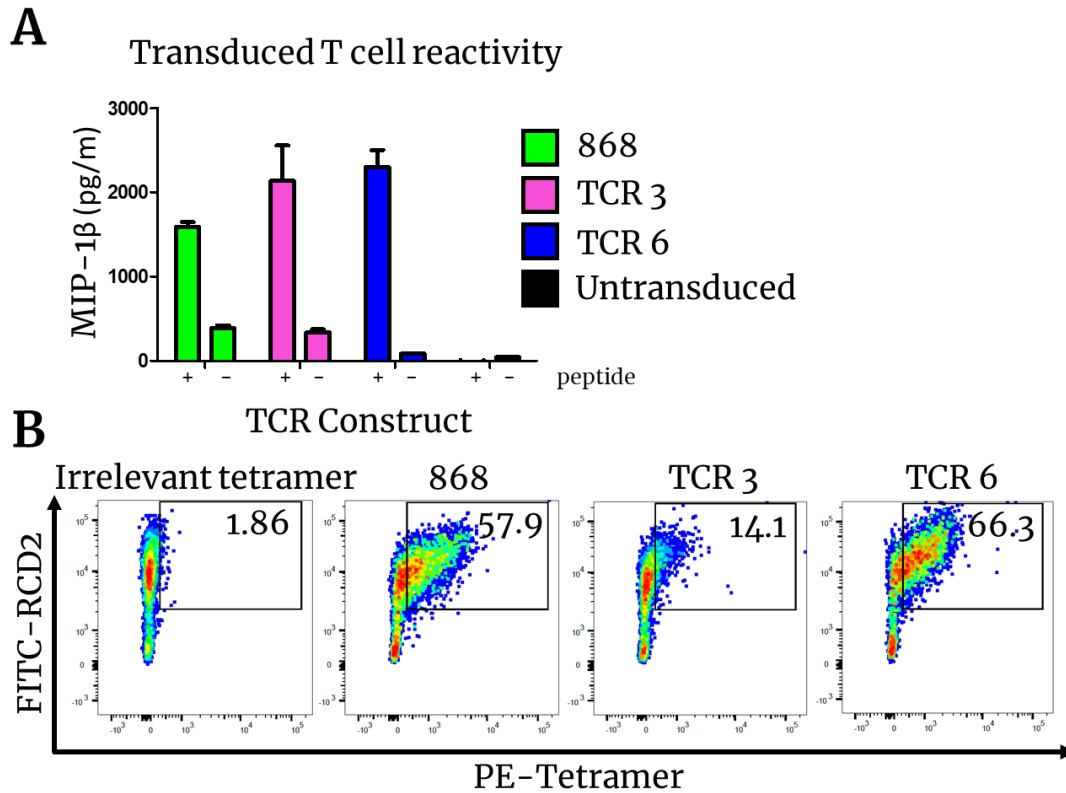
**Figure 4.4: Validating expression of the lentiviral constructs.** **A)** Jurkat cells were cultured in 10 mL R10 suspension flasks for 1 week following spinfection. Cells previously gated on live CD3<sup>+</sup> single lymphocytes. Surface expression of lentiviral constructs was monitored based on simultaneous expression of marker gene, rCD2, and TRBV 5-6 chain. **B)** 50,000 868-transduced Jurkat cells were stained with 0.5 μg HLA-A2-SLYNTVATL-tetramer (relative to the pMHC component), with a PE-conjugated backbone. Cells previously gated on live CD3<sup>+</sup> single lymphocytes. **C)** 868-transduced Jurkat cells were cultured in 10 mL R10 suspension flasks for 1 month following spinfection. Cells previously gated on live CD3<sup>+</sup> single lymphocytes. Surface expression of lentiviral constructs was monitored based on simultaneous expression of marker gene, rCD2, and TRBV 5-6 chain. **D)** CD8<sup>+</sup> T cells isolated from donated PBMCs and cultured in transduction media for 1 week prior to flow cytometry analysis. Cells previously gated on CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Surface expression of lentiviral constructs was monitored based on simultaneous expression of marker gene, rCD2, and TRBV 5-6 chain. Numbers on dot plots correspond to the percentage of cells in the gated population. 10,000 events recorded for each plot.

#### 4.2.3 – Minimal differences between the recognition footprints of 868, TCR 3, and TCR 6

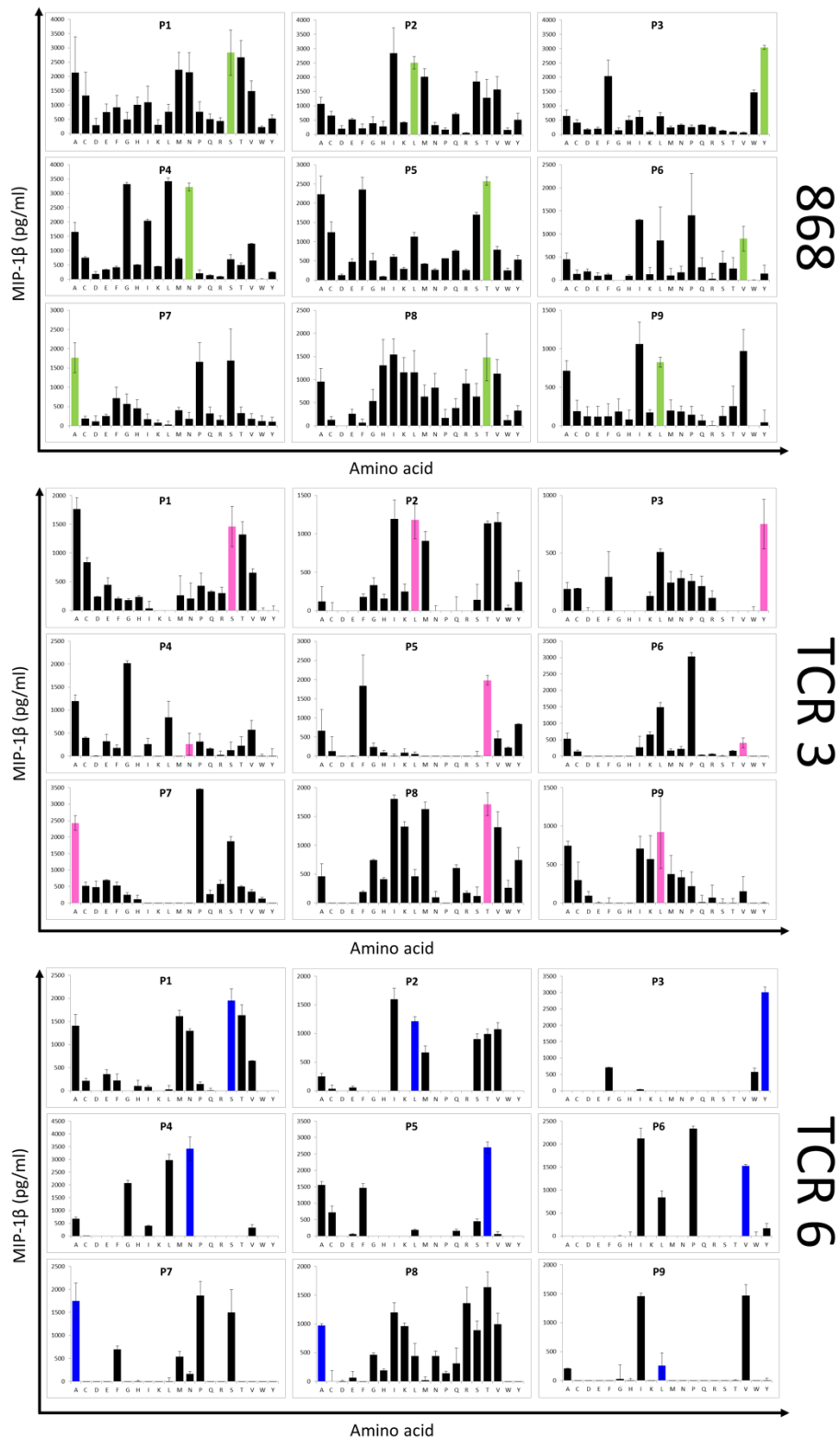
TCRs 3 and 6 were the first constructs I successfully cloned into the pELNS vector. Functional testing suggested there was little difference between the peptide recognition footprints of 868 and either TCR 3 or TCR 6. Reactivity to SLYNTVATL, as measured by MIP-1 $\beta$  production, showed no obvious difference between the mutants, although I noted an unusually high level of background MIP-1 $\beta$  production by 868 and TCR 3 in response to the T2 cells used as APCs, **Figure 4.5A**.

All three TCRs stained with the SLYNTVATL tetramer, **Figure 4.5B**, suggesting that the amino acid substitutions had not drastically altered TCR specificity for the HIV-1 epitope. Staining of TCR 3 appeared to be slightly reduced (14.1% Tetramer<sup>+</sup> rCD2<sup>+</sup>, compared to 57.9% and 66.3% for 868 and TCR 6 respectively), **Figure 4.5B**.

The three TCRs were subject to CPL screening (**Figure 4.6**), but few differences were noted between their reactivity profiles, suggesting that the  $\beta$ T96F and  $\beta$ V97T substitutions were insufficient to alter the TCRs peptide preferences. These amino acid substitutions represent significant alterations in the chemical properties at the respective residue, to retain specificity despite these substitutions demonstrates the remarkable plasticity of the TCR. As such these data were not used to search the proteome databases because finding novel specificities was deemed to be unlikely. These TCRs were not subject to any further functionality testing.



**Figure 4.5: TCRs 3 and 6 show little variation in their ability to recognise the cognate HLA-A2-SLYNTVATL epitope. A)** 30,000 CD8<sup>+</sup> T cells transduced with TCR constructs were cultured in the presence of 60,000 T2 cells as APC and 10<sup>-5</sup> M SLYNTVATL peptide overnight before the supernatant was harvested for measurement of MIP-1β presence by ELISA analysis. **B)** 50,000 CD8<sup>+</sup> T cells transduced with TCR constructs were stained with 0.5 μg HLA-A2-SLYNTVATL tetramer (relative to the pMHC component), with a PE-conjugated backbone. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Numbers on dot plots represent percentages of tetramer-positive cells, 10,000 events measured.



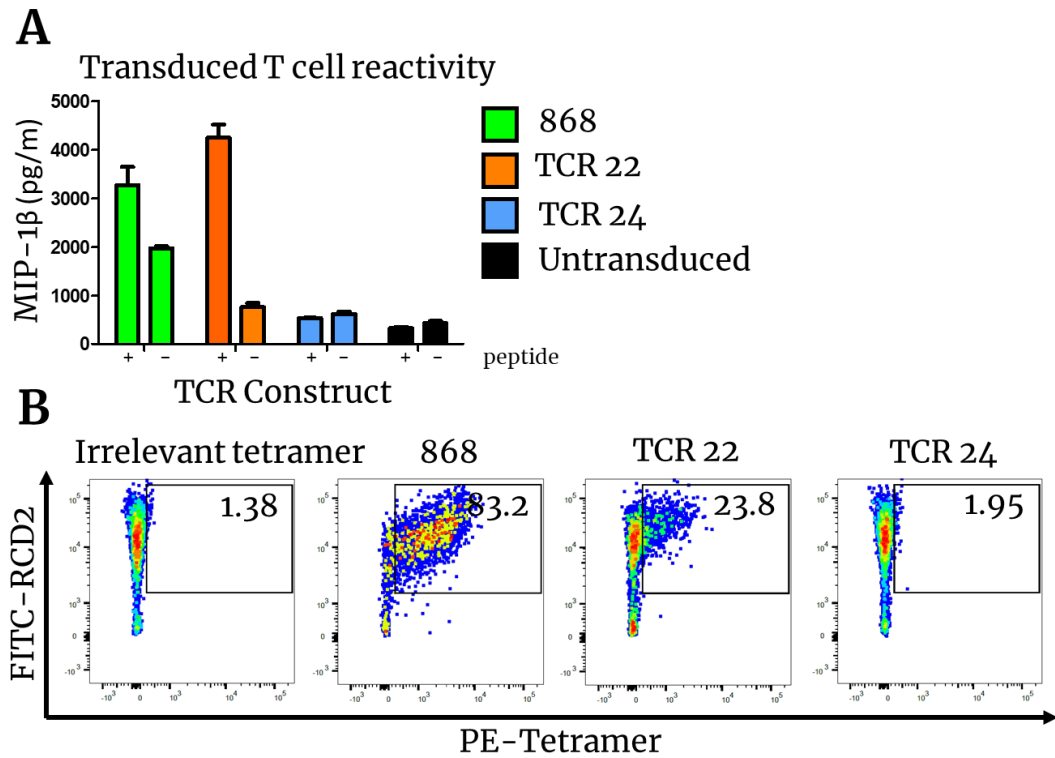
**Figure 4.6: TCRs 3 and 6 show little variation under CPL screening.** 30,000 CD8<sup>+</sup> T cells transduced with either 868 (green), TCR 3 (pink), or TCR 6 (blue) constructs were co-cultured with 60,000 T2 cells as APCs and 100 µg/mL peptide mix from a 9-mer CPL screen. Supernatants were harvested and ELISA analysis performed to measure MIP-1β presence as a proxy for T cell response. Coloured bars indicate the sequence of the index SLYNTVATL peptide. All assays performed once.

#### 4.2.4 – TCR 22 and TCR 24 show differing specificity than the parent 868 TCR

I next moved on to assessment of TCRs 22 and 24. Both 868- and TCR 22-transduced cells responded comparably to the SLYNTVATL peptide as measured by MIP-1 $\beta$  production. In contrast, TCR 24-transduced cells did not respond to the SLYNTVATL peptide suggesting TCR 24 might have altered specificity (**Figure 4.7A**). This observation was supported by tetramer staining data showing that 868- and TCR 22-transduced cells can both be stained by HLA-A2-SLYNTVATL tetramers but TCR 24-transduced cells cannot, **Figure 4.7B**. The three amino acid substitutions in the TCR 24 construct ( $\beta$ D95I T96R V97S) appeared to have eradicated recognition of the index peptide.

As noted above, I again observed an unusually high level of background MIP-1 $\beta$  production by 868 TCR-transduced cells in response to T2 cells using this very sensitive readout. This background recognition was not observed for TCR 22- or TCR 24-transduced cells (**Figure 4.7A**). As the 868-transduced cells exhibited background activity and cells transduced with TCRs 22 and 24 did not, this provided an opportunity to potentially identify the epitope on T2 cells to which T cells with the 868 TCR appeared to be responding.



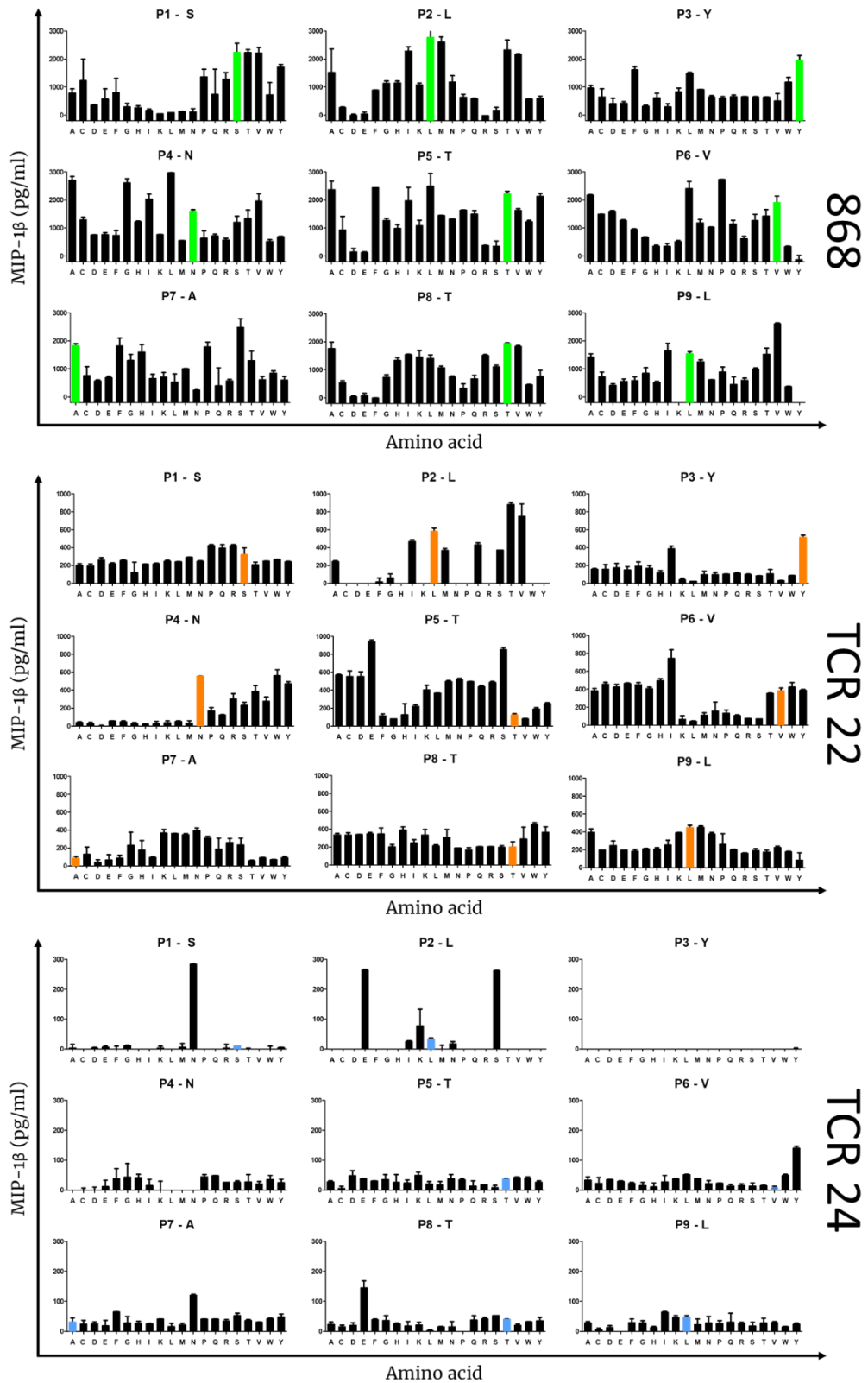


**Figure 4.7: 868, TCRs 22 and 24 show differences in peptide preference. A)** 30,000 CD8<sup>+</sup> T cells transduced with TCR constructs were cultured in the presence of 60,000 T2 cells as APCs and 10<sup>-5</sup> M SLYNTVATL peptide overnight before the supernatant was harvested for measurement of MIP-1β presence by ELISA analysis. **B)** 50,000 CD8<sup>+</sup> T cells transduced with TCR constructs were stained with 0.5 μg HLA-A2-SLYNTVATL tetramer (relative to the pMHC component), with a PE-conjugated backbone. Cells were previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Numbers on dot plots represent percentages of tetramer-positive cells, 10,000 events measured.

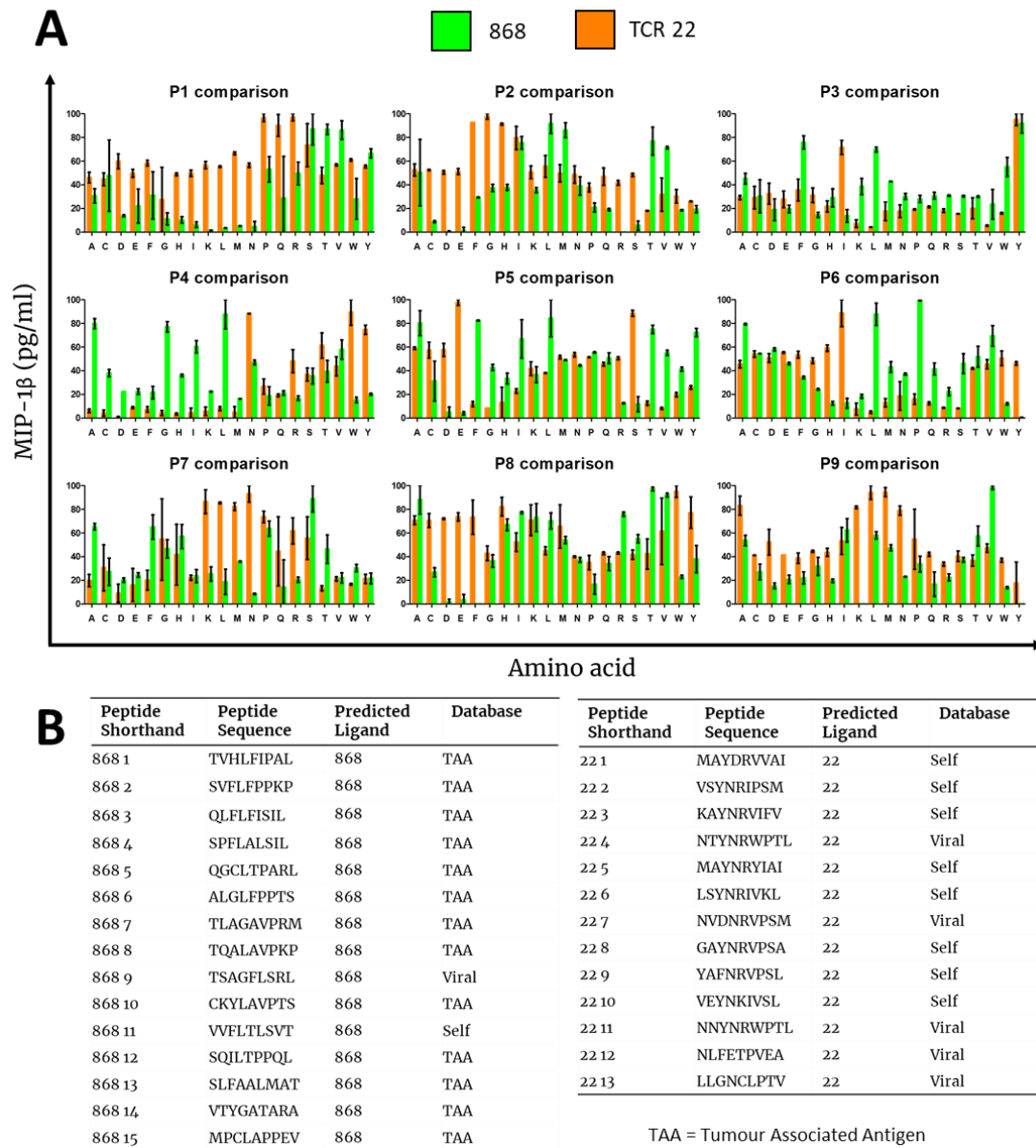
4.2.5 – Identifying the HLA-A2 self-epitope that the 868 TCR responds to 868, TCR 22, and TCR 24 were subject to CPL screening. Aside from a few isolated responses at peptide positions 1 and 2, TCR 24 showed minimal reactivity at most amino acid positions (**Figure 4.8**). Given that the amino acid substitutions in TCR 24 abrogated recognition of SLYNTVATL it is possible that the substantial structural rearrangement caused by these three amino acid substitutions (**Figure 4.3E**) may have interfered with HLA-A2 binding or completely ablated the function of this TCR.

In contrast, 868 and TCR 22 showed patterns of amino acid preferences at all peptide residue positions, **Figure 4.8**. Responses at each position were normalised to allow comparative assessment of amino acid preference, **Figure 4.9A**. This visual analysis depicts clear differences between the preferences of the two TCRs. For example, TCR 22 has preferences for glutamic acid, isoleucine, and phenylalanine at positions 5, 6, and 8 respectively that are not shared by the 868 TCR. In contrast, the 868 TCR displays a preference for leucine, threonine, and proline residues at positions 4, 5, and 6 respectively that are not shared by TCR 22 (**Figure 4.9A**).

These data were used to screen proteome databases using a webtool created by Szomolay *et al.* (Szomolay *et al.*, 2016). This tool uses CPL data from each peptide position to search proteomic databases for epitopes that the TCR in question would likely recognise and ranks them in order of predicted preference. Each TCR was screened against five databases covering the proteomes of bacterial, fungal, viral, self, and tumour-associated antigens (TAA). The top scoring peptides overall were collated and are shown, along with the database from which each peptide originates, in **Figure 4.9B**. Interestingly, in a combined search of self, pathogenic and TAA databases, 12 of the top 15 scoring peptides for the wildtype 868 TCR originated in the TAA database.



**Figure 4.8: CPL screening of TCR 22 and 24 in comparison to 868.** 30,000 CD8<sup>+</sup> T cells transduced with either 868 (green), TCR 22 (orange), or TCR 24 (light blue) constructs co-cultured with 60,000 T2 cells as APC and 100  $\mu$ g/mL peptide mix from a commercially available 9-mer CPL screen. Supernatants were harvested and ELISA analysis performed to measure MIP-1 $\beta$  presence as a proxy for T cell response. Coloured bars indicate the sequence of the index SLYNTVATL peptide. All assays performed once.



**Figure 4.9: 868 and TCR 22 are predicted to have varying peptide preference footprints. A)** a comparative overlay of the 868 and TCR 22 CPL data, showing each response as a percentage of the maximum response at that peptide position to highlight variation in amino acid preference. **B)** data in A were used to search proteomic databases for agonist ligands using a web tool developed by Szomlay *et al.* Said tool generated ranked lists of peptides predicted to be favoured by each TCR.

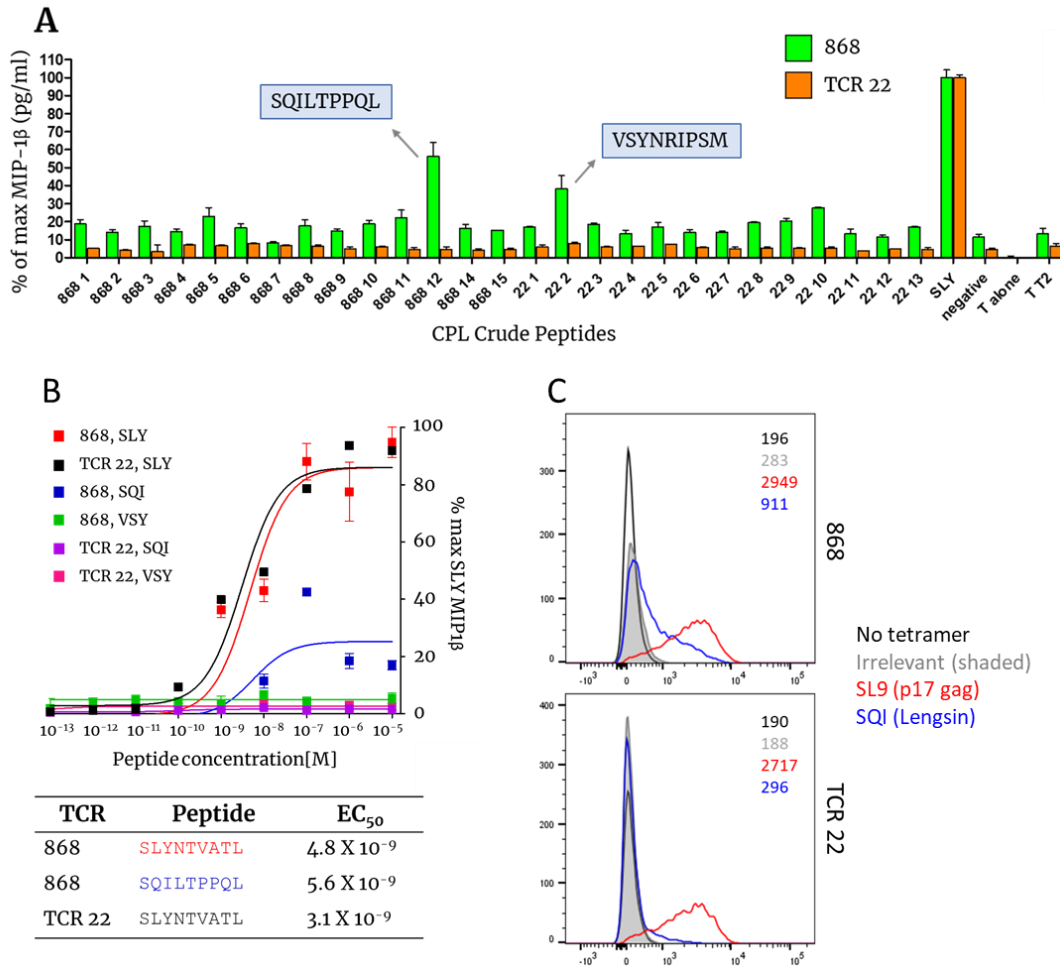
#### 4.2.6 – 868 TCR recognises epitopes that are not seen by TCR 22

The peptides identified by CPL-based database screening of peptide databases were ordered in crude form (~40 % purity) and cells transduced with the two TCRs were assessed for reactivity against  $10^{-5}$  M of each peptide. Notably, TCR 22 saw none of the peptides that were predicted from the screen data, **Figure 4.10A**. I therefore failed to identify a new specificity for this mutant TCR.

868 recognised two peptides from the predicted peptide lists, one of which was expected to be an 868 ligand (SQLTTPPQL) and another that was predicted by the webtool to be a strong agonist ligand for TCR 22 (VSYNRIPSM), **Figure 4.10A**. These peptides were derived from the TAA- and self-proteomic databases respectively (Szomolay *et al.*, 2016). The SQLTTPPQL peptide comes from a protein expressed in the optical lens called lengsin. The VSYNRIPSM peptide is contained within amphoterin-induced protein 2 precursor (AMIGO2). The two peptides were ordered as pure preparations to confirm response by T cells expressing the 868 TCR but not by TCR 22 (**Figure 4.10B**). The lengsin-derived peptide was recognised by T cells expressing the 868 TCR. The AMIGO2-derived peptide, in contrast, did not produce a comparable response from 868-expressing T cells (**Figure 4.10B**).

Soluble monomers were prepared for HLA-A2-SQLTTPPQL and HLA-A2-VSYNRIPSM as described in **Section 2.1.7**. Repeated attempts to refold HLA-A2-VSYNRIPSM were unsuccessful. This was little surprise as the 2S and 9M amino acids are suboptimal for HLA-A2 binding. Given that T cells expressing the 868 TCR also failed to respond pure VSYNRIPSM peptide in antigen titrations (**Figure 4.10B**), I concluded that the VSYNRIPSM sequence was unlikely to be a true epitope recognised by 868-transduced T cells. In contrast, the lengsin-derived sequence SQLTTPPQL was well recognised and refolded well with HLA-A2.

Both 868- and TCR 22-transduced cells stained comparatively well with the HLA-A2-SLYNTVATL tetramer (MFI values of 2949 and 2717 respectively, blue) but only 868-transduced cells stained with the lengsin tetramer, HLA-A2-SQLTTPPQL (MFI value of 911 compared to 296 for TCR 22, red) in parallel assays (**Figure 4.10C**). Thus, the single  $\beta$ D95S amino acid change in TCR 22 appears remove the response to a putative lengsin-derived epitope that can be recognised by the 868 TCR. I next aimed to validate the lengsin SQLTTPPQL epitope.



**Figure 4.10: Recognition of two putative self-derived epitopes by 868 TCR-expressing cells appears to have been ablated by the  $\beta$ D95S mutation in TCR 22.** **A)** 30,000 CD8<sup>+</sup> T cells transduced with TCR constructs were cultured in the presence of 60,000 T2 cells as APCs and 10<sup>-5</sup> M of the crude peptide and incubated overnight before the supernatant was harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis. Response is shown as a percentage of response to the index SLYNTVATL peptide. **B)** 30,000 CD8<sup>+</sup> T cells transduced with TCR constructs were cultured overnight in the presence of 60,000 T2 cells as APC and varying concentrations (10<sup>-13</sup> M to 10<sup>-5</sup> M) of the three peptides recognised in A (at >95% purity) overnight before the supernatants were harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis. The EC<sub>50</sub> for each viable T cell-peptide combination is shown in the accompanying table. EC<sub>50</sub> based on GraphPad Prism software analysis. Measurements performed in duplicate, error bars depict SEM. **C)** 50,000 CD8<sup>+</sup> T cells transduced with 868 or TCR 22 constructs were stained with 0.5  $\mu$ g tetramer (relative to the pMHC component), with a PE-conjugated backbone. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Numbers on histograms represent MFI values of 10,000 events.

#### 4.2.7 – Attempts to validate the lengsin epitope

Lengsin is a 57 kDa protein expressed in the ocular lens (Grassi *et al.*, 2006; Wistow, 2006), an immune-privileged site that escapes the majority of immune scrutiny (Martin *et al.*, 1995; Streilein, 2003; Nakatsugawa *et al.*, 2009). The lengsin protein plays a role in cytoskeletal organisation (Wyatt *et al.*, 2008). Lengsin expression is tightly regulated to the lens and in healthy scenarios it is expressed in no other adult tissues (Wistow, 2006). Despite this tight regulation, ectopic expression of a lengsin splice variant was described as a TAA in lung cancers in 2009 (Nakatsugawa *et al.*, 2009). Lengsin is discussed in detail in **Section 4.3.6**.

Given its potential as a TAA, I next wanted to validate that the lengsin SQILTPPQL peptide as a legitimate epitope that is correctly processed and presented at the cell surface. To do so, as before, I cloned the parent protein into a lentiviral vector, pELNS, (**Section 2.3.3**) and transduced it into MOLT-3 cells (**Section 2.4.2**). Lengsin transduced well into both the wildtype HLA-A2<sup>-</sup> MOLT-3 line, and a MOLT-3 line that had previously been stably transduced with HLA-A2. Both wildtype and HLA-A2<sup>+</sup> MOLT-3 lines were >80% positive for marker gene rCD2, confirming successful transduction (**Figure 4.11A**).

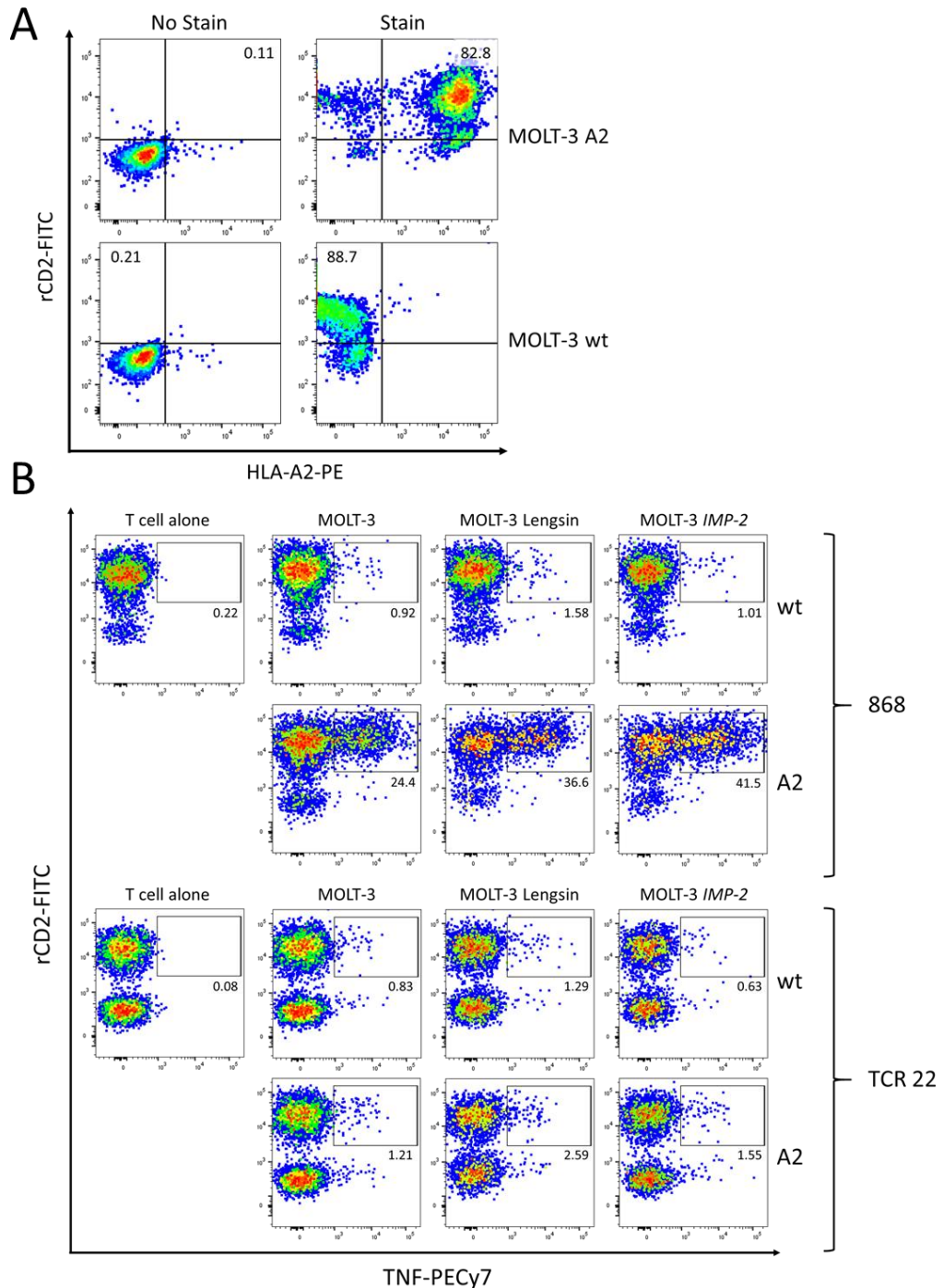
Response of 868- or TCR 22-transduced CD8<sup>+</sup> T cells to transduced MOLT-3 lines was then analysed by TAPI-0 assay (**Figure 4.11B**). 868-transduced CD8<sup>+</sup> T cells responded to not only lengsin-transduced MOLT-3 cells, but unexpectedly to untransduced MOLT-3 cells and MOLT-3 cells transduced with an irrelevant protein, insulin-like growth factor 2 mRNA binding protein (IMP-2). This response was HLA-A2-dependent and did not occur in T cells transduced with TCR 22 (**Figure 4.11B**). The observations in **Figure 4.11B** could be replicated (**Supplementary Figure 7.2** and **Figure 4.12A**), in fact 868-transduced CD8<sup>+</sup> T cells produced TNF in response to HLA-A2<sup>+</sup> MOLT-3 cells transduced with a range of proteins (**Figure 4.12B**). Furthermore, 868-transduced CD8<sup>+</sup> T cells produced TNF in response to SAR26 (an HLA-A2<sup>+</sup> sarcoma cell line) but not MM909.27 (an HLA-A2<sup>-</sup> melanoma cell line) (**Figure 4.12C**). From these data, it appears that that 868-transduced CD8<sup>+</sup> T cells respond to an epitope inherently expressed in the context of HLA-A2 by MOLT-3 cells. This epitope could potentially be shared with T2 cells and SAR26, and might be lengsin-derived. The Protein Atlas contains data suggesting that MOLT-4 cells, a cell line derived from the same parental line as MOLT-3 cells, express lengsin at a low level (Protein Atlas, 2020). Confirmation that MOLT-3 cells express lengsin will require Western Blot analyses. Unfortunately, I did not have time to undertake these studies. Verification that MOLT-3 cells are being recognised via the new HLA-A2 lengsin epitope I identified could be also achieved by examining recognition of lengsin knockout

MOLT-3 cells. Alternatively, multiple epitopes could be being recognised by 868 transduced T cells, the TCR could be specific for more than one TAA.

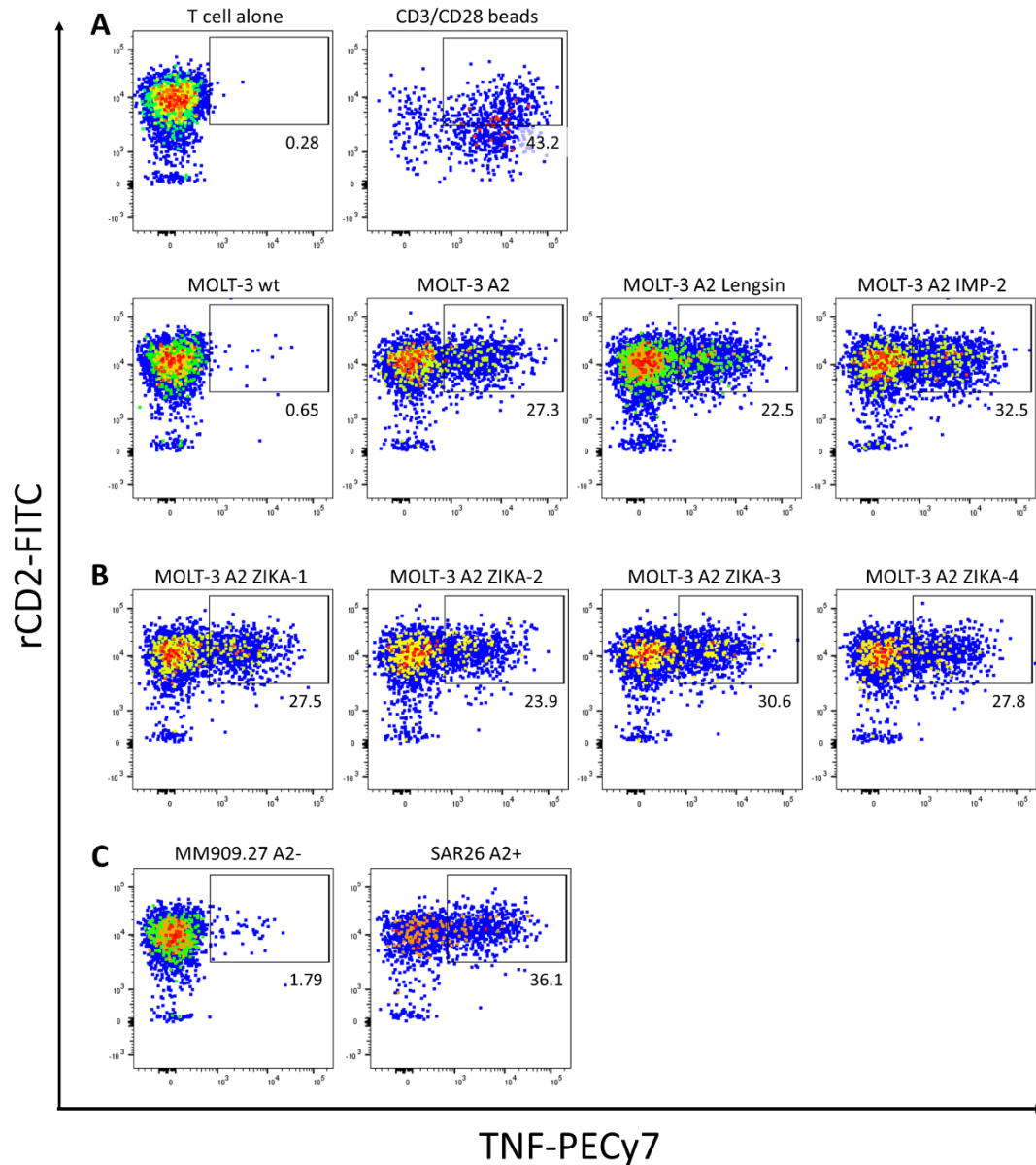
Retaining the goal of validating the lengsin epitope, I next assessed the response of 868-transduced CD8<sup>+</sup> T cells to a panel of three other immortalised lines, with the hope of finding a line that could be used to verify the lengsin epitope. I found that 868-transduced CD8<sup>+</sup> T cells showed a moderate response to each immortal cell line, in an HLA-A2-dependent manner (**Figure 4.13**). This was not observed for CD8<sup>+</sup> T cells expressing TCR 22 (**Figure 4.13**). These data were replicable using transduced T cells from two further donors (**Supplementary Figure 7.3**).

My data suggest that this TCR might recognise a self-antigen that is expressed in a range of immortalised cell lines. As these cell lines are cancerous, it is possible that the HIV-1-specific 868 T cells might crossreact with a common tumour antigen.

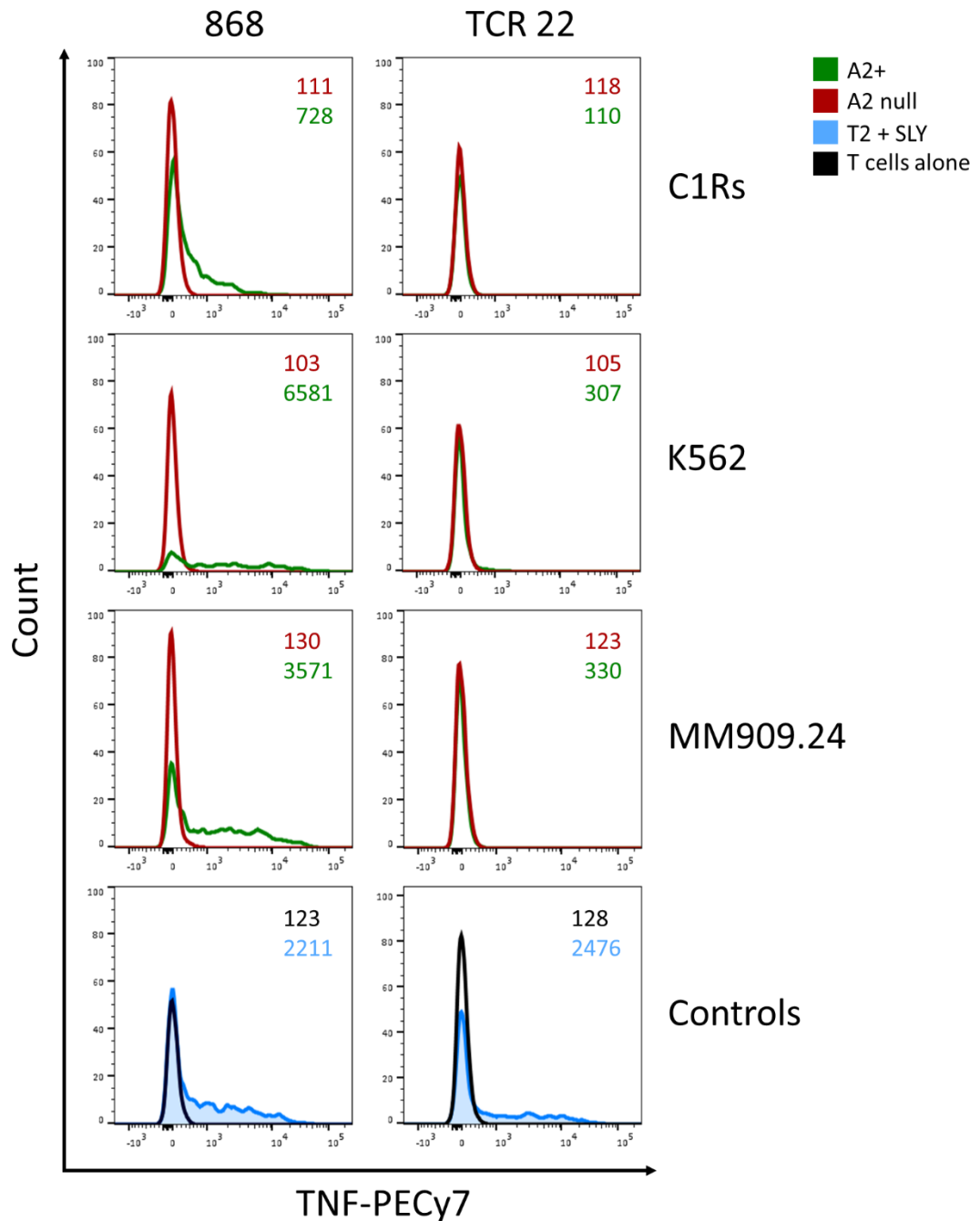




**Figure 4.11: Attempts to validation the lengsin-derived epitope SQILTPPQL.** **A)** Transduced MOLT-3 HLA-A2 or MOLT-3 wt cells were cultured in 10 mL R10 suspension flasks for 1 week following spinfection. Cells previously gated on live, single lymphocytes. Surface expression of lentiviral constructs was monitored based on expression of marker gene, rCD2. Numbers on dot plots correspond to the percentage of cells in the gated population, 10,000 events recorded. **B)** 30,000 CD8<sup>+</sup> T cells transduced with either 868 wt or TCR 22 were cultured in the presence of 60,000 MOLT-3 cell lines overnight before cells were harvested and assessed for surface expression of TNF. Cells previously gated on live CD3<sup>+</sup> CD8<sup>+</sup> single lymphocytes. Numbers on dot plots correspond to the percentage of cells in the gated population, mean of 10,000 events.



**Figure 4.12: 868-transduced CD8<sup>+</sup> T cells respond to MOLT-3 cells expressing HLA-A2.** 30,000 CD8<sup>+</sup> T cells transduced with 868 wt were cultured in the presence of 60,000 **A**) MOLT-3 cells lines transduced with lengsin parental protein, or an irrelevant protein (insulin-like growth factor 2 mRNA binding protein, IMP-2) **B**) MOLT-3 cells transduced with portions of the ZIKA virus genome, and thus expressing a range of potential viral epitopes, or **C**) HLA-A2<sup>+</sup> or HLA-A2<sup>-</sup> cancer cell lines. Cells were co-cultured overnight before being harvested and assessed for surface expression of TNF. Cells previously gated on live CD3<sup>+</sup> CD8<sup>+</sup> single lymphocytes. Numbers on dot plots correspond to the percentage of cells in the gated population, mean of 10,000 events.

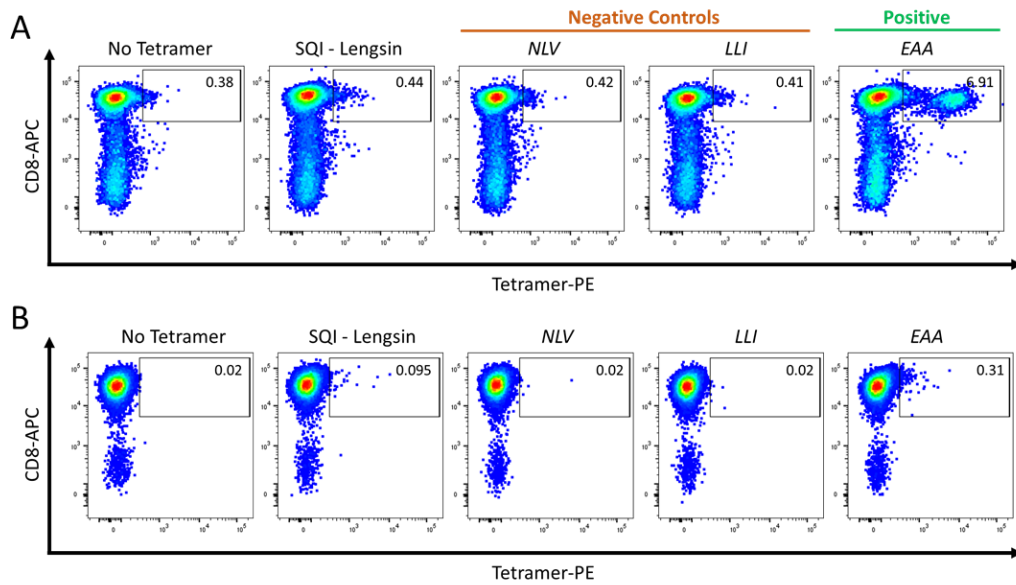


**Figure 4.13: 868-transduced CD8<sup>+</sup> T cells respond to a panel of potential APCs in an HLA-A2-dependent manner.** 30,000 CD8<sup>+</sup> T cells transduced with TCR constructs were cultured in the presence of 60,000 immortalised HLA-A2<sup>+</sup> and HLA-A2<sup>-</sup> cell lines overnight before cells were harvested and assessed for surface expression of TNF. Cells previously gated on live CD3<sup>+</sup> CD8<sup>+</sup> rCD2<sup>+</sup> single lymphocytes. Numbers on histograms represent MFI values of 10,000 events.

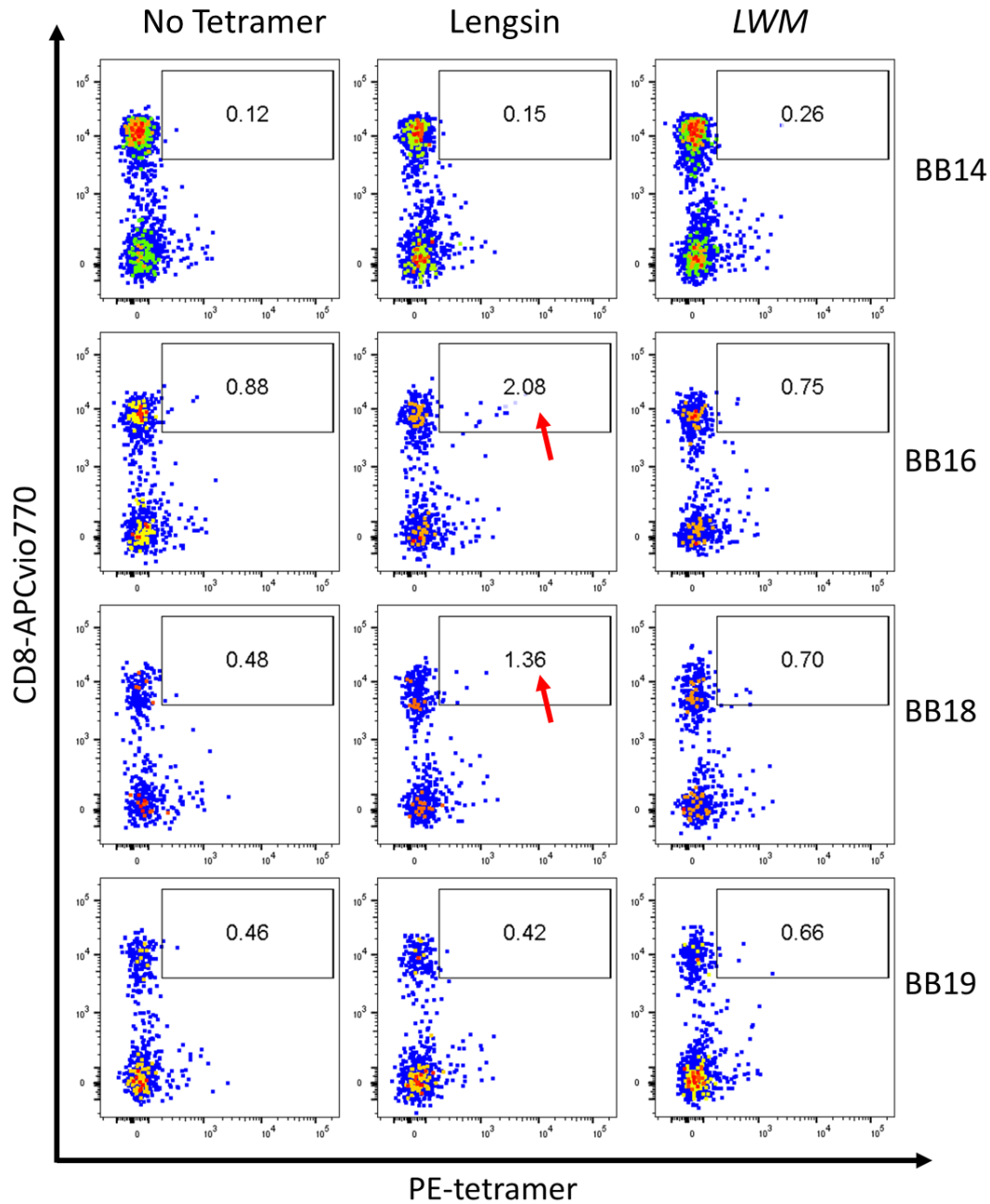
#### 4.2.8 – Attempts to isolate lengsin-reactive T cells

The antigen that 868-transduced T cells are responding to in the panel of cell lines may or may not be the lengsin epitope identified by CPL-driven database screening of the TAA database. Confirmation that the recognition of T2, MOLT-3 and other HLA-A2<sup>+</sup> cell lines is as a result of lengsin expression will require confirmation that these cells express lengsin. To confirm that the lengsin peptide is a truly processed epitope I would ideally need to find a cell that does not express lengsin and then transduce in the lengsin gene. It would also be helpful to have T cells that were primed with the lengsin peptide. As the SQILTPPQL lengsin peptide originates from a known TAA, my first thought was to look for responses to this peptide within two tumour-infiltrating lymphocyte (TIL) lines from HLA-A2<sup>+</sup> patients that my laboratory routinely works with. However, I was unable to identify any lengsin-specific T cells within a sarcoma TIL sample (**Figure 4.14A**) or a melanoma TIL sample (**Figure 4.14**), as measured by tetramer staining.

Given the difficulty of obtaining fresh PBMC donations during the 2020 COVID-19 pandemic, I was fortunate to benefit from access to stored PBMC samples from 4 healthy donors. Each was defrosted and, after a culturing for a short period, stained with HLA-A2-SQILTPPQL tetramer (**Figure 4.15**). Whilst not entirely convincing, two donors (BB16 and BB18) showed some increased staining compared to the irrelevant tetramer. These two lines have been enriched for binding to HLA-A2-SQILTPPQL (**Section 2.4.5**) and are currently being further examined.



**Figure 4.14: Lengsin-reactive T cells were not found in TIL samples. A)** 50,000 MM909.42 TILs and **B)** 50,000 SAR26 TILs were stained with 0.5  $\mu$ g (relative the pMHC component) HLA-A2-SQILTTPPQL tetramer, with a PE-conjugated backbone. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Numbers on dot plots represent MFI values of 10,000 events. Controls HLA-A2-EAAGIGILTV, HLA-A2-NLVPMVATV, and HLA-A2-LLIEGIFFI derived from human Melan-A, cytomegalovirus pp65, and EBV BARF-1 respectively.



**Figure 4.15: Potential lengsin-reactive T cells found in healthy PBMC samples.** CD8<sup>+</sup> T cells were isolated from four frozen healthy PBMC samples, 50,000 from each donor were stained with 0.5  $\mu$ g (relative the pMHC component) HLA-A2-SQILTPPQL tetramer, with a PE-conjugated backbone. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup> single lymphocytes. Numbers on dot plots represent percentage of cells in the gated population, of 10,000 events. Red arrows indicated potentially notable staining above background. Irrelevant tetramer HLA-A24-LWMRLLPLL derived from preproinsulin-signal peptide (residues 3-11).

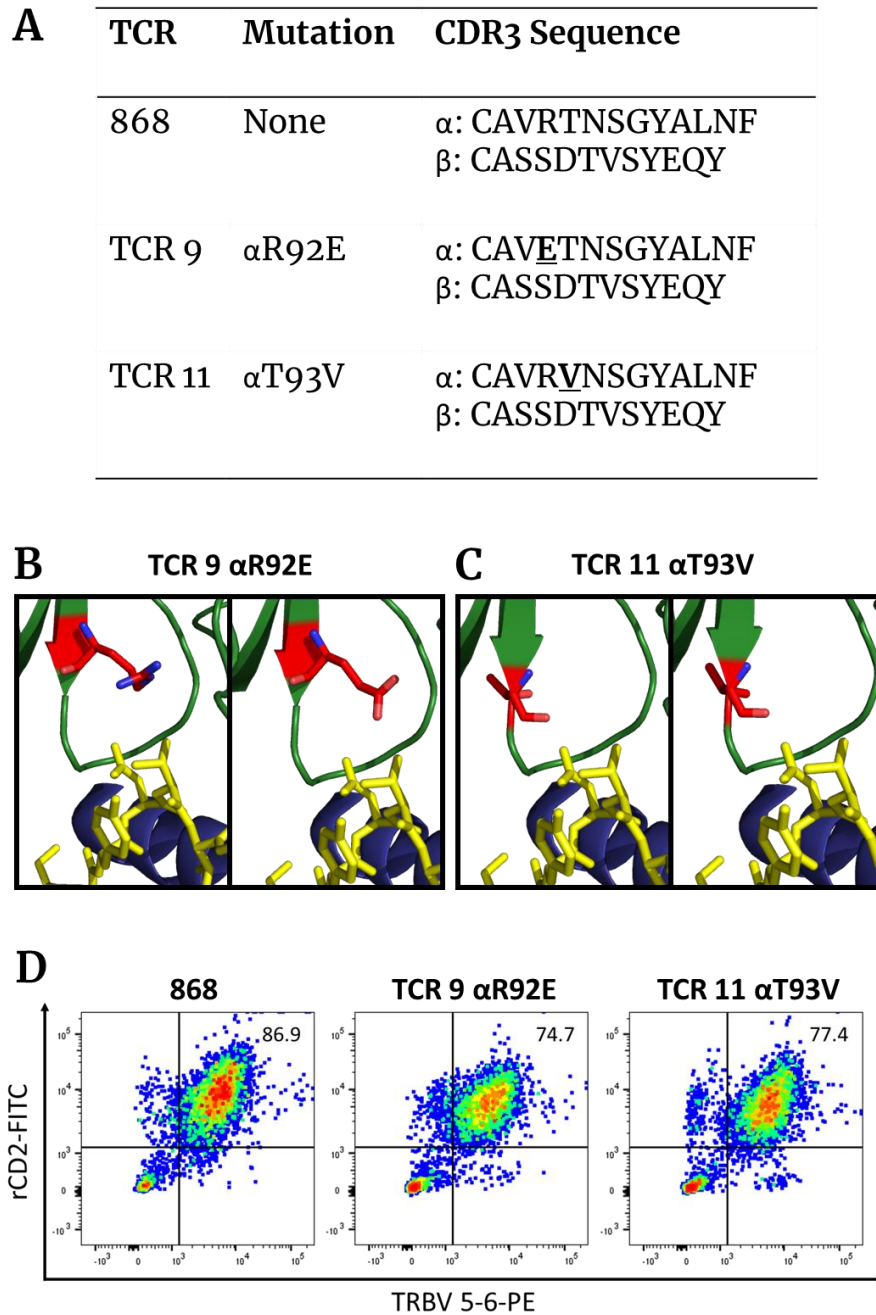
4.2.9 – Preliminary characterisation of two further mutants of the 868 TCR  
Since my return to the laboratory following the COVID-19 summer lockdown I was able to begin the analysis of two further mutants of the 868 TCR, TCRs 9 and 11 (see **Table 4.2**). In contrast to the TCR mutants that I have already studied, TCRs 9 and 11 both contain substitutions in their CDR3 $\alpha$  loop (**Figure 4.16A**) allowing me to assess the role of the TCR $\alpha$  chain in peptide discrimination. TCRs 9 and 11 both have amino acid substitutions in key peptide contact residues (**Table 4.1**). Residue 92 in the 868  $\alpha$  chain is an arginine, a positively charged amino acid that makes a total of five contacts with two peptide residues –asparagine (P4) and threonine (P5) (**Figure 4.2**). In TCR 9 arginine 92 was substituted for glutamic acid to reverse the charge of this residue (**Figure 4.16B**). Residue 93 in the 868  $\alpha$  chain is a threonine, a polar amino acid that contacts the asparagine at position 4 in the peptide (**Figure 4.2**). In TCR 11 this residue has been substituted for valine, a hydrophobic amino acid (**Figure 4.16C**). Prior to any analysis the surface expression of the transgene was confirmed, the rCD2<sup>+</sup>TRBV5-6<sup>+</sup> fraction of CD8<sup>+</sup> T cells transduced with the 868, TCR 9, and TCR-11 was 86.9 %, 74.7 %, and 77.4 % respectively (**Figure 4.16D**).

Cells transduced with either TCR 9 or TCR 11 were then subject to CPL screening (**Figure 4.17**, green and blue respectively). Each showed varying amino acid preferences in comparison with their wildtype parent TCR (grey bars) at a number of peptide positions (**Figure 4.17**). These data were then used to screen the 9-mer peptide universe (screening kindly performed by Dr Barbara Szomolay) for peptides predicted to be recognised by the mutant TCRs but not the 868 parental TCR; predicted peptides are shown in (**Table 4.3**).

The peptides identified by CPL-based database screening of the universe database were ordered in crude form (~40 % purity) and the three TCRs (868, TCR 9, and TCR 11) were assessed for reactivity against 10<sup>-5</sup> M of each peptide, **Figure 4.18**. The graph for each mutant TCR is overlaid with that of 868 (grey bars) in **Figure 4.19** for ease of visual analysis. **Figure 4.19** shows some clear incidences where peptides are seen by the respective mutant TCRs, but not by wildtype 868. These data suggest that the single amino acid substitutions in TCRs 9 and 11 have resulted in the gain of specificity for peptides that are not seen by the wildtype 868 TCR.

Further work will be needed to confirm that TCRs 9 and 11 exhibit reactivity to the peptides highlighted in **Figure 4.18**, and to confirm that they are not recognised by the 868 TCR. Unfortunately, I was unable to extend my PhD funding further to cover the period that I lost in the laboratory due to the COVID-19 lockdown. I am now contracted to work on a different

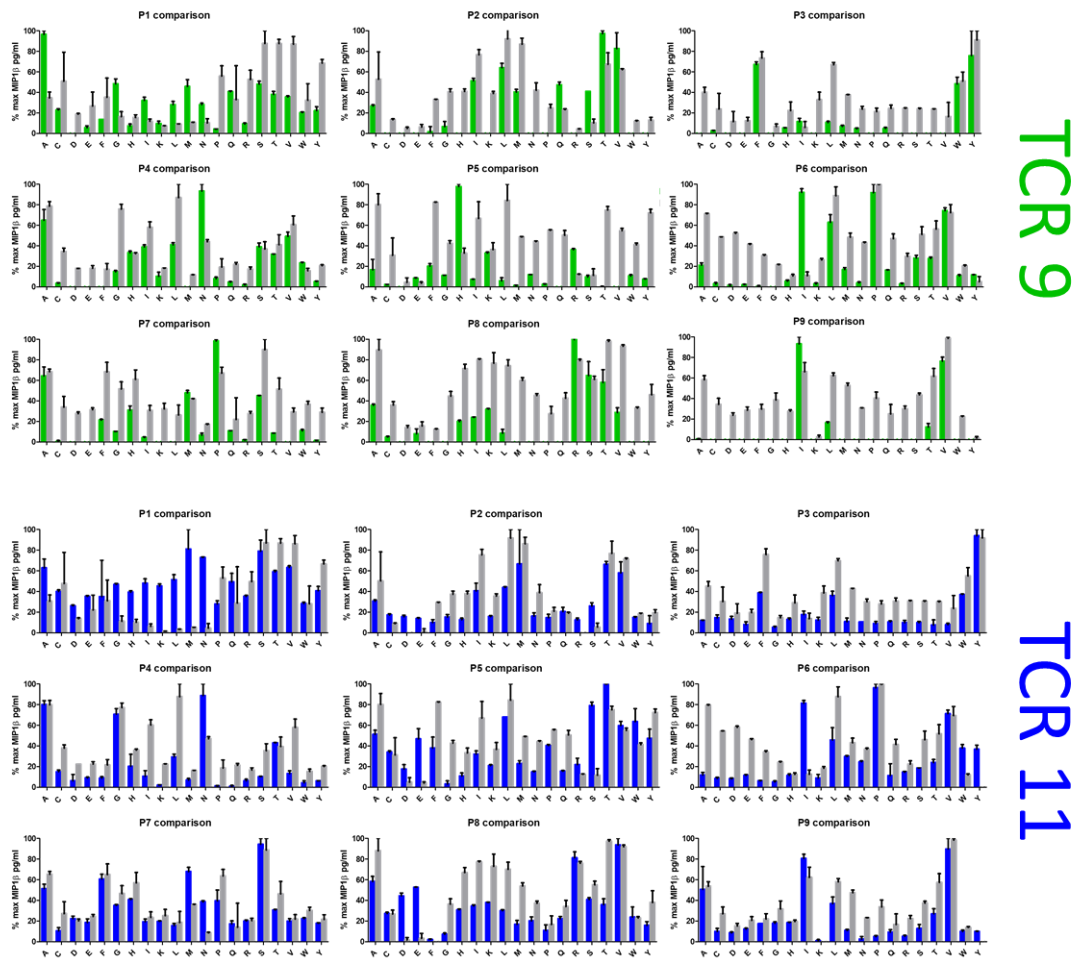
project so I have very limited time in which I can undertake further experiments on the 868 TCR.



**Figure 4.16: TCR 9 and TCR 11.** **A)** shows the CDR3 sequences of each of TCRs 9 and 11 in comparison with the wildtype 868 sequence. Both TCRs carry amino acid substitutions in their CDR3 $\alpha$  sequences, these substitutions are underlined. **B)** Model prediction of the  $\alpha$ R92E substitution in TCR 11. **C)** Model prediction of the  $\alpha$ T93V substitution in TCR 11. **D)** Surface expression of lentiviral constructs was confirmed based on simultaneous expression of marker gene, rCD2, and TRBV 5-6 chain. Numbers on dot plots correspond to the percentage of cells in the gated population, of 10,000 events.



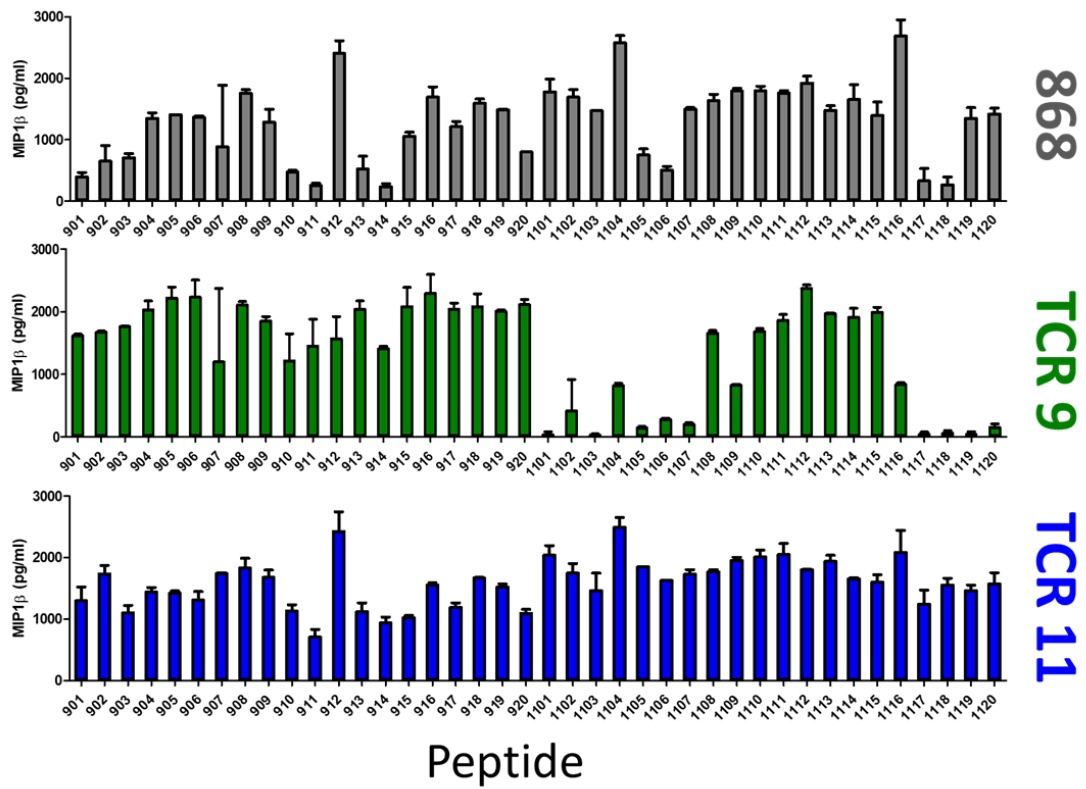
# 868 Comparison



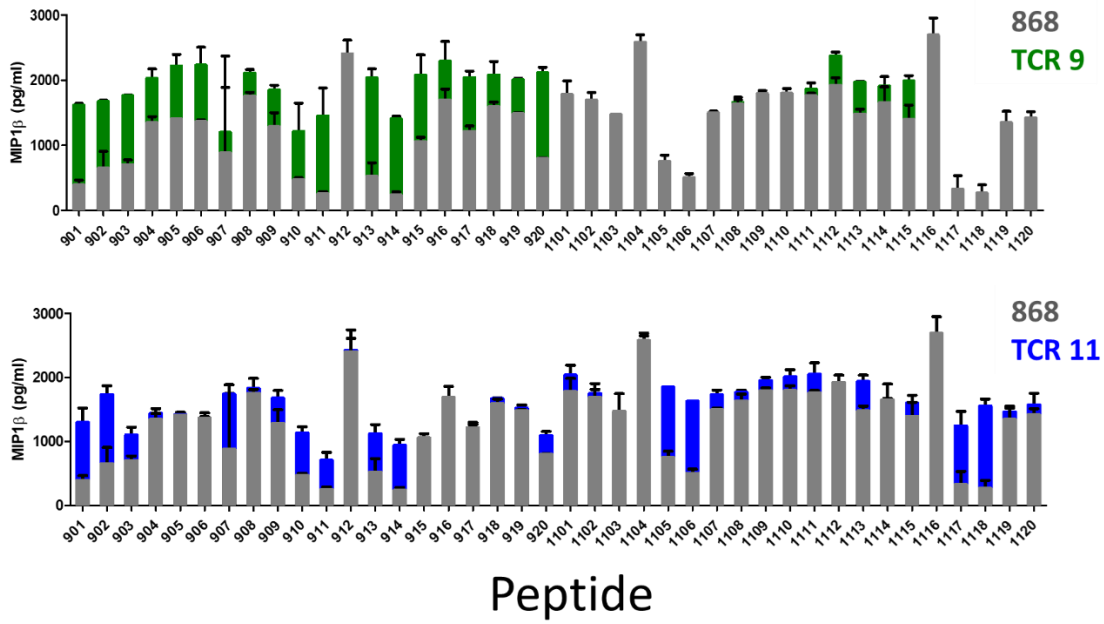
**Figure 4.17: TCRs 9 and 11 exhibit different peptide preferences than the 868 parental TCR.** 30,000 CD8<sup>+</sup> T cells transduced with either TCR 9 (green) or TCR 11 (blue) constructs co-cultured with 60,000 T2 cells as APC and 100 µg/mL peptide mix from a 9-mer CPL screen. Supernatants were harvested and ELISA analysis performed to measure MIP-1β presence as a proxy for T cell response. Grey bars indicate the peptide preferences of the wildtype 868 TCR for comparison. All assays performed once.

TCR 9		TCR 11	
Peptide ID	Sequence	Peptide ID	Sequence
901	ATYNHIPRI	1101	MMYNTPSVV
902	ATYNHPPRI	1102	MTYNTPSVV
903	ATFNHIPRI	1103	MMYATPSVV
904	ATYNHIPRV	1104	NMYNTPSVV
905	AVYNHIPRI	1105	MMYNTPSVI
906	ATFNHPPRI	1106	MTYATPSVV
907	ATYNHPPRV	1107	NTYNTPSVV
908	AVYNHPPRI	1108	MTYNTPSVI
909	ATYNHVPRI	1109	MVYNTPSVV
910	ATFNHIPRV	1110	MMYNTPSRV
911	AVFNHIPRI	1111	MTYNTPSRV
912	AVYNHIPRV	1112	MMYNTISVV
913	ATFNHPPRV	1113	MTYNTISVV
914	AVFNHPPRI	1114	SMYNTISVV
915	ATYNHIPSI	1115	STYNTISVV
916	AVYNHPPRV	1116	NMYATPSVV
917	ALYNHIPRI	1117	MMYATPSVI
918	ATYAHIPRI	1118	NMYNTPSVI
919	ATYNHPPSI	1119	NTYATPSVV
920	ATFNHVPRI	1120	MTYATPSVI

**Table 4.3: CPL data was used to screen the 9-mer peptide universe for the top 20 predicted peptide agonists of each TCR. Peptides predicted to be top agonists for 868 were removed.**



**Figure 4.18: Recognition of putative epitopes unique to mutant TCRs.** 30,000 CD8<sup>+</sup> T cells transduced with TCR constructs were cultured in the presence of 60,000 T2 cells as APCs and 10<sup>-5</sup> M of the crude peptide and incubated overnight before the supernatant was harvested for measurement of MIP-1β presence by ELISA analysis. The response of 868-, TCR 9-, and TCR 11-transduced T cells is depicted by grey, green, and blue bars respectively.



**Figure 4.19: Novel specificities in mutant TCRs.** Response of TCRs 9 and 11 to each peptide overlaid with that of 868 (grey) to aid visual analysis suggests some peptides produce response in T cells transduced with mutant TCRs but not with 868 wildtype.

## 4.3 – Discussion

In this chapter, I have demonstrated broad crossreactivity of 868-transduced CD8<sup>+</sup> T cells. I have shown that these cells respond to peptides derived from viral, self, and TAA proteomes in the context of HLA-A2. I have further shown that a single amino acid change in the CDR3 loops of a TCR can alter the repertoire of peptides that a T cell can respond to.

Data such as that presented here provides fuel for ongoing efforts to predict what pMHC targets a given T cell will be able to respond to based on the CDR3 sequences of its TCR (Udyavar *et al.*, 2009; Roomp and Domingues, 2011; Zoete *et al.*, 2013; Smith *et al.*, 2014). Greater understanding of how CDR3 loop sequences influence the peptide recognition repertoire, and how small changes to the CDR3 might alter TCR function, will provide a foundation for the rational design of TCRs against a nominal epitope. A recent model created by Jurtz *et al.* was able to predict a cognate antigen for a TCR based on CDR3 $\beta$  sequence (Jurtz *et al.*, 2017). However, this model was limited to the training data supplied and could not be used to predict novel agonist epitopes (Jurtz *et al.*, 2017). Uncovering further information regarding the plasticity of the TCR-pMHC interaction, and particularly how CDR sequence relates to peptide preference will lead to improvement in predictive tools.

### 4.3.1 – TCR transduced T cells

I chose the 868 TCR to begin investigation into how TCR sequence relates to function because it is biochemically and structurally well-characterised (Varela-Rohena *et al.*, 2008; Cole, Fuller, *et al.*, 2017). Repeated attempts to generate a clone from the patient 868 polyclonal HIV-1-specific line were unsuccessful (Varela-Rohena *et al.*, 2008), I therefore turned to TCR gene transfer to deliver this TCR to a polyclonal population of primary T cells.

The ability of TCR gene transfer to redirect the specificity of T cells was first demonstrated by Dembic *et al.* in 1986 (Dembic *et al.*, 1986). There have since been many studies investigating ways in which to optimise expression of the transgene in order to more effectively redirect the recipient cells (Emerman and Temin, 1984; De Felipe *et al.*, 1999; Mizuguchi *et al.*, 2000; Szymczak *et al.*, 2004; Leisegang *et al.*, 2008; Yang *et al.*, 2008). To transduce the TCRs described in this chapter, I used a single tricistronic transfer vector to achieve stoichiometric expression of both TCR genes and a marker gene. Within the vector, the three genes were separated by 2A ‘self-cleaving’ peptide sequences which has previously been shown to be optimal for the expression of genes that function with a 1:1 stoichiometry.

The advantages and disadvantages of the various available expression systems are discussed further in **Appendix Section 7.2**.

#### 4.3.2 – TCRs 3 and 6 showed little change in peptide preference

My initial aim was to study the peptide recognition repertoire of the 868 TCR and 24 mutant versions of the TCR carrying amino acid substitutions in their CDR3 loops, as described in **Table 4.2**. At the start of my studies, I wanted to move the 868 TCR $\alpha$ -2A-TCR $\beta$ -2A-rCD2 construct from the pELNS lentivirus used by Riley and colleagues (Varela-Rohena *et al.*, 2008) to the pSF lentivirus because the pELNS virus had been sold to Novartis by its creator Jim Riley. While my laboratory retained an academic license to use this virus, we were unable to share it with other laboratories. The pSF lentivirus was purchased from Addgene and did not have this limitation. Unfortunately, despite repeated attempts at cloning, the 868 TCR $\alpha$ -2A-TCR $\beta$ -2A-rCD2 construct did not work in pSF. This was later tracked down to a lack of stop codon within the transduced fragment (the stop codon in pELNS lies outside of the restriction site and our historic use of this vector meant that constructs were designed without their own stop codon). Trying to troubleshoot this issue wasted ~5 months of my lab time. My supervisors then decided that I should just continue with pELNS rather than waste any more time. I have since successfully cloned 19/24 mutant TCRs into the pELNS lentivirus and expressed 12 of these constructs in T cells. I was able to screen seven TCRs by CPL.

Two of the mutant TCRs I studied, TCR 3 and TCR 6, showed little difference in their peptide specificity compared to the wildtype sequence. This was unexpected, as both TCRs bear amino acid substitutions to amino acids with differing chemical properties at key peptide contact residues, **Table 4.1** and **Figure 4.3**. The  $\beta$ T96F mutation in TCR 3 involves the substitution of a small polar amino acid, threonine, to a bulky hydrophobic amino acid with a large aromatic ring, phenylalanine. As the threonine at position 96 of the wildtype 868 sequence is a key contact residue, forming two peptide contacts one of which is a hydrogen bond, I expected that this substitution would have a more drastic phenotype. The  $\beta$ V97T mutation in TCR 6 involves the substitution of a hydrophobic amino acid, valine, to a polar amino acid, threonine. In the wildtype TCR this residue also makes two peptide contacts, alongside an additional four contacts with HLA-A2. The retainment of TCR specificity despite such amino acid substitutions demonstrates the substantial plasticity within the TCR-pMHC interaction.

I demonstrated that both TCR 3 and TCR 6 were correctly processed and expressed at the T cell surface, achieving measurable post-purification surface expression on 81% and 82% of T cells respectively (**Figure 4.4**). Both mutant TCRs were able to bind and respond to the cognate SLYNTVATL peptide in the context of HLA-A2, as demonstrated by MIP-1 $\beta$  ELISA (**Figure 4.5A**), and tetramer binding (**Figure 4.5B**). Examination of the CPL data for these TCRs in comparison to the wildtype 868 (**Figure 4.6**) suggested little change in peptide preference. These results confirmed that CPL screens can be used to probe the peptide recognition landscape of mutated TCRs. However, it appears that the single amino acid substitutions in TCR 3 and TCR 6 did not result in the acquisition of novel reactivities. Given the limited timescale of my project these TCRs were set aside in favour of more interesting mutants.

It is interesting to ponder why substitutions that alter chemical properties did not alter the peptide preferences of TCRs 3 and 6. It could be that the selected assay failed to detect novel peptide reactivity. CPL screens are powerful tools to assess ligand preferences with no prior knowledge of likely candidates (Houghten *et al.*, 1991; Pinilla *et al.*, 1992). CPL screens can rapidly assess almost the whole proteomic universe and have been used successfully in many incidences (Houghten *et al.*, 1991; Pinilla *et al.*, 1992; Hemmer *et al.*, 1997, 1998; Hiemstra *et al.*, 1997; Wooldridge *et al.*, 2012). However, CPL screens are not without caveat, while cysteine is used as a fixed amino acid in each position it is excluded from the amino acid pool due to its propensity to form disulphide bonds. Thus, each library is devoid of peptides containing two or more cysteines. Furthermore, such libraries do not include post-translationally modified amino acids and are likely to be suboptimal for screening of highly hydrophobic peptides due to aqueous solubility issues. Alternative methods for T cell ligand discovery are discussed in **Section 6.2**.

It is also possible that the CPL assay is not at fault. The substitutions employed to create TCRs 3 and 6 may not have altered the peptide preference of the TCR from that of the wildtype 868 due to the natural plasticity of the TCR, and the formation of compensatory interactions. Amino acids changes in the peptide residues do not always alter TCR binding and recognition, as seen in the case of the common naturally-occurring SLYNTVATL escape mutants (Sewell *et al.*, 1997; Cole, Fuller, *et al.*, 2017). Furthermore, when Burrows *et al.* mutated key TCR contact residues on the MHC molecules they found that compensatory interactions occurred to maintain binding (Burrows *et al.*, 2010). The TCR-pMHC interaction is extraordinarily plastic. If amino acid substitutions in both peptide and MHC molecule can be tolerated, it stands to reason, that certain single amino acid substitutions in the TCR might also retain the

peptide recognition repertoire, if TCR plasticity allows for suitable compensatory interactions.

It is also notable that the 868 TCR binds to the cognate HLA-A2-SLYNTVATL epitope with an extremely high affinity for a naturally-occurring TCR (Cole, Fuller, *et al.*, 2017). This strong binding might allow weaker binding mutants of this TCR to still appear fully functional at the T cell surface. It could be that if I had started with a weaker binding TCR, single amino acid substitutions in CDR3 may have had much greater functional effects.

#### 4.3.3 – TCRs 22 and 24 have altered peptide preferences

TCRs 3 and 6 proved useful in validating that my experimental set up was functional and demonstrated that I could successfully redirect the specificity of polyclonal primary CD8<sup>+</sup> T cells using lentiviral transfer of a TCR construct. These experiments also showed that the TCRs could still remain functional when key contact residues were mutated to residues with different chemistries. Given the limited evidence of any novel specificity with TCRs 3 and 6, I turned my focus to the next two TCRs that I was able to clone into pELNS, TCRs 22 and 24.

For TCR 22, I altered a non-contact residue, residue 95, in order to assess the impact of potential knock-on effects on the recognition footprint of the TCR. The negatively charged aspartic acid residue present in the wildtype chain was mutated to a polar, uncharged, serine -  $\beta$ D95S (**Figure 4.3D**). TCR 24, on the other hand, is the only mutant TCR that I was able to screen that contained more than a single amino acid substitution. TCR 24 contains three amino acid substitutions at residues 95, 96, and 97. The wildtype sequence consists of aspartic acid, threonine, and valine (DTV). These residues were mutated to isoleucine, arginine, and serine (IRS,  $\beta$ D95I T96R V97S) (**Figure 4.3E**). As described in **Section 4.2.1** these amino acid substitutions are based on the published structure of a HLA-A2-restricted, influenza-specific TCR, JM22 (PDB:5HHO) (Valkenburg *et al.*, 2016). The JM22 TCR recognises an epitope from the M1 influenza protein (GILGFVFTL, residues 58-66) (Valkenburg *et al.*, 2016). TCR 24 did not recognise the GILGFVFTL peptide in the context of HLA-A2 (**Supplementary Figure 7.1**); this is likely due to the remaining differences in the TCR- $\alpha$  chain.

I first assessed the ability of the mutant TCRs to bind and respond to the wildtype SLYNTVATL. TCR 22 but not TCR 24 could respond to SLYNTVATL, as measured by MIP-1 $\beta$  ELISA, **Figure 4.7A**. Similarly, T cells expressing TCR 22 but not TCR 24 could bind HLA-A2-SLYNTVATL tetramer, **Figure 4.7B**. T cells expressing wildtype 868 TCR responded to the T2 cells used as APCs in the assay. This background response was not observed with T cells expressing TCR



22 or TCR 24 suggesting that the amino acid substitutions between them and the wildtype TCR were sufficient to ablate this reactivity, **Figure 4.7A**. These results strongly suggested that the recognition repertoire differed between the 868 wildtype- and mutant TCR-transduced CD8<sup>+</sup> T cells.

With this observation in mind, I turned to the CPL data for each TCR to see if they could be used to identify the peptide that was recognised by the 868 TCR-transduced T cells but not T cells transduced with TCRs 22 or 24, that might be responsible for background recognition of T2 cells **Figure 4.8**. Both the 868 wildtype TCR and TCR 22 assayed well, showing variable MIP-1 $\beta$  outputs to different peptide pools, reflecting variable peptide preferences. TCR 24 however, produced very low levels of MIP-1 $\beta$  in response to most peptide pools. At some positions, almost no MIP-1 $\beta$  was produced in response to any peptide pool. My work validating the surface expression of each construct, **Figure 4.4**, suggests this lack of response is unlikely to be due to failure of the construct expression or surface trafficking. Post-purification CD8<sup>+</sup> T cells transduced with TCR 24 were 81.2 % positive for both the TCR  $\beta$  chain and the marker gene rCD2, **Figure 4.4D**. It is, therefore, likely that function rather than expression has been impeded by the triple CDR3 amino acid substitution in TCR 24, resulting in the minimal response observed across the 9-mer peptide universe, **Figure 4.8**. The triple substitution in TCR 24 may have ablated its ability to bind pMHCs and function as a TCR at all. It is further possible that the peptide length preference of the TCR might have been altered so that T cells transduced with this mutant no longer responded to 9-mer peptides. The triple substitution could have had knock on effects that were translated to other parts of the protein including the TCR  $\alpha$  chain. In the absence of an atomic resolution structure for the  $\beta$ D95I T96R V97S TCR, it is impossible to know the extent to which these substitutions affected the antigen-binding site.

Fortunately, TCR 22 retained recognition of pMHC complexes but exhibited different amino acid preferences at some peptide positions compared to the 868 TCR. Peptide preference differences between TCR 22 and the 868 TCR are illustrated by the overlay graphs in **Figure 4.9A**. These data were input into the public webtool for analysis of CPL data created by Szomolay *et al.* (Szomolay *et al.*, 2016), the output of which is shown in **Figure 4.9B**. The webtool ranks peptides in a predicted order of preference, whilst I did not see this predicted order manifest in terms of response (**Figure 4.10A**) I was able to identify a novel peptide ligand of the 868 wildtype TCR that was not recognised by TCR 22, which differs by just a single amino acid substitution, SQLTPPPQL (**Figure 4.10B**).

I concluded that the VSYNRIPSM peptide flagged in my initial studies with crude peptides was not a true ligand for the 868 TCR as purified peptide was neither recognised nor capable of forming a stable complex with HLA-A2 (**Figure 4.10**). In contrast HLA-A2-SQILTPPQL refolded well and was shown to bind the wildtype 868 TCR but not TCR 22 (**Figure 4.10C**), demonstrating that it is possible to alter the specificity of a natural TCR with a single amino acid substitution.

4.3.4 – The 868 TCR may crossreact with a tumour-associated antigen  
Throughout my studies, I noticed that T cells expressing the 868 TCR exhibited background recognition of the HLA-A2<sup>+</sup> T2 cell line. I reasoned that this reactivity may be due to expression of lengsin in the T2 cells I used in my experiments. As such I began attempts to validate the SQILTPPQL peptide as a legitimate epitope that is processed and presented at the cell surface. To achieve this, I transduced the parental lengsin protein (along with the rCD2 marker gene) into MOLT-3 cells, both wildtype MOLT-3 cells and MOLT-3 cells that had been previously stably transduced with HLA-A2, **Figure 4.11A**.

I found that 868-transduced CD8<sup>+</sup> T cells responded to MOLT-3 HLA-A2<sup>+</sup> regardless of, or even in the absence of a, transduced protein (**Figures 4.11** and **4.12**). Furthermore, in an attempt to find a different APC that could be used to validate lengsin I found that 868-transduced CD8<sup>+</sup> T cells responded to a range of cancer cell lines in an HLA-A2-dependent manner (**Figures 4.12C** and **4.13**). This was not observed for TCR 22, which differs from the wildtype 868 by just a single amino acid substitution (**Figure 4.13**).

It is important to note that T cells expressing the 868 TCR have not previously been shown to respond to HLA-A2<sup>+</sup> cell lines such as the autologous B cell line derived from patient 9300868 (Sewell *et al.*, 1997; Varela-Rohena *et al.*, 2008). A previous study from my laboratory demonstrated that it was not possible to transduce CD8<sup>+</sup> T cells with a TCR that responded to a peptide presented at the T cell surface (Tan *et al.*, 2015). High affinity HLA-A2-restricted, self-reactive TCRs could be transduced into HLA-A2<sup>-</sup> T cells but failed to express in HLA-A2<sup>+</sup> T cells. It was concluded that transduction with self-reactive TCRs induced T cell fratricide (Tan *et al.*, 2015). Previous studies with the 868 TCR showed that it expressed and functioned well in HLA-A2<sup>+</sup> T cells (Varela-Rohena *et al.*, 2008). These findings suggest that although T cells expressing the 868 TCR recognise many HLA-A2<sup>+</sup> cancer cell lines they do not respond to all human HLA-A2<sup>+</sup> cells. These findings fit with the fact that there was no obvious pathology in patient 9300868 from which the HIV-1-specific 868 T cell line was grown. I

concluded that T cells expressing the 868 TCR might respond to an epitope from a TAA, **Figure 4.9B**.

As mentioned above, 868-transduced T cells responded to a number of cancer cell lines in a HLA-A2<sup>+</sup>-dependent manner. To my understanding, there are two likely explanations for these observations. The first being that endogenous expression of lengsin, a TAA, is shared between all of the cancer cell lines tested. The alternative being that multiple epitopes could be being recognised by the 868 TCR. Further work will be required to distinguish between these possibilities.

Lengsin was identified as part of the national eye institute bank (NEIBank) project in 2002, the project aimed to profile the gene expression in the human adult lens (Wistow *et al.*, 2002). The NEIBank project found a novel protein that had sequence homology to the ancient glutamine-synthetase family, hence naming it *LENS* *Glutamine-SYN*thetase-like (Wistow *et al.*, 2002). Despite its heritage, lengsin has no enzymatic activity (Wistow, 2006; Wyatt *et al.*, 2006, 2008). The 57kDa lengsin polypeptide forms a catalytically inactive dodecamer structure (Grassi *et al.*, 2006; Wyatt *et al.*, 2006) that co-ordinates the reorganisation of the cytoskeleton during terminal differentiation (Wyatt *et al.*, 2008). Expression of lengsin is extremely tissue-specific, being tightly regulated within the lens (in terminally differentiating cells) and no other adult tissues (Wistow, 2006). Orthologs of the protein were found in mouse, rat, dog, and zebrafish (Wistow, 2006).

The tightly-regulated lens-specific expression pattern is noteworthy as in 2009 a splice variant of lengsin was described as a novel TAA expressed ectopically in lung cancers (Nakatsugawa *et al.*, 2009). Knockdown of lengsin mRNA using RNA interference resulted in heightened cell death indicating an essential role in cancer cell survival (Nakatsugawa *et al.*, 2009). The Human Protein Atlas suggests that lengsin mRNA is expressed by several cancer types including stomach, renal, liver, pancreatic and ovarian cancer while being most highly expressed in lung cancers (Protein Atlas, 2020). Nakatsugawa *et al.* later described lengsin-derived HLA-A2-presented peptide epitopes that could be recognised by specific clones grown from healthy HLA-A2<sup>+</sup> donors (Nakatsugawa *et al.*, 2011). They demonstrated that one of their peptide epitopes (FLPEFGISSA) was naturally processed and presented by HLA-A2, thus highlighting the real potential for lengsin-derived epitopes to be clinically-relevant TAAs (Nakatsugawa *et al.*, 2011).

The lens is an immune-privileged site, meaning that – like the testis – it escapes the majority of immune scrutiny (Martin *et al.*, 1995; Streilein, 2003; Nakatsugawa *et al.*, 2009). Immune

privileged is achieved through a number of mechanisms including the tight junction-regulated blood-organ barriers, a lack of lymphatic drainage, low level expression of MHC molecules, and an immunosuppressive microenvironment abundant in components such as TGF- $\beta$  (Martin *et al.*, 1995; Streilein, 2003; Nakatsugawa *et al.*, 2009). Immune privilege serves to protect vision, a sense that is essential for survival in most higher animals, from the potentially damaging effects of inflammation (Streilein, 2003). Anti-lengsin antibodies were detected in the lung cancer patients involved in the aforementioned 2009 study, with none experiencing vision disturbances (Nakatsugawa *et al.*, 2009, 2011). This immune-protection makes lengsin a highly relevant, immunogenic cancer-antigen (Nakatsugawa *et al.*, 2009), a relevance that has been recognised clinically – a number of lengsin peptides have been patented, as HLA-A2 complexes, for the use in anti-cancer immunotherapies. This patent covers the 8-mer lengsin-derived peptide, QILTPPQL that overlaps with the longer putative epitope I discovered (patent number W02017089777). Thus, the CPL-discovered 868 peptide ligand, SQILTPPQL, could be a clinically relevant cancer-associated immunotherapy target. I have therefore demonstrated HIV/cancer crossreactivity on the part of the 868 TCR and, moreover, showed how this crossreactivity could be eradicated with a single amino acid substitution in TCR 22.

As lengsin-derived peptides are already recognised as potentially clinically-relevant TAAs future work on this project could attempt to characterise lengsin-specific T cell clones (**Figures 4.14** and **4.1**). If I had had time, I would have liked to have undertaken lengsin antibody Western blots and knock-out studies to determine whether the cell lines recognised by T cells expressing the 868 TCR express lengsin endogenously.

It also remains possible that the 868 TCR binds to a different cancer-derived epitope and that lengsin expression is not responsible for the observed recognition of HLA-A2<sup>+</sup> cancer cell lines by T cells expressing 868. Given that most of the top scoring peptides from the CPL-database screen for the 868-transduced T cells originated in the TAA database (**Figure 4.9B**), it could be that the 868 has capacity to interact with a range of TAAs – which could explain why T cells expressing the TCR exhibit recognition of many HLA-A2<sup>+</sup> cell lines.

To briefly consider the original aim of this project – to use amino acid substitutions to begin assessing how TCR sequence relates to function – it is striking that the ability of T cells transduced with the 868 TCR to respond to the panel of HLA-A2<sup>+</sup> cell lines described in this chapter can be completely abrogated by a single amino acid substitution,  $\beta$ D95S. This demonstrates the extraordinarily precise influence of TCR sequence on TCR function.

4.3.5 – T cells expressing TCRs 9 and 11 might exhibit new specificities  
During the last few months of my PhD, I was able to begin analysis of two further mutant TCRs, TCRs 9 and 11. TCRs 9 and 11 both contain substitutions in key contact residues of their CDR3 $\alpha$  loops (**Figure 4.16A** and **Table 4.1**). TCR 9 contains the  $\alpha$ R92E substitution, exchanging a positively charged amino acid for a negatively charged amino acid (**Figure 4.16B**). TCR 11 contains the  $\alpha$ T93V substitution, exchanging a polar (hydrophilic) amino acid for a hydrophobic amino acid. Both TCR constructs were expressed well at the surface of transduced T cells (**Figure 4.16D**).

CPL screening of TCR 9 and TCR 11 suggested differences in amino acid preferences at various peptide positions compared to the 868 wildtype TCR and to each other (**Figure 4.16**). These data were used to screen a 9-mer peptide universe database for potential ligands for each TCR. Preliminary analysis with crude peptides suggests the amino acid substitutions in TCRs 9 and 11 have resulted in the gain of specificity for some peptides, meaning that T cells transduced with the mutant TCRs respond to some peptides that T cells transduced with the 868 wildtype TCR do not (**Figure 4.17**). These data will need confirmation with pure peptide via peptide titration and tetramer staining, as demonstrated for the HLA-A2-SQILTPPQL ligand in **Section 4.2.6**. Ultimately any potential ligand of TCR 9 or 11 would ideally be confirmed by atomic resolution structure of the co-complex, and affinity measurements by SPR.

#### 4.3.6 – Future optimisation to reduce polyclonal background noise

Given that the aim of this project was to investigate the influence of TCR sequence on TCR function within the context of a particular TCR, 868, it is important to consider the influence that endogenous TCRs might have on the CPL data, as – in the absence of an 868 T cell clone – the TCR was transduced into a polyclonal CD8<sup>+</sup> T cell line.

By using a polyclonal population of CD8<sup>+</sup> T cells in this work, I may have minimised the ‘background’ specificities from endogenous TCRs, because my transduced TCR will be present almost every cell, whereas other TCRs will only be present in single or small copy numbers. However, it is possible to enhance the expression of the introduced TCR at the expense of the endogenous TCR by maximising chain pairing between the two introduced chains, allowing the introduced TCR a competitive advantage over the endogenous TCR (Heemskerk *et al.*, 2007).

When a TCR construct is introduced into a T cell, the transduced 'exogenous'  $\alpha$  and  $\beta$  chains could potentially pair with the endogenous TCR chains, creating chimeric TCRs with unintentional specificities (Heemskerk *et al.*, 2007). A single T cell could simultaneously express the introduced TCR, the endogenous TCR, and two chimeric TCRs (Heemskerk *et al.*, 2007). Van Loenen *et al.* demonstrated that the chimeric TCRs formed after the introduction of an exogenous TCR can be autoreactive (Van Loenen *et al.*, 2010) and chimeric TCRs have actually been implicated in autoimmunity (Ji, Perchellet and Goverman, 2010). Furthermore, each of these TCR pairs will compete for a limited number of CD3 subunits, providing a bottleneck to surface expression (Heemskerk *et al.*, 2007). Heemskerk *et al.* demonstrated that introduction of an exogenous TCR into a T cell clone markedly reduced the tetramer staining of the cognate ligand, as the introduced TCR competed with the endogenous TCR for the CD3 subunits (Heemskerk *et al.*, 2007). Improving the chain pairing between the two exogenously introduced TCR chains can give the introduced TCR a competitive edge over the endogenous TCR. Several strategies have been utilised in an attempt to improve TCR chain pairing of transduced TCRs. These are described in **Appendix Section 7.3**.

Optimisations to enhance surface expression of transduced TCRs clearly have substantial potential to reduce the background noise created by residual endogenous TCRs and chimeric TCRs and are something that should be considered for future work of this kind. Even if considered surplus to requirements for initial investigations (in total, 24 mutant TCR constructs were initially designed for the work described in this chapter, **Table 4.2**, and not all proved interesting for full analysis) these optimisations are certainly something I would include in more in-depth investigative work on constructs that prove themselves to be interesting, such as 868, TCR 9, TCR 11, and TCR 22.

#### 4.3.7 – Summary

In this chapter I aimed to explore the crossreactive nature of the HIV-specific 868 TCR and assess the impact of amino acid substitutions in the sequences of its CDR3 loops. I have shown that 868, and mutants thereof, can be stably transferred into polyclonal populations of CD8<sup>+</sup> T cells and expressed at the T cell surface (**Figure 4.4**). Transduction of the 868 TCR could redirect primary CD8<sup>+</sup> T cells to recognise the SLYNTVATL epitope (**Figure 4.5**).

I showed that some amino acid substitutions do not significantly alter the peptide recognition footprint of the 868 TCR (**Figure 4.6**). The interaction is highly plastic and this retention of specificity despite alteration of a key contact residues could potentially be due

to the formation of compensatory interactions. Some amino acid substitutions can ablate peptide recognition completely (**Figure 4.7 A and B**, and **Figure 4.8**), possibly by inhibiting the function of the TCR, by altering its peptide-length preference, or ablating its ability to bind pMHC targets at all. Furthermore, I showed that some amino acid substitutions may result in the gain of new specificities (**Figure 4.18**). The data presented in this chapter highlight plasticity within the TCR-pMHC interactions between the 868 TCR and its ligands.

In this chapter, I also demonstrated the potential for a patient-derived HIV-specific TCR (Sewell *et al.*, 1997) to crossreact with a cancer-specific epitope, and showed that this recognition can be removed by a single amino acid substitution.

It is important to note that all of my amino acid substitutions were made to the CDR3 loops of the 868 TCR and were designed with the aim of altering peptide specificity (Garboczi *et al.*, 1996; Garcia *et al.*, 1996; Ding *et al.*, 1998). Peptide specificity is, however, not exclusively determined by the CDR3 loops (Dunn, 2006; Cole *et al.*, 2009) and according to the published 868 TCR structure, CDR1 $\alpha$  makes two peptide contacts (Cole, Fuller, *et al.*, 2017). It would be interesting to learn how amino acid substitutions in the CDR1 and 2 loops alter the peptide recognition footprint of the 868 TCR.

My studies provide data that increase understanding of how TCR sequence can govern TCR function and endeavour towards the ultimate aim of predicting TCR function from TCR sequence. Whilst each individual piece of experimental work can provide only information regarding the model system used, they can collectively contribute as training data for bioinformatical ventures aiming at prediction of TCR specificity. One such venture, (Jurtz *et al.*, 2018), a webtool known as Net TCR, aims to predict, from TCR sequence alone, which HLA-A2-peptide complexes a selected TCR will recognise. The tool was able to successfully pick out the cognate antigen when challenged with TCRs that were included in the training data, but failed to do so for uncharacterised TCRs (Jurtz *et al.*, 2018). Predictive models such as this draw on data from databases such as the VDJ database (VDJdb) (Shugay *et al.*, 2018). The VDJdb collects data from published T cell specificity assays (Shugay *et al.*, 2018). The tool is being continuously improved as more training data becomes available. Furthermore, compared with the publication of positive interactions there is a substantial lack of negative data for this purpose. Here I provide not only predicted ligands for the TCRs but propose a novel ligand for the 868 TCR and provide conclusive negative data. TCR 24 does not see SLYNTVATL and TCR 22 recognises neither SQILTPPQL nor VSYNRIPSM. Similarly, the preliminary data in **Figure 4.19** suggests that the amino acid substitutions in TCRs 9 and

11 respectively have resulted in the gain of specificity for peptides unseen by T cells expressing the 868 parental TCR.

To conclude, my studies provide the beginnings of investigation into the plasticity of the interaction between the 868 TCR and its peptide ligands. I have demonstrated that the specificity of the 868 TCR can be modulated by amino acid substitutions and I have shown that novel peptide specificity can both arise and be ablated by single amino acid substitutions in TCR CDR3 loops. I have further demonstrated the potential for HIV/cancer crossreactivity in a naturally-occurring, patient-derived TCR. I demonstrated that T cells expressing the 868 TCR may recognise both the index HIV-1-epitope and a TAA peptide whilst simultaneously illustrating the fragility of this crossreactivity, which can be completely eradicated by a single amino acid substitution.



## 5 – Peptide cargo modulates pMHC flexibility

---

### 5.1 – Introduction

#### 5.1.1 – The flexibility of the pMHC is generally overlooked

As part of my studies I wanted to look more closely at the other side of the TCR-pMHC interaction, specifically the molecular flexibility of the pMHC-I molecule. The conditions of my Great Western 4 (GW4) Medical Research Council PhD funding required collaboration with another GW4 university, which gave me the opportunity to utilise the expertise, and technical equipment, of the Pudney laboratory. Dr Chris Pudney and colleagues regularly employ a phenomenon called the red edge excitation shift (REES, discussed below) to inspect the molecular flexibility of proteins.

Despite its well conserved binding mode (Rudolph and Wilson, 2002; Rudolph, Stanfield and Wilson, 2006) the TCR-pMHC interaction is not a rigid one, many incidences of TCR flexibility have been observed (Garcia *et al.*, 1998; Wagner *et al.*, 1999; Willcox *et al.*, 1999; Reiser *et al.*, 2002, 2003; Armstrong, Piepenbrink and Baker, 2008). Flexibility of the TCR CDR loops contributes to the crossreactive nature of the TCR, which helps provide comprehensive immune coverage to the host (Bhati *et al.*, 2014). In contrast to the wealth of evidence concerning TCR flexibility, pMHCs have previously been viewed as rigid targets for the more dynamic TCR to interact with (Rashin and Jernigan, 2016). Yet evidence to the contrary is rapidly amounting (Hawse *et al.*, 2013, 2014; Kass, Buckle and Borg, 2014; Van Hateren *et al.*, 2017).

#### 5.1.2 – The pMHC as a dynamic molecule

Two key methods that have been employed to investigate pMHC flexibility are nuclear magnetic resonance (NMR) and hydrogen/deuterium exchange (HDX).

NMR has been used study the flexible nature of proteins for decades (Snyder *et al.*, 1975; Wüthrich and Wagner, 1978). NMR is possible because many nuclei present in organic molecules have a characteristic spin and, as a result, generate their own magnetic field. When a strong, external magnetic field is applied to the molecule the dipoles of the nuclei will align, and the different types of nuclei will change the orientation of their rotational axis, or “precess” in characteristic ways (Kwan *et al.*, 2011). Pulses of electromagnetic radiation are then used to disrupt the dipole alignment, the disruption is measured by a detector and gives rise to the characteristic NMR spectra (Kwan *et al.*, 2011). NMR is able to give structural

information regarding the arrangement of the nuclei in the molecule because each nucleus will be affected by the disruption to a different degree, and thus give off a different signal. The disruption that a particular nucleus experiences depends on the chemical environment in which it sits. A nucleus in an electron dense area is shielded from a larger proportion of the disruption than a nucleus in an electron sparse area (Kwan *et al.*, 2011). Thus, NMR can provide information regarding both the nuclei that are present and their arrangement.

Importantly for studies of molecular motion, NMR is recorded over a time-scale and shifts in the spectra indicate changes in the arrangement of nuclei – in other words, conformational change. In this manner, NMR has been used to demonstrate the conformational plasticity that  $\beta_2M$  can utilise in order to accommodate different MHC alleles (Beerbaum *et al.*, 2013). NMR has also been used to highlight how conformational fluctuation of HLA-B\*35:01 can subtly differ dependent on the peptide it is presenting (Yanaka *et al.*, 2014).

HDX, on the other hand, monitors the exchange of hydrogen for deuterium in the amide backbone of a protein (Giladi and Khananshvili, 2020). Small quantities of protein (< 0.1 mg) are diluted in a  $D_2O$  buffer and HDX occurs. After a predetermined amount of time has elapsed the reaction can be quenched by altering the pH from a neutral pH to an acidic pH and reducing the temperature, this slows the reaction to an almost complete stop. The protein is then digested with protease and the fragments analysed by mass spectrometry (Giladi and Khananshvili, 2020). HDX is particularly amenable to analysis of molecular motions because the degree of HDX that can occur within a set time is dependent on the solvent accessibility and structural rigidity of the protein. HDX of different proteins (or MHC molecules holding different peptides) can be compared as a proxy for flexibility (Giladi and Khananshvili, 2020).

HDX was used by Van Hateren *et al.* to demonstrate that the peptide-receptive (unbound) MHC molecule is more dynamic than the peptide-bound complex (Van Hateren *et al.*, 2017). Using UV hydrolysable peptides, they showed that HDX increased following peptide hydrolysis, and decreased upon addition of peptide. HDX changes were observed in the peptide-binding domain, as may be expected, but also throughout the molecule - including the  $\alpha_3$  domain and  $\beta_2M$  (Van Hateren *et al.*, 2017).

Hawse *et al.* also used HDX to analyse the peptide-dependence of MHC flexibility. They showed that varying the peptide cargo alters the HDX behaviour of HLA-A2. The variation in exchange behaviour was attributed to peptide-dependant motions within the HLA-A2 binding groove (Hawse *et al.*, 2013). They suggested that varying the peptide cargo may

result in knock-on effects that alter the thermodynamic barriers between conformational states – facilitating or impeding conformational change. Differing peptide cargo might be responsible for an alteration in the range of available conformational states that exist and are accessible to the MHC molecule, or might influence the entropic cost for receptor binding (Hawse *et al.*, 2013). Ultimately, varying the peptide cargo of an MHC molecule might produce consequent variations in its free energy landscape, which may have functional consequence. Like the Van Hateren study, Hawse and colleagues demonstrate that dynamic changes occur throughout the pMHC molecule (Hawse *et al.*, 2013).

A dynamic pMHC could theoretically influence the TCR-pMHC interaction in a number of ways. A more dynamic pMHC may sample the ‘optimal’ conformation for TCR binding more frequently and therefore display improved TCR binding. In contrast, a more dynamic pMHC may confer a higher entropic cost when binding a TCR, and as a result make a more difficult target (Ayres, Corcelli and Baker, 2017). This characteristic is therefore an important consideration to the overall understanding of the TCR-pMHC interaction.

To this end, I set about furthering the work started by Van Hateren *et al.* and Hawse *et al.* in exploring pMHC flexibility. Benefiting from a collaboration with the Pudney laboratory at Bath University, I aimed to investigate whether the red edge excitation shift (REES) phenomenon and pressure/temperature ( $p/T$ ) dependant fluorimetry could probe the role of the peptide cargo in modulating pMHC flexibility, thereby adding to the experimental toolkit that can be used to explore this characteristic.

### 5.1.3 – The REES phenomenon

The REES phenomenon, first noted in 1970 (Galley and Purkey, 1970; Weber and Shinitzky, 1970), describes the red shift in the maximum emission intensity wavelength (centre of spectral mass, CSM) that is measured when a fluorophore is excited with wavelengths towards the red edge of the spectrum (Demchenko, 2002; Catici *et al.*, 2016). The observation did not adhere to the commonly accepted model known as Kasha’s rule (Demchenko, 2002). Kasha’s rule states that the emission spectra of a fluorophore will be independent of the excitation wavelength used provided that the excitation wavelength is within the absorption range for that fluorophore (Demchenko, 2002).

Kasha’s rule holds true when the fluorescence lifetime is longer than the time taken for solvent relaxation (Chattopadhyay and Halder, 2014). When a fluorophore is raised to an excited state it and the surrounding solvent (the molecule and buffer) will rapidly relax into

the lowest vibrational energy level of the excited state (Chattopadhyay and Haldar, 2014). In a fully solvated environment (such as a denatured protein or a free fluorophore) solvent relaxation is much faster than the fluorescence lifetime, so emission occurs from a fully relaxed state, following Kasha's rule. In contrast, if there are environment-induced restrictions on solvent relaxation, such as a rigid protein backbone, solvent relaxation is retarded and may be incomplete (Demchenko, 2002; Sourav and Chattopadhyay, 2007; Chattopadhyay and Haldar, 2014). If relaxation to the lowest vibrational energy does not occur within the fluorescent lifetime it results in higher energy emission following excitation at the standard wavelength (Chattopadhyay and Haldar, 2014). Different excitation wavelengths will result in different emission spectra. Increasing the excitation wavelength to longer wavelengths, and therefore using lower energy excitation, photoselects for specific solvation states within the range of available conformational states that exists for that protein (described by its free energy landscape) (Chattopadhyay and Haldar, 2014). Using longer excitation wavelengths selects for fluorophores that are in a more solvent-relaxed state as they need less energy to become activated (Chattopadhyay and Haldar, 2014). They therefore emit at a lower energy. Experimentally, increasing the excitation wavelength results in a red shift in the CSM (Chattopadhyay and Haldar, 2014).

There are a range of conformational states available to any given protein, the REES phenomenon provides information about the dynamic behaviour of a protein by giving an approximation of the breadth of this range (Demchenko, 2002; Catici *et al.*, 2016). Thus, in the context of this study we can use REES to investigate whether changing the peptide cargo alters the range of conformational states available to HLA-A2.

The REES effect was shown to demonstrate pressure/temperature ( $p/T$ ) dependence in 2016 (Catici *et al.*, 2016). The aforementioned range of available conformational states can be perturbed by non-denaturing pressures and temperatures. Temperature perturbs the range of conformational states due to differences in heat capacity between the discrete conformational states available to the protein (Akasaka, 2006). Pressure effects the range of conformational states through volume differences of the different conformational states (Akasaka, 2006). Thus, this perturbation can alter the free energy landscape of a protein (Akasaka, 2006; Catici *et al.*, 2016). An analysis tool developed by Catici *et al.* (Catici *et al.*, 2016) allows extraction of thermodynamic parameters, such as Gibb's free energy ( $\Delta G$ ), from fluorescence emission data taken under varying pressure and temperature conditions.

Using REES and  $p/T$  dependant fluorimetry, I probed the free energy landscapes of six pMHC complexes that can be recognised by T cells expressing the 1E6 TCR (see **Table 5.1**). The 1E6 TCR, which was isolated from a patient with type 1 diabetes, recognises a peptide derived from preproinsulin (PPI) (Skowera *et al.*, 2008) in the context of the diseases risk allele HLA-A2 (Nejentsev *et al.*, 2007). Our laboratory previously solved the structure of this TCR in complex with its cognate PPI-derived peptide (Bulek *et al.*, 2012). This was the first ever human autoimmune TCR-pMHC class I structure. The 1E6 T cell is known to recognise well over a million different peptides as well as, or better than, the PPI ‘index’ sequence (Wooldridge *et al.*, 2012). Structural studies showed that recognition of antigen by the 1E6 TCR is focussed around a ‘hotspot’ in the centre of the peptide (Cole *et al.*, 2016). This hotspot was used to identify pathogen-derived peptides that could bind to HLA-A2 and act as potent activators of 1E6 T cells; and thereby provide a potential triggering mechanism of the autoimmune disease (Cole *et al.*, 2016). This model system was selected due to this abundance of previously collected structural and biochemical data, and my particular interest in pathogenic-causes of autoimmune disease (explored further in **Chapter 3**). Here, I made use of these well-described ligands to examine whether the peptide cargo could tune HLA-A2 flexibility.

#### 5.1.4 – Aims

The data presented here follows on from work by Van Hateren *et al.* (Van Hateren *et al.*, 2017) and Hawse *et al.* (Hawse *et al.*, 2013), using an autoimmune-relevant system. Their work demonstrated that the dynamic regions of the MHC that are not limited to the small stretch of MHC surrounding the peptide, but occur throughout the helices and beta sheet – including in areas known to interact with the TCR and its co-receptor, CD8 (Hawse *et al.*, 2013; Van Hateren *et al.*, 2017). These studies describe two major observations that are important to my continuation with this investigation. Firstly, that the peptide cargo – small relative to the much larger MHC molecule – can modulate the dynamic behaviour of the MHC in which it is held. Secondly, that this alteration in dynamic behaviour occurs throughout the pMHC molecule and thus may have functional consequence within the TCR-pMHC interaction.

My overall aim for this project was to investigate the potential dynamic nature of the pMHC complex using fluorescent techniques, and to assess whether the peptide would influence the dynamic behaviour of the complex. To do this I planned to compare dynamic behaviour

of the same MHC molecule (HLA-A2) when presenting different peptides. The specific aims where to:

- Investigate whether the REES phenomenon may be an applicable technique to study protein flexibility within the TCR-pMHC interaction, in particular the peptide-dependent molecular dynamics of HLA-A2. It has previously been used to investigate enzymes (Catici *et al.*, 2016).
- Determine whether HLA-A2 complexes demonstrate a REE shift, thus acting as proxy to indicate that the structure has access to a range of conformational states.
- Investigate the influence of the peptide on the peptide-HLA-A2 complex as a whole, particularly whether altered peptide ligands (APLs) can influence the flexible nature of the HLA-A2 molecule in which they are held.

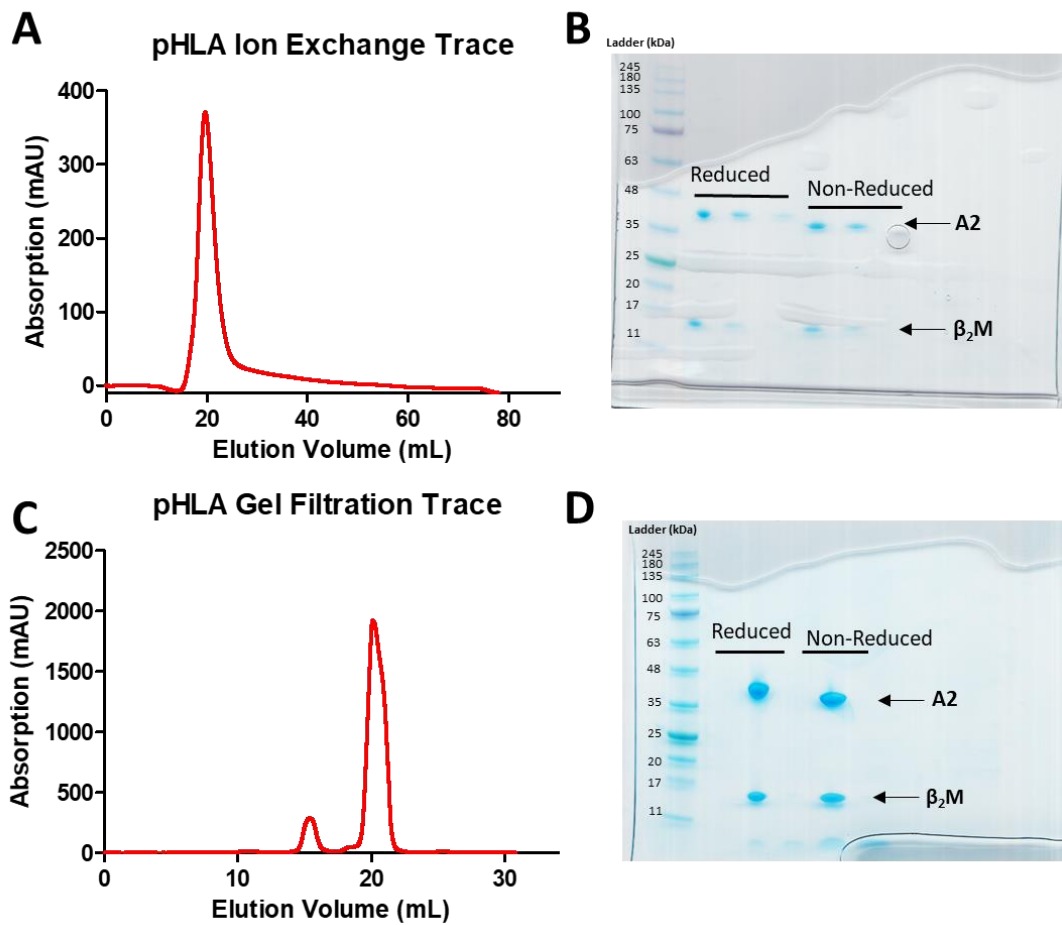
## 5.2 – Results

### 5.2.1 – Producing stable pMHC complexes

For this project six soluble pMHC complexes (**Table 5.1**) were refolded and purified. This involved: (a) expression of the HLA-A2 and  $\beta_2$ M constructs separately in *Escherichia coli*; (b) purifying the resulting inclusion bodies; (c) refolding the two protein chains together with a peptide by slow dilution of denaturing compounds; and (d) purification of the refolded protein by anion exchange chromatography followed by gel filtration size exclusion chromatography. **Figure 5.1** shows a representative example of the purification steps.

<i>Amino Acid Sequence</i>										<i>Affinity for 1E6 TCR (<math>K_D</math>, <math>\mu</math>M)</i>
A	L	W	<b>G</b>	<b>P</b>	<b>D</b>	P	A	A	A	208
R	Q	W	<b>G</b>	<b>P</b>	<b>D</b>	P	A	A	V	7.8
Y	Q	F	<b>G</b>	<b>P</b>	<b>D</b>	F	P	T	A	7.4
M	V	W	<b>G</b>	<b>P</b>	<b>D</b>	P	L	Y	V	~600
R	Q	F	<b>G</b>	<b>P</b>	<b>D</b>	W	I	V	A	44.4
R	Q	F	<b>G</b>	<b>P</b>	<b>D</b>	F	P	T	I	0.5

**Table 5.1: The peptides used in Chapter 5.** All were refolded with HLA-A\*0201 and  $\beta_2$ M. All can be recognised by T cells expressing the 1E6 TCR to various degrees as indicated (Cole *et al.*, 2016). Affinity data as previously described (Cole *et al.*, 2016). The conserved “hotspot” central GPD motif is indicated in bold text.



**Figure 5.1: Production of pure pMHC complexes.** **A)** Representative anion exchange chromatography of pMHC refolded via *E. coli* expression on an 8 mL POROS 50HQ column. After binding to the column complexes were eluted by means of a NaCl gradient. Complexes elute as a single peak, fractions corresponding to this peak are then analysed by SDS-PAGE. **B)** Coomassie-stained SDS-PAGE of anion exchange fractions. Samples are analysed under both reducing and non-reducing conditions. Two bands are seen if a pMHC is present, one at ~34 kDa corresponding to the HLA-A2 heavy chain and one at ~12 kDa corresponding to  $\beta_2M$ . **C)** Following SDS-PAGE analysis, fractions observed to have the correct bands are concentrated and further purified by size exclusion. Gel filtration chromatography using a Superdex S200 column is used for further purification of refolded pMHC. **D)** The resulting gel filtration peak is analysed on Coomassie-stained SDS-PAGE to confirm the presence of the pMHC with no contaminants. Fractions are then pooled, concentrated, and frozen at  $-80^\circ\text{C}$ .



5.2.2 – HLA-A2-ALWGPDPAAA complexes show a significant REES signal  
 In order to test whether: (a) this approach could be used to characterise pMHC molecules; and (b) my peptide-HLA-A2 complexes (**Table 5.1**) had access to multiple different conformational states, I generated soluble HLA-A2 in complex with a peptide (ALWGPDPAAA) derived from human preproinsulin. This epitope is known to be a key target on pancreatic beta cells for autoreactive CD8<sup>+</sup> T cells in type 1 diabetes patients (Nejentsev *et al.*, 2007; Skowera *et al.*, 2008; Bulek *et al.*, 2012; Wooldridge *et al.*, 2012; Cole *et al.*, 2016). Prior to all experiments in Bath, dynamic light scattering (**Section 2.2.1**) was used to confirm that freeze-thawed proteins had not aggregated or unfolded. Peaks between 1-10 nm represented refolded proteins.

For REES experiments I used tryptophan fluorescence as a molecular reporter. Tryptophan was selected as it is present in the HLA-A2 molecule (12 tryptophan in the MHC itself, with some of the selected peptides adding a 13<sup>th</sup>, see **Table 5.1**) and is the dominant natural fluorophore in proteins. As standard, tryptophan was excited at 295 nm to avoid energy transfer to any tyrosine residues in the protein (generally excited at 280 nm) (Moon and Fleming, 2011). For this reason, a wavelength range of 292-310 nm was selected for REES experiments.

**Figure 5.2** shows the effect of increasing the excitation wavelength on HLA-A2-ALWGPDPAAA tryptophan emission. Maximum emission intensity ( $\Delta\lambda_{Em}^{max}$ ) of the 13 tryptophan fluorophores within the HLA-A2-ALWGPDPAAA complex fell substantially over the 292-310 nm excitation wavelength range (**Figure 5.2A**). The complete fluorescence intensity spectra for all pMHC molecules are shown in **Figure 5.4**.

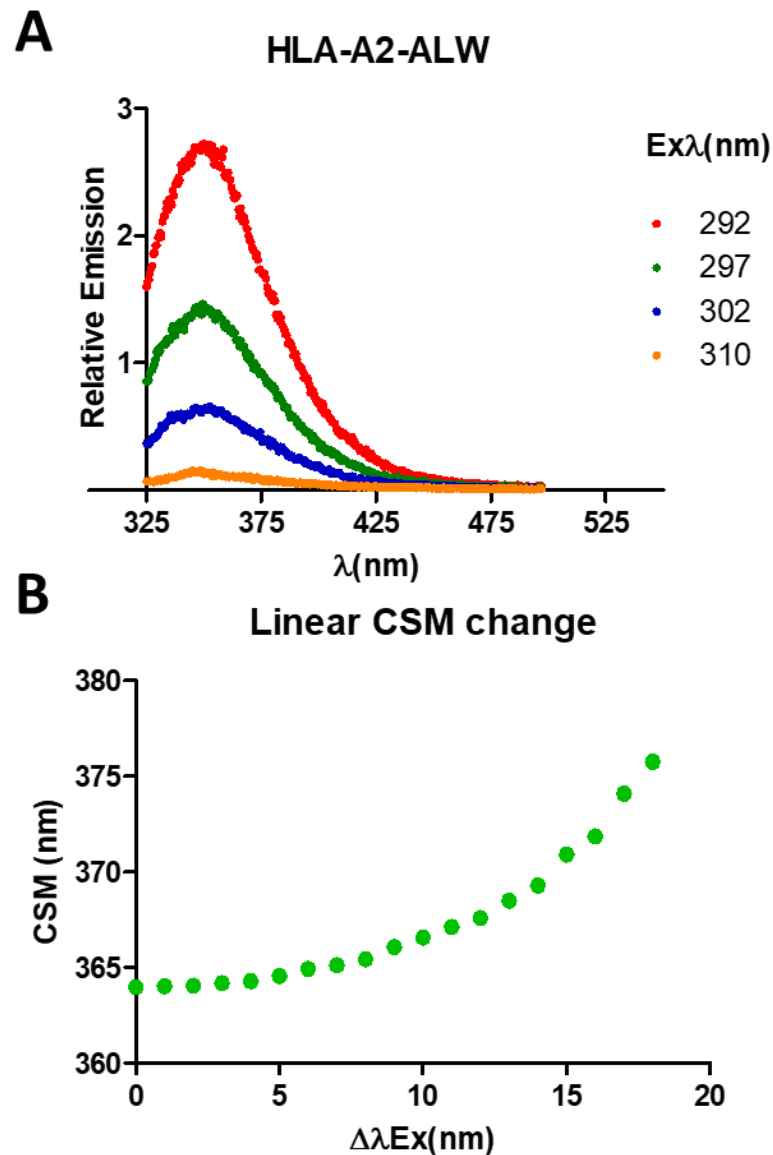
The tryptophan emission underwent a significant redshift across this excitation wavelength range (**Figure 5.2B**). This is demonstrated by a change in CSM, equation 1.

$$CSM = \frac{\sum(fi \times \lambda Em)}{\sum(fi)} \quad (1)$$

In which *fi* is the measured fluorescence intensity, and  $\lambda em$  is the emission wavelength.

For HLA-A2-ALWGPDPAAA, I observed a  $\Delta CSM$  of 11.78 nm, see **Table 5.2**. This indicates that the complex is unable to fully relax from its excited state during the fluorescent lifetime, this was expected as the complex is a quaternary folded protein structure. The shift observed also suggests that HLA-A2-ALWGPDPAAA can access a range of different solvation states, which can be photoselected for using different excitation wavelengths. These data therefore

demonstrated that the REES approach may provide a useful tool in investigating the flexible nature of pMHC complexes, and validated my continuation with the project.



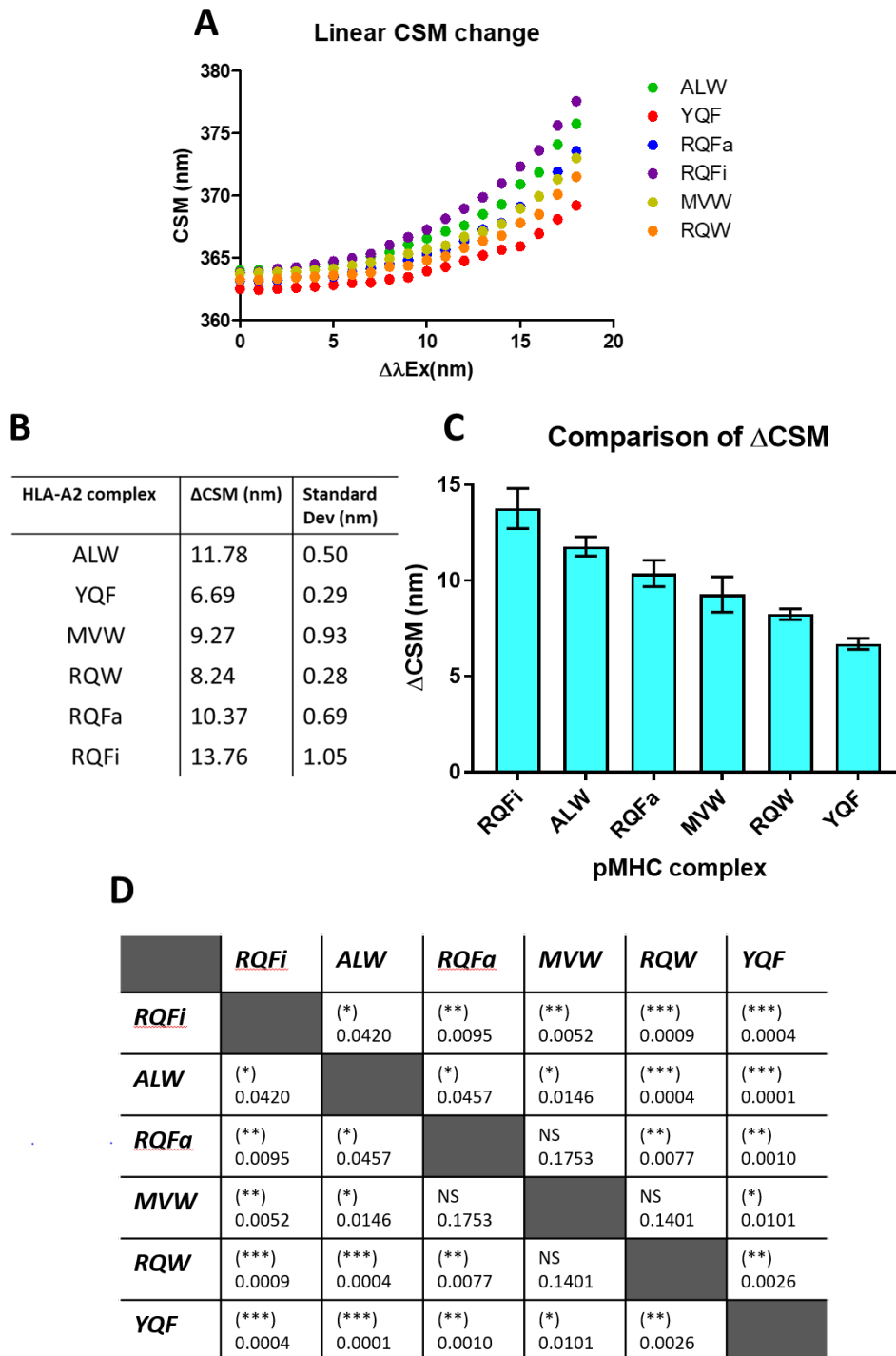
**Figure 5.2: HLA-A2 complexes show a significant REES signal. A)** Relative change in emission intensity of HLA-A2-ALWGPDPAAA (HLA-A2-ALW) complex across an excitation range of 292-310 nm,  $\Delta\lambda_{Em}^{max}$  fell significantly over the range of excitation wavelengths used. **B)** Change in CSM over the same excitation wavelength range. CSM calculated according to equation 1. ALW= HLA-A2-ALWGPDPAAA.

### 5.2.3 – $\Delta$ CSM varies with different peptides suggesting the molecular flexibility of each complex is different

My initial REES results demonstrated the value of the REES technique in probing the dynamic nature of the pMHC. The data indicated that HLA-A2-ALWGPDPAAA has access to a range of conformational states. As such, I next investigated whether the same was true for other HLA-A2 complexes, and importantly for my hypothesis, whether any differences in flexibility exist between them. I used five well characterised altered peptide ligands of HLA-A2-ALWGPDPAAA which can all be recognised by the HLA-A2-ALWGPDPAAA-specific TCR, 1E6 (Cole *et al.*, 2016) (**Table 5.1**). The complete fluorescence intensity spectra for each complex are shown in **Figure 5.4**. Each spectrum is the average of data collected in triplicate.

For each complex a significant redshift was observed in CSM, demonstrating that each complex experiences a degree of structural restriction in its ability to relax around the excited fluorophores (**Figure 5.3**). Furthermore, they are likely able to access a range of conformational states within their conformational equilibria. Importantly the  $\Delta$ CSM of the complex varied with peptide cargo, demonstrating that peptide cargo can alter the flexibility of the whole complex.  $\Delta$ CSM of 11.78, 6.69, 9.26, 8.24, 10.39, 11.77 nm were observed for HLA-A2-ALWGPDPAAA, HLA-A2-YQFGPDPAAV, HLA-A2-MVWGPDPPLYV, HLA-A2-RQWGPDPAAV, HLA-A2-RQFGPDWIVA, and HLA-A2-RQFGPDGPTI complexes respectively (**Figure 5.3**). The CSM for each complex at each excitation wavelength is shown in **Table 5.2**.

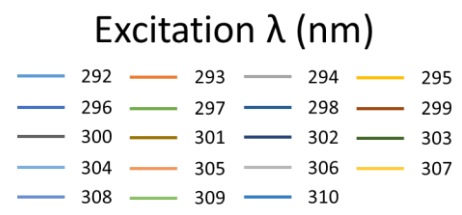
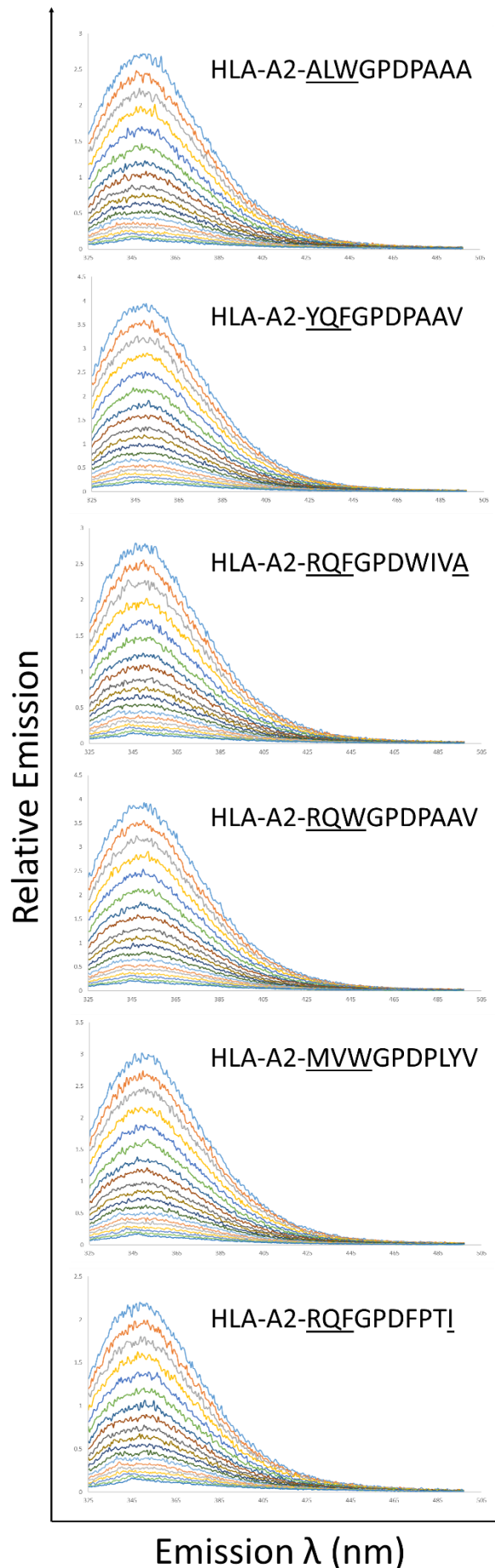
The complexes demonstrated differences in their REES behaviour, indicating that they have different dynamic characteristics. As all complexes are HLA-A2, with only a differing peptide cargo, this difference in dynamic behaviour demonstrates the influence of the bound peptide on pMHC flexibility. Furthermore, the HLA-A2 complexes are structurally similar (Cole *et al.*, 2016), and therefore the solvent accessible surface area (SASA) available for fluorescent excitation is similar, **Supplementary Table 7.1**. Thus, the difference in REES effect between the complexes observed here may indicate a difference in the availability of conformational states to each complex.



**Figure 5.3:  $\Delta$ CSM varies for pMHC complexes carrying different peptide cargo. A)** Change in CSM over the same excitation wavelength range for each pMHC complex. CSM calculated according to equation 1. **B)**  $\Delta$ CSM of 11.78, 6.69, 9.26, 8.24, 10.39, 11.77 nm were observed for ALW, YQF, MVW, RQW, RQFa, and RQFi complexes respectively. **C)**  $\Delta$ CSM data for each pMHC complexes as a histogram allows visual depiction of the differences. **D)** Statistical significance for observed differences according to unpaired two-sample T-test. RQFi = HLA-A2-RQFGPDGPTI. ALW= HLA-A2-ALWGPDPAAA. RQFa = HLA-A2-RQFGPDWIVA. MVW = HLA-A2-MVWGPDPPLYV. RQW = HLA-A2-RQWGPDPAAV. YQF = HLA-A2-YQFGPDPAAV.

	ALW	YQF	RQFa	RQFi	MVW	RQW
dEx(nm)						
0.0001	363.9714	362.5138	363.1715	363.8147	363.7469	363.2583
1	364.0236	362.4368	363.166	363.8664	363.7908	363.2389
2	364.0555	362.5134	363.2079	364.1293	363.8601	363.3324
3	364.1857	362.6098	363.4577	364.2485	363.8905	363.4188
4	364.2868	362.6924	363.4963	364.4822	364.0316	363.4759
5	364.5501	362.8325	363.5149	364.7304	364.1322	363.5948
6	364.9223	362.9942	363.7949	364.9785	364.384	363.6811
7	365.117	363.0174	364.1335	365.3159	364.6276	363.8299
8	365.436	363.2724	364.4807	366.0476	364.9234	364.2925
9	366.0682	363.4398	364.8083	366.6646	365.3219	364.3782
10	366.5828	363.9184	365.2722	367.2613	365.6973	364.8083
11	367.1234	364.2783	365.6192	368.1449	365.9969	365.1253
12	367.5902	364.7599	366.3532	368.9496	366.669	365.8061
13	368.493	365.2083	367.2738	369.8616	367.1111	366.3699
14	369.2997	365.6555	367.7869	370.9683	367.7268	366.7893
15	370.9056	365.9251	369.0839	372.3284	368.9611	367.8077
16	371.8568	366.9338	369.9415	373.6321	369.935	368.4971
17	374.094	368.089	371.9123	375.6312	371.3041	370.0844
18	375.7557	369.2044	373.5568	377.5811	373.0154	371.5005

**Table 5.2: The change in CSM over the 292-310 nm excitation range for each protein.** Each peptide-HLA-A2 complex demonstrated a red-shift in the maximum emission intensity when excited with wavelengths of decreasing energy. Table shows the CSM measured at each excitation wavelength within the range of 292-310 nm. RQFi = HLA-A2-RQFGPDGPTI. ALW= HLA-A2-ALWGPDPAAA. RQFa = HLA-A2-RQFGPDWIVA. MVW = HLA-A2-MVWGPDPPLYV. RQW = HLA-A2-RQWGPDPAAV. YQF = HLA-A2-YQFGPDPAAV.



**Figure 5.4: Fluorescence intensity spectra for each HLA-A2 complex.** Protein-intrinsic tryptophan fluorescence was excited using increasing excitation wavelengths (ranging 292-310) and the emission spectra measured using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). Spectra are the average of triplicate collection.

#### 5.2.4 – Peptide cargo modulates energy barriers between conformational states

Thus far, I had shown that the REES phenomenon could be used to investigate the dynamic behaviour of the pMHC, and that this behaviour varied dependant on the peptide cargo. Each complex demonstrated a REE shift, but the degree of the shift was different for different complexes. This indicated that each complex had access to a range of conformational states, but that this range is likely different for different complexes.

Prompted by this, I hoped to further investigate the flexible nature of the pMHC complexes. I was fortunate to benefit from the extensive expertise of Dr Dragana Catichi and Dr Chris Pudney from my collaborator group who not only taught me to perform the experiments described in this chapter, but employed their novel analysis tool (see **Supplementary Equation 7.1**, published only a few months before I began my PhD) to my data to extract critical thermodynamic information (Catichi *et al.*, 2016).

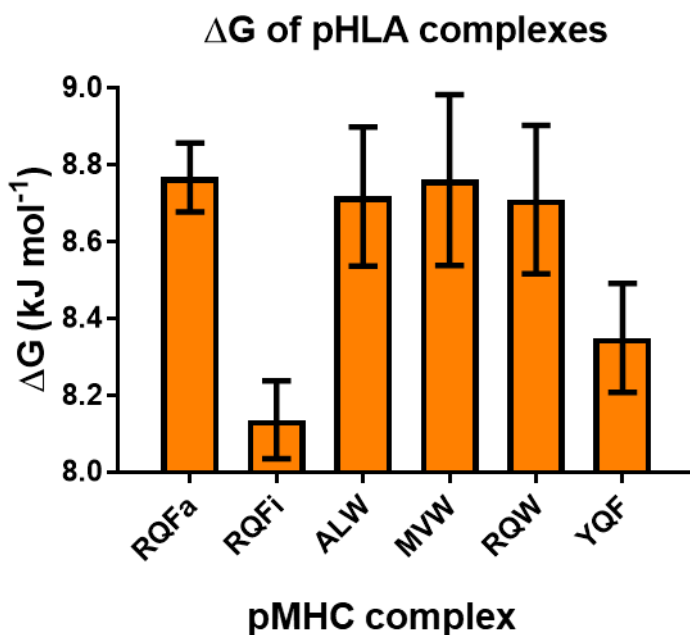
For use with the Pudney analysis tool, I next collected emission spectra from each complex, excited at the standard wavelength, over a range of non-denaturing varying pressures and temperatures. The full *fi* data is shown in **Figures 7.4 to 7.9**. Each spectrum, at each pressure and each temperature, was collected in triplicate.

I hoped to use this pressure/temperature (*p/T*)-dependant fluorimetry to probe how easily a pMHC molecule might switch between the conformational states available to it. As demonstrated by Catichi *et al.* (Catichi *et al.*, 2016), varying the pressure and temperature of the system alters the range of the conformational states available to the molecules, and allows extraction of a number of thermodynamic parameters using the Pudney group analysis tool (Catichi *et al.*, 2016).

Fluorescence intensity data collected under varying, non-denaturing pressures and temperatures was inputted into the analysis tool. Analysis kindly performed by Dr Chris Pudney. The tool generates a number of thermodynamic parameters – some of which can be used as proxy for flexibility. Of particular interest to this study was a  $\Delta G$  value for each complex,  $\Delta G$  describes the energy barriers between conformational states experienced by the complex. **Figure 5.5** shows the predicted  $\Delta G$  values generated by this method for each pMHC complex.  $\Delta G$  values ranged from 8.1 kJ mol<sup>-1</sup> (HLA-A2-RQFGPDGPTI) as the lowest) to 8.7 kJ mol<sup>-1</sup> (HLA-A2-RQFGPDWIVA) as the highest). Each complex had a different predicted  $\Delta G$  value, some of which differed outside of standard error. This indicates that the energy barriers faced by the pMHC complexes attempting to change conformational states vary

dependent on the peptide cargo. For example, it may be easier for HLA-A2-RQFGPDGPTI to change conformational state, than for HLA-A2-RQFGPDWIVA. These data highlight that the relatively small string of ten amino acids sat in the binding groove can have a measurable impact on the entire ~45 kDa complex.

pMHC complex	$\Delta G$	SE
RQFa	8.76726	0.09019
RQFi	8.13560	0.10125
ALW	8.71765	0.18125
MVW	8.76007	0.22244
RQW	8.70993	0.19335
YQF	8.34947	0.14200

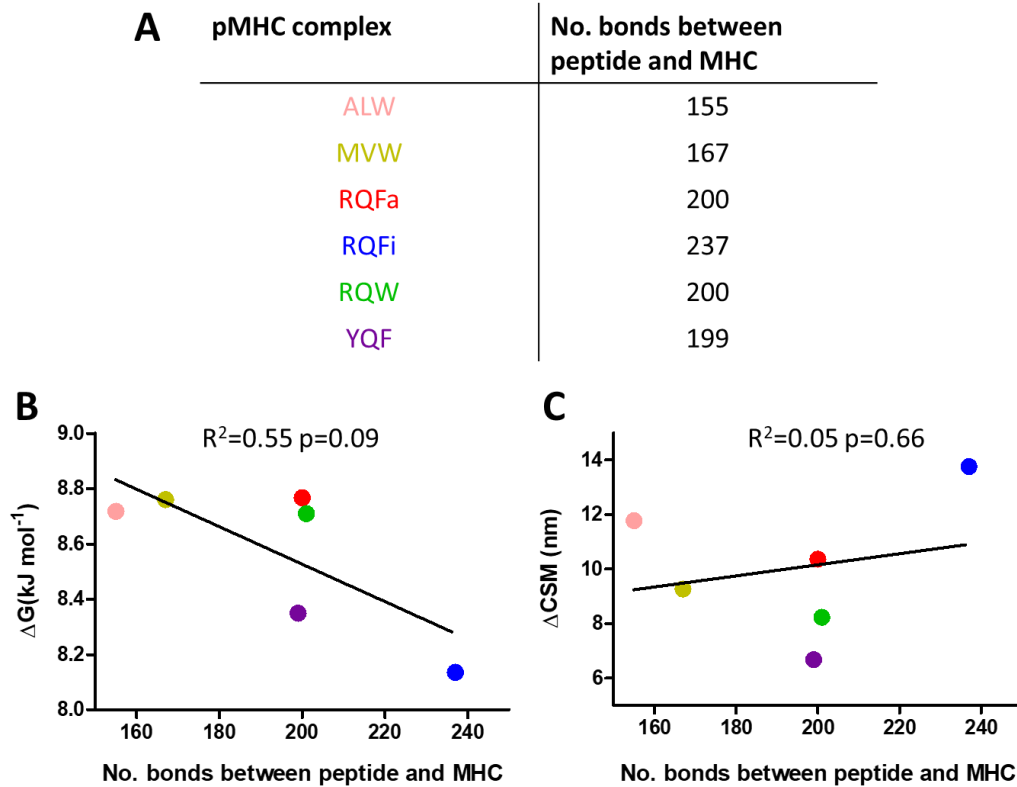


**Figure 5.5:  $\Delta G$  varies for pMHC complexes carrying different peptide cargo.**  $\Delta G$  values generated from  $p/T$ -dependent fluorimetry data inputted into the Pudney group analysis tool demonstrate that  $\Delta G$  varies dependent on the peptide cargo carried by the HLA-A2 molecule. Some of this variation is outside of error suggesting that these pMHC complexes face true differences in the thermodynamic barriers between conformation states. RQFi = HLA-A2-RQFGPDGPTI. ALW= HLA-A2-ALWGPDPAAA. RQFa = HLA-A2-RQFGPDWIVA. MVW = HLA-A2-MVWGPDPLYV. RQW = HLA-A2-RQWGPDPAAV. YQF = HLA-A2-YQFGPDPAAV. Analysis performed by Dr Chris Pudney.



5.2.5 – Peptide-MHC contacts might drive global flexibility of the complex  
The data I have presented in this chapter supports previous reports (Hawse *et al.*, 2013; Van Hateren *et al.*, 2017) that the pMHC is a flexible structure, and that that flexibility can be tuned by the peptide cargo it carries. As such I wondered if the degree of connectivity between the peptide and the MHC molecule itself, namely the number of bonds formed between them, could be a driving force behind these observations.

CCP4 analysis performed by Aaron Wall revealed that, amongst the 6 pMHCs discussed here, the peptide sequence significantly impacted the number of bonds between the MHC and its cargo, **Figure 5.6A**. The cognate, PPI-derived peptide ALWGPDAAA formed 155 bonds (defined as atoms that are less than 4 Å apart) with HLA-A2, whereas RQFGPDGPTI forms over 80 more. However, linear regression analysis of the data revealed a non-significant correlation between  $\Delta G$  and the number of bonds between peptide and MHC, **Figure 5.6B**, (correlation according to linear regression,  $p=0.09$ , R square 0.55). Furthermore, the range of the available conformational states (as defined by  $\Delta CSM$ ) appears to be similarly unaffected by the number of bonds between MHC and peptide cargo, **Figure 5.6B**.



**Figure 5.6: Peptide-MHC contacts do not correlate with flexibility of the complex.** **A)** The number of bonds between peptide cargo and the HLA-A2 molecule for each complex was quantified using CCP4 software by Aaron Wall. **B)** A notable, but non-significant trend was noted between number of bonds and the  $\Delta G$  (correlation according to linear regression,  $p=0.093$ , R square 0.55) but **C)** number of bonds did not influence  $\Delta CSM$ . RQFi = HLA-A2-RQFGPDGPTI. ALW= HLA-A2-ALWGPDPA<sup>AAA</sup>. RQFa = HLA-A2-RQFGPDWIVA. MVW = HLA-A2-MVWGPDPLYV. RQW = HLA-A2-RQWGPDPA<sup>AAV</sup>. YQF = HLA-A2-YQFGPDPA<sup>AAV</sup>.

## 5.3 – Discussion

In general, crystal structures of numerous pMHC complexes have strong similarities (Wieczorek *et al.*, 2017). This has understandably propagated a view that the pMHC is a rigid structure (Wilson and Fremont, 1993). However, conformational plasticity within the pMHC is being increasingly noticed. Thus, it is likely that there is inconspicuous conformational plasticity within the pMHC that is undetectable by standard crystallographic methods.

### 5.3.1 – Evidence for a flexible pMHC

In addition to the work discussed in **Section 5.1.2** (Hawse *et al.*, 2013; Van Hateren *et al.*, 2017), differences in conformational plasticity of the pMHC molecule have been demonstrated between different, but closely related MHCs, with the observed dynamical difference being attributed to the polymorphism (Pöhlmann *et al.*, 2004; Bailey *et al.*, 2014). In this study, I compared the same MHC molecule with varying peptide cargo, in an attempt to investigate what effect the peptide may have on the complex as a whole.

Peptide-dependant conformational fluctuations in the context of HLA-B\*35:01 have been demonstrated using NMR spectroscopy (Yanaka *et al.*, 2014). NMR is one of the most powerful tools for studying protein flexibility. Yet the size of the pMHC molecule can present challenges (Hawse *et al.*, 2013). As such, we and others have sought different techniques for investigating the dynamics of the pMHC molecule.

Two key studies of pMHC dynamics by Hawse *et al.* and Van Hateren *et al.* both used HDX to demonstrate flexibility within the pMHC (Hawse *et al.*, 2013; Van Hateren *et al.*, 2017). Peptide-dependant effects on pMHC dynamics have the potential to influence the interaction of that pMHC with its TCR (Borbulevych *et al.*, 2009; Hawse *et al.*, 2013). As suggested by Hawse *et al.* the knock-on effects of the peptide cargo might alter the thermodynamic barriers between conformational states – facilitating or impeding conformational change. Differing peptide cargo might be responsible for an alteration in the range of available conformational states that exist and are accessible to the pMHC molecule, contributing to which pMHCs can be recognised and which cannot. Some peptides may restrict the available conformational states in such a way to limit or impede access to conformations capable of binding the TCR by raising the energy barriers between states. In addition, peptide-dependent dynamics might influence the entropic cost for receptor binding (Hawse *et al.*, 2013). Ultimately, varying the peptide cargo of an MHC molecule might produce consequent variations in its free energy landscape.

### 5.3.2 – REES as a tool for exploring pMHC flexibility

With the aim of further probing the role of the peptide in MHC flexibility, I turned to the REES phenomenon. REES has been previously used to qualitatively measure changes to the free energy landscape of a protein, which describes the range of available conformational states that exists for that protein (Catici *et al.*, 2016). Catici *et al.* used REES to describe the ‘ruggedness’ of the free energy landscape of a metabolic enzyme (Catici *et al.*, 2016).

REES has also been used to demonstrate rigidity within an endocrine receptor (Mishra and Jha, 2019). Having the advantage of a single-tryptophan molecule, Mishra and Jha were able to show that a very specific loop of the molecule was held in a motionally-restricted conformation, as it displayed a REE shift of just 4 nm (Mishra and Jha, 2019). The ability to describe the dynamic behaviour of such a small region of the molecule demonstrates the strength of REES as a structural biology tool.

Here, I used the REES phenomenon to probe the free energy landscape of six HLA-A2-peptide complexes. The six complexes each carried different peptide cargo. The peptides chosen were all altered peptide ligands based off a fragment of preproinsulin that is presented to the 1E6 TCR and implicated in T1D (Cole *et al.*, 2016), **Table 5.1**. First, using HLA-A2-ALWGPDPAAA, I demonstrated that the REES phenomenon was applicable to the study of pMHCs. HLA-A2-ALWGPDPAAA displayed a clear red-edge shift implying that the complex cannot fully relax within the fluorescent lifetime and can access a range of different solvation states, which can be photoselected for by increasing the excitation wavelength used.

Repeating this experiment for all six HLA-A2 complexes I showed that whilst all complexes demonstrated a shift, there were differences as to the degree of the shift. As all complexes are HLA-A2, with differing peptide, this difference in dynamic behaviour may be attributed to the influence of the peptide cargo. Given that the HLA-A2 complexes are structurally similar, and therefore the SASA for fluorescent excitation is similar, the difference in REES effect between the complexes observed here may indicate a difference in the availability of conformational states to each complex. This supports the peptide-dependant effect on pMHC motion that was observed previously (Hawse *et al.*, 2013; Van Hateren *et al.*, 2017).

This suggestion that the free energy landscape might vary between HLA-A2 complexes prompted further investigation, for which I was directed to *p/T*-dependant fluorimetry. The pressure-dependence of the REES effect had recently been demonstrated for the first time by the Catici *et al.* (Catici *et al.*, 2016), who showed that *p/T*-dependant fluorimetry is a

powerful tool for tackling issues in structural biology, derived from the unwaveringly static nature of crystallographic data (Catici *et al.*, 2016).

Using non-denaturing pressures and temperatures it is possible to perturb the range of conformational states that is available to the protein being studied. The resulting fluorescence intensity data can be inputted into an analysis tool developed by the Pudney group (Catici *et al.*, 2016) to extract parameters that describe the thermodynamic behaviour of proteins. Of particular interest to my study was the  $\Delta G$  values, describing the energy barriers each complex experiences between the conformational states available to it. I collected emission spectra for each complex under varying non-denaturing pressures and temperatures. These data were used to calculate a singular predicted  $\Delta G$  value for each complex. These values represent a proxy estimate for the ease with which a particular complex can move between its available conformational states.  $\Delta G$  values differed between the six pMHC complexes, suggesting that the complexes might face different energy barriers between conformational states depending on the peptide they present.

The peptide, being a single short chain of amino acids and not a quaternary folded structure like the MHC itself, is well-documented as being a flexible component of the interaction. It can be both flattened into (Tynan *et al.*, 2007) and pulled out of the peptide binding groove (Madura *et al.*, 2015). I wondered whether the peptide influenced the MHC flexibility by 'pulling' it into a state of greater molecular motion, and whether increased contacts between peptide and MHC would result in a more flexible complex. CCP4 analysis revealed a non-significant but notable negative trend between  $\Delta G$  and the quantity of bonds between the two parts of the complex. This would suggest that increased connectivity between the MHC and its peptide cargo favors a more dynamic molecule, however more data is required to verify this conjecture.

Whilst there are numerous contributing factors to the efficacy of a TCR-pMHC interaction, I have demonstrated that pMHC flexibility could potentially be one of them and that the REES phenomenon might be employed to investigate it.

### 5.3.3 – Summary

In summary, I showed that the REES phenomenon could be used as a tool to investigate the dynamic behaviours of immune proteins. Furthermore, these data demonstrated that pMHC complexes are subject to peptide-driven modulation of the free energy landscape. Altering the peptide cargo of the same MHC molecule can modify the free energy barriers between

the conformational states that are accessible to that molecule (described by  $\Delta G$ ). These data would benefit from corroboration by other methodologies such as NMR and HDX.

Furthermore, the data presented in this chapter show HLA-A2-RQFGPDGPTI as having the greatest  $\Delta\text{CSM}$ , 13.76 nm (see **Figure 5.3**), suggesting it has the widest breath of available conformational states (states in different stages of folding can be photoselected using lower energy excitation). HLA-A2-RQFGPDGPTI also had the lowest  $\Delta G$  value, 8.13 kJ mol<sup>-1</sup>, suggesting it experiences the lowest energy barrier between conformational states of the six complexes I studied. Together this would suggest that HLA-A2-RQFGPDGPTI both has the widest range of available conformational states, and finds it easier to switch between them. It is fairly reasonable, therefore, to say that HLA-A2-RQFGPDGPTI is the most flexible of the complexes assessed here. However, HLA-A2-YQFGPDPAAV had the smallest  $\Delta\text{CSM}$ , 6.69 nm, suggesting it has the narrowest breath of available conformational states. Yet,  $p/T$  analysis predicted it to have the second lowest  $\Delta G$  value, 8.35 kJ mol<sup>-1</sup>, suggesting it experiences the second lowest energy barrier between conformational states. This seemingly contrasting information implies that the concept of 'molecular flexibility' is more complicated than originally appreciated during the conception of this project. A complex can have access to a relatively limited range of conformations but move easily between them, and thus should still be considered flexible.

The data presented in this chapter suggest that, within the 1E6 TCR-PII-HLA-A2 interaction, the peptide cargo can influence the molecular dynamics of the pMHC complex. Whilst it is beyond the scope of these data to demonstrate a biologically relevant functional consequence for this observation, it is none-the-less interesting to postulate. I, and others, have shown that the peptide – small relative to the much larger MHC molecule – can have a measurable impact on the complex as a whole. This could, at least in part, help to explain why small changes in peptide sequence can alter the T cell response that can be triggered (Valmori *et al.*, 1998; Cole *et al.*, 2010; Madura *et al.*, 2015)

Differences in the flexibility of the pMHC target may influence the ability of the TCR to bind in a number of different ways. A more flexible target may sample the optimal conformation for binding more frequently, and thus form higher affinity interactions. On the other hand, in other systems, a more rigid pMHC molecule – one that naturally favours the optimal conformation for binding - might provide a superior TCR target, as the entropic cost required to bind might be lower.

I have shown that REES and  $p/T$ -dependent fluorimetry can be added to the increasing number of tools useful for investigating the dynamic nature of immune proteins, supporting existing techniques such as NMR and HDX. Furthermore, these data demonstrate that the peptide can tune MHC flexibility. The full functional consequences of these observations remains to be seen.

## 6 – General Discussion

---

### 6.1 – Understanding the TCR-pMHC interaction

In this thesis, I have used three clinically relevant TCR-pMHC interactions to investigate the biochemical foundation underpinning the highly plastic interaction between CD8<sup>+</sup> T cells and their targets. In **Chapter 3**, I profiled the peptide recognition repertoire of an auto-reactive, T1D-implicated T cell clone, InsB4. I demonstrated that the InsB4 T cell clone could strongly recognise pathogen-derived peptides and proposed three viral peptides that could potentially have broken immune tolerance and activated this T cell clone in the T1D patient from which it was isolated. In **Chapter 4**, I investigated the contribution of TCR CDR3 sequence to the TCR-pMHC interaction. Using the same technique as in **Chapter 3**, I demonstrated that the same crossreactive characteristic that enabled the autoimmune activities of InsB4, allowed for cross-disease crossreactivity in another patient-derived TCR, 868. I showed that the HIV-specific 868 TCR could recognise and respond to a TAA, potentially accounting for the observed response to a number of cancer cell lines. I subsequently highlighted the fragile and extremely specific nature of TCR-peptide recognition by demonstrating that recognition of the TAA in question could be completely eradicated by a single amino acid substitution in the CDR3 $\beta$ . I further demonstrated that new specificities could be conferred on the TCR using other single amino acid substitutions. Finally, in **Chapter 5**, I explored the potential contribution pMHC dynamics to the TCR-pMHC interaction. I outlined a novel technique for use in exploring pMHC dynamics, and found that pMHC dynamics can be altered by its peptide cargo. Full understanding of plasticity within the TCR-pMHC, to the extent where interactions can be predicted, will require detailed understanding of both complexes in the interaction. Future predictive tools will need to consider both TCR sequence, and the dynamic characteristics of potential pMHC targets.

### 6.2 – Investigations into the TCR-pMHC interaction

In this thesis, I utilised the strength of combinatorial peptide library (CPL) screens, to uncover TCR peptide preferences. CPL screens are discussed in detail in **Section 3.1.2**. Their suitability for this work lies in their almost unparalleled ability to predict novel TCR epitopes without any prior knowledge of likely candidates, almost the entire peptide universe can be scrutinised in a single assay. CPL screens provide large quantities of data to fuel antigen prediction tools such as NetTCR (Jurtz *et al.*, 2018). The necessary exclusion of cysteine from



the non-fixed amino acid pools however, may limit discovery of peptides with more than one cysteine residue, warranting the use of other investigative methods to explore the TCR-pMHC interaction on a smaller scale, such as peptide display systems.

### 6.2.1 – Display systems for studying the TCR-pMHC interface

A peptide display system was used to demonstrate the impact of CDR3 amino acid substitutions in a 2005 study in which Li *et al.* subjected two HLA-A2-restricted TCRs to affinity maturation – the A6 TCR which is specific for the HTLV-1-derived Tax peptide (LLFGYPVYV, residues 11-19) (Yi Li *et al.*, 2005), and the 1G4 TCR specific for NY-ESO-1 peptide (SLLMWITQC, residues 157-165) (Jäger *et al.*, 1998). Libraries of mutant TCRs were generated containing amino acid substitutions in their CDR loops. These were displayed on the surface of M13 phage by conjugation to a phage surface protein, and subjected to repeated rounds of selection and competitive binding assays to identify variants with enhanced affinity for the pMHC target (Yi Li *et al.*, 2005). In **Chapter 4**, I used amino acid substitutions at key CDR3 contact positions to alter the peptide recognition repertoire of a TCR. In 2013, the structure of a high affinity A6 variant was solved in complex with Tax-HLA-A2, it showed how just 4 altered residues in the CDR3 $\beta$  loop enabled enhanced contacts with the peptide that resulted in a nearly 1000-fold increase in the affinity of the interaction (Cole, 2013).

In **Chapter 4**, amino acid substitutions were made in the CDR3 loops of the 868 TCR. Whilst the CDR3 loops generally predominate peptide contacts, it is important to note that amino acid alterations in the other CDR loops can also generate enhanced TCRs. Dunn *et al.* used phage display to show that amino acid substitutions in the CDR2 loops, which predominantly contact the MHC (Rudolph, Stanfield and Wilson, 2006), could enhance the affinity within the 1G4-A2-NY-ESO-1 interaction without apparent loss of specificity (Dunn, 2006). My own laboratory also demonstrated that CDR2 amino acid substitutions could be used to enhance TCR affinity whilst maintaining exquisite peptide specificity (Cole *et al.*, 2014). Thus, it would be interesting to evaluate the impact of amino acid substitutions in other CDR loops of the 868 TCR in future to gain a more complete understanding of how sequence relates to function within the context of the 868 TCR.

In addition to phage display systems, modified TCRs can be displayed for selection on yeast cells and mammalian cells, which offer more human-like post-translational modifications (Nguyen, Le and Maynard, 2018).

Gee *et al.* used yeast display to uncover the pMHC ligands for 'orphan' TCRs. Orphan TCRs were multimerised using MACS beads and incubated with a yeast display library featuring over 400 million unique peptides. The peptides were displayed by single chain HLA-A2- $\beta_2$ M complexes that were linked to yeast surface protein Aga2. Yeast displaying target pMHC were pulled out by repeated rounds of magnetic separation (Gee *et al.*, 2018). This technique represents a powerful way to screen large peptide libraries without prior knowledge of the likely ligand. However, Gee *et al.* restricted their library at the P2 and C-terminal anchor positions to reflect the anchor preference of the MHC molecule they were investigating (Gee *et al.*, 2018). Adams *et al.* also fixed the peptide anchors when investigating a H-2Ld-restricted TCR (Adams *et al.*, 2016). Restricting the anchor residues during peptide display could fail to identify physiologically relevant epitopes that do not use the favoured anchor residues such as the HLA-A2-restricted Melan-A peptide EAAGIGILTV (Speiser *et al.*, 2008; Madura *et al.*, 2019). Indeed, my own group showed that the EAAGIGILTV peptide acted as a much stronger agonist for T cells expressing the MEL5 TCR than the position 2 heteroclitic variant peptide, ELAGIGILTV, once used in the several clinical trials (Ayyoub *et al.*, 2003; Liénard *et al.*, 2004; Speiser *et al.*, 2008). Recognition of the preproinsulin (PPI)-derived epitope ALWGPDAAA by the 1E6 TCR provides a further example of how a weak TCR anchor can generate a substantially more potent peptide antigen. Substitution of the HLA-A2 P2 anchor residue from leucine to glutamine (representing a substitution from the optimal anchor residue to a poor HLA-A2 anchor residue) generated one of the best recognised singly substituted variant of ALWGPDAAA (see figure 2 here, (Bulek *et al.*, 2012)). Indeed all the best agonists of the 1E6 TCR contained the suboptimal glutamine anchor at P2 (Cole *et al.*, 2016) that would not have been discovered using a display system with fixed optimal MHC anchors.

Display systems have been widely and successfully used to identify TCR epitopes (Crawford *et al.*, 2004; Birnbaum *et al.*, 2014; Adams *et al.*, 2016; Gee *et al.*, 2018) and offer the advantage of being able to freely incorporate all 20 proteinogenic amino acids (Gee *et al.*, 2018). Selection is, however, usually limited by the size of the display library, often designed around or bias towards a known epitope (Crawford *et al.*, 2004; Birnbaum *et al.*, 2014; Gee *et al.*, 2018). CPL screens are not limited by the size of the peptide library so do not have to incorporate such biases.

6.2.2 – Rational design based on knowledge of the TCR-pMHC interaction  
Affinity-enhanced TCRs that are used in clinical trials have not been through the rigours of thymic selection and therefore may have the capacity to recognise self-antigens. This possibility was tragically demonstrated during trials of an enhanced TCR that targeted the multi-cancer associated MAGE-A3. Adoptive transfer of patient T cells expressing a MAGE A3-specific TCR, that had been produced by phage display, resulted in undesirable off-target crossreactivity leading to fatalities (Cameron *et al.*, 2013; Linette *et al.*, 2013; Raman *et al.*, 2016). These unfortunate events followed previous studies where a different MAGE A3-specific TCR had induced cancer-regression and neurological toxicity (Morgan *et al.*, 2013). In a further example, affinity enhancement of the MART-1-specific DMF5 TCR led to a 400-fold increase in its affinity for the HLA-A2-ELAGIGILTV epitope, but brought about additional self-reactivity (Hellman *et al.*, 2019). Epitopes that were not seen, or seen only weakly by the wild-type TCR were strong ligands for the mutant, such as the AVGIGIAVV peptide from the human CD9 protein, and the IGGIGTVPV epitope from human elongation factor 1 $\alpha$  (Hellman *et al.*, 2019). These studies highlight the potential dangers of T cell crossreactivity and how important it is to screen for it prior to clinical use.

The danger of off-target crossreactivity led to suggestions that, instead of random mutagenesis, more rational TCR alterations should be made. In 2010, Bennett *et al.* collated previously described public TCRs that recognise the HIV-1 epitope HLA-A2-SLYNTVATL, and designed CDR mutants based on motifs present in existing TCRs (Bennett *et al.*, 2010). This more rational approach is based on two assumptions: 1) that the prevalence of public TCRs is indicative of their efficacy at recognising the desired antigen and; 2) that TCRs that are 'public' are less likely to encompass dangerous crossreactivities (Bennett *et al.*, 2010). The study resulted in mutant TCRs with moderately increased affinity for the HLA-A2-SLYNTVATL epitope, and a broader recognition of SLYNTVATL escape mutants (Bennett *et al.*, 2010).

The above described studies demonstrate the importance of understanding the TCR-pMHC interaction for the rational design of therapeutics. In **Chapter 4**, I used structural data published by Cole *et al.* (Cole, Fuller, *et al.*, 2017) to rationally design a series of mutant TCRs based on the HIV-specific 868 TCR. This involved substituting key TCR-peptide contact residues with the hope, not of increasing the specificity of the 868-A2-SLYNTVATL interaction but of altering the specificity of the TCR completely.

### 6.2.3 – Further methods for peptide discovery

Understanding how TCRs contribute to disease requires knowledge of what they recognise so it is no surprise that several new techniques for discovering ligands for so-called “orphan TCRs” have arisen during the course of my PhD studies as described below.

#### 6.2.3.1 – T-Scan

T-Scan is a method for epitope discovery that was described by Kula *et al.* in 2019 (Kula *et al.*, 2019). T-Scan uses lentiviral delivery of genome-wide protein libraries, peptides are then processed endogenously and presented on MHC-I molecules at the surface of the cell, this gives the method a distinct advantage over CPL screens and peptide display methods as T-scan takes into account endogenous processing of peptides (Kula *et al.*, 2019). In **Chapter 3**, I compensated for this caveat by validating any peptide of interest by lentiviral transfer of the parent gene, to determine if that antigen was genuinely processed and presented. In T-Scan, the target cell library is co-cultured with T cells. Like CPL screening and unlike peptide display systems, T-scan requires both binding and functional activation for a positive readout. The library cells carry a reporter of granzyme B activity, a protein that fluoresces following cleavage by granzyme B, allowing the target cells to be sorted from the non-target cells via FACS (Kula *et al.*, 2019). Identified target cells are then sequenced to determine the peptide antigen they carry (Kula *et al.*, 2019).

#### 6.2.3.2 – Trogocytosis

In 2019, David Baltimore’s group described a method of T cell ligand discovery that draws on the phenomenon of trogocytosis. Trogocytosis describes the transfer of membrane proteins when T cells and target cells are brought into close proximity by the TCR-pMHC interaction (Joly and Hudrisier, 2003; G. Li *et al.*, 2019). Using Jurkat cells transduced with TCRs of interest, and K562 antigen-presenting cells expressing single chain pMHC they were able to identify TCR epitopes based on the presence of T cell surface markers on the K562 cells (G. Li *et al.*, 2019). However, the system has a number of artificialities that limit its physiological relevance. Like CPL screens, it does not rely on endogenous peptide processing. Unlike CPL screens, it uses modified, single chain pMHCs and supraphysiological levels of expression were required (G. Li *et al.*, 2019).

#### 6.2.3.3 – SABR

David Baltimore's group also developed a chimeric antigen receptor called a signalling and antigen-presenting bifunctional receptor (SABR) (Joglekar *et al.*, 2019). SABRs consist of a MHC molecule associated with CD3 $\zeta$  and CD28, upon binding a TCR the CD3 $\zeta$  dimerise and induce T cell-like signalling in the target cell (Joglekar *et al.*, 2019). This signalling promotes upregulation of early activation markers such as CD69 and CD107a which can be used to identify target cells (Weiss and Littman, 1994). CD3 signalling also results in the activation of the nuclear factor of activated T cells (NFAT) transcription factor which was exploited by using NFAT-GFP in Jurkat reporter cells. In the NFAT-GFP Jurkat cell line, NFAT activation promotes the expression of green fluorescent protein (GFP) which can be used to identify target cells by FACS (Joglekar *et al.*, 2019). Extracting the DNA from these cells and amplifying the SABR region was used to identify target epitopes (Joglekar *et al.*, 2019).

There are many methodologies that can be used to probe T cell specificity, each with their own strengths and caveats. The three methodologies described above were published during my own studies. I selected CPL screening due to the high throughput nature of CPL-based peptide discovery, and the lack of requirement for prior knowledge or bias with regards to potential peptide ligands. Furthermore, this methodology benefited from the recently described CPL-driven database searching webtool which ranks peptide ligands (Szomolay *et al.*, 2016). CPL screening provided me with large quantities of detailed data. It would be interesting to compare the ligand preferences of a TCR using these newly available antigen-discovery pipelines and our own CPL methodology.

#### 6.2.4 – Peptide-MHC plasticity: The other side of the coin

It is now well recognised that the TCR-pMHC interface is dynamic and yet almost all studies to date have treated the pMHC as a fixed entity around which the more fluid TCR can mould. The plasticity of the pMHC is, however, being increasingly recognised and in **Chapter 5**, I turned my attention to this side of the interaction and added to the ever-increasing library of data that proves that TCR ligands are also dynamic. I showed that the pMHC is a dynamic structure whose molecular motions are fine-tuned by the peptide cargo that it carries. Understanding the dynamic behaviour of pMHC molecules is an important addition to the toolkit needed for therapeutic exploitation of the TCR-pMHC interaction. As mentioned above, Madura *et al.* showed that a modified melanoma peptide with enhanced binding to HLA-A2 made for a worse T cell antigen (Madura *et al.*, 2015). The increased affinity between peptide and MHC molecule reduced the plasticity of the complex and prevented the TCR

from “pulling” the peptide out of the groove slightly, as was seen in the wildtype complex. Thus, reducing the flexibility of the pMHC made the pMHC an inferior TCR ligand (Madura *et al.*, 2015) highlighting the importance of taking into account molecular flexibility during future endeavours to utilise TCR-pMHC interactions therapeutically.

There are a number of methods that can be employed to investigate molecular flexibility. Chiefly amongst these methods is the use of molecular dynamics (MD) simulations. This computational technique allows for high-throughput analysis of molecular flexibility, as demonstrated by the use of MD simulations to characterise the flexibility of over 50 pMHC molecules (Ayres *et al.*, 2019). Ayres *et al.* found that peptide-dependent dynamic effects are transmitted throughout the MHC molecule, principally affecting the peptide-binding groove itself, but also extending out of it to influence distal regions of the molecule (Ayres *et al.*, 2019). Despite lacking experimental supporting data, high-throughput methods such as MD simulations can be used to build large libraries of data from which future conclusions and comparisons can be drawn.

Computational data demands support in the form of experimental data. A simple way to experimentally assess molecular flexibility is by the examination of static atomic resolution crystal structures. Borbulevych *et al.* used crystal structures to demonstrate peptide-dependent flexibility of HLA-A2 using two peptides (Tax (LLFGYPVYV) and Tel1p (MLWGYLQYV)) that could both be recognised by the A6 TCR (Borbulevych *et al.*, 2009). The pMHCs were almost complete molecular mimics in their unbound state, but formed a substantially different interface of contacts when bound to the A6 TCR. Comparison of the TCR-bound and -unbound structures pinpointed regions of molecular movement within the pMHC (Borbulevych *et al.*, 2009).

Molecular flexibility of proteins in solution, including pMHC, can also be examined by nuclear magnetic resonance (NMR) and hydrogen/deuterium exchange (HDX). NMR and HDX are discussed in detail in **Section 5.1.2**. Beerbaum *et al.* used NMR to demonstrate remarkable plasticity at the MHC-heavy chain: $\beta_2M$  interface in complexes with different MHC molecules and the invariant  $\beta_2M$ . Structural variation in  $\beta_2M$  occurs at the residues involved at the  $\beta_2M$ -MHC interface, allowing  $\beta_2M$  to tolerate different MHC molecules without significant change to its overall structure (Beerbaum *et al.*, 2013).

I assess pMHC dynamics in **Chapter 5**, benefiting from an institutional collaboration with the University of Bath, and satisfying the requirements of my GW4 PhD funding from the UK Medical Research Council. With the aid of equipment and expertise from the Bath

laboratory, I was able to use the red-edge excitation shift (REES) phenomenon to follow on from two key publications, Hawse *et al.* 2013 and Van Hateren *et al.* 2017, which use HDX to demonstrate peptide-dependent molecular motility throughout the pMHC molecule (Hawse *et al.*, 2013; Van Hateren *et al.*, 2017). I utilised the REES phenomenon, representing its application to pMHC molecules for the first time to my knowledge, to confirm observations of peptide-dependent MHC dynamics in an autoimmune-relevant model system (Wooldridge *et al.*, 2012; Cole *et al.*, 2016) as discussed in detail in **Chapter 5**.

### 6.3 –T cell therapy

Greater understanding of the TCR-pMHC interaction achieved through methods such as those described in this thesis will facilitate the development of future T cell therapies. T cell-based therapeutics have been used successfully in a variety of clinical settings as described below:

#### 6.3.1 – Adoptive cell transfer (ACT)

One of the most prevalent forms of T cell therapy in current use is adoptive cell transfer (ACT). ACT involves the extraction and *ex vivo* expansion of human T cells, followed by their reinfusion into patients (Savoldo, Heslop and Rooney, 2000; Ellebaek *et al.*, 2012). ACT has been used in a number of different clinical settings, principally to treat viral infections and tumours. For example, the infusions of donor-derived CMV-specific CD8<sup>+</sup> T cells can be used to reconstitute cellular immunity in transplant patients following bone marrow transplant (Riddell *et al.*, 1992). One study found that infusions of CMV-specific clones from their donors could increase CMV immunity without observable toxic side effects in 78% (11 of a cohort of 14) of patients (Walter *et al.*, 1995).

Another complication common in transplant recipients is EBV lymphoproliferative disease. In immunocompetent individuals EBV infection is mild, but the viral genome can persist in a latent form in infected B cells (Savoldo, Heslop and Rooney, 2000). Post-transplant immunosuppression in these patients impairs the normal CD8<sup>+</sup> T cell response that keeps the infection at bay leading to uncontrolled expansion of EBV-infected B cells (Savoldo, Heslop and Rooney, 2000). *Ex vivo* produced EBV-specific CD8<sup>+</sup> T cells can be used both prophylactically and therapeutically. An early attempt to treat transplant patients with donor-derived CD3<sup>+</sup> cells successfully achieved disease regression but two patients died from respiratory failure caused by graft vs host disease (Papadopoulos *et al.*, 1994). Later, in an

attempt to circumvent the alloreactivity problem, Savoldo *et al.* used patient B cells to generate EBV-transformed lymphoblastic cell lines (LCLs). The autologous LCLs were then used to activate EBV-specific donor T cells which were expanded for reinfusion (Savoldo, Heslop and Rooney, 2000). 59 prospective transplant patients were given prophylactic treatment in this manner, none of whom developed EBV lymphoma compared with an incidence rate of 11.5% in the control group (Savoldo, Heslop and Rooney, 2000). Such bespoke, personalised therapy can be lengthy in development and patients may die while awaiting treatment. To strike the balance between rejection of donor T cells and completely autologous therapy Dorothy H. Crawford and colleagues established a bank of EBV-specific T cells from a range of donors (Vickers *et al.*, 2014). Patients are given EBV-specific T cell infusions from the closest HLA-matched donor in the bank. This approach bypassed the need for individual bespoke therapy for each parents to create an off-the-shelf therapy that has been used successfully, with no adverse effects, to treat patients with EBV lymphoproliferative disease (a response rate of 65%) (Haque *et al.*, 2007; Vickers *et al.*, 2014).

In addition to the treatment of viral infections, ACT is frequently employed to treat cancer patients. This form of the therapy involves the infusion of tumour-specific T cells, often with accompanying supportive cytokines such as IL-2 and IL-15 (Overwijk *et al.*, 2003; Klebanoff *et al.*, 2004). Dudley *et al.* treated 35 metastatic melanoma patients with autologous tumour-reactive ex vivo expanded tumour-infiltrating lymphocytes (TILs) and IL-2 achieving a 51% clinical response rate, including three complete responses (Dudley *et al.*, 2005). The treatment could effectively mediate clinical regression in both the original tumour and metastases (Dudley *et al.*, 2005).

The above examples demonstrate the strength of ACT as a therapy to bolster endogenous CD8<sup>+</sup> T cell activity for clinical benefit. However, the need to generate autologous T cells for each patient is labour-intensive and time-consuming. Furthermore, numerous studies have shown that not all TIL respond to tumour (Scheper *et al.*, 2019) so alternative approaches have involved equipping T cells with receptors that do recognise tumour to ensure that all infused T cells have the capacity to contribute to tumour clearance (Yannelli *et al.*, 1996; Zhu *et al.*, 2017; Horton and Gajewski, 2018; Horton *et al.*, 2018).



### 6.3.2 – TCR gene transfer

TCR gene transfer involves the transfer of *TCR $\alpha$*  and *TCR $\beta$*  genes from a TCR of known specificity in order to confer that specificity on a population of T cells (D. Li *et al.*, 2019). Unlike ACT, for use in therapy, T cell genetic engineering does not rely on the presence of pre-existing disease-specific T cells. Transfer of TCR genes can instead confer that specificity on the patient's own peripheral lymphocytes, offering it a significant advantage over ACT (Morgan *et al.*, 2006). Disease-associated epitopes and the T cells specific for them can be identified in ongoing peptide discovery efforts such as those described in this thesis. The identified TCRs can then be cloned and 'banked' for use in future therapies. For example, TCRs specific for a number of TAAs have been identified, cloned, and successfully used to redirect non-specific T cells (Morgan *et al.*, 2003; Hughes *et al.*, 2005; Zhao *et al.*, 2005).

T cell genetic engineering was used in **Chapter 4** of this thesis to redirect a polyclonal population of donor-derived CD8<sup>+</sup> T cells to specifically recognise the HIV-1 epitope, SLYNTVATL. I, like many others (Morgan *et al.*, 2006; Rapoport *et al.*, 2015), used lentiviral vectors to achieve high, stable TCR expression at the T cell surface.

Lentiviruses naturally excel at delivering genetic material to cells and boast an advantage over other types of viral vector in their heightened ability to infect non-proliferating cells (Milone and O'Doherty, 2018). Lentiviruses have also been cited as being safer than alternatives like gammaretroviruses on account of a lower propensity to cause insertional mutagenesis (Oldham, Berinstein and Medin, 2015). Lentiviral vectors first featured in clinical trials in 2003 (Levine *et al.*, 2006), despite the replication-competency of these vectors no adverse effects were noted in an 8-year follow up (McGarrity *et al.*, 2013). Since then, T cells transduced to express TCRs specific for tumour antigens have been used as a successful therapy (Milone and O'Doherty, 2018). In a 2015 trial, 16 of 20 myeloma patients experienced clinical responses after receiving T cells expressing an affinity-enhanced, HLA-A2-restricted NY-ESO-1-specific TCR (Rapoport *et al.*, 2015). Methods for the optimised delivery of TCR genes are discussed in detail in **Appendix Sections 7.2** and **7.3**.

I believe that T cell genetic engineering offers many benefits over the use of the patient's endogenous T cell repertoire as in ACT. As discussed above, the genetic engineering of patient T cells does not rely on the existence of disease-specific T cells within the patient, nor does it necessitate the time-consuming expansion of T cells on a patient-by-patient basis. Additionally, engineered T cells can be subjected to extensive crossreactivity analysis, without the time pressure of an awaiting patient. Large libraries of HLA-typed T cells can be

generated and stored for future 'off-the-shelf' allogenic T cell therapies (Mo *et al.*, 2020; Perez, Gruber and Arber, 2020). Furthermore, Mo *et al.* recently described a method for off-the-shelf cellular therapy in which they designed an alloimmune defence receptor (ADR) with which a readily-available bank of disease-specific allogenic T cells can be equipped (Mo *et al.*, 2020). The ADR recognised 4-1BB which is transiently expressed on the surface of activated lymphocytes, thus protecting against graft vs host disease (Mo *et al.*, 2020). Such cells could be subjected to extensive safety testing, far more than bespoke therapies would have time for, and be immediately available when required (Mo *et al.*, 2020).

In addition to not relying on the endogenous T cell repertoire in a given patient, T cell genetic engineering benefits from the ability to improve upon naturally occurring disease specific TCRs. Transduced TCRs can be subject to affinity enhancement (described in detail in **Section 6.1.2**) that can both enhance the effectiveness of the therapy, and broaden the specificity of the TCR to capture any potential escape mutants that may arise.

## 6.4 – Concluding remarks

The repertoire of TCRs on expanded, antigen-experienced T cells contains a record of all past infections and information on some potential future pathologies. There is currently much interest in using TCRs as predictors of health and disease but full realisation of this goal will require means of determining TCR function based on TCR sequence (Attaf, Huseby and Sewell, 2015). In this thesis, I present specificity data, both positive and negative, for two disease-relevant human TCRs that would provide suitable fuel to these predictive endeavours. The ultimate goal of being able to rationally design a TCR to target any given peptide ligand relies on the ability to predict positive interactions, which in turn relies on large volumes of published TCR specificity data – a small contribution to which is contained in this thesis.

I believe that the future treatment of many human diseases will rely heavily on the therapeutic application of the TCR-pMHC interaction. During my PhD project I have employed multiple techniques to investigate the interaction in disease-relevant systems. I have demonstrated that a single CDR3 amino acid substitution can provide the difference between recognition and ignorance, and I have highlighted hitherto unanticipated viral-autoimmunity and viral-cancer crossreactivity. Furthermore, I have demonstrated that extremely similar pMHC ligands can exhibit differing molecular motions.

Whilst each individual study, both here and in the literature, strictly provides information regarding the model system used, collectively they build up the framework from which future T cell therapeutics can draw the strength needed to harness one of evolution's greatest weapons against disease.

## 7 – Appendix and supplementary information

---

### 7.1 – Human immunodeficiency virus

HIVs are typically grouped into two categories: HIV type 1 (HIV-1) and HIV type 2 (HIV-2) (Coffin *et al.*, 1986; Gelderblom, Özel and Pauli, 1989). HIV-1 is prevalent worldwide (Beloukas *et al.*, 2016), whereas HIV-2 tends to be limited to regions in Africa (Visseaux *et al.*, 2016). HIV primarily infects CD4<sup>+</sup> T cells (Lasky *et al.*, 1987; Gelderblom, Özel and Pauli, 1989; Weiss, 1993; Wilen, Tilton and Doms, 2012). Infected CD4<sup>+</sup> T cells migrate to T cell-dense lymphoid tissues such as the lymph nodes, offering prime opportunity to spread the infection (Fanales-Belasio *et al.*, 2010). This results in infected individuals experiencing a steady decline in the number of circulating CD4<sup>+</sup> T cells over time, leading to life-threatening immunocompromisation (Gottlieb *et al.*, 1981; Siegal *et al.*, 1981; Friedman-Kien *et al.*, 1982; Mildvan *et al.*, 1982; Schroff *et al.*, 1983; Melbye *et al.*, 1986; Kahn and Walker, 1998). Viral replication and chronic immune activation steadily destroy lymphoid tissue (Ford, Puroden and Sereti, 2009; Fanales-Belasio *et al.*, 2010). This progressive immune impairment puts the patient at risk of opportunistic infections and neoplasms such as Kaposi sarcoma (Gottlieb *et al.*, 1981; Siegal *et al.*, 1981; Friedman-Kien *et al.*, 1982; Schroff *et al.*, 1983; Gupta, 1993; Hoover *et al.*, 1993; Brooks *et al.*, 2009; Clifford and Franceschi, 2009).

HIV has a characteristic pattern of infection consisting of an initial acute, symptomatic infection followed, after a few weeks, by a long period of asymptomatic chronic infection (Deeks and Walker, 2007; Fanales-Belasio *et al.*, 2010). Symptoms that manifest during the acute phase include flu-like fever, rash, oral ulcers, weight loss, and lymphadenopathy (swelling of the lymph nodes) (Kahn and Walker, 1998; Deeks and Walker, 2007; Fanales-Belasio *et al.*, 2010). Viral RNA becomes detectable in the blood approximately 10-12 days post-infection (Fiebig *et al.*, 2003) which can be used for diagnosis (Lindbäck *et al.*, 2000; Hecht *et al.*, 2002). Viremia then experiences an exponential rise (Clark *et al.*, 1991; Daar *et al.*, 1991; Little *et al.*, 1999) before it peaks around 21 days post infection (Little *et al.*, 1999). Untreated, the following clinically asymptomatic period is typically 10 years and features a steady decline of CD4<sup>+</sup> T cells culminating in death of the patient (Gupta, 1993; Deeks and Walker, 2007; Ford, Puroden and Sereti, 2009; Fanales-Belasio *et al.*, 2010).

HIV is a retrovirus; it has two copies of its single-stranded RNA genome that must be reverse transcribed into DNA before it can be incorporated into the host genome (Fanales-Belasio *et al.*, 2010). This genome encodes, amongst others, the *gag*, *pol*, and *env* genes. The *gag* gene

encodes core structural proteins (p24, p7, p6) and matrix protein, p17 (Gelderblom, Özel and Pauli, 1989; Fanales-Belasio *et al.*, 2010). The *env* gene encodes the viral envelope glycoproteins gp120 and gp41. The *pol* gene encodes enzymes required for viral replication such as reverse transcriptase, which converts viral RNA into DNA, integrase which incorporates the viral DNA into the host genome, and a protease which cleaves viral precursor proteins into the proteins necessary for assembling new viral particles (Veronese *et al.*, 1987; Gelderblom, Özel and Pauli, 1989; Fanales-Belasio *et al.*, 2010).

The viral genome is housed, along with vital viral enzymes (reverse transcriptase, integrase, and protease) within a capsid made of polymerised p24 core antigen. This core antigen associates with the p17 matrix protein which anchors the capsid to the lipid bilayer that encases the viral particle (Gelderblom, Özel and Pauli, 1989). The virus acquires its lipid bilayer as it exits the host cell, so it often also incorporates host proteins such as intercellular adhesion molecule (ICAM)-1, MHC-I, and MHC-II (Gelderblom, Özel and Pauli, 1989). A typical viral particle measures ~110nm in diameter (Gelderblom, Özel and Pauli, 1989).

The viral life cycle of HIV can be summarised in six steps: 1) binding and entry; 2) uncoating; 3) reverse transcription; 4) provirus integration; 5) virus protein synthesis and assembly; and 6) budding (Fanales-Belasio *et al.*, 2010). A viral particle binds to a host cell through the interaction of gp120 with CD4 on the surface of T cells (Lasky *et al.*, 1987; Gelderblom, Özel and Pauli, 1989; Straus and Weiss, 1993; Wilen, Tilton and Doms, 2012). This adhesion triggers a conformational change in gp120 that allows it to interact with other surface receptors on the host cell such as CXCR4 and CCR5 thus stabilising the binding (Chackerian *et al.*, 1997; Agrawal *et al.*, 2004; Alkhatib, 2009). CXCR4/CCR5 interaction brings the virus particle into close proximity with the host cell causing the lipid bilayers to merge allowing the virus to enter the cell (Gelderblom, Özel and Pauli, 1989). Naturally occurring mutations in chemokine receptors CXCR4 and CCR5 can confer some degree of resistance to HIV-1 infection (Carmichael *et al.*, 1993; Balotta *et al.*, 1997; O'Brien *et al.*, 1997; Agrawal *et al.*, 2004; Q. Wang *et al.*, 2017; Allen *et al.*, 2018). Furthermore, conventional ligands for these receptors such as MIP-1 $\alpha$  and MIP-1 $\beta$  can suppress HIV-1 infection *in vitro* by competing for the CCR5 receptor (Cocchi *et al.*, 1995; Price *et al.*, 1998; Garzino-Demo, 2006; Fanales-Belasio *et al.*, 2010). CD8<sup>+</sup> T cells are a key source of these  $\beta$ -chemokines (Conlon *et al.*, 1995), and produce them in response to specific MHC-bound HIV-1 peptide epitopes (Price *et al.*, 1998). Price *et al.* showed that the release of  $\beta$ -chemokines was epitope specific (it did not occur in response to some HIV escape mutants), and – like cytotoxic activity – mediated through activation of the TCR engaging relevant pMHC (Price *et al.*, 1998). Thus, they

demonstrated that HIV escape mutants (discussed in detail below) not only serve to evade the cytotoxic immune response but can enhance the propagation of the infection by preventing  $\beta$ -chemokine-mediated inhibition (Price *et al.*, 1998).

Once inside the cell, HIV loses its capsid— a process known as uncoating (Cosnefroy, Murray and Bishop, 2016) – and transcription of the viral RNA genome can begin. The newly produced DNA is incorporated into the host DNA by the integrase enzyme (Fanales-Belasio *et al.*, 2010). Replication of the viral DNA is then delayed until the host cell is activated and dividing. Quiescent immune cells are a long-lived source of latent virus (Chun, Carruth, *et al.*, 1997; Chun, Stuyver, *et al.*, 1997; Herzig *et al.*, 2019). This latent virus is often referred to as the latent reservoir and can be difficult to eliminate through treatment (Chun, Carruth, *et al.*, 1997; Herzig *et al.*, 2019).

When an infected CD4<sup>+</sup> T cell is activated it begins the process of cell division, replicating both its own and the viral DNA (Herzig *et al.*, 2019). Some of the earliest HIV-1 proteins to be produced include the regulatory proteins, Tat and Rev, these serve to specifically promote the transcription of HIV-1 DNA. The proteins encoded by *gag* and *pol* then begin to form the new viral particle (Fanales-Belasio *et al.*, 2010). *Env* encodes a precursor protein, gp160, which is cleaved by protease into the envelope glycoproteins gp120 and gp41 (Kantanen, Leinikki and Kuismanen, 1995). Once the immature particle is assembled it migrates to the cell surface for budding and release, acquiring a new lipid bilayer coat from the host cell membrane (Fanales-Belasio *et al.*, 2010).

There is ample evidence of CD8<sup>+</sup> T cell involvement in the antiviral response to HIV-1. During the acute phase cytotoxic activity is detected before the appearance of anti-HIV antibodies (Borrow *et al.*, 1994; Koup, 1994; Price *et al.*, 1997; Allen *et al.*, 2000; Bangham, 2009). In 1994, Borrow *et al.* demonstrated specific cytotoxic activity against five HIV-1 proteins (*Env*, *Gag*, *Pol*, *Nef*, and *Tat*) in five patients (Borrow *et al.*, 1994). Many specific CD8<sup>+</sup> T cell MHC-restricted epitopes have been described in the literature and are collated by the Los Alamos National Laboratory Database (T-Cell Epitope Database, 2020).

The cytotoxic immune response is sufficient to reduce viremia and bring about the chronic asymptomatic phase (Mellors *et al.*, 1995; O'Brien, 1996). Yet despite this, the CD8<sup>+</sup> T cell response is unable to fully clear the virus. A number of factors are responsible for this including the emergence of escape mutants (Jamieson *et al.*, 2003; Petrova, Naumova and Gorski, 2011; Wright *et al.*, 2012), a reduction in available CD4<sup>+</sup> T cell help (Hoover *et al.*, 1993; Rosenberg, Anderson and Pabst, 1998; Rodés *et al.*, 2004), CD8<sup>+</sup> T cell exhaustion

(Oxenius *et al.*, 2002; Shin and Wherry, 2007; Cella *et al.*, 2010), and activation-induced downregulation of the CD8<sup>+</sup> TCR (Oxenius *et al.*, 2002).

Another hurdle for the anti-HIV CD8<sup>+</sup> T cell response and antiretroviral therapy is what is known as the latent reservoir. Mentioned briefly above, these are quiescently infected CD4<sup>+</sup> T cells that retain the HIV-1 provirus integrated into their genome but do not express viral proteins, rendering them invisible to the immune system and difficult to remove (Chun *et al.*, 1995; Chun, Carruth, *et al.*, 1997; Chun, Stuyver, *et al.*, 1997; Finzi *et al.*, 1997). The greater the size of this reservoir of quiescently infected cells the faster the virus will rebound if antiretroviral therapy is interrupted, this means that patients must adhere strictly to lifelong treatment (Li *et al.*, 2016). Reducing or eliminating the latent reservoir presents a significant challenge as the infected cells are effectively invisible (Herzig *et al.*, 2019). One of the main methods of intervention is known as “shock and kill” (Archin *et al.*, 2012). This approach involves the use of latency reversing agents (LRAs) to reactivate viral replication. Once the viral genome is actively being transcribed, viral proteins will be displayed on the surface of the infected host cell rendering it vulnerable to immune attack once again (Herzig *et al.*, 2019; Jean *et al.*, 2019).

Successful “shock and kill” interventions depend, therefore, on the effective killing of reactivated viral-infected CD4<sup>+</sup> T cells by specific CD8<sup>+</sup> T cells (Herzig *et al.*, 2019). Whilst the specific CD8<sup>+</sup> response to HIV-1 is well documented (Borrow *et al.*, 1994; Koup, 1994; Price *et al.*, 1997; Allen *et al.*, 2000; Bangham, 2009; Kim *et al.*, 2018), the chronic immune landscape that HIV-1 infection causes is generally associated with exhausted CD8<sup>+</sup> T cells (Shin and Wherry, 2007; Cella *et al.*, 2010). Attempts have been made to overcome the problem of exhausted indigenous T cells through use of adoptive T cell therapy and chimeric antigen receptors (CARs) (Deeks *et al.*, 2002; Sung *et al.*, 2018; Wagner, 2018; Guedan *et al.*, 2019).

In 2019, Herzig *et al.* designed a 2-part CAR system to target the latent reservoir. Termed ‘convertibleCAR T cells’ they aimed to simultaneously overcome the problem of exhausted immune cells and deliver a therapy that could be turned on and off through administration (and natural clearance) of a broadly neutralising antibody (bNAb) (Herzig *et al.*, 2019). Their CAR was specific for a modified natural molecule that was tagged to the Fc region of selected bNAbs, ensuring that their adoptively transferred T cells would only be active if and when the bNAb was also administered (Herzig *et al.*, 2019). A further advantage of this technique lies in the wide availability of different anti-HIV-1 bNAbs, meaning that alternatives can be

selected should resistance arise, and that – theoretically – this therapy would be just as effective at targeting other aberrant cells provided there were suitable bNAbs available (Sok *et al.*, 2016; Mayer *et al.*, 2017; Dashti *et al.*, 2019; Julg and Barouch, 2019). A limitation of this technique is the ineffectiveness of many of the available LRAs – 100% reactivation of the latent reservoir is rarely achieved and side-effect toxicity has been reported (Herzig *et al.*, 2019; Jean *et al.*, 2019).

A further challenge for the immune response to HIV-1 infection is the emergence of escape mutants. Viral replication is highly error-prone; this high mutational rate is beneficial to the virus as it facilitates evasion of the host immune system (Roberts, Bebenek and Kunkel, 1988; Goodenow *et al.*, 1989). Rapidly introduced mutations are subject to host-applied selective pressure that promotes viral variants that are ‘hidden’ from the host’s immune system, these are known as escape mutants (Price *et al.*, 1997; Menéndez-Arias, 2002; Sarafianos *et al.*, 2009). There are a number of ways that mutations in viral proteins can facilitate immune evasion.

Escape mutations may result in peptides that cannot bind to MHCs (Voeten *et al.*, 2000; Goulder, Brander, *et al.*, 2001; Kelleher *et al.*, 2001; Boon *et al.*, 2002; Feeney *et al.*, 2004; Rimmelzwaan *et al.*, 2004; Casazza *et al.*, 2005). For example, HLA-B27<sup>+</sup> HIV-1 patients often mount an immunodominant response against an epitope from the Gag-derived p24 protein (KRWIILGLNK<sub>263-272</sub>) (Nixon *et al.*, 1988; Goulder, Phillips, *et al.*, 1997; Kelleher *et al.*, 2001). Immune escape can occur through mutation of the lysine or glycine residues within this epitope, this results in a peptide that doesn’t bind HLA-B27 well (Nietfield *et al.*, 1995; Goulder, Phillips, *et al.*, 1997; Kelleher *et al.*, 2001). Occurrence of this mutation often coincides with disease progression (Kelleher *et al.*, 2001), and removal of the CD8-mediated selective pressure causes reversion to the wild-type sequence (Kelleher *et al.*, 2001).

Escape mutations may produce peptides that bind to MHCs but the epitopes are no longer recognised by the host’s CD8<sup>+</sup> T cell repertoire (Phillips *et al.*, 1991). In 1997, Price *et al.* showed selective pressure drove immune escape in a HLA-B8-restricted Nef peptide (FLKEKGGL), with genomic variation being largely confined to this epitope (Price *et al.*, 1997). They demonstrated how positively selected variants either diminished or eradicated CD8<sup>+</sup> T cell recognition. In further evidence that this selective pressure was driven by the CD8<sup>+</sup> T cell immune response they demonstrated that the same variation did not occur in HLA-B8-negative donors (Price *et al.*, 1997).



Alternatively, the peptides that are produced following mutation of the viral genome might be suboptimally bound by TCRs, engaging the TCR but not activating it – this serves to prevent the TCR from encountering and potentially responding to other antigen, thus having an antagonistic effect (Bertoletti *et al.*, 1994; Sewell *et al.*, 1997).

In 2017, Cole *et al.* investigated the escape mutants that arise from a HLA-A2-restricted immunodominant Gag epitope, SLYNTVATL (Cole, Fuller, *et al.*, 2017). A simple strategy often employed during viral escape is to mutate the positions in the epitope that anchor the peptide to its MHC, in this case positions 2 and 9 (the C-terminus). In the SLYNTVATL epitope, the Y and L residues (positions 79 and 85 in the Gag protein sequence) are crucial for the function of the Gag-derived p17 protein and thus cannot be mutated without significant fitness cost to the virus (Cannon *et al.*, 1997; Morikawa *et al.*, 1998; Martinez-Hackert *et al.*, 2006). Thus, it was assumed that the escape mutants that arise from SLYNTVATL must instead abrogate T cell recognition. In fact, Cole *et al.* demonstrate structurally that escape mutants are still recognised by the cognate TCR. They show that the mutants achieve their escape not by preventing T cell recognition, or by fully preventing MHC binding, but by destabilising the pMHC structure at the surface of the cell (Cole, Fuller, *et al.*, 2017).

Strategies employed for immune escape demonstrate how variations in the TCR-pMHC interaction between individuals can influence how their disease progresses. The MHC background an infectious agent faces in any individual host can dictate which escape mutants will arise, and different escape mutants will have varying impacts on viral fitness (Carlson *et al.*, 2012) For example, both HLA-B\*42:01 and HLA-B\*81:01 present an immunodominant epitope from the Gag protein (Gag<sub>180–188</sub>, TPQDLNTML) (Honeyborne *et al.*, 2006). Despite the two alleles being closely related members of the B7 superfamily, the escape mutation selected in individuals expressing either allele differs with drastic effects on viral fitness (Wright *et al.*, 2012; Kløverpris, McGregor, *et al.*, 2015). In HLA-B\*42:01 positive individuals the most common escape mutation arises at position 3 (Q) of the peptide, relating to position 182 in the Gag protein. In contrast, in HLA-B\*81:01-expressing individuals mutations more frequently arise in Gag-186 (position 7 of the peptide) (Wright *et al.*, 2012; Kløverpris, McGregor, *et al.*, 2015). Unlike the mutation at Gag-182, which has negligible impact on the function of the Gag protein, mutations at Gag-186 have a substantial negative impact on viral fitness. As a result of the reduced viral replicative capacity, HLA-B\*81:01-positive individuals cope relatively well with the infection and experience delayed progression to acquired immunodeficiency syndrome (AIDS) (Wright *et al.*, 2012; Kløverpris, McGregor, *et al.*, 2015).

Even micropolymorphisms in the MHC alleles expressed by different individuals can have a significant influence on how well they control the disease. For example, single amino acid differences between HLA-B\*35 alleles can influence progression to AIDS (Gao *et al.*, 2001; Jin *et al.*, 2002). Furthermore, of the closely related HLA-B\*58:01, HLA-B\*57:02, and HLA-B\*57:03 alleles the latter is associated with lower viral loads than the two former. Each phenotype triggers a unique cohort of CD8<sup>+</sup> T cells in response to infection (Kløverpris *et al.*, 2012).

Variation in how individuals control HIV-1 infection has led to patients being classified based on their ability to deal with the chronic infection, terms such as “rapid progressors” and “long-term non-progressors” or “elite controllers” are used (Fanales-Belasio *et al.*, 2010). Approximately 0.5% of HIV-1-infected patients are elite controllers, meaning that in the absence of treatment their own immune response suppresses viral replication to below detection limits (Deeks and Walker, 2007). Furthermore, a proportion of regularly exposed people do not contract HIV, suggesting their anti-viral immune activity can prevent the virus from establishing an infection (Rowland-Jones *et al.*, 1995; Stranford *et al.*, 1999). Understanding why these patients are able to respond well to the virus may aid treatment of less fortunate patients.

In 2007, Blankson and Siliciano reported that the viruses infecting a cohort of elite controllers had normal virulence (Blankson and Siliciano, 2008), and in 2008 Bailey *et al.* showed that elite controllers could still transmit the virus to other people (Bailey *et al.*, 2008). This combined evidence suggests that elite controllers are not infected with an attenuated virus, but that their immune response is more effective than that of standard patients (Blankson and Siliciano, 2008). They are able to effectively control the infection despite the emergence of viral mutants, suggesting that novel CD8<sup>+</sup> T cell responses continue to be initiated across the course of the infection (Bailey *et al.*, 2006). Elite controllers are thought to have CD8<sup>+</sup> T cells with a higher proliferative capacity against immunodominant epitopes (Migueles *et al.*, 2002). They have also been shown to express higher levels of perforin, IFN $\gamma$ , MIP-1- $\beta$ , and TNF (Migueles *et al.*, 2002; Betts *et al.*, 2006).

A number of studies have suggested that certain MHC-I alleles, such as HLA-B\*57 and HLA-B\*27, are overrepresented in elite controllers compared to other HIV-1 patients (Lambotte *et al.*, 2005; Bailey *et al.*, 2006; Betts *et al.*, 2006; Deeks and Walker, 2007). Suggesting that possession of particular MHC backgrounds enables the patients to present optimal peptide epitopes to their CD8<sup>+</sup> T cells. Furthermore, there is evidence that selective pressure from

HIV-1 prevalence in the human population can drive the accumulation of protective MHC-I alleles (Moore *et al.*, 2002; Leslie *et al.*, 2005). Whole-genome association scan analysis of HIV-1 patients actually showed MHC-I alleles to be one of the strongest polymorphisms associated with protection against the disease (Fellay *et al.*, 2007; Catano *et al.*, 2008).

It is clear, therefore, that vital understanding can be gleaned by studying the specific TCR-pMHC interactions that occur during the course of HIV-1 infection. One TCR-pMHC interaction of particular interest to this thesis is the 868-HLA-A2-SLYNTVATL interaction. This interaction is used as a model system to investigate how TCR sequence relates to peptide preference, it has a rich history within the field of HIV-1 research, see **Section 3.1.1**.

## 7.2 – Optimised expression of transduced TCRs at the T cell surface

2A self-cleaving peptide sequences are found in the genomes of many viruses (Hahn and Palmenberg, 1996; Szymczak *et al.*, 2004; Leisegang *et al.*, 2008). During translation, the sequence of these short peptides causes a ribosomal 'skip' between the penultimate and N-terminal residues of the 2A sequence. The formation of a peptide bond is inhibited, thus breaking the continuous polypeptide chain (De Felipe *et al.*, 1999; Szymczak *et al.*, 2004; Leisegang *et al.*, 2008). This mechanism results in the majority of the 2A sequence being tagged onto the end of the upstream gene, whilst the N-terminal residue, a glycine, remains attached to the downstream gene (De Felipe *et al.*, 1999; Leisegang *et al.*, 2008).

2A-mediated cleavage has been shown to be almost 100% efficient (Szymczak *et al.*, 2004). In 2004, Szymczak *et al.* used a 2A-based vector system to restore T cell function of CD3-deficient mice. Their tetracistronic vector contained the four CD3 genes ( $\epsilon$ ,  $\gamma$ ,  $\delta$ ,  $\zeta$ ) separated by 2A sequences (Szymczak *et al.*, 2004). Western blot analysis of the resulting proteome revealed successful expression of all four genes. Almost no uncleaved bands were visible – indicating close to 100% cleavage (Szymczak *et al.*, 2004).

2A peptides are, however, not the only option for multicistronic expression. Other options include the use of multiple vectors (containing a single gene each), the use of internal promoters, and the use of internal ribosomal entry sites (IRES). IRES sequences provide an additional ribosome binding site within the mRNA transcript, allowing the second polypeptide to be translated independently of the first (Mizuguchi *et al.*, 2000; Morgan *et al.*, 2006). Expression of the downstream genes is often poor compared to expression of the gene preceding the IRES element (Mizuguchi *et al.*, 2000; Osti *et al.*, 2006). Mizuguchi *et al.*

demonstrated that expression of downstream genes is approximately 20-50% that of the upstream gene (Mizuguchi *et al.*, 2000). This is an undesirable caveat when expressing genes that act with 1:1 stoichiometry like an  $\alpha\beta$  TCR heterodimer at the T cell surface. Enhanced expression of downstream genes in 2A systems compared to IRES elements has been frequently noted (De Felipe *et al.*, 1999; Klump *et al.*, 2001). Furthermore, the efficiency of IRES elements has been shown to vary between different cell types (Borman *et al.*, 1997), and they can be subject to competition for translation factors (Szymczak *et al.*, 2004). IRES elements are also much larger than 2A peptides which can cause difficulties in viral vectors with limited gene carrying capacity, such as lentiviruses (De Felipe *et al.*, 1999).

A useful comparative study of the different transfer vector options was provided by Leisegang *et al.* in 2008, in which they compared expression of TCR $\alpha$  and TCR $\beta$  when introduced by either two separate vectors, a single vector with an IRES, or a single vector with a 2A peptide (Leisegang *et al.*, 2008). Use of two separate vectors was found to be hampered by the necessity of ensuring both vectors are taken up by the same cell (Leisegang *et al.*, 2008; Banu *et al.*, 2014). The 2A system was found to be less susceptible to competition from an endogenous TCR than the IRES expression method, producing enhanced surface expression and improved peptide-specific IFN $\gamma$  production (Leisegang *et al.*, 2008).

When the expression of the second gene is driven by a second, internal, promoter it invites the possibility of promoter interference, or competition between the two promoters for transcriptional machinery (Emerman and Temin, 1984; Leisegang *et al.*, 2008). In 1984, Emerman and Temin showed that in a two-promoter vector one of the two genes was often suppressed. They suggested that the suppression was epigenetic as they could alter which gene was suppressed by pre-exposing the cells to different conditions (Emerman and Temin, 1984). For example, if cells were given two antibiotic resistance genes and cultured in the presence of antibiotic A, when subsequently exposed to antibiotic B most cells died despite possessing the resistance gene. This was also true in reverse, regardless of the order of the genes in the vector (Emerman and Temin, 1984). Thus, neither an IRES nor a two-promotor vector achieves the stoichiometric expression optimal for expression of T cell receptors.

A potential disadvantage of the 2A system is that the short peptide that remains attached to the upstream polypeptide might interfere with its biological function or contribute to immune recognition of the transduced protein. However, the system has been widely used without any noteworthy interference (Yang *et al.*, 2008). In 1999, de Felipe *et al.* used 2A bicistronic vectors to confer resistance to two antibiotics, the upstream tag did not interfere

with the function of the upstream gene (De Felipe *et al.*, 1999). In a tricistronic vector the function of the second gene was similarly unaffected, and expression of the third gene (GFP) was noted for up to three months before repurification was needed (De Felipe *et al.*, 1999). The 2A sequence was also shown not to interfere with the expected subcellular locations of transgenes (Klump *et al.*, 2001). Szymczak *et al.* suggest that the tag might actually provide an advantageous distinction between the transduced protein and endogenous protein (Szymczak *et al.*, 2004).

The 2A autocleavage site can be further optimised by the addition of upstream spacer sequences (Yang *et al.*, 2008). Yang *et al.* demonstrated that the addition of an amino acid spacer sequence (GSG or SGSG) just before the 2A sequence could enhance the expression of two different TCRs, as measured by pMHC tetramer staining (Yang *et al.*, 2008). My construct system incorporates this spacer. There were, however, further optimisations noted by Yanh *et al.* that could be included and tested in future. For example, they found the addition of a furin protease recognition site prior to the 2A peptide enhanced surface expression (Yang *et al.*, 2008).

Interestingly, a 2014 study found that the order of genes within a P2A construct could influence the expression efficiency. Using 10 virus-specific TCRs Banu *et al.* demonstrated that 9/10 TCRs favoured a beta-2A-alpha orientation over an alpha-2A-beta orientation, as indicated by surface expression measured by  $\beta$ -chain-specific antibodies and pMHC-multimers (Banu *et al.*, 2014). This optimal expression manifested functionally as a significant increase in the target-specific release of IFN $\gamma$ , IL-2, and TNF (Banu *et al.*, 2014). This observation is not unique to the Banu study (Leisegang *et al.*, 2008). In this chapter, I used an alpha-2A-beta orientation. It would, therefore, be insightful to perform parallel comparisons of my TCRs in both orientations in future.

### 7.3 – Optimising TCR gene transfer

In 2007, Kuball *et al.* described a method for improving chain pairing by introducing non-natural cysteine residues in the  $\alpha$  and  $\beta$  TCR chains, thus enhancing chain pairing akin to the manner described for soluble TCR production (Boulter *et al.*, 2003). This study demonstrated that the introduction of non-natural cysteine residues promoted preferential pairing between the two introduced chains, increased desirable surface expression, and reduced mispairing resulting in greater functional activity against the target antigen (Kuball *et al.*, 2007). A paper published by the same group later the same year demonstrated that the

addition of the non-native disulphide bond was broadly applicable. Using three additional TCRs they showed that the presence of the disulphide bond improved pMHC tetramer staining, and specific killing by transduced T cells (Cohen *et al.*, 2007).

TCR constructs can also be designed to have murine constant domains to promote interchain recognition and binding, enhancing the formation of the correct pairs (Stanislowski *et al.*, 2001; Cohen *et al.*, 2006; Kuball *et al.*, 2007). Cohen *et al.* reported enhanced *in vitro* surface expression and cytokine production of murinized TCRs compared to their fully human counterparts that translated into an enhanced anti-tumour response *in vivo* (Cohen *et al.*, 2006). Murinization may also enhance association with human CD3. Cohen *et al.* demonstrated (using detergents of different strengths) that the murinized-TCR/CD3 complex was more stable than the human TCR/CD3 complex (Cohen *et al.*, 2006). TCR murinization has been frequently used to enhance TCR expression at the T cell surface (Leko *et al.*, 2019; Ren *et al.*, 2019; Tan *et al.*, 2019) however, it does not come without caveats if such TCRs are used therapeutically as murine constant domains have the potential to be immunogenic, causing any adoptively transferred cells to be targeted by the immune system of the patient (Riddell *et al.*, 1996).

Improving chain pairing can significantly reduce the likelihood of chimeric TCRs and resulting off-target immunity. However, the strategies employed rarely eliminate mispairing completely. For this, many have turned to downregulating the endogenous TCR. In 2009, Okamoto *et al.* described the down regulation of the endogenous TCR using small interfering RNA (siRNA) (Okamoto *et al.*, 2009). The introduced TCR is subject to codon optimisation and thus differs sufficiently from the endogenous TCR to be protected from the siRNA (Okamoto *et al.*, 2009).

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein-9 (Cas9) techniques can also be used to remove, or 'replace', the endogenous TCR (Legut *et al.*, 2018). In 2018, Legut *et al.* described a CRISPR/Cas9 strategy for use during simultaneous TCR gene transduction. This 'TCR replacement' strategy was applicable to both  $\alpha\beta$  and  $\gamma\delta$  TCRs, and produced enhanced functionality (increased tetramer staining, increased multi-cytokine responsiveness, and increased specific killing) (Legut *et al.*, 2018). Indeed, the removal of endogenous TCR chains was observed to increase T cell function in peptide titrations by up to three orders of magnitude (Legut *et al.*, 2018).

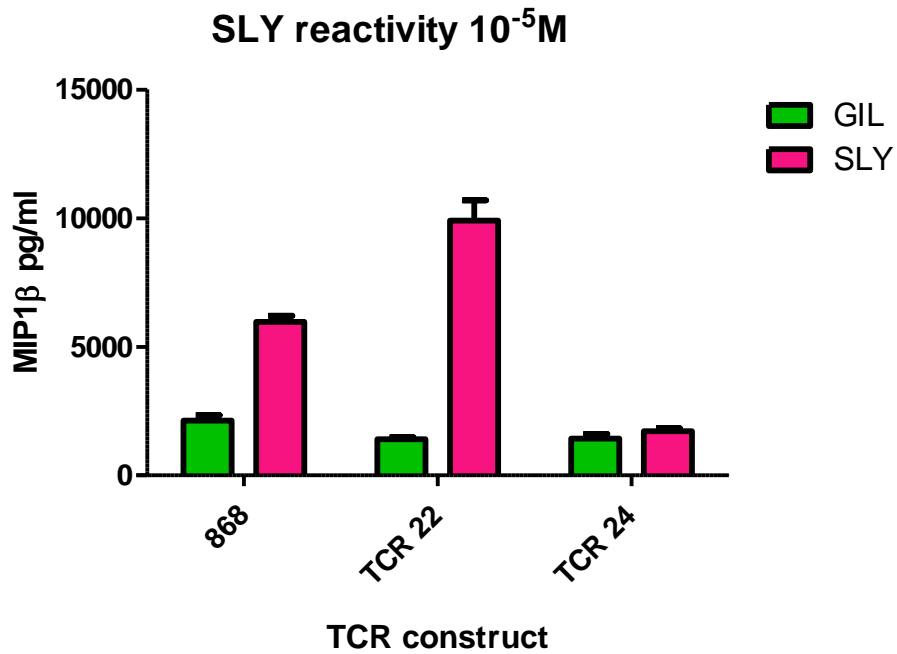
Roth *et al.* described a CRISPR-Cas9 methodology that does not rely on the use of viral vectors, citing their production as lengthy and expensive. Instead, Roth *et al.* introduce the

Cas9 ribonucleoprotein, the guide RNA, and the 'replacement' linear double-stranded DNA to the cell by electroporation (Roth *et al.*, 2018). DNA could be inserted at precise locations within the genome without damaging cell viability or function. TCR replacement resulted in the effective redirection of T cells to specifically target a cancer antigen, producing both *in vitro* and *in vivo* responses. Importantly, they found no evidence of off-target crossreactivities (Roth *et al.*, 2018).

Competition for the available endogenous CD3 has been shown to be another rate-limiting factor in TCR gene therapy (Ahmadi *et al.*, 2011). Ahmadi *et al.* co-delivered the four genes required for expression of CD3 (CD3 $\gamma$ , CD3 $\delta$ , CD3 $\epsilon$ , and CD3 $\zeta$ ) on a single vector, separated by 2A sequences with their introduced TCR. In comparison to the delivery of TCR alone, these recipient cells expressed higher levels of the desired TCR, produced increased amounts of IFN $\gamma$  in response to their target peptide, and recognised lower concentration of peptide (Ahmadi *et al.*, 2011).

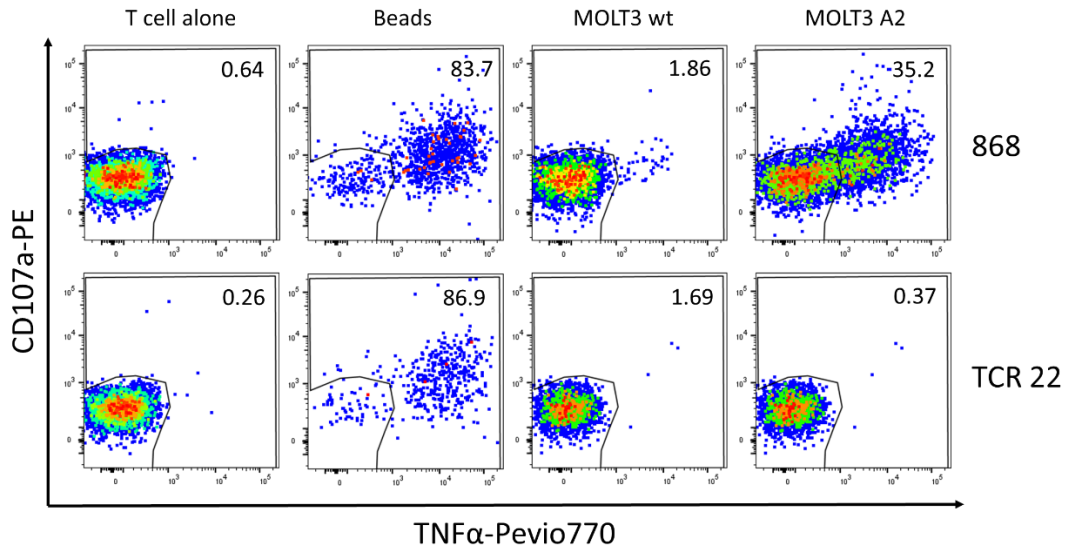
Scholten *et al.* used codon-optimisation of TCRs to improve surface expression. Their approach increased the portion of tetramer positive cells, and increased the specific IFN $\gamma$  production in response to target cells (Scholten *et al.*, 2006). Codon optimisation was employed in the work described in this chapter.

## 7.4 – Supplementary figures

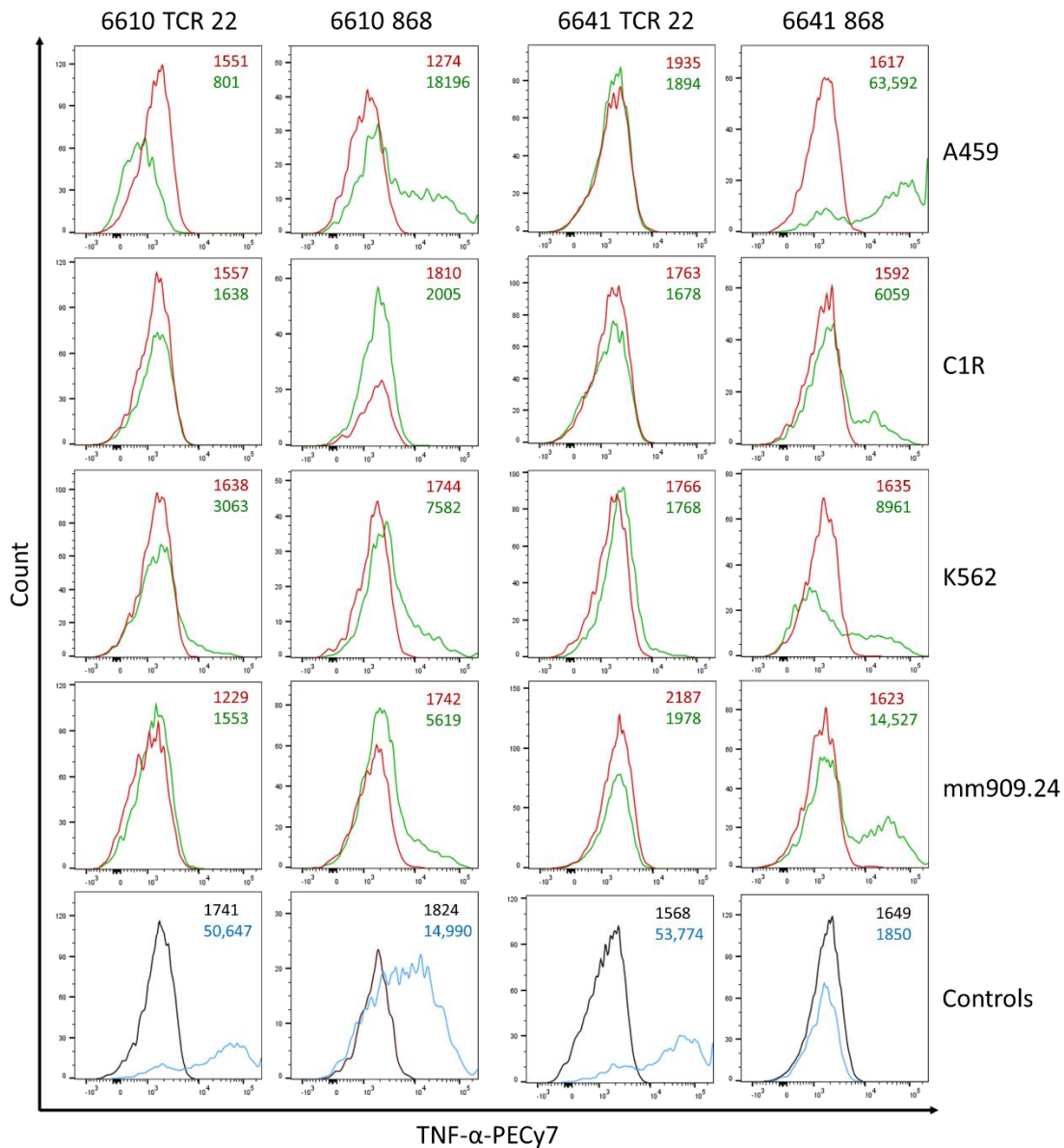


**Supplementary Figure 7.1: TCR 24-transduced  $CD8^+$  T cells do not respond to the M1 influenza-derived GILGFVFTL epitope.** 30,000  $CD8^+$  T cells transduced with TCR constructs were cultured in the presence of 60,000 T2 cells as APCs and  $10^{-5} M$  GILGFVFTL (green) or SLYNTVATL (pink) peptides overnight before the supernatant was harvested for measurement of MIP-1 $\beta$  presence by ELISA analysis.





**Supplementary Figure 7.2: 868-transduced CD8<sup>+</sup> T cells respond to untransduced HLA-A2<sup>+</sup> MOLT-3 cells, repeat assay.** 30,000 CD8<sup>+</sup> T cells transduced with TCR constructs were cultured in the presence of 60,000 either MOLT-3 wildtype cells or HLA-A2<sup>+</sup> MOLT-3 cells overnight before cells were harvested and assessed for surface expression of TNF and CD107a. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup>CD2<sup>+</sup> single lymphocytes. Numbers on dot plots correspond to the percentage of cells (10,000 events) in the gated population.



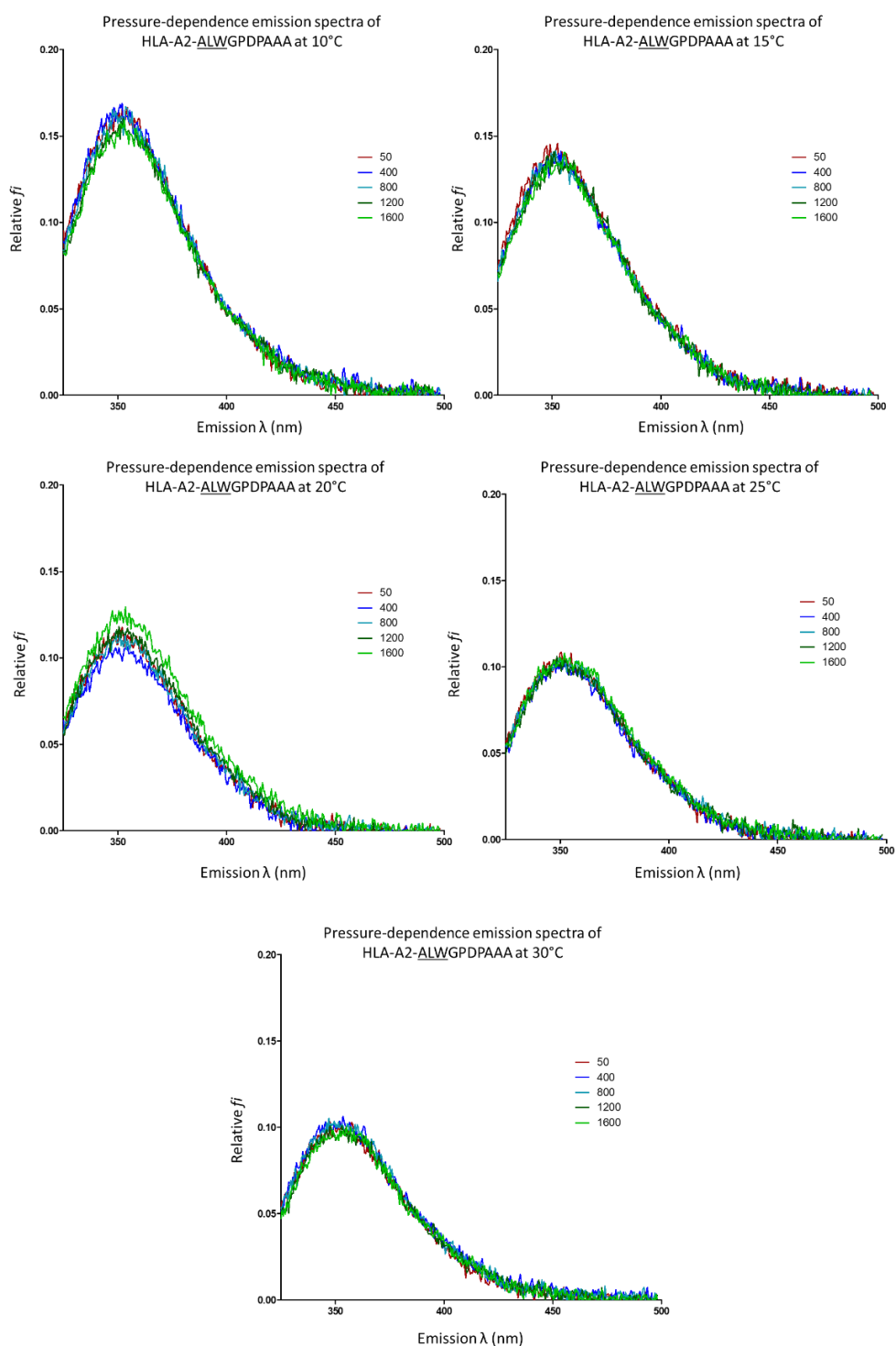
**Supplementary Figure 7.3: 868-transduced CD8<sup>+</sup> T cells respond to a range of HLA-A2<sup>+</sup> immortalised cell lines.** 30,000 CD8<sup>+</sup> T cells transduced with either 868 or TCR 22 were cultured in the presence of 60,000 of a range of cell lines (either HLA-A2<sup>+</sup> or HLA-A2<sup>-</sup>) overnight before cells were harvested and assessed for surface expression of TNF. Cells previously gated on live CD3<sup>+</sup>CD8<sup>+</sup>rCD2<sup>+</sup> single lymphocytes. Numbers on dot plots correspond to the MFI values of 10,000 events.

pMHC complex	Solvent Accessible Surface Area (Å <sup>2</sup> )
ALW	19262.674
MVW	19406.383
YQF	19044.336
RQW	19141.076
RQFa	19266.105
RQFi	19181.838

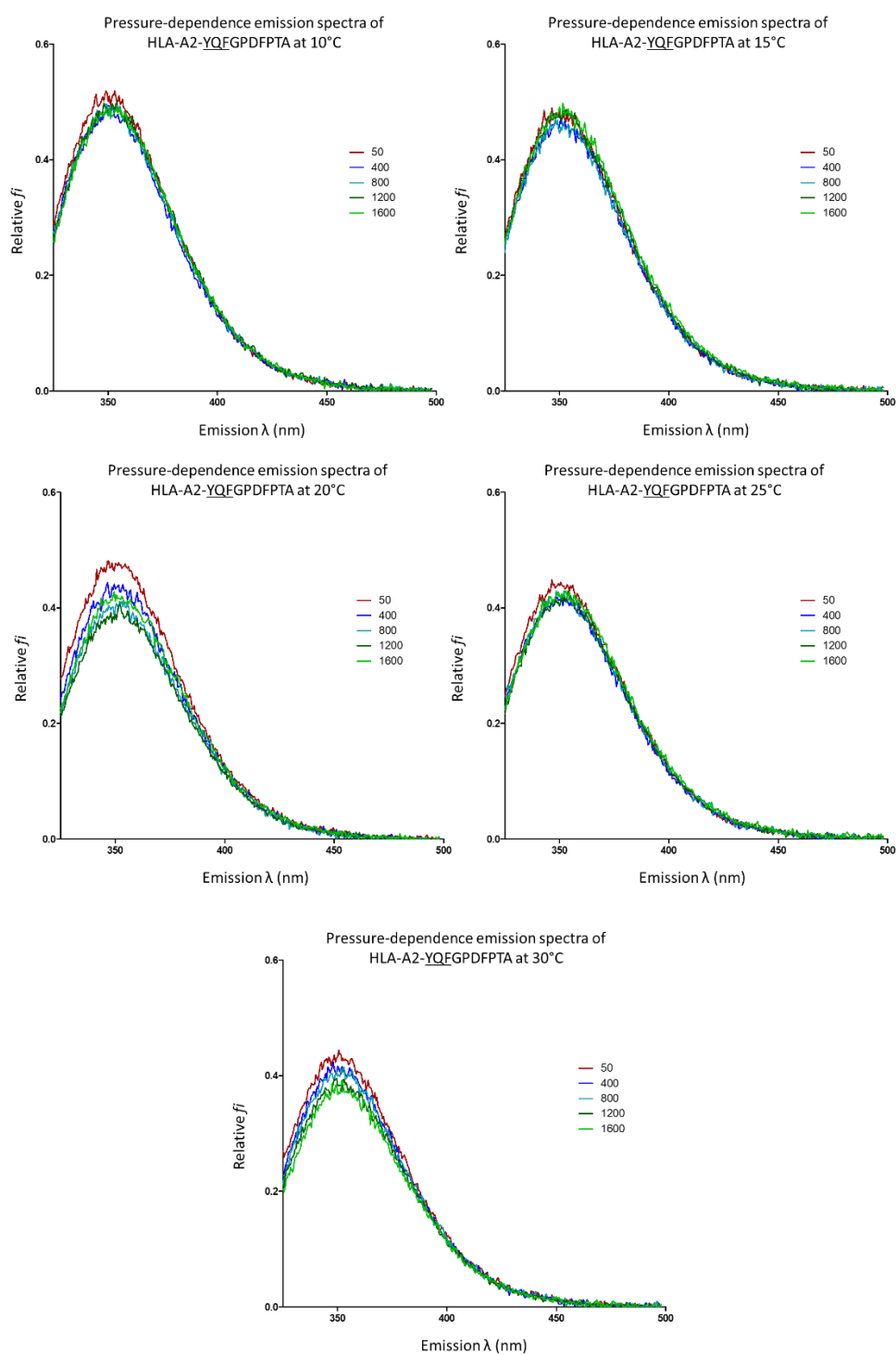
**Supplementary Table 7.1: The SASA of the 6 pMHC complexes is similar.** Calculated using PyMOL get\_area function.

$$\Delta G_{P,T} = \Delta G_0 + \Delta V_0(P - P_0) + \Delta \alpha' (P - P_0)(T - T_0) + \frac{\Delta \beta'}{2} (P - P_0)^2 - \Delta S_0 (T - T_0) - \left[ T \left( \ln \left( \frac{T}{T_0} \Delta C_P \right) - 1 \right) + T_0 \right]$$

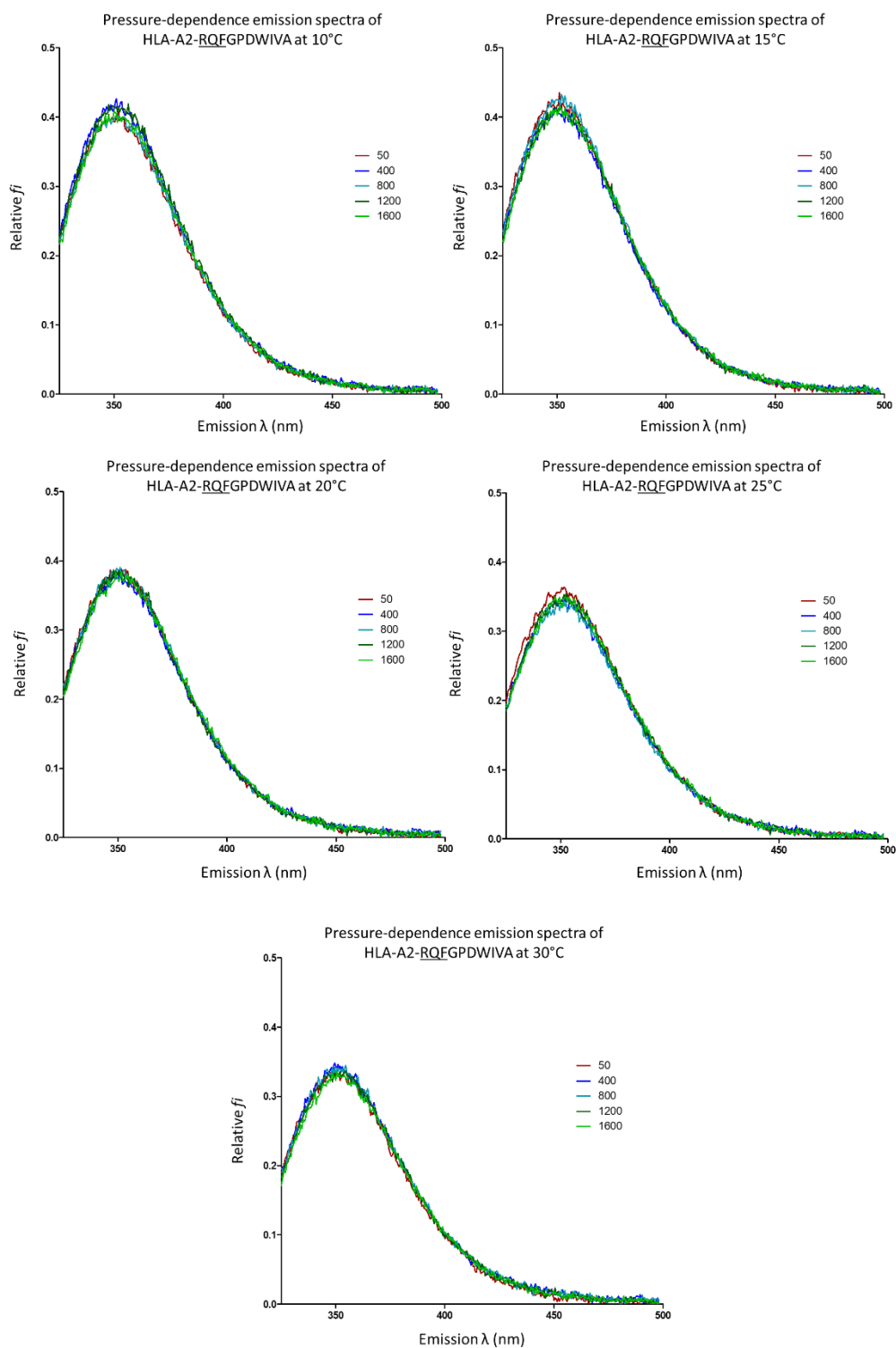
**Supplementary Equation 7.1: Algorithm developed by the Pudney laboratory for the extraction of thermodynamic parameters from emission spectra collected under perturbing pressures and temperatures.**  $\Delta G$  was of particular use to this work.



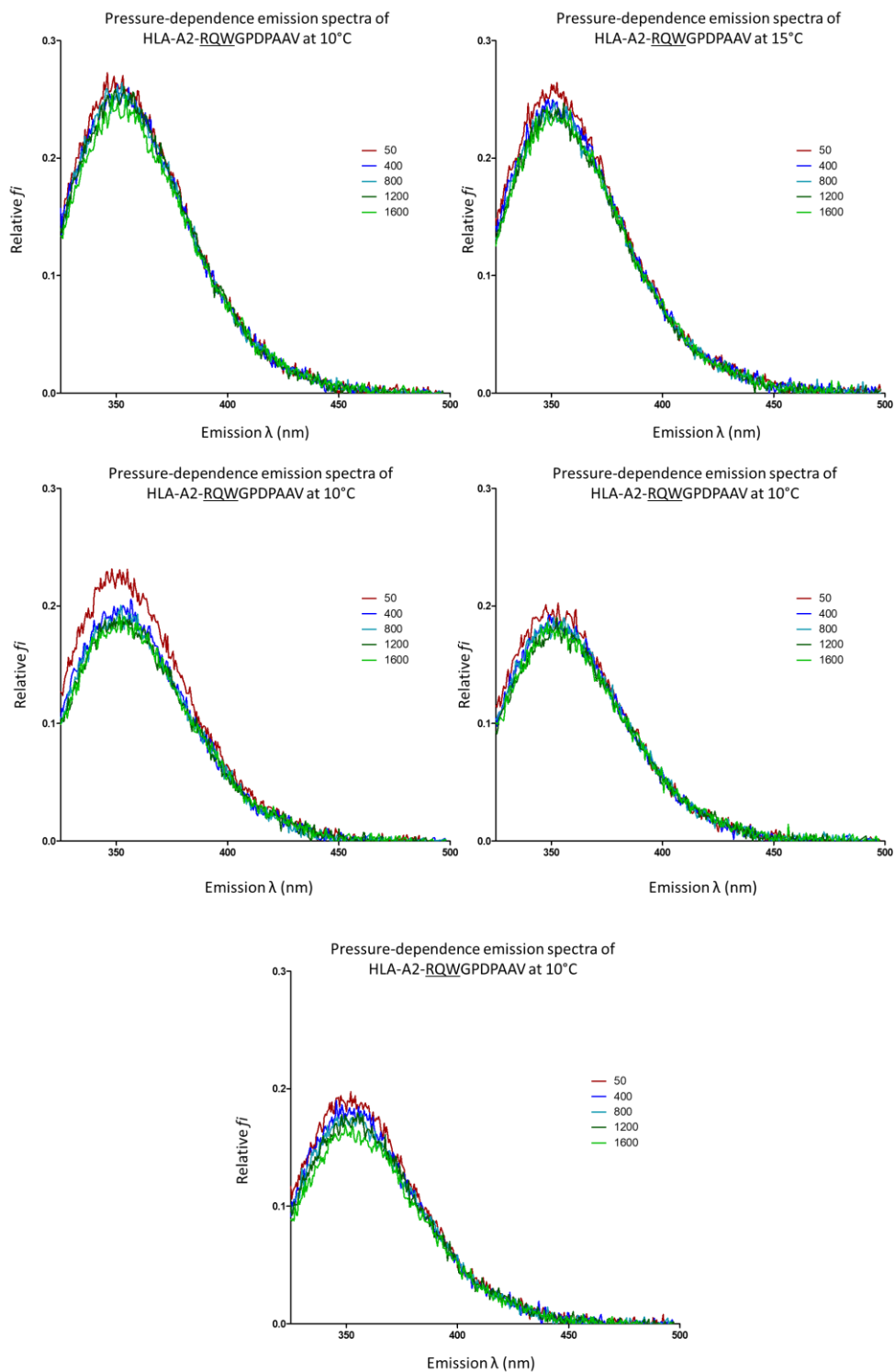
**Supplementary Figure 7.4: Pressure/temperature-dependence of HLA-A2-ALWGPDPAAA complex.** Protein-intrinsic tryptophan fluorescence was excited at 292nm and the emission spectra measured using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). Pressure was applied using an ISS high-pressure cell (ISS, Champaign, IL, USA) fitted with a custom fibre optic mounting connecting to the fluorimeter and a circulating water bath to control temperature ( $\pm 1^\circ\text{C}$ ). Spectra are the average of triplicate collection.



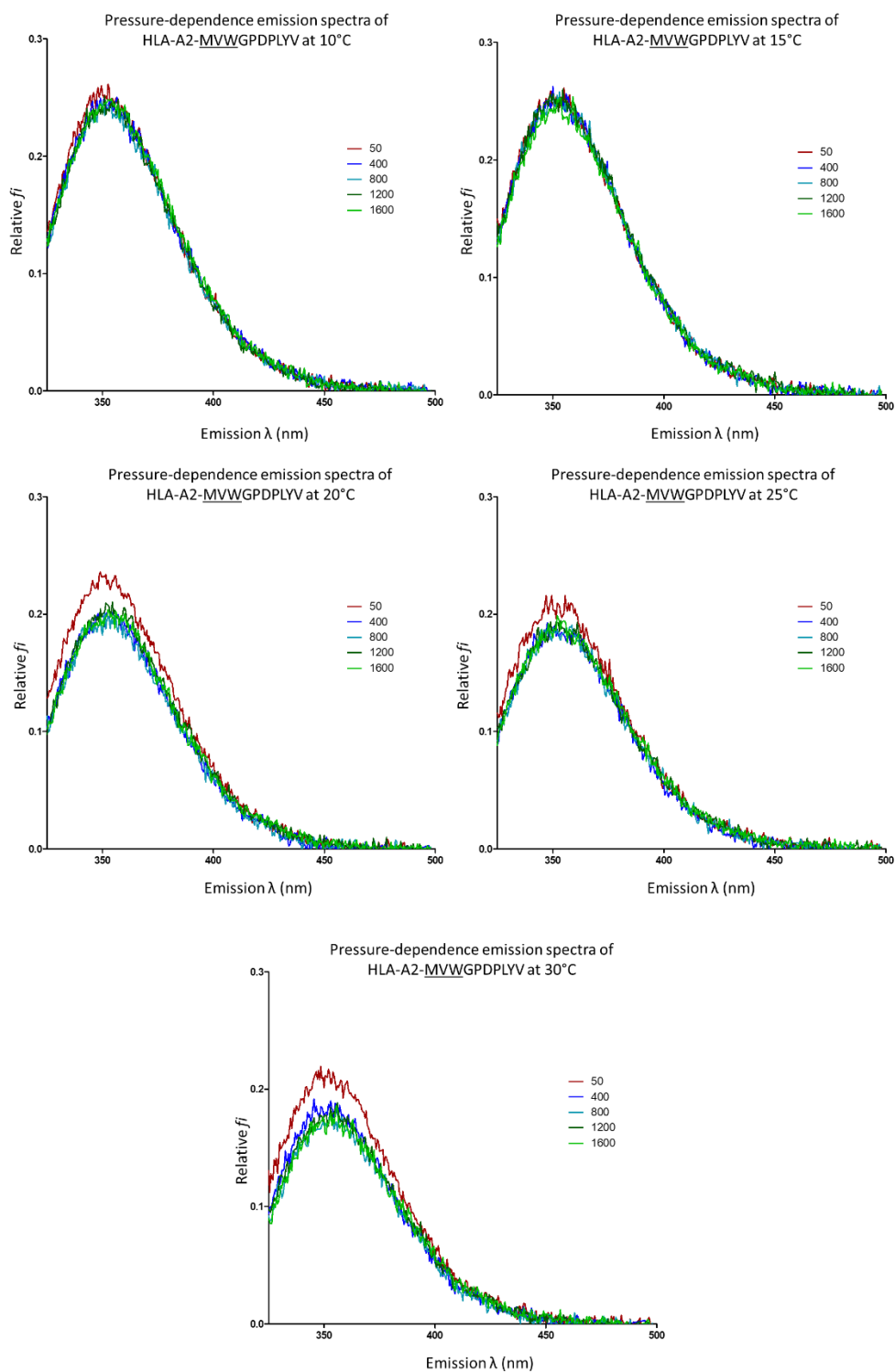
**Supplementary Figure 7.5: Pressure/temperature-dependence of HLA-A2-YQEGPDFPTA complex.** Protein-intrinsic tryptophan fluorescence was excited at 292nm and the emission spectra measured using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). Pressure was applied using an ISS high-pressure cell (ISS, Champaign, IL, USA) fitted with a custom fibre optic mounting connecting to the fluorimeter and a circulating water bath to control temperature ( $\pm 1^\circ\text{C}$ ). Spectra are the average of triplicate collection.



**Supplementary Figure 7.6: Pressure/temperature-dependence of HLA-A2-RQFGPDWIVA complex.** Protein-intrinsic tryptophan fluorescence was excited at 292nm and the emission spectra measured using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). Pressure was applied using an ISS high-pressure cell (ISS, Champaign, IL, USA) fitted with a custom fibre optic mounting connecting to the fluorimeter and a circulating water bath to control temperature ( $\pm 1^\circ\text{C}$ ). Spectra are the average of triplicate collection.

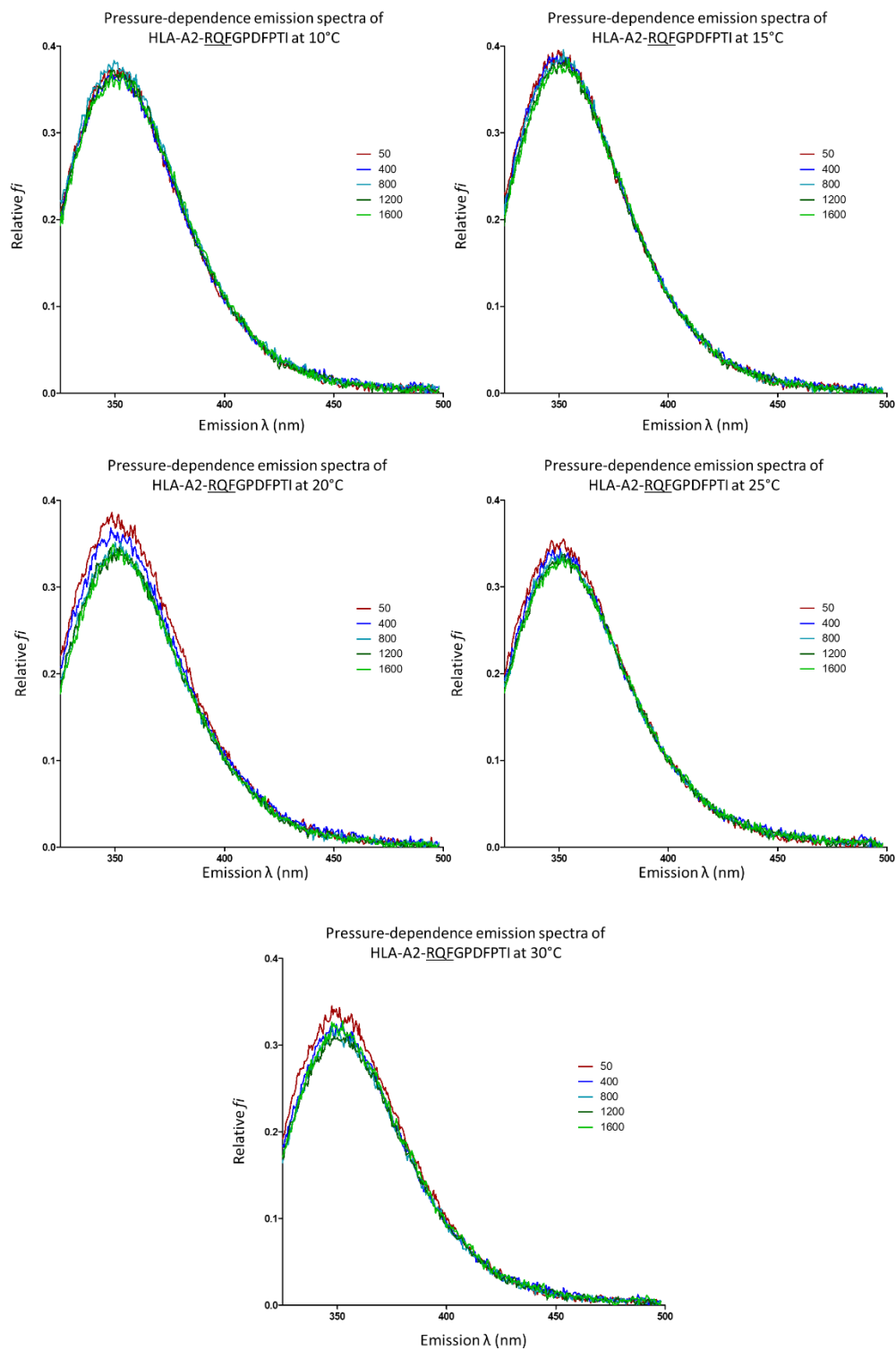


**Supplementary Figure 7.7: Pressure/temperature-dependence of HLA-A2-RQWGPDPAAV complex.** Protein-intrinsic tryptophan fluorescence was excited at 292nm and the emission spectra measured using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). Pressure was applied using an ISS high-pressure cell (ISS, Champaign, IL, USA) fitted with a custom fibre optic mounting connecting to the fluorimeter and a circulating water bath to control temperature ( $\pm 1^\circ\text{C}$ ). Spectra are the average of triplicate collection.



**Supplementary Figure 7.8: Pressure/temperature-dependence of HLA-A2-MVWGPDPlyV complex.** Protein-intrinsic tryptophan fluorescence was excited at 292nm and the emission spectra measured using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). Pressure was applied using an ISS high-pressure cell (ISS, Champaign, IL, USA) fitted with a custom fibre optic mounting connecting to the fluorimeter and a circulating water bath to control temperature ( $\pm 1^\circ\text{C}$ ). Spectra are the average of triplicate collection.





**Supplementary Figure 7.9: Pressure/temperature-dependence of HLA-A2-RQFGPDFPTI complex.**

Protein-intrinsic tryptophan fluorescence was excited at 292nm and the emission spectra measured using a Perkin Elmer LS50B Luminescence Spectrometer (Perkin Elmer, Waltham, MA, USA). Pressure was applied using an ISS high-pressure cell (ISS, Champaign, IL, USA) fitted with a custom fibre optic mounting connecting to the fluorimeter and a circulating water bath to control temperature ( $\pm 1^\circ\text{C}$ ). Spectra are the average of triplicate collection.

## References

- Abdul-Rasoul, M., Habib, H. and Al-Khouly, M. (2006) "The honeymoon phase" in children with type 1 diabetes mellitus: frequency, duration, and influential factors', *Pediatric Diabetes*, 7(2), pp. 101–107. doi: 10.1111/j.1399-543X.2006.00155.x.
- Abraham, N. *et al.* (1991) 'Enhancement of T-cell responsiveness by the lymphocyte-specific tyrosine protein kinase p56lck', *Nature*, 350(6313), pp. 62–66. doi: 10.1038/350062a0.
- Acuto, O. *et al.* (1983) 'The human T cell receptor: Appearance in ontogeny and biochemical relationship of  $\alpha$  and  $\beta$  subunits on IL-2 dependent clones and T cell tumors', *Cell*, 34(3), pp. 717–726. doi: 10.1016/0092-8674(83)90528-7.
- Adams, J. J. *et al.* (2016) 'Structural interplay between germline interactions and adaptive recognition determines the bandwidth of TCR-peptide-MHC cross-reactivity', *Nature Immunology*. Nature Publishing Group, 17(1), pp. 87–94. doi: 10.1038/ni.3310.
- Agata, Y. *et al.* (1996) 'Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes', *International Immunology*. Oxford University Press, 8(5), pp. 765–772. doi: 10.1093/intimm/8.5.765.
- Agrawal, L. *et al.* (2004) 'Role for CCR5 32 Protein in Resistance to R5, R5X4, and X4 Human Immunodeficiency Virus Type 1 in Primary CD4+ Cells', *Journal of Virology*. American Society for Microbiology, 78(5), pp. 2277–2287. doi: 10.1128/jvi.78.5.2277-2287.2004.
- Ahmadi, M. *et al.* (2011) 'CD3 limits the efficacy of TCR gene therapy in vivo', *Blood*, 118(13), pp. 3528–3537. doi: 10.1182/blood-2011-04-346338.
- Akasaka, K. (2006) 'Probing Conformational Fluctuation of Proteins by Pressure Perturbation', *Chemistry Reviews*. doi: 10.1021/cr040440z.
- Aki, M. *et al.* (1994) 'Interferon- $\gamma$  Induces Different Subunit Organizations and Functional Diversity of Proteasomes<sup>1</sup>', *The Journal of Biochemistry*. Oxford University Press, 115(2), pp. 257–269. doi: 10.1093/oxfordjournals.jbchem.a124327.
- Akira, S., Uematsu, S. and Takeuchi, O. (2006) 'Pathogen recognition and innate immunity', *Cell*, pp. 783–801. doi: 10.1016/j.cell.2006.02.015.
- Akkaraju, S. *et al.* (1997) 'A range of CD4 T cell tolerance: Partial inactivation to organ-specific antigen allows nondestructive thyroiditis or insulinitis', *Immunity*. Cell Press, 7(2), pp. 255–271. doi: 10.1016/S1074-7613(00)80528-2.
- Alam, S. M. *et al.* (1996) 'T-cell-receptor affinity and thymocyte positive selection', *Nature*. Nature Publishing Group, 381(6583), pp. 616–620. doi: 10.1038/381616a0.
- Alegre, M. L., Frauwirth, K. A. and Thompson, C. B. (2001) 'T-cell regulation by CD28 and CTLA-4', *Nature Reviews Immunology*. European Association for Cardio-Thoracic Surgery, pp. 220–228. doi: 10.1038/35105024.
- Alkhatib, G. (2009) 'The biology of CCR5 and CXCR4', *Current Opinion in HIV and AIDS*. NIH Public Access, pp. 96–103. doi: 10.1097/COH.0b013e328324bbec.
- Allen, A. G. *et al.* (2018) 'Gene Editing of HIV-1 Co-receptors to Prevent and/or Cure Virus Infection', *Frontiers in Microbiology*. Frontiers Media SA, 9. doi: 10.3389/fmicb.2018.02940.

- Allen, P. M., Babbitt, B. P. and Unanue, E. R. (1987) 'T-Cell Recognition of Lysozyme: The Biochemical Basis of Presentation', *Immunological Reviews*. John Wiley & Sons, Ltd, 98(1), pp. 171–187. doi: 10.1111/j.1600-065X.1987.tb00524.x.
- Allen, T. M. *et al.* (2000) 'Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia', *Nature*. *Nature*, 407(6802), pp. 386–390. doi: 10.1038/35030124.
- Allison, J. P., Hurwitz, A. A. and Leach, D. R. (1995) 'Manipulation of costimulatory signals to enhance antitumor T-cell responses', *Current Opinion in Immunology*, 7(5), pp. 682–686. doi: 10.1016/0952-7915(95)80077-8.
- Allison, J. P., McIntyre, B. W. and Bloch, D. (1982) 'Tumor-specific antigen of murine T-lymphoma defined with monoclonal antibody.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 129(5), pp. 2293–300. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/6181166> (Accessed: 24 February 2020).
- Alt, F. W. *et al.* (1992) 'VDJ recombination', *Immunology Today*, pp. 306–314. doi: 10.1016/0167-5699(92)90043-7.
- Alt, F. W. and Baltimore, D. (1982) 'Joining of immunoglobulin heavy chain gene segments: Implications from a chromosome with evidence of three DJH fusions (recognition elements/Abelson murine leukemia virus/N gene segment/terminal deoxynucleotidyltransferase)', *Proc. Natl Acad. Sci. USA*, 79, p. 41184122.
- Altfeld, M. *et al.* (2005) 'The Majority of Currently Circulating Human Immunodeficiency Virus Type 1 Clade B Viruses Fail To Prime Cytotoxic T-Lymphocyte Responses against an Otherwise Immunodominant HLA-A2-Restricted Epitope: Implications for Vaccine Design', *Journal of Virology*. American Society for Microbiology, 79(8), pp. 5000–5005. doi: 10.1128/jvi.79.8.5000-5005.2005.
- Androlewicz, M. J., Anderson, K. S. and Cresswell, P. (1993) 'Evidence that transporters associated with antigen processing translocate a major histocompatibility complex class I-binding peptide into the endoplasmic reticulum in an ATP-dependent manner', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 90(19), pp. 9130–9134. doi: 10.1073/pnas.90.19.9130.
- Ansari, M. J. I. *et al.* (2003) 'The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice', *Journal of Experimental Medicine*. The Rockefeller University Press, 198(1), pp. 63–69. doi: 10.1084/jem.20022125.
- Archin, N. M. *et al.* (2012) 'Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy', *Nature*. *Nature*, 487(7408), pp. 482–485. doi: 10.1038/nature11286.
- Arends, J. *et al.* (2006) 'T Cell Epitope Mimicry in Antiglomerular Basement Membrane Disease', *The Journal of Immunology*. The American Association of Immunologists, 176(2), pp. 1252–1258. doi: 10.4049/jimmunol.176.2.1252.
- Armstrong, K. M., Piepenbrink, K. H. and Baker, B. M. (2008) 'Conformational changes and flexibility in T-cell receptor recognition of peptide-MHC complexes.', *The Biochemical journal*. Portland Press Ltd, 415(2), pp. 183–96. doi: 10.1042/BJ20080850.
- Arstila, T. P. *et al.* (1999) 'A direct estimate of the human  $\alpha\beta$  T cell receptor diversity', *Science*. American Association for the Advancement of Science, 286(5441), pp. 958–961. doi: 10.1126/science.286.5441.958.

- Artyomov, M. N. *et al.* (2010) 'CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 107(39), pp. 16916–16921. doi: 10.1073/pnas.1010568107.
- Aruffo, A. and Seed, B. (1987) 'Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system.', *Proceedings of the National Academy of Sciences of the United States of America*, 84(23), pp. 8573–8577. doi: 10.1073/pnas.84.23.8573.
- Ashton-Rickardt, P. G. *et al.* (1994) 'Evidence for a differential avidity model of T cell selection in the thymus', *Cell*, 76(4), pp. 651–663. doi: 10.1016/0092-8674(94)90505-3.
- Atkinson, M. A., Eisenbarth, G. S. and Michels, A. W. (2014) 'Type 1 diabetes', *The Lancet*. Lancet Publishing Group, pp. 69–82. doi: 10.1016/S0140-6736(13)60591-7.
- Attaf, M. *et al.* (2015) 'The T cell antigen receptor: The Swiss army knife of the immune system', *Clinical and Experimental Immunology*. Blackwell Publishing Ltd, 181(1), pp. 1–18. doi: 10.1111/cei.12622.
- Attaf, M., Huseby, E. and Sewell, A. K. (2015) 'αβ T cell receptors as predictors of health and disease', *Cellular and Molecular Immunology*. Chinese Soc Immunology, 12(4), pp. 391–399. doi: 10.1038/cmi.2014.134.
- Ayres, C. M. *et al.* (2016) 'Differential utilization of binding loop flexibility in T cell receptor ligand selection and cross-reactivity', *Scientific Reports*. Nature Publishing Group, 6(1), pp. 1–14. doi: 10.1038/srep25070.
- Ayres, C. M. *et al.* (2019) 'Dynamically driven allostery in MHC proteins: Peptide-dependent tuning of class I MHC global flexibility', *Frontiers in Immunology*. Frontiers Media S.A., 10(MAY), p. 966. doi: 10.3389/fimmu.2019.00966.
- Ayres, C. M., Corcelli, S. A. and Baker, B. M. (2017) 'Peptide and peptide-dependent motions in MHC proteins: Immunological implications and biophysical underpinnings', *Frontiers in Immunology*. Frontiers Media S.A., p. 935. doi: 10.3389/fimmu.2017.00935.
- Ayyoub, M. *et al.* (2003) 'Activation of Human Melanoma Reactive CD8+ T Cells by Vaccination With an Immunogenic Peptide Analog Derived From Melan-A/melanoma Antigen Recognized by T cells-1', *Clinical cancer research : an official journal of the American Association for Cancer Research*. Clin Cancer Res, 9(2).
- Babad, J. *et al.* (2015) 'Generation of β cell-specific human cytotoxic T cells by lentiviral transduction and their survival in immunodeficient human leucocyte antigen-transgenic mice', *Clinical and Experimental Immunology*. Wiley-Blackwell, 179(3), p. 398. doi: 10.1111/CEI.12465.
- Babbitt, B. P. *et al.* (1985) 'Binding of immunogenic peptides to Ia histocompatibility molecules', *Nature*, 317(6035), pp. 359–361. doi: 10.1038/317359a0.
- Bach, F. H. *et al.* (1977) 'Antigenic Requirements for Triggering of Cytotoxic T Lymphocytes', *Immunological Reviews*. John Wiley & Sons, Ltd, 35(1), pp. 76–96. doi: 10.1111/j.1600-065X.1977.tb00236.x.
- Bachmann, M. F. *et al.* (1999) 'Developmental regulation of Lck targeting to the CD8 coreceptor controls signaling in naive and memory T cells', *Journal of Experimental Medicine*. The Rockefeller University Press, 189(10), pp. 1521–1529. doi: 10.1084/jem.189.10.1521.

- Bachmann, M. F. *et al.* (2007) 'Differential role of IL-2R signaling for CD8+ T cell responses in acute and chronic viral infections', *European Journal of Immunology*, 37(6), pp. 1502–1512. doi: 10.1002/eji.200637023.
- Badovinac, V. P., Haring, J. S. and Harty, J. T. (2007) 'Initial T Cell Receptor Transgenic Cell Precursor Frequency Dictates Critical Aspects of the CD8+ T Cell Response to Infection', *Immunity*, 26(6), pp. 827–841. doi: 10.1016/j.immuni.2007.04.013.
- Bagaev, D. V. *et al.* (2020) 'VDJdb in 2019: Database extension, new analysis infrastructure and a T-cell receptor motif compendium', *Nucleic Acids Research*. Oxford University Press, 48(D1), pp. D1057–D1062. doi: 10.1093/nar/gkz874.
- Bai, A. and Forman, J. (1997) 'The effect of the proteasome inhibitor lactacystin on the presentation of transporter associated with antigen processing (TAP)-dependent and TAP-independent peptide epitopes by class I molecules.', *The Journal of Immunology*, 159(5).
- Bailey, A. *et al.* (2014) 'Two Polymorphisms Facilitate Differences in Plasticity between Two Chicken Major Histocompatibility Complex Class I Proteins', *PLoS ONE*. Edited by J. Wang, 9(2), p. e89657. doi: 10.1371/journal.pone.0089657.
- Bailey, J. R. *et al.* (2006) 'Maintenance of viral suppression in HIV-1-infected HLA-B\*57 + elite suppressors despite CTL escape mutations', *Journal of Experimental Medicine*. J Exp Med, 203(5), pp. 1357–1369. doi: 10.1084/jem.20052319.
- Bailey, J. R. *et al.* (2008) 'Transmission of Human Immunodeficiency Virus Type 1 from a Patient Who Developed AIDS to an Elite Suppressor', *Journal of Virology*. American Society for Microbiology, 82(15), pp. 7395–7410. doi: 10.1128/jvi.00800-08.
- Balomenos, D. *et al.* (1995) 'Incomplete T cell receptor V beta allelic exclusion and dual V beta-expressing cells.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 155(7), pp. 3308–12. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7561023> (Accessed: 16 April 2020).
- Balotta, C. *et al.* (1997) 'Homozygous  $\Delta 32$  deletion of the CCR-5 chemokine receptor gene in an HIV-1-infected patient', *AIDS*. AIDS, 11(10). doi: 10.1097/00002030-199710000-00001.
- Bangert, C., Brunner, P. M. and Stingl, G. (2011) 'Immune functions of the skin', *Clinics in Dermatology*, 29(4), pp. 360–376. doi: 10.1016/j.clindermatol.2011.01.006.
- Bangham, C. R. M. (2009) 'CTL quality and the control of human retroviral infections', *European Journal of Immunology*. Eur J Immunol, pp. 1700–1712. doi: 10.1002/eji.200939451.
- Banham, A. H. and Smith, G. L. (1993) 'Characterization of vaccinia virus gene B12R', *Journal of General Virology*. Microbiology Society, 74(12), pp. 2807–2812. doi: 10.1099/0022-1317-74-12-2807.
- Bank, I. *et al.* (1986) 'A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes', *Nature*, 322(6075), pp. 179–181. doi: 10.1038/322179a0.
- Banting, F. G. *et al.* (1922) 'Pancreatic Extracts in the Treatment of Diabetes Mellitus.', *Canadian Medical Association journal*. Canadian Medical Association, 12(3), pp. 141–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/20314060> (Accessed: 17 April 2020).
- Banu, N. *et al.* (2014) 'Building and optimizing a virus-specific T cell receptor library for

- targeted immunotherapy in viral infections', *Scientific Reports*. Nature Publishing Group, 4. doi: 10.1038/srep04166.
- Barber, E. K. *et al.* (1989) 'The CD4 and CD8 antigens are coupled to a protein-tyrosine kinase (p56(lck)) that phosphorylates the CD3 complex', *Proceedings of the National Academy of Sciences of the United States of America*, 86(9), pp. 3277–3281. doi: 10.1073/pnas.86.9.3277.
- Barber, L. D. *et al.* (1995) 'Overlap in the repertoires of peptides bound in vivo by a group of related class I HLA-B allotypes', *Current Biology*, 5(2), pp. 179–190. doi: 10.1016/S0960-9822(95)00039-X.
- Barber, L. D. *et al.* (2001) 'The quantity of naturally processed peptides stably bound by HLA-A\*0201 is significantly reduced in the absence of tapasin', *Tissue Antigens*. John Wiley & Sons, Ltd, 58(6), pp. 363–368. doi: 10.1034/j.1399-0039.2001.580604.x.
- Barnd, D. L. *et al.* (1989) 'Specific, major histocompatibility complex-unrestricted recognition of tumor-associated mucins by human cytotoxic T cells', *Proceedings of the National Academy of Sciences of the United States of America*, 86(18), pp. 7159–7163. doi: 10.1073/pnas.86.18.7159.
- Baskar, S. *et al.* (1993) 'Constitutive expression of B7 restores immunogenicity of tumor cells expressing truncated major histocompatibility complex class II molecules', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 90(12), pp. 5687–5690. doi: 10.1073/pnas.90.12.5687.
- Bassing, C. H., Swat, W. and Alt, F. W. (2002) 'The mechanism and regulation of chromosomal V(D)J recombination', *Cell*. Cell Press. doi: 10.1016/S0092-8674(02)00675-X.
- Beck, S. *et al.* (1999) 'Complete sequence and gene map of a human major histocompatibility complex', *Nature*. Macmillan Magazines Ltd, 401(6756), pp. 921–923. doi: 10.1038/44853.
- Beerbaum, M. *et al.* (2013) 'NMR spectroscopy reveals unexpected structural variation at the protein-protein interface in MHC class I molecules', *Journal of Biomolecular NMR*. Springer, 57(2), pp. 167–178. doi: 10.1007/s10858-013-9777-z.
- Bell, M. J. *et al.* (2009) 'The peptide length specificity of some HLA class I alleles is very broad and includes peptides of up to 25 amino acids in length', *Molecular Immunology*, 46(8–9), pp. 1911–1917. doi: 10.1016/j.molimm.2008.12.003.
- Beloukas, A. *et al.* (2016) 'Molecular epidemiology of HIV-1 infection in Europe: An overview', *Infection, Genetics and Evolution*. Elsevier B.V., 46, pp. 180–189. doi: 10.1016/j.meegid.2016.06.033.
- Benhar, I., London, A. and Schwartz, M. (2012) 'The privileged immunity of immune privileged organs: The case of the eye', *Frontiers in Immunology*. Frontiers Media SA, 3(SEP). doi: 10.3389/fimmu.2012.00296.
- Bennett, M. S. *et al.* (2010) 'Fine-tuning of T-cell receptor avidity to increase HIV epitope variant recognition by cytotoxic T lymphocytes', *AIDS*. NIH Public Access, 24(17), pp. 2619–2628. doi: 10.1097/QAD.0b013e32833f7b22.
- Bentley, G. A. and Mariuzza, R. A. (1996) *The Structure of the T Cell Antigen Receptor*, *Annu. Rev. Immunol.* Available at: [www.annualreviews.org](http://www.annualreviews.org) (Accessed: 10 December 2019).
- Bentzen, A. K. *et al.* (2016) 'Large-scale detection of antigen-specific T cells using peptide-

- MHC-I multimers labeled with DNA barcodes', *Nature Biotechnology*. Nature Publishing Group, 34(10), pp. 1037–1045. doi: 10.1038/nbt.3662.
- Berglund, P. *et al.* (2007) 'Viral alteration of cellular translational machinery increases defective ribosomal products.', *Journal of virology*, 81(13), pp. 7220–9. doi: 10.1128/JVI.00137-07.
- Berenger, D. X. *et al.* (2015) 'T cell receptor reversed polarity recognition of a self-antigen major histocompatibility complex', *Nature Immunology*. Nature Publishing Group, 16(11), pp. 1153–1161. doi: 10.1038/ni.3271.
- Berke, G. (1995) 'The CTL's kiss of death', *Cell*, pp. 9–12. doi: 10.1016/0092-8674(95)90365-8.
- Bertoletti, A. *et al.* (1994) 'Natural variants of cytotoxic epitopes are T-cell receptor antagonists for antiviral cytotoxic T cells', *Nature*. Nature, 369(6479), pp. 407–410. doi: 10.1038/369407a0.
- Betts, M. R. *et al.* (2003) 'Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation', *Journal of Immunological Methods*. Elsevier, 281(1–2), pp. 65–78. doi: 10.1016/S0022-1759(03)00265-5.
- Betts, M. R. *et al.* (2006) 'HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells', *Blood*. Blood, 107(12), pp. 4781–4789. doi: 10.1182/blood-2005-12-4818.
- Beutler, B. (2004) 'Innate immunity: An overview', *Molecular Immunology*. Elsevier Ltd, 40(12), pp. 845–859. doi: 10.1016/j.molimm.2003.10.005.
- Bevan, M. J. (1977) 'Killer cells reactive to altered self antigens can also be alloreactive', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 74(5), pp. 2094–2098. doi: 10.1073/pnas.74.5.2094.
- Bhardwaj, V. *et al.* (1993) 'Degenerate recognition of a dissimilar antigenic peptide by myelin basic protein-reactive T cells. Implications for thymic education and autoimmunity.', *Journal of immunology (Baltimore, Md. : 1950)*, 151(9), pp. 5000–10. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7691962> (Accessed: 4 February 2020).
- Bhati, M. *et al.* (2014) 'The versatility of the  $\alpha\beta$  T-cell antigen receptor.', *Protein science : a publication of the Protein Society*. Wiley-Blackwell, 23(3), pp. 260–72. doi: 10.1002/pro.2412.
- Bilal, M. Y., Vacaflares, A. and Houtman, J. C. D. (2015) 'Optimization of methods for the genetic modification of human T cells', *Immunology and Cell Biology*. Nature Publishing Group, 93(10), pp. 896–908. doi: 10.1038/icb.2015.59.
- Billingham, R. E., Brent, L. and Medawar, P. B. (1954) 'Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of actively and adoptively acquired immunity', *Proceedings of the Royal Society of London. Series B - Biological Sciences*. The Royal Society London, 143(910), pp. 58–80. doi: 10.1098/rspb.1954.0054.
- Birnbaum, M. E. *et al.* (2014) 'Deconstructing the peptide-MHC specificity of t cell recognition', *Cell*. Cell Press, 157(5), pp. 1073–1087. doi: 10.1016/j.cell.2014.03.047.
- Björk, S. (2001) 'The cost of diabetes and diabetes care', in *Diabetes Research and Clinical Practice*. Elsevier Ireland Ltd, pp. 13–18. doi: 10.1016/s0168-8227(01)00304-7.
- Bjorkman, P. J., Saper, M A, *et al.* (1987) 'Structure of the human class I histocompatibility

antigen, HLA-A2', *Nature*, 329, pp. 506–512.

Bjorkman, P. J., Saper, M. A., *et al.* (1987) 'The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens', *Nature*, 329(6139), pp. 512–518. doi: 10.1038/329512a0.

Bjorkman, P. J. and Parham, P. (1990) 'Structure, Function, and Diversity of Class I Major Histocompatibility Complex Molecules', *Annual Review of Biochemistry*. Annual Reviews, 59(1), pp. 253–288. doi: 10.1146/annurev.bi.59.070190.001345.

Björnberg, F. *et al.* (1994) 'Mechanisms involved in the processing of the p55 and the p75 tumor necrosis factor (TNF) receptors to soluble receptor forms.', *Lymphokine and cytokine research*, 13(3), pp. 203–11. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7948429> (Accessed: 29 October 2019).

Blankson, J. N. and Siliciano, R. F. (2008) 'Elite Suppression of HIV-1 Replication', *Immunity*. *Immunity*, pp. 845–847. doi: 10.1016/j.immuni.2008.12.002.

Van Bleek, G. M. and Nathenson, S. G. (1991) 'The structure of the antigen-binding groove of major histocompatibility complex class I molecules determines specific selection of self-peptides', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 88(24), pp. 11032–11036. doi: 10.1073/pnas.88.24.11032.

Bluestone, J. A. (2011) 'Mechanisms of tolerance', *Immunological Reviews*, 241(1), pp. 5–19. doi: 10.1111/j.1600-065X.2011.01019.x.

Bluestone, J. A., Herold, K. and Eisenbarth, G. (2010) 'Genetics, pathogenesis and clinical interventions in type 1 diabetes', *Nature*. NIH Public Access, pp. 1293–1300. doi: 10.1038/nature08933.

Boehm, U. *et al.* (1997) 'Cellular responses to interferon- $\gamma$ ', *Annual Review of Immunology*. Annual Reviews, 15(1), pp. 749–795. doi: 10.1146/annurev.immunol.15.1.749.

Boehmer, H. V (1988) 'The Developmental Biology of T Lymphocytes', *Annual Review of Immunology*, 6(1), pp. 309–326. doi: 10.1146/annurev.iy.06.040188.001521.

Von Boehmer, H., Haas, W. and Jerne, N. K. (1978) 'Major histocompatibility complex-linked immune-responsiveness is acquired by lymphocytes of low-responder mice differentiating in thymus of high-responder mice', *Proceedings of the National Academy of Sciences of the United States of America*, 75(5), pp. 2439–2442. doi: 10.1073/pnas.75.5.2439.

Von Boehmer, H., Teh, H. S. and Kisielow, P. (1989) 'The thymus selects the useful, neglects the useless and destroys the harmful', *Immunology Today*, 10(2), pp. 57–61. doi: 10.1016/0167-5699(89)90307-1.

Bolton, W. K. *et al.* (1987) 'T-cells and macrophages in rapidly progressive glomerulonephritis: Clinicopathologic correlations', *Kidney International*, 32(6), pp. 869–876. doi: 10.1038/ki.1987.288.

Boniface, J. J. *et al.* (1999) 'Thermodynamics of T cell receptor binding to peptide-MHC: Evidence for a general mechanism of molecular scanning', *Proceedings of the National Academy of Sciences of the United States of America*, 96(20), pp. 11446–11451. doi: 10.1073/pnas.96.20.11446.

Boon, A. C. M. *et al.* (2002) 'Sequence Variation in a Newly Identified HLA-B35-Restricted Epitope in the Influenza A Virus Nucleoprotein Associated with Escape from Cytotoxic T



- Lymphocytes', *Journal of Virology*. American Society for Microbiology, 76(5), pp. 2567–2572. doi: 10.1128/jvi.76.5.2567-2572.2002.
- Borbulevych, O. Y. *et al.* (2007) 'Structures of MART-126/27-35 Peptide/HLA-A2 Complexes Reveal a Remarkable Disconnect between Antigen Structural Homology and T Cell Recognition', *Journal of Molecular Biology*. NIH Public Access, 372(5), pp. 1123–1136. doi: 10.1016/j.jmb.2007.07.025.
- Borbulevych, O. Y. *et al.* (2009) 'T Cell Receptor Cross-reactivity Directed by Antigen-Dependent Tuning of Peptide-MHC Molecular Flexibility', *Immunity*. NIH Public Access, 31(6), pp. 885–896. doi: 10.1016/j.immuni.2009.11.003.
- Borbulevych, O. Y. *et al.* (2011) 'TCRs Used in Cancer Gene Therapy Cross-React with MART-1/Melan-A Tumor Antigens via Distinct Mechanisms', *The Journal of Immunology*, 187(5), pp. 2453–2463. doi: 10.4049/jimmunol.1101268.
- Borman, A. M. *et al.* (1997) 'Comparison of picornaviral IRES-driven internal initiation of translation in cultured cells of different origins', *Nucleic Acids Research*, 25(5).
- Borrow, P. *et al.* (1994) 'Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection.', *Journal of Virology*. American Society for Microbiology, 68(9), pp. 6103–6110. doi: 10.1128/jvi.68.9.6103-6110.1994.
- Bottazzo, G. F. *et al.* (1985) 'In Situ Characterization of Autoimmune Phenomena and Expression of HLA Molecules in the Pancreas in Diabetic Insulinitis', *New England Journal of Medicine*, 313(6), pp. 353–360. doi: 10.1056/NEJM198508083130604.
- Bouley, D. M. *et al.* (1995) 'Characterization of herpes simplex virus type-1 infection and herpetic stromal keratitis development in IFN-gamma knockout mice.', *Journal of immunology (Baltimore, Md. : 1950)*, 155(8), pp. 3964–71. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7561104> (Accessed: 13 January 2020).
- Boulter, J. M. *et al.* (2003) 'Stable, soluble T-cell receptor molecules for crystallization and therapeutics.', *Protein engineering*, 16(9), pp. 707–11. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/14560057> (Accessed: 20 November 2017).
- Boyle, L. H. *et al.* (2013) 'Tapasin-related protein TAPBPR is an additional component of the MHC class I presentation pathway', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 110(9), pp. 3465–3470. doi: 10.1073/pnas.1222342110.
- Boyle, M. J. *et al.* (2015) 'Human antibodies fix complement to inhibit plasmodium falciparum invasion of erythrocytes and are associated with protection against malaria', *Immunity*. Cell Press, 42(3), pp. 580–590. doi: 10.1016/j.immuni.2015.02.012.
- Brady, B. L., Steinel, N. C. and Bassing, C. H. (2010) 'Antigen Receptor Allelic Exclusion: An Update and Reappraisal', *The Journal of Immunology*. The American Association of Immunologists, 185(7), pp. 3801–3808. doi: 10.4049/jimmunol.1001158.
- Brander, C. *et al.* (1998) 'Lack of strong immune selection pressure by the immunodominant, HLA-A\*0201-restricted cytotoxic T lymphocyte response in chronic human immunodeficiency virus-1 infection', *Journal of Clinical Investigation*. American Society for Clinical Investigation, 101(11), pp. 2559–2566. doi: 10.1172/JCI2405.
- Brenner, M. B. *et al.* (1986) 'Identification of a putative second T-cell receptor', *Nature*, 322(6075), pp. 145–149. doi: 10.1038/322145a0.

- Bretscher, P. and Cohn, M. (1970) 'A theory of self-nonsel self discrimination', *Science*, 169(3950), pp. 1042–1049. doi: 10.1126/science.169.3950.1042.
- Bridgeman, J. S. *et al.* (2011) 'Structural and biophysical determinants of  $\alpha\beta$  T-cell antigen recognition.', *Immunology*. Wiley-Blackwell, 135(1), pp. 9–18. doi: 10.1111/j.1365-2567.2011.03515.x.
- van den Broek, M. F. *et al.* (1995) 'Antiviral defense in mice lacking both alpha/beta and gamma interferon receptors.', *Journal of virology*, 69(8), pp. 4792–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7609046> (Accessed: 13 January 2020).
- Brooks, J. T. *et al.* (2009) 'HIV-Associated Opportunistic Infections—Going, Going, But Not Gone: The Continued Need for Prevention and Treatment Guidelines', *Clinical Infectious Diseases*. Oxford University Press (OUP), 48(5), pp. 609–611. doi: 10.1086/596756.
- Brown, J. A. *et al.* (2003) 'Blockade of Programmed Death-1 Ligands on Dendritic Cells Enhances T Cell Activation and Cytokine Production', *The Journal of Immunology*. The American Association of Immunologists, 170(3), pp. 1257–1266. doi: 10.4049/jimmunol.170.3.1257.
- Brown, J. H. *et al.* (1993) 'Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1', *Nature*, 364(6432), pp. 33–39. doi: 10.1038/364033a0.
- Brown, M. G., Driscoll, J. and Monaco, J. J. (1991) 'Structural and serological similarity of MHC-linked LMP and proteasome (multicatalytic proteinase) complexes', *Nature*. Nature Publishing Group, 353(6342), pp. 355–357. doi: 10.1038/353355a0.
- Bu, J. Y., Shaw, A. S. and Chan, A. C. (1995) 'Analysis of the interaction of ZAP-70 and syk protein-tyrosine kinases with the T-cell antigen receptor by plasmon resonance', *Proceedings of the National Academy of Sciences of the United States of America*, 92(11), pp. 5106–5110. doi: 10.1073/pnas.92.11.5106.
- Bulek, A. M. *et al.* (2012) 'Structural basis for the killing of human beta cells by CD8+ T cells in type 1 diabetes', *Nature Immunology*, 13(3), pp. 283–289. doi: 10.1038/ni.2206.
- Burkhardt, J. K. *et al.* (1993) 'Lytic granules from cytotoxic T cells exhibit kinesin-dependent motility on microtubules in vitro.', *Journal of cell science*, 104 ( Pt 1), pp. 151–62. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8449993> (Accessed: 13 January 2020).
- Burkhardt, C. *et al.* (1999) 'Peptide-induced T cell regulation of experimental autoimmune encephalomyelitis: A role for IL-10', *International Immunology*. Int Immunol, 11(10), pp. 1625–1634. doi: 10.1093/intimm/11.10.1625.
- Burnet, F. M. (1959) *The clonal selection theory of acquired immunity.*, *The clonal selection theory of acquired immunity*. Nashville,: Vanderbilt University Press,. doi: 10.5962/bhl.title.8281.
- Burnet, F. M. (1976) 'A Modification of Jerne's Theory of Antibody Production using the Concept of Clonal Selection', *CA: A Cancer Journal for Clinicians*, 26(2), pp. 119–121. doi: 10.3322/canjclin.26.2.119.
- Burrows, J. M. *et al.* (2008) 'Preferential binding of unusually long peptides to MHC class I and its influence on the selection of target peptides for T cell recognition', *Molecular Immunology*, 45(6), pp. 1818–1824. doi: 10.1016/j.molimm.2007.09.026.
- Burrows, S. R. *et al.* (2010) 'Hard wiring of T cell receptor specificity for the major

histocompatibility complex is underpinned by TCR adaptability', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 107(23), pp. 10608–10613. doi: 10.1073/pnas.1004926107.

Butz, E. A. and Bevan, M. J. (1998) 'Massive expansion of antigen-specific CD8+ T cells during an acute virus infection', *Immunity*. Cell Press, 8(2), pp. 167–175. doi: 10.1016/S1074-7613(00)80469-0.

Bystry, R. S. *et al.* (2001) 'B cells and professional APCs recruit regulatory T cells via CCL4', *Nature Immunology*, 2(12), pp. 1126–1132. doi: 10.1038/ni735.

Cameron, B. J. *et al.* (2013) 'Identification of a titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE A3-directed T cells', *Science Translational Medicine*, 5(197), p. 197ra103. doi: 10.1126/scitranslmed.3006034.

Campbell, R. D. and Trowsdale, J. (1993) 'Map of the human MHC', *Immunology Today*, pp. 349–352. doi: 10.1016/0167-5699(93)90234-C.

Cannon, P. M. *et al.* (1997) 'Structure-function studies of the human immunodeficiency virus type 1 matrix protein, p17.', *Journal of virology*. American Society for Microbiology, 71(5), pp. 3474–3483. doi: 10.1128/jvi.71.5.3474-3483.1997.

Cantor, H. and Boyse, E. A. (1977) 'Lymphocytes as Models for the Study of Mammalian Cellular Differentiation', *Immunological Reviews*. John Wiley & Sons, Ltd, 33(1), pp. 105–124. doi: 10.1111/j.1600-065X.1977.tb00364.x.

Carlson, J. M. *et al.* (2012) 'Widespread Impact of HLA Restriction on Immune Control and Escape Pathways of HIV-1', *Journal of Virology*. American Society for Microbiology, 86(9), pp. 5230–5243. doi: 10.1128/jvi.06728-11.

Carmichael, A. *et al.* (1993) 'Quantitative Analysis of the Human Immunodeficiency Virus Type 1 (HIV-1)-specific Cytotoxic T Lymphocyte (CTL) Response at Different Stages of HIV-1 Infection: Differential CTL Responses to HIV-1 and Epstein-Barr Virus in Late Disease', *Journal of Experimental Medicine*, 177(2), pp. 249–256.

Carreno, B. M. and Collins, M. (2002) 'The B7 Family of ligands and its receptors : New Pathways for Costimulation and Inhibition of Immune Responses', *Annual Review of Immunology*. Annual Reviews, 20(1), pp. 29–53. doi: 10.1146/annurev.immunol.20.091101.091806.

Carter, L. *et al.* (2002) 'PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2.', *European journal of immunology*, 32(3), pp. 634–43. doi: 10.1002/1521-4141(200203)32:3<634::AID-IMMU634>3.0.CO;2-9.

Casazza, J. P. *et al.* (2005) 'Immunologic Pressure within Class I-Restricted Cognate Human Immunodeficiency Virus Epitopes during Highly Active Antiretroviral Therapy', *Journal of Virology*. American Society for Microbiology, 79(6), pp. 3653–3663. doi: 10.1128/jvi.79.6.3653-3663.2005.

Casey Crooks, M. E. and Littman, D. R. (1994) 'Disruption of T lymphocyte positive and negative selection in mice lacking the CD8  $\beta$  chain', *Immunity*, 1(4), pp. 277–285. doi: 10.1016/1074-7613(94)90079-5.

Catano, G. *et al.* (2008) 'HIV-1 disease-influencing effects associated with ZNRD1, HCP5 and HLA-C alleles are attributable mainly to either HLA-A10 or HLA-B\*57 alleles', *PLoS ONE*. PLoS One, 3(11). doi: 10.1371/journal.pone.0003636.

- Catici, D. A. M. *et al.* (2016) 'The red edge excitation shift phenomenon can be used to unmask protein structural ensembles: implications for NEMO-ubiquitin interactions', *The FEBS Journal*, 283(12), pp. 2272–2284. doi: 10.1111/febs.13724.
- Cella, M. *et al.* (2010) 'Loss of DNAM-1 contributes to CD8+ T-cell exhaustion in chronic HIV-1 infection', *European Journal of Immunology*. Eur J Immunol, 40(4), pp. 949–954. doi: 10.1002/eji.200940234.
- Cerundolo, V. *et al.* (1991) 'The binding affinity and dissociation rates of peptides for class I major histocompatibility complex molecules', *European Journal of Immunology*. John Wiley & Sons, Ltd, 21(9), pp. 2069–2075. doi: 10.1002/eji.1830210915.
- Cerundolo, V. *et al.* (1995) 'Genes encoded in the major histocompatibility complex affecting the generation of peptides for TAP transport', *European Journal of Immunology*. John Wiley & Sons, Ltd, 25(2), pp. 554–562. doi: 10.1002/eji.1830250238.
- Chabas, D. *et al.* (2003) 'The genetics of arcolepsy', *Annual Review of Genomics and Human Genetics*. Annual Reviews, 4(1), pp. 459–483. doi: 10.1146/annurev.genom.4.070802.110432.
- Chackerian, B. *et al.* (1997) 'Human immunodeficiency virus type 1 coreceptors participate in postentry stages in the virus replication cycle and function in simian immunodeficiency virus infection.', *Journal of virology*. American Society for Microbiology, 71(5), pp. 3932–3939. doi: 10.1128/jvi.71.5.3932-3939.1997.
- Chan, A. C. *et al.* (1992) 'ZAP-70: A 70 kd protein-tyrosine kinase that associates with the TCR  $\zeta$  chain', *Cell*, 71(4), pp. 649–662. doi: 10.1016/0092-8674(92)90598-7.
- Chapiro, J. *et al.* (2006) 'Destructive Cleavage of Antigenic Peptides Either by the Immunoproteasome or by the Standard Proteasome Results in Differential Antigen Presentation', *The Journal of Immunology*. The American Association of Immunologists, 176(2), pp. 1053–1061. doi: 10.4049/jimmunol.176.2.1053.
- Chattopadhyay, A. and Haldar, S. (2014) 'Dynamic Insight into Protein Structure Utilizing Red Edge Excitation Shift', *Accounts of Chemical Research*. American Chemical Society, 47(1), pp. 12–19. doi: 10.1021/ar400006z.
- Chemin, K. *et al.* (2016) 'A Novel HLA-DRB1\*10:01-Restricted T Cell Epitope from Citrullinated Type II Collagen Relevant to Rheumatoid Arthritis', *Arthritis and Rheumatology*. John Wiley and Sons Inc., 68(5), pp. 1124–1135. doi: 10.1002/art.39553.
- Chen, G. *et al.* (2017) 'Sequence and Structural Analyses Reveal Distinct and Highly Diverse Human CD8+ TCR Repertoires to Immunodominant Viral Antigens', *Cell Reports*. Elsevier B.V., 19(3), pp. 569–583. doi: 10.1016/j.celrep.2017.03.072.
- Chen, J. L. *et al.* (2005) 'Structural and kinetic basis for heightened immunogenicity of T cell vaccines', *Journal of Experimental Medicine*. The Rockefeller University Press, 201(8), pp. 1243–1255. doi: 10.1084/jem.20042323.
- Chen, L. *et al.* (1992) 'Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4.', *Cell*, 71(7), pp. 1093–102. doi: 10.1016/s0092-8674(05)80059-5.
- Chen, M. and Bouvier, M. (2007) 'Analysis of interactions in a tapasin/class I complex provides a mechanism for peptide selection', *EMBO Journal*. European Molecular Biology Organization, 26(6), pp. 1681–1690. doi: 10.1038/sj.emboj.7601624.

- Chen, Y. *et al.* (1994) 'Naturally processed peptides longer than nine amino acid residues bind to the class I MHC molecule HLA-A2.1 with high affinity and in different conformations.', *Journal of immunology (Baltimore, Md. : 1950)*, 152(6), pp. 2874–81. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8144888> (Accessed: 12 January 2018).
- Chicz, R. M. *et al.* (1992) 'Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size', *Nature*, 358(6389), pp. 764–768. doi: 10.1038/358764a0.
- Chiu, C. *et al.* (2014) 'Broadly Reactive Human CD8 T Cells that Recognize an Epitope Conserved between VZV, HSV and EBV', *PLoS Pathogens*. Public Library of Science, 10(3). doi: 10.1371/journal.ppat.1004008.
- Choudhuri, K. *et al.* (2005) 'T-cell receptor triggering is critically dependent on the dimensions of its peptide-MHC ligand', *Nature*. Nature Publishing Group, 436(7050), pp. 578–582. doi: 10.1038/nature03843.
- Choudhuri, K. *et al.* (2009) 'Peptide-major histocompatibility complex dimensions control proximal kinase-phosphatase balance during T cell activation', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 284(38), pp. 26096–26105. doi: 10.1074/jbc.M109.039966.
- Christie, N. M. *et al.* (2009) 'Viral fitness implications of variation within an immunodominant CD8+ T-cell epitope of HIV-1', *Virology*. Virology, 388(1), pp. 137–146. doi: 10.1016/j.virol.2009.03.003.
- Chujo, D. *et al.* (2013) 'ZnT8-Specific CD4+ T Cells Display Distinct Cytokine Expression Profiles between Type 1 Diabetes Patients and Healthy Adults', *PLoS ONE*, 8(2), p. e55595. doi: 10.1371/journal.pone.0055595.
- Chun, T. W. *et al.* (1995) 'In vivo fate of HIV-1-infected T cells: Quantitative analysis of the transition to stable latency', *Nature Medicine*. Nature Publishing Group, 1(12), pp. 1284–1290. doi: 10.1038/nm1295-1284.
- Chun, T. W., Stuyver, L., *et al.* (1997) 'Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy', *Proceedings of the National Academy of Sciences of the United States of America*. Proc Natl Acad Sci U S A, 94(24), pp. 13193–13197. doi: 10.1073/pnas.94.24.13193.
- Chun, T. W., Carruth, L., *et al.* (1997) 'Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection', *Nature*. Nature, 387(6629), pp. 183–188. doi: 10.1038/387183a0.
- Cibotti, R. *et al.* (1992) 'Tolerance to a self-protein involves its immunodominant but does not involve its subdominant determinants', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 89(1), pp. 416–420. doi: 10.1073/pnas.89.1.416.
- Clark, S. J. *et al.* (1991) 'High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection', *New England Journal of Medicine*. N Engl J Med, 324(14), pp. 954–960. doi: 10.1056/NEJM199104043241404.
- Clifford, G. M. and Franceschi, S. (2009) 'Cancer risk in HIV-infected persons: Influence of CD4+ count', *Future Oncology*. Future Oncol, pp. 669–678. doi: 10.2217/fon.09.28.
- Cocchi, F. *et al.* (1995) 'Identification of RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$  as the major HIV-suppressive factors produced by CD8+ T cells', *Science*. Science, 270(5243), p. 1811. doi:

10.1126/science.270.5243.1811.

Coffin, J. *et al.* (1986) 'Human immunodeficiency viruses [1]', *Science*. Science, p. 697. doi: 10.1126/science.3008335.

Cohen, C. J. *et al.* (2006) 'Enhanced antitumor activity of murine-human hybrid T-cell receptor (TCR) in human lymphocytes is associated with improved pairing and TCR/CD3 stability', *Cancer Research*. NIH Public Access, 66(17), pp. 8878–8886. doi: 10.1158/0008-5472.CAN-06-1450.

Cohen, C. J. *et al.* (2007) 'Enhanced antitumor activity of T cells engineered to express T-cell receptors with a second disulfide bond', *Cancer Research*. NIH Public Access, 67(8), pp. 3898–3903. doi: 10.1158/0008-5472.CAN-06-3986.

Cole, D. K. *et al.* (2007) 'Human TCR-Binding Affinity is Governed by MHC Class Restriction', *The Journal of Immunology*. American Association of Immunologists, 178(9), pp. 5727–5734. doi: 10.4049/jimmunol.178.9.5727.

Cole, D. K. *et al.* (2008) 'T cell receptor engagement of peptide-major histocompatibility complex class I does not modify CD8 binding', *Molecular Immunology*. Pergamon, 45(9), pp. 2700–2709. doi: 10.1016/j.molimm.2007.12.009.

Cole, D. K. *et al.* (2009) 'Germ line-governed recognition of a cancer epitope by an immunodominant human T-cell receptor', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 284(40), pp. 27281–27289. doi: 10.1074/jbc.M109.022509.

Cole, D. K. *et al.* (2010) 'Modification of MHC anchor residues generates heteroclitic peptides that alter TCR binding and T cell recognition.', *Journal of immunology (Baltimore, Md. : 1950)*. Europe PMC Funders, 185(4), pp. 2600–10. doi: 10.4049/jimmunol.1000629.

Cole, D. K. (2013) 'Increased peptide contacts govern high affinity binding of a modified TCR whilst maintaining a native pMHC docking mode', *Frontiers in Immunology*. Frontiers Research Foundation, 4(JUN), p. 168. doi: 10.3389/fimmu.2013.00168.

Cole, D. K. *et al.* (2014) 'T-cell Receptor (TCR)-peptide specificity overrides affinity-enhancing TCR-major histocompatibility complex interactions', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 289(2), pp. 628–638. doi: 10.1074/jbc.M113.522110.

Cole, D. K. *et al.* (2016) 'Hotspot autoimmune T cell receptor binding underlies pathogen and insulin peptide cross-reactivity', *The Journal of Clinical Investigation*. American Society for Clinical Investigation, 126(6), pp. 2191–2204. doi: 10.1172/JCI85679.

Cole, D. K., Fuller, A., *et al.* (2017) 'Dual Molecular Mechanisms Govern Escape at Immunodominant HLA A2-Restricted HIV Epitope', *Frontiers in Immunology*, 8, p. 1503. doi: 10.3389/fimmu.2017.01503.

Cole, D. K., Van Den Berg, H. A., *et al.* (2017) 'Structural mechanism underpinning cross-reactivity of a CD8+ T-cell clone that recognizes a peptide derived from human telomerase reverse transcriptase', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology Inc., 292(3), pp. 802–813. doi: 10.1074/jbc.M116.741603.

Colf, L. A. *et al.* (2007) 'How a Single T Cell Receptor Recognizes Both Self and Foreign MHC', *Cell*, 129(1), pp. 135–146. doi: 10.1016/j.cell.2007.01.048.

Conlon, K. *et al.* (1995) 'CD8+ and CD45RA+ human peripheral blood lymphocytes are

potent sources of macrophage inflammatory protein 1 $\alpha$ , interleukin-8 and RANTES', *European Journal of Immunology*. Eur J Immunol, 25(3), pp. 751–756. doi: 10.1002/eji.1830250319.

Connolly, J. M. *et al.* (1988) 'The Lyt-2 molecule recognizes residues in the class I  $\alpha$ 3 domain in allogeneic cytotoxic t cell responses', *Journal of Experimental Medicine*. The Rockefeller University Press, 168(1), pp. 325–341. doi: 10.1084/jem.168.1.325.

Conte, L. Lo, Chothia, C. and Janin, J. (1999) 'The atomic structure of protein-protein recognition sites', *Journal of Molecular Biology*. Academic Press, 285(5), pp. 2177–2198. doi: 10.1006/jmbi.1998.2439.

Coppieters, K. T. *et al.* (2012) 'Demonstration of islet-autoreactive CD8 T cells in insulinitic lesions from recent onset and long-term type 1 diabetes patients.', *The Journal of experimental medicine*. Rockefeller University Press, 209(1), pp. 51–60. doi: 10.1084/jem.20111187.

Corr, M. *et al.* (1994) 'T cell receptor-MHC class I peptide interactions: Affinity, kinetics, and specificity', *Science*. American Association for the Advancement of Science, 265(5174), pp. 946–949. doi: 10.1126/science.8052850.

Cosnefroy, O., Murray, P. J. and Bishop, K. N. (2016) 'HIV-1 capsid uncoating initiates after the first strand transfer of reverse transcription', *Retrovirology*. BioMed Central Ltd., 13(1), p. 58. doi: 10.1186/s12977-016-0292-7.

Craiu, A. *et al.* (1997) 'Two distinct proteolytic processes in the generation of a major histocompatibility complex class I-presented peptide', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 94(20), pp. 10850–10855. doi: 10.1073/pnas.94.20.10850.

Crawford, F. *et al.* (2004) 'Mimotopes for alloreactive and conventional T cells in a peptide-MHC display library', *PLoS Biology*. PLoS Biol, 2(4). doi: 10.1371/journal.pbio.0020090.

Culina, S. *et al.* (2018) 'Islet-reactive CD8+ T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors', *Science Immunology*. American Association for the Advancement of Science, 3(20), p. 4013. doi: 10.1126/sciimmunol.aao4013.

Daar, E. S. *et al.* (1991) 'Transient High Levels of Viremia in Patients with Primary Human Immunodeficiency Virus Type 1 Infection', *New England Journal of Medicine*. N Engl J Med, 324(14), pp. 961–964. doi: 10.1056/NEJM199104043241405.

Dai, S. *et al.* (2008) 'Crossreactive T Cells Spotlight the Germline Rules for  $\alpha\beta$  T Cell-Receptor Interactions with MHC Molecules', *Immunity*, 28(3), pp. 324–334. doi: 10.1016/j.immuni.2008.01.008.

Dale, J. B. and Beachey, E. H. (1986) 'Sequence of myosin-crossreactive epitopes of streptococcal M protein', *Journal of Experimental Medicine*, 164(5), pp. 1785–1790. doi: 10.1084/jem.164.5.1785.

Dashti, A. *et al.* (2019) 'Broadly Neutralizing Antibodies against HIV: Back to Blood', *Trends in Molecular Medicine*. Elsevier Ltd, pp. 228–240. doi: 10.1016/j.molmed.2019.01.007.

David-Watine, B., Israël, A. and Kourilsky, P. (1990) 'The regulation and expression of MHC class I genes', *Immunology Today*, 11(C), pp. 286–292. doi: 10.1016/0167-5699(90)90114-O.

- Davis, M. M. and Bjorkman, P. J. (1988) 'T-cell antigen receptor genes and T-cell recognition', *Nature*, pp. 395–402. doi: 10.1038/334395a0.
- Davis, S. J. and van der Merwe, P. A. (1996) 'The structure and ligand interactions of CD2: implications for T-cell function.', *Immunology today*, 17(4), pp. 177–87. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8871350> (Accessed: 11 October 2019).
- Davis, S. J. and van der Merwe, P. A. (2006) 'The kinetic-segregation model: TCR triggering and beyond', *Nature Immunology*. Nature Publishing Group, pp. 803–809. doi: 10.1038/ni1369.
- Davodeau, F. *et al.* (1995) 'Dual T cell receptor  $\beta$  chain expression on human T lymphocytes', *Journal of Experimental Medicine*. The Rockefeller University Press, 181(4), pp. 1391–1398. doi: 10.1084/jem.181.4.1391.
- Deeks, S. G. *et al.* (2002) 'A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy', *Molecular Therapy*. Academic Press Inc., 5(6), pp. 788–797. doi: 10.1006/mthe.2002.0611.
- Deeks, S. G. and Walker, B. D. (2007) 'Human Immunodeficiency Virus Controllers: Mechanisms of Durable Virus Control in the Absence of Antiretroviral Therapy', *Immunity*. Immunity, pp. 406–416. doi: 10.1016/j.immuni.2007.08.010.
- Degano, M. *et al.* (2000) 'A functional hot spot for antigen recognition in a superagonist TCR/MHC complex.', *Immunity*, 12(3), pp. 251–61. doi: 10.1016/s1074-7613(00)80178-8.
- Degen, E. and Williams, D. B. (1991) 'Participation of a novel 88-kD protein in the biogenesis of murine class I histocompatibility molecules', *Journal of Cell Biology*. Rockefeller University Press, 112(6), pp. 1099–1115. doi: 10.1083/jcb.112.6.1099.
- Delong, T. *et al.* (2011) 'Islet amyloid polypeptide is a target antigen for diabetogenic CD4 + T cells', *Diabetes*, 60(9), pp. 2325–2330. doi: 10.2337/db11-0288.
- Demić, Z. *et al.* (1986) 'Transfer of specificity by murine  $\alpha$  and  $\beta$  T-cell receptor genes', *Nature*. Nature, 320(6059), pp. 232–238. doi: 10.1038/320232a0.
- Demchenko, A. P. (2002) 'The red-edge effects: 30 years of exploration', *Luminescence*, 17(1), pp. 19–42. doi: 10.1002/bio.671.
- Derry, C. J. *et al.* (2008) 'Analysis of T cell responses to the autoantigen in Goodpasture's disease', *Clinical & Experimental Immunology*. Wiley, 100(2), pp. 262–268. doi: 10.1111/j.1365-2249.1995.tb03663.x.
- Desfrancois, J. *et al.* (2010) 'Double Positive CD4CD8  $\alpha\beta$  T Cells: A New Tumor-Reactive Population in Human Melanomas', *PLoS ONE*. Edited by P. R. Lowenstein, 5(1), p. e8437. doi: 10.1371/journal.pone.0008437.
- Deverson, E. V. *et al.* (1990) 'MHC class II region encoding proteins related to the muKidrug resistance family of transmembrane transporters', *Nature*, 348(6303), pp. 738–741. doi: 10.1038/348738a0.
- DiLorenzo, T. P. and Serreze, D. V. (2005) 'The good turned ugly: immunopathogenic basis for diabetogenic CD8+ T cells in NOD mice', *Immunological Reviews*. John Wiley & Sons, Ltd, 204(1), pp. 250–263. doi: 10.1111/j.0105-2896.2005.00244.x.
- Ding, Y.-H. *et al.* (1998) 'Two Human T Cell Receptors Bind in a Similar Diagonal Mode to the HLA-A2/Tax Peptide Complex Using Different TCR Amino Acids', *Immunity*, 8, pp. 403–411.



- Ding, Y.-H. *et al.* (1999) 'Four A6-TCR/peptide/HLA-A2 structures that generate very different T cell signals are nearly identical', *Immunity*. Cell Press, 11(1), pp. 45–56. doi: 10.1016/S1074-7613(00)80080-1.
- Doherty, P. C., Blanden, R. V. and Zinkernagel, R. M. (1976) 'Specificity of virus-immune effector T cells for H-2K or H-2D compatible interactions: implications for H-antigen diversity.', *Transplantation reviews*, 29, pp. 89–124. doi: 10.1111/j.1600-065x.1976.tb00198.x.
- Doherty, P. C. and Zinkernagel, R. M. (1976) 'Specific immune lysis of paramyxovirus-infected cells by H-2-compatible thymus-derived lymphocytes.', *Immunology*. Wiley-Blackwell, 31(1), pp. 27–32. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/194830>.
- Doherty, P. C., Zinkernagel, R. M. and Ramshaw, I. A. (1974) 'Specificity and development of cytotoxic thymus-derived lymphocytes in lymphocytic choriomeningitis.', *Journal of immunology (Baltimore, Md. : 1950)*, 112(4), pp. 1548–52. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/4592605> (Accessed: 17 March 2020).
- Dolton, G. *et al.* (2015) 'More tricks with tetramers: a practical guide to staining T cells with peptide-MHC multimers.', *Immunology*, 146(1), pp. 11–22. doi: 10.1111/imm.12499.
- Dolton, G. *et al.* (2018) 'Optimized peptide-MHC multimer protocols for detection and isolation of autoimmune T-cells', *Frontiers in Immunology*. Frontiers Media S.A., 9(JUN), p. 29. doi: 10.3389/fimmu.2018.01378.
- Donello, J. E., Loeb, J. E. and Hope, T. J. (1998) 'Woodchuck hepatitis virus contains a tripartite posttranscriptional regulatory element.', *Journal of virology*, 72(6), pp. 5085–92. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9573279> (Accessed: 14 October 2019).
- Dong, D. *et al.* (2019) 'Structural basis of assembly of the human T cell receptor–CD3 complex', *Nature*. Nature Publishing Group, 573(7775), pp. 546–552. doi: 10.1038/s41586-019-1537-0.
- Dong, G. *et al.* (2009) 'Insights into MHC Class I Peptide Loading from the Structure of the Tapasin-ERp57 Thiol Oxidoreductase Heterodimer', *Immunity*. Cell Press, 30(1), pp. 21–32. doi: 10.1016/j.immuni.2008.10.018.
- Dong, L. and Bouey, J. (2020) 'Public Mental Health Crisis During COVID-19 Pandemic, China', *Emerging infectious diseases*. Emerg Infect Dis, 26(7). doi: 10.3201/EID2607.200407.
- Doyle, C. and Strominger, J. L. (1987) 'Interaction between CD4 and class II MHC molecules mediates cell adhesion', *Nature*. Nature Publishing Group, 330(6145), pp. 256–259. doi: 10.1038/330256a0.
- Dudley, M. E. *et al.* (2005) 'Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma', *Journal of Clinical Oncology*. J Clin Oncol, 23(10), pp. 2346–2357. doi: 10.1200/JCO.2005.00.240.
- Dunn, S. M. (2006) 'Directed evolution of human T cell receptor CDR2 residues by phage display dramatically enhances affinity for cognate peptide-MHC without increasing apparent cross-reactivity', *Protein Science*. Wiley, 15(4), pp. 710–721. doi: 10.1110/ps.051936406.
- Dustin, M. L. *et al.* (1997) 'Low Affinity Interaction of Human or Rat T Cell Adhesion Molecule CD2 with Its Ligand Aligns Adhering Membranes to Achieve High Physiological Affinity', *Journal of Biological Chemistry*, 272(49), pp. 30889–30898. doi:

10.1074/jbc.272.49.30889.

Ebnet, K. *et al.* (1995) 'Granzyme A-deficient mice retain potent cell-mediated cytotoxicity.', *The EMBO journal*, 14(17), pp. 4230–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7556064> (Accessed: 13 January 2020).

Eiselein, L., Schwartz, H. J. and Rutledge, J. C. (2004) 'The challenge of type 1 diabetes mellitus.', *ILAR journal / National Research Council, Institute of Laboratory Animal Resources*. ILAR J, pp. 231–236. doi: 10.1093/ilar.45.3.231.

Eisenbarth, G. S. (1986) 'Type I diabetes mellitus. A chronic autoimmune disease.', *New England Journal of Medicine*, 314, pp. 1360–1368. Available at: <https://www.nejm.org/doi/pdf/10.1056/NEJM198605223142106> (Accessed: 18 April 2020).

Ekeruche-Makinde, J. *et al.* (2012) 'T-cell receptor-optimized peptide skewing of the T-cell repertoire can enhance antigen targeting', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 287(44), pp. 37269–37281. doi: 10.1074/jbc.M112.386409.

Ekeruche-Makinde, J. *et al.* (2013) 'Peptide length determines the outcome of TCR/peptide-MHCI engagement', *Blood*. The American Society of Hematology, 121(7), pp. 1112–1123. doi: 10.1182/blood-2012-06-437202.

Ellebaek, E. *et al.* (2012) 'Adoptive cell therapy with autologous tumor infiltrating lymphocytes and low-dose Interleukin-2 in metastatic melanoma patients', *Journal of Translational Medicine*. J Transl Med, 10(1). doi: 10.1186/1479-5876-10-169.

Elliott, J. I. and Altmann, D. M. (1995) 'Dual T cell receptor  $\alpha$  chain T cells in autoimmunity', *Journal of Experimental Medicine*. The Rockefeller University Press, 182(4), pp. 953–960. doi: 10.1084/jem.182.4.953.

Elliott, T. *et al.* (1991) 'Peptide-induced conformational change of the class I heavy chain', *Nature*. Nature Publishing Group, 351(6325), pp. 402–406. doi: 10.1038/351402a0.

Elliott, T. *et al.* (1992) 'Structural requirements for the peptide-induced conformational change of free major histocompatibility complex class I heavy chains', *European Journal of Immunology*. John Wiley & Sons, Ltd, 22(8), pp. 2085–2091. doi: 10.1002/eji.1830220819.

Elliott, T. *et al.* (1995) 'Processing of major histocompatibility class I-restricted antigens in the endoplasmic reticulum', *Journal of Experimental Medicine*. The Rockefeller University Press, 181(4), pp. 1481–1491. doi: 10.1084/jem.181.4.1481.

Elliott, T., Bodmer, H. and Townsend, A. (1996) 'Recognition of out-of-frame major histocompatibility complex class I-restricted epitopes *in vivo*', *European Journal of Immunology*. John Wiley & Sons, Ltd, 26(5), pp. 1175–1179. doi: 10.1002/eji.1830260532.

Van Den Elsen, P. J. *et al.* (2003) 'DNA methylation and expression of major histocompatibility complex class I and class II transactivator genes in human developmental tumor cells and in T cell malignancies', *Clinical Immunology*. Academic Press Inc., 109(1), pp. 46–52. doi: 10.1016/S1521-6616(03)00200-6.

Van Den Elsen, P. J. (2011) 'Expression regulation of major histocompatibility complex class I and class II encoding genes', *Frontiers in Immunology*. doi: 10.3389/fimmu.2011.00048.

Emerman, M. and Temin, H. M. (1984) 'Genes with Promoters in Retrovirus Vectors Can Be Independently Suppressed by an Epigenetic Mechanism', *Cell*, 39, pp. 459–467.

- Van Endert, P. M. *et al.* (1994) 'A sequential model for peptide binding and transport by the transporters associated with antigen processing', *Immunity*. Cell Press, 1(6), pp. 491–500. doi: 10.1016/1074-7613(94)90091-4.
- Van Endert, P. M. *et al.* (1995) 'The peptide-binding motif for the human transporter associated with antigen processing', *Journal of Experimental Medicine*. The Rockefeller University Press, 182(6), pp. 1883–1895. doi: 10.1084/jem.182.6.1883.
- Van Endert, P. M. (2011) 'Post-proteasomal and proteasome-independent generation of MHC class I ligands', *Cellular and Molecular Life Sciences*. Springer, pp. 1553–1567. doi: 10.1007/s00018-011-0662-1.
- Énée, E. *et al.* (2008) 'Equivalent Specificity of Peripheral Blood and Islet-Infiltrating CD8 + T Lymphocytes in Spontaneously Diabetic HLA-A2 Transgenic NOD Mice', *The Journal of Immunology*. The American Association of Immunologists, 180(8), pp. 5430–5438. doi: 10.4049/jimmunol.180.8.5430.
- Énée, É. *et al.* (2012) 'ZnT8 is a major CD8 + T cell-recognized autoantigen in pediatric type 1 diabetes', *Diabetes*, 61(7), pp. 1779–1784. doi: 10.2337/db12-0071.
- Engleman, E. G. *et al.* (1981) 'Activation of human T lymphocyte subsets: helper and suppressor/cytotoxic T cells recognize and respond to distinct histocompatibility antigens.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 127(5), pp. 2124–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/6457863> (Accessed: 18 March 2020).
- Eppihimer, M. J. *et al.* (2002) 'Expression and regulation of the PD-L1 immunoinhibitory molecule on micro vascular endothelial cells', *Microcirculation*, 9(2), pp. 133–145. doi: 10.1038/sj.mn.7800123.
- Erb, P. and Feldmann, M. (1975a) 'The role of macrophages in the generation of T-helper cells. I. The requirement for macrophages in helper cell induction and characteristics of the macrophage-T cell interaction', *Cellular Immunology*. Academic Press, 19(2), pp. 356–367. doi: 10.1016/0008-8749(75)90217-8.
- Erb, P. and Feldmann, M. (1975b) 'The role of macrophages in the generation of T helper cells. II. The genetic control of the macrophage T cell interaction for helper cell induction with soluble antigens', *Journal of Experimental Medicine*, 142(2), pp. 460–472. doi: 10.1084/jem.142.2.460.
- Erb, P. and Feldmann, M. (1975c) 'The role of macrophages in the generation of T helper cells. III. Influence of macrophage-derived factors in helper cell induction', *European Journal of Immunology*. John Wiley & Sons, Ltd, 5(11), pp. 759–766. doi: 10.1002/eji.1830051106.
- Ettaro, L. *et al.* (2004) 'Cost-of-illness studies in diabetes mellitus', *Pharmacoeconomics*. Springer, pp. 149–164. doi: 10.2165/00019053-200422030-00002.
- Fabio, G. *et al.* (1992) 'HLA-associated susceptibility to HIV-1 infection', *Clinical & Experimental Immunology*, 87(1), pp. 20–23. doi: 10.1111/j.1365-2249.1992.tb06407.x.
- Fahl, S. P., Coffey, F. and Wiest, D. L. (2014) 'Origins of  $\gamma\delta$  T Cell Effector Subsets: A Riddle Wrapped in an Enigma', *The Journal of Immunology*. The American Association of Immunologists, 193(9), pp. 4289–4294. doi: 10.4049/jimmunol.1401813.
- Fairchild, P. J., Pope, H. and Wraith, D. C. (1996) 'The nature of cryptic epitopes within the self-antigen myelin basic protein', *International Immunology*, 8(7), pp. 1035–1043. doi:

10.1093/intimm/8.7.1035.

Falk, K. *et al.* (1991) 'Identification of naturally processed viral nonapeptides allows their quantification in infected cells and suggests an allele-specific T cell epitope forecast', *Journal of Experimental Medicine*. Rockefeller University Press, 174(2), pp. 425–434. doi: 10.1084/jem.174.2.425.

Falk, K. *et al.* (1994) 'Peptide motifs of HLA-A1,-A11,-A31, and-A33 molecules', *Immunogenetics*. Springer-Verlag, 40(3), pp. 238–241. doi: 10.1007/BF00167086.

Fanales-Belasio, E. *et al.* (2010) 'HIV Virology and Pathogenetic Mechanisms of Infection: A Brief Overview', *Annali dell'Istituto superiore di sanita*. Ann Ist Super Sanita, 46(1). doi: 10.4415/ANN\_10\_01\_02.

Feeney, M. E. *et al.* (2004) 'Immune Escape Precedes Breakthrough Human Immunodeficiency Virus Type 1 Viremia and Broadening of the Cytotoxic T-Lymphocyte Response in an HLA-B27-Positive Long-Term-Nonprogressing Child', *Journal of Virology*. American Society for Microbiology, 78(16), pp. 8927–8930. doi: 10.1128/jvi.78.16.8927-8930.2004.

De Felipe, P. *et al.* (1999) 'Use of the 2A sequence from foot-and-mouth disease virus in the generation of retroviral vectors for gene therapy', *Gene Therapy*. Nature Publishing Group, 6(2), pp. 198–208. doi: 10.1038/sj.gt.3300811.

Fellay, J. *et al.* (2007) 'A whole-genome association study of major determinants for host control of HIV-1', *Science*. Science, 317(5840), pp. 944–947. doi: 10.1126/science.1143767.

Feng, D. *et al.* (2007) 'Structural evidence for a germline-encoded T cell receptor-major histocompatibility complex interaction "codon"', *Nature Immunology*, 8(9), pp. 975–983. doi: 10.1038/ni1502.

Fidelis Maier, B. *et al.* (1994) 'Peptide motifs of HLA-A3,-A24, and-B7 molecules as determined by pool sequencing', *Immunogenetics*, 40, pp. 306–308. doi: 10.1007/BF00189978.

Fiebig, E. W. *et al.* (2003) 'Dynamics of HIV viremia and antibody seroconversion in plasma donors: Implications for diagnosis and staging of primary HIV infection', *AIDS*. AIDS, 17(13), pp. 1871–1879. doi: 10.1097/00002030-200309050-00005.

Fink, P. J. and Bevan, M. J. (1978) 'H-2 antigens of the thymus determine lymphocyte specificity\*', *Journal of Experimental Medicine*, 148(3), pp. 766–775. doi: 10.1084/jem.148.3.766.

Finke, D. *et al.* (1995) 'Gamma interferon is a major mediator of antiviral defense in experimental measles virus-induced encephalitis.', *Journal of virology*, 69(9), pp. 5469–74. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7636992> (Accessed: 13 January 2020).

Finzi, D. *et al.* (1997) 'Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy', *Science*. Science, 278(5341), pp. 1295–1300. doi: 10.1126/science.278.5341.1295.

Fodor, S. P. A. *et al.* (1991) 'Light-directed, spatially addressable parallel chemical synthesis', *Science*, 251(4995), pp. 767–773. doi: 10.1126/science.1990438.

Fonolleda, M. *et al.* (2017) 'Remission Phase in Paediatric Type 1 Diabetes: New Understanding and Emerging Biomarkers', *Hormone Research in Paediatrics*. S. Karger AG, 88(5), pp. 307–315. doi: 10.1159/000479030.

- Ford, E. S., Puronen, C. E. and Sereti, I. (2009) 'Immunopathogenesis of asymptomatic chronic HIV Infection: The calm before the storm', *Current Opinion in HIV and AIDS*. NIH Public Access, pp. 206–214. doi: 10.1097/COH.0b013e328329c68c.
- Forrest, C. *et al.* (2018) 'Proteome-wide analysis of CD8+ T cell responses to EBV reveals differences between primary and persistent infection', *PLoS Pathogens*. Public Library of Science, 14(9). doi: 10.1371/journal.ppat.1007110.
- Foulis, A. K., Farquharson, M. A. and Meager, A. (1987) 'Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus.', *Lancet (London, England)*, 2(8573), pp. 1423–7. doi: 10.1016/s0140-6736(87)91128-7.
- Fowell, D. and Mason, D. (1993) 'Evidence that the t cell repertoire of normal rats contains cells with the potential to cause diabetes. Characterization of the CD4+ T cell subset that inhibits this autoimmune potential', *Journal of Experimental Medicine*, 177(3), pp. 627–636. doi: 10.1084/jem.177.3.627.
- Frahm, M. A. *et al.* (2012) 'CD4<sup>+</sup> CD8<sup>+</sup> T Cells Represent a Significant Portion of the Anti-HIV T Cell Response to Acute HIV Infection', *The Journal of Immunology*, 188(9), pp. 4289–4296. doi: 10.4049/jimmunol.1103701.
- Frank, S. J. *et al.* (1990) 'Structural mutations of the T cell receptor  $\zeta$  chain and its role in T cell activation', *Science*, 249(4965), pp. 174–177. doi: 10.1126/science.2371564.
- Freed, E. O. *et al.* (1994) 'Single amino acid changes in the human immunodeficiency virus type 1 matrix protein block virus particle production.', *Journal of Virology*. American Society for Microbiology, 68(8), pp. 5311–5320. doi: 10.1128/jvi.68.8.5311-5320.1994.
- Freeman, G. J., Gribben, J. G., *et al.* (1993) 'Cloning of B7-2: A CTLA-4 counter-receptor that costimulates human T cell proliferation', *Science*, 262(5135), pp. 909–911. doi: 10.1126/science.7694363.
- Freeman, G. J., Borriello, F., *et al.* (1993) 'Murine b7-2, an alternative ctla4 counter-receptor that costimulates t cell proliferation and interleukin 2 production', *Journal of Experimental Medicine*, 178(6), pp. 2185–2192. doi: 10.1084/jem.178.6.2185.
- Freeman, G. J. *et al.* (2000) 'Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation', *Journal of Experimental Medicine*. The Rockefeller University Press, 192(7), pp. 1027–1034. doi: 10.1084/jem.192.7.1027.
- Fremont, D. H. *et al.* (1992) 'Crystal structures of two viral peptides in complex with murine MHC class I H-2Kb', *Science*, 257(5072), pp. 919–927. doi: 10.1126/science.1323877.
- Frey, C. R. *et al.* (2003) 'Identification of CD8 + T Cell Epitopes in the Immediate Early 62 Protein (IE62) of Varicella-Zoster Virus, and Evaluation of Frequency of CD8 + T Cell Response to IE62, by Use of IE62 Peptides after Varicella Vaccination', *The Journal of Infectious Diseases*. Oxford University Press (OUP), 188(1), pp. 40–52. doi: 10.1086/375828.
- Friedman-Kien, A. E. *et al.* (1982) 'Disseminated Kaposi's sarcoma in homosexual men', *Annals of Internal Medicine*. Ann Intern Med, 96(6 I), pp. 693–700. doi: 10.7326/0003-4819-96-6-693.
- Fujinami, R. S. and Oldstone, M. B. A. (1985) 'Amino acid homology between the encephalitogenic site of myelin basic protein and virus: Mechanism for autoimmunity', *Science*. Cambridge University Press, 230(4729), pp. 1043–1045. doi: 10.1126/science.2414848.

- Fung-Leung, W. P. *et al.* (1991) 'CD8 is needed for development of cytotoxic T but not helper T cells', *Cell*, 65(3), pp. 443–449. doi: 10.1016/0092-8674(91)90462-8.
- Furutani, M. *et al.* (1989) 'Post-transcriptional allelic exclusion of two functionally rearranged T cell receptor alpha genes.', *International immunology*, 1(3), pp. 281–8. doi: 10.1093/intimm/1.3.281.
- Gabert, J. *et al.* (1987) 'Reconstitution of MHC class I specificity by transfer of the T cell receptor and Lyt-2 genes', *Cell*, 50(4), pp. 545–554. doi: 10.1016/0092-8674(87)90027-4.
- Gaczynska, M. *et al.* (1994) 'Peptidase activities of proteasomes are differentially regulated by the major histocompatibility complex-encoded genes for LMP2 and LMP7 (proteolysis/antigen presentation/major histocompatibility complex class I molecule)', *Proc. Nati. Acad. Sci. USA*, 91, pp. 9213–9217. Available at: <https://www.pnas.org/content/91/20/9213.short> (Accessed: 3 March 2020).
- Gallagher, P. F., Fazekas de, S. G. V and Miller, J. F. A. P. (1989) 'CD4 and CD8 molecules can physically associate with the same T-cell receptor', *Proceedings of the National Academy of Sciences of the United States of America*, 86(24), pp. 10044–10048. doi: 10.1073/pnas.86.24.10044.
- Galley, W. C. and Purkey, R. M. (1970) 'Role of heterogeneity of the solvation site in electronic spectra in solution.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 67(3), pp. 1116–21. doi: 10.1073/PNAS.67.3.1116.
- Gallimore, A. *et al.* (1998) 'Protective immunity does not correlate with the hierarchy of virus-specific cytotoxic T cell responses to naturally processed peptides', *Journal of Experimental Medicine*. *J Exp Med*, 187(10), pp. 1647–1657. doi: 10.1084/jem.187.10.1647.
- Galloway, S. A. E. *et al.* (2019) 'Peptide super-agonist enhances T-cell responses to melanoma', *Frontiers in Immunology*. Frontiers Media S.A., 10(MAR). doi: 10.3389/fimmu.2019.00319.
- Gammon, G. and Sercarz, E. (1989) 'How some T cells escape tolerance induction', *Nature*, 342(6246), pp. 183–185. doi: 10.1038/342183a0.
- Gao, G. F. *et al.* (1997) 'Crystal structure of the complex between CD8 $\alpha$  human and HLA-A2', *Nature*. Nature Publishing Group, 387(6633), pp. 630–634. doi: 10.1038/42523.
- Gao, X. *et al.* (2001) 'Effect of a single amino acid change in MHC class I molecules on the rate of progression to aids', *New England Journal of Medicine*, 344(22), pp. 1668–1675. doi: 10.1056/NEJM200105313442203.
- Garboczi, D. N. *et al.* (1996) 'Structure of the complex between human T-cell receptor, viral peptide and HLA-A2', *Nature*, 384(6605), pp. 134–141. doi: 10.1038/384134a0.
- Garboczi, D. N., Hung, D. T. and Wiley, D. C. (1992) 'HLA-A2-peptide complexes: refolding and crystallization of molecules expressed in *Escherichia coli* and complexed with single antigenic peptides.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 89(8), pp. 3429–33. doi: 10.1073/PNAS.89.8.3429.
- Garcia, K. C. *et al.* (1996) 'An  $\alpha\beta$  T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex', *Journal of Immunology*, 156(11), pp. 209–219. doi: 10.1126/science.274.5285.209.

- Garcia, K. C. *et al.* (1997) 'αβ T cell receptor interactions with syngeneic and allogeneic ligands: Affinity measurements and crystallization', *Proceedings of the National Academy of Sciences of the United States of America*, 94(25), pp. 13838–13843. doi: 10.1073/pnas.94.25.13838.
- Garcia, K. C. *et al.* (1998) 'Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen.', *Science (New York, N.Y.)*, 279(5354), pp. 1166–72. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9469799> (Accessed: 14 November 2017).
- Garcia, K. C. *et al.* (2001) 'Kinetics and thermodynamics of T cell receptor-autoantigen interactions in murine experimental autoimmune encephalomyelitis', *Proceedings of the National Academy of Sciences of the United States of America*, 98(12), pp. 6818–6823. doi: 10.1073/pnas.111161198.
- Garcia, K. C. *et al.* (2009) 'The molecular basis of TCR germline bias for MHC is surprisingly simple', *Nature Immunology*, 10(2), pp. 143–147. doi: 10.1038/ni.f.219.
- Garcia, K. C., Teyton, L. and Wilson, I. A. (1999) 'Structural Basis of T Cell Recognition', *Annual Review of Immunology*. Annual Reviews, 17(1), pp. 369–397. doi: 10.1146/annurev.immunol.17.1.369.
- Garzino-Demo, A. (2006) 'Chemokines and Defensins as HIV Suppressive Factors: An Evolving Story', *Current Pharmaceutical Design*. Bentham Science Publishers Ltd., 13(2), pp. 163–172. doi: 10.2174/138161207779313696.
- Gascoigne, N. R. J. (2014) 'Immunology: Tolerance lies in the timing', *Nature*. Nature Publishing Group, pp. 502–503. doi: 10.1038/515502a.
- Gavichandran, K. S. and Burakoff, S. J. (1994) 'Evidence for differential intracellular signaling via CD4 and CD8 Molecules', *Journal of Experimental Medicine*, 179(2), pp. 727–732. doi: 10.1084/jem.179.2.727.
- Gee, M. H. *et al.* (2018) 'Antigen identification for orphan T cell receptors expressed on tumor-infiltrating lymphocytes HHS Public Access', *Cell*, 172(3), pp. 549–563. doi: 10.1016/j.cell.2017.11.043.
- Gelderblom, H. R., Özel, M. and Pauli, G. (1989) 'Morphogenesis and morphology of HIV structure-function relations', *Archives of Virology*. Springer-Verlag, pp. 1–13. doi: 10.1007/BF01311033.
- Geldmacher, C. *et al.* (2009) 'Minor viral and host genetic polymorphisms can dramatically impact the biologic outcome of an epitope-specific CD8 T-cell response', *Blood*, 114(8), pp. 1553–1562. doi: 10.1182/blood-2009-02-206193.
- Gepts, W. (1965) 'Pathologic anatomy of the pancreas in juvenile diabetes mellitus', *Am Diabetes Assoc*. Available at: <https://diabetes.diabetesjournals.org/content/14/10/619.short> (Accessed: 18 April 2020).
- Germain, R. N. (1994) 'MHC-dependent antigen processing and peptide presentation: Providing ligands for T lymphocyte activation', *Cell*, pp. 287–299. doi: 10.1016/0092-8674(94)90336-0.
- Geysen, H. M., Meloen, R. H. and Barteling, S. J. (1984) 'Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid', *Proceedings of the National Academy of Sciences of the United States of America*, 81(13 l), pp. 3998–4002. doi: 10.1073/pnas.81.13.3998.

- Ghendler, Y. *et al.* (1998) 'One of the CD3 $\epsilon$  subunits within a T cell receptor complex lies in close proximity to the C $\beta$  FG loop', *Journal of Experimental Medicine*, 187(9), pp. 1529–1536. doi: 10.1084/jem.187.9.1529.
- Gialeli, C., Gungor, B. and Blom, A. M. (2018) 'Novel potential inhibitors of complement system and their roles in complement regulation and beyond', *Molecular Immunology*. Elsevier Ltd, 102, pp. 73–83. doi: 10.1016/j.molimm.2018.05.023.
- Giladi, M. and Khananshvil, D. (2020) 'Hydrogen-Deuterium Exchange Mass-Spectrometry of Secondary Active Transporters: From Structural Dynamics to Molecular Mechanisms', *Frontiers in Pharmacology*. Frontiers Media S.A., 11, p. 70. doi: 10.3389/fphar.2020.00070.
- Gilfillan, S. *et al.* (1993) 'Mice lacking TdT: Mature animals with an immature lymphocyte repertoire', *Science*, 261(5125), pp. 1175–1178. doi: 10.1126/science.8356452.
- Gill, D. *et al.* (2001) 'Increased persistence of lung gene expression using plasmids containing the ubiquitin C or elongation factor1 $\alpha$  promoter', *Gene Therapy*, 8(20), pp. 1539–1546. doi: 10.1038/sj.gt.3301561.
- Glas, R. *et al.* (1998) 'A proteolytic system that compensates for loss of proteasome function', *Nature*. Nature Publishing Group, 392(6676), pp. 618–622. doi: 10.1038/33443.
- Glimcher, L. H. and Kara, C. J. (1992) 'Sequences and Factors: A Guide to MHC Class-II Transcription', *Annual Review of Immunology*, 10(1), pp. 13–49. doi: 10.1146/annurev.iy.10.040192.000305.
- Glithero, A. *et al.* (1999) 'Crystal structures of two H-2D(b)/glycopeptide complexes suggest a molecular basis for CTL cross-reactivity', *Immunity*. Cell Press, 10(1), pp. 63–74. doi: 10.1016/S1074-7613(00)80007-2.
- Glynne, R. *et al.* (1991) 'A proteasome-related gene between the two ABC transporter loci in the class II region of the human MHC', *Nature*. Nature Publishing Group, 353(6342), pp. 357–360. doi: 10.1038/353357a0.
- Gmünder, H. and Lesslauer, W. (1984) 'A 45-kDa human T-cell membrane glycoprotein functions in the regulation of cell proliferative responses', *European Journal of Biochemistry*, 142(1), pp. 153–160. doi: 10.1111/j.1432-1033.1984.tb08263.x.
- Godfrey, D. I. *et al.* (2015) 'The burgeoning family of unconventional T cells', *Nature Immunology*. Nature Publishing Group, pp. 1114–1123. doi: 10.1038/ni.3298.
- Gold, M. C. *et al.* (2010) 'Human mucosal associated invariant T cells detect bacterially infected cells', *PLoS Biology*, 8(6), p. e1000407. doi: 10.1371/journal.pbio.1000407.
- Goldberg, A. L. and Rock, K. L. (1992) 'Proteolysis, proteasomes and antigen presentation', *Nature*, pp. 375–379. doi: 10.1038/357375a0.
- Goldman, E. R. *et al.* (1997) 'Analysis of binding interactions in an idiotope-antiidiotope protein-protein complex by double mutant cycles', *Biochemistry*, 36(1), pp. 49–56. doi: 10.1021/bi961769k.
- Golubovskaya, V. and Wu, L. (2016) 'Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy', *Cancers*. MDPI AG. doi: 10.3390/cancers8030036.
- Goodenow, M. *et al.* (1989) 'HIV-1 isolates are rapidly evolving quasispecies: Evidence for viral mixtures and preferred nucleotide substitutions', *Journal of Acquired Immune Deficiency Syndromes*, 2(4), pp. 344–352.



- Goodnow, C. C. (2007) 'Multistep Pathogenesis of Autoimmune Disease', *Cell*. Cell Press, pp. 25–35. doi: 10.1016/j.cell.2007.06.033.
- Gorer, P. A. (1936) 'The detection of a hereditary antigenic difference in the blood of mice by means of human group a serum', *Journal of Genetics*, 32(1), pp. 17–31. doi: 10.1007/BF02982499.
- Gottlieb, M. S. *et al.* (1981) 'Pneumocystis carinii Pneumonia and Mucosal Candidiasis in Previously Healthy Homosexual Men: Evidence of a New Acquired Cellular Immunodeficiency', *New England Journal of Medicine*. N Engl J Med, 305(24), pp. 1425–1431. doi: 10.1056/NEJM198112103052401.
- Goulder, P. J. R., Phillips, R. E., *et al.* (1997) 'Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS', *Nature Medicine*. Nat Med, 3(2), pp. 212–217. doi: 10.1038/nm0297-212.
- Goulder, P. J. R., Sewell, A. K., *et al.* (1997) 'Patterns of immunodominance in HIV-1-specific cytotoxic T lymphocyte responses in two human histocompatibility leukocyte antigens (HLA)-identical siblings with HLA-A\*0201 are influenced by epitope mutation', *Journal of Experimental Medicine*. J Exp Med, 185(8), pp. 1423–1433. doi: 10.1084/jem.185.8.1423.
- Goulder, P. J. R., Brander, C., *et al.* (2001) 'Evolution and transmission of stable CTL escape mutations in HIV infection', *Nature*. Macmillan Magazines Ltd, 412(6844), pp. 334–338. doi: 10.1038/35085576.
- Goulder, P. J. R., Altfeld, M. A., *et al.* (2001) 'Substantial differences in specificity of HIV-specific cytotoxic T cells in acute and chronic HIV infection', *Journal of Experimental Medicine*. Rockefeller University Press, 193(2), pp. 181–193. doi: 10.1084/jem.193.2.181.
- Gourraud, P. A. *et al.* (2015) 'High-resolution HLA-A, HLA-B, and HLA-DRB1 haplotype frequencies from the French Bone Marrow Donor Registry', *Human Immunology*. Elsevier Inc., 76(5), pp. 381–384. doi: 10.1016/j.humimm.2015.01.028.
- Grant, E. J. *et al.* (2013) 'Nucleoprotein of influenza A virus is a major target of immunodominant CD8 + T-cell responses', *Immunology and Cell Biology*. Immunol Cell Biol, 91(2), pp. 184–194. doi: 10.1038/icb.2012.78.
- Grant, E. J. *et al.* (2014) 'T-cell immunity to influenza A viruses', *Critical Reviews in Immunology*. Crit Rev Immunol, 34(1), pp. 15–39. doi: 10.1615/CritRevImmunol.2013010019.
- Grant, E. J. *et al.* (2016) 'Human influenza viruses and CD8+ T cell responses', *Current Opinion in Virology*. Elsevier B.V., pp. 132–142. doi: 10.1016/j.coviro.2016.01.016.
- Gras, S. *et al.* (2016) 'Reversed T Cell Receptor Docking on a Major Histocompatibility Class I Complex Limits Involvement in the Immune Response', *Immunity*. Cell Press, 45(4), pp. 749–760. doi: 10.1016/j.immuni.2016.09.007.
- Grassi, F. *et al.* (2006) 'Structural and functional properties of lensin, a pseudo-glutamine synthetase in the transparent human lens', *Biochemical and Biophysical Research Communications*. Biochem Biophys Res Commun, 350(2), pp. 424–429. doi: 10.1016/j.bbrc.2006.09.062.
- Griesser, H. *et al.* (1988) 'The human t cell receptor  $\alpha$ - $\delta$  locus: a physical map of the variable, joining and constant region genes', *European Journal of Immunology*, 18(4), pp. 641–644. doi: 10.1002/eji.1830180424.

- Griffin, T. *et al.* (1998) 'Immunoproteasome assembly: cooperative incorporation of interferon  $\gamma$  (IFN- $\gamma$ )-inducible subunits', *Journal of Experimental Medicine*, 187(1), pp. 97–104. Available at: <https://rupress.org/jem/article-abstract/187/1/97/25375> (Accessed: 3 March 2020).
- Grommé, M. *et al.* (1997) 'The rational design of TAP inhibitors using peptide substrate modifications and peptidomimetics', *European Journal of Immunology*. John Wiley & Sons, Ltd, 27(4), pp. 898–904. doi: 10.1002/eji.1830270415.
- Gross, J. A., St John, T. and Allison, J. P. (1990) 'The murine homologue of the T lymphocyte antigen CD28. Molecular cloning and cell surface expression.', *Journal of immunology (Baltimore, Md. : 1950)*, 144(8), pp. 3201–10. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2157764> (Accessed: 15 April 2020).
- La Gruta, N. L. *et al.* (2018) 'Understanding the drivers of MHC restriction of T cell receptors', *Nature Reviews Immunology*. Nature Publishing Group, pp. 467–478. doi: 10.1038/s41577-018-0007-5.
- Guedan, S. *et al.* (2019) 'Engineering and Design of Chimeric Antigen Receptors', *Molecular Therapy - Methods and Clinical Development*. Cell Press, pp. 145–156. doi: 10.1016/j.omtm.2018.12.009.
- Guillet, J. G. *et al.* (1986) 'Interaction of peptide antigens and class II major histocompatibility complex antigens', *Nature*, 324(6094), pp. 260–262. doi: 10.1038/324260a0.
- Guo, H. C. *et al.* (1992) 'Different length peptides bind to HLA-Aw68 similarly at their ends but bulge out in the middle.', *Nature*, 360(6402), pp. 364–6. doi: 10.1038/360364a0.
- Gupta, K. K. (1993) 'Acute Immunosuppression with HIV Seroconversion', *New England Journal of Medicine*. N Engl J Med, pp. 288–289. doi: 10.1056/NEJM199301283280419.
- Hagerty, D. T. and Allen, P. M. (1995) 'Intramolecular mimicry. Identification and analysis of two cross-reactive T cell epitopes within a single protein.', *Journal of immunology (Baltimore, Md. : 1950)*, 155(6), pp. 2993–3001. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7673717> (Accessed: 4 February 2020).
- Hahn, H. and Palmenberg, A. C. (1996) 'Mutational analysis of the encephalomyocarditis virus primary cleavage.', *Journal of Virology*, 70(10).
- Hahn, M. *et al.* (2005) 'Unconventional topology of self peptide-major histocompatibility complex binding by a human autoimmune T cell receptor', *Nature Immunology*, 6(5), pp. 490–496. doi: 10.1038/ni1187.
- Hamaoka, T. and Ono, S. (1986) 'Regulation of B-Cell Differentiation: Interactions of Factors and Corresponding Receptors', *Annual Review of Immunology*, 4(1), pp. 167–204. doi: 10.1146/annurev.iy.04.040186.001123.
- Hanada, K. I. *et al.* (2011) 'Molecular identification of an MHC-independent ligand recognized by a human  $\alpha/\beta$  T-cell receptor', *Blood*, 117(18), pp. 4816–4825. doi: 10.1182/blood-2010-11-317743.
- Haney, D. *et al.* (2011) 'Isolation of viable antigen-specific CD8+ T cells based on membrane-bound tumor necrosis factor (TNF)- $\alpha$  expression.', *Journal of immunological methods*, 369(1–2), pp. 33–41. doi: 10.1016/j.jim.2011.04.003.
- Haque, T. *et al.* (2007) 'Allogeneic cytotoxic T-cell therapy for EBV-positive

- posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial'. doi: 10.1182/blood-2006-12-063008.
- Harding, H. P. and Ron, D. (2002) 'Endoplasmic reticulum stress and the development of diabetes: A review', in *Diabetes*. American Diabetes Association, pp. S455–S461. doi: 10.2337/diabetes.51.2007.s455.
- Harkioliaki, M. *et al.* (2009) 'T Cell-Mediated Autoimmune Disease Due to Low-Affinity Crossreactivity to Common Microbial Peptides', *Immunity*. Cell Press, 30(3), pp. 348–357. doi: 10.1016/j.immuni.2009.01.009.
- Harley, J. B. *et al.* (2018) 'Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity', *Nature Genetics*. Nature Publishing Group, 50(5), pp. 699–707. doi: 10.1038/s41588-018-0102-3.
- Harper, J. *et al.* (2018) 'An approved in vitro approach to preclinical safety and efficacy evaluation of engineered T cell receptor anti-CD3 bispecific (ImmTAC) molecules', *PLoS ONE*. Public Library of Science, 13(10). doi: 10.1371/journal.pone.0205491.
- Harris, D. T. *et al.* (2016) 'An Engineered Switch in T Cell Receptor Specificity Leads to an Unusual but Functional Binding Geometry', *Structure*. Cell Press, 24(7), pp. 1142–1154. doi: 10.1016/j.str.2016.04.011.
- Haskins, K. *et al.* (1983) 'The major histocompatibility complex-restricted antigen receptor on T cells: I. Isolation with a monoclonal antibody', *Journal of Experimental Medicine*. The Rockefeller University Press, 157(4), pp. 1149–1169. doi: 10.1084/jem.157.4.1149.
- Van Hateren, A. *et al.* (2017) 'Direct evidence for conformational dynamics in major histocompatibility complex class I molecules', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology Inc., 292(49), pp. 20255–20269. doi: 10.1074/jbc.M117.809624.
- Hausmann, S. *et al.* (1999) 'Peptide recognition by two HLA-A2/Tax11-19-specific T cell clones in relationship to their MHC/peptide/TCR crystal structures.', *Journal of immunology (Baltimore, Md. : 1950)*, 162(9), pp. 5389–97. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10228016> (Accessed: 14 November 2017).
- Hawse, W. F. *et al.* (2013) 'Peptide modulation of class I major histocompatibility complex protein molecular flexibility and the implications for immune recognition', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 288(34), pp. 24372–24381. doi: 10.1074/jbc.M113.490664.
- Hawse, W. F. *et al.* (2014) 'TCR scanning of peptide/MHC through complementary matching of receptor and ligand molecular flexibility.', *Journal of immunology (Baltimore, Md. : 1950)*. NIH Public Access, 192(6), pp. 2885–91. doi: 10.4049/jimmunol.1302953.
- Heath, W. R. *et al.* (1995) 'Expression of two T cell receptor  $\alpha$  chains on the surface of normal murine T cells', *European Journal of Immunology*, 25(6), pp. 1617–1623. doi: 10.1002/eji.1830250622.
- Heath, W. R. and Miller, J. F. A. P. (1993) 'Expression of two  $\pm$  chains on the surface of T cells in T cell receptor transgenic mice', *Journal of Experimental Medicine*, 178(5), pp. 1807–1811. doi: 10.1084/jem.178.5.1807.
- Heber-Katz, E., Hansburg, D. and Schwartz, R. H. (1983) 'The Ia molecule of the antigen-presenting cell plays a critical role in immune response gene regulation of T cell activation.', *The Journal of molecular and cellular immunology : JMCI*, 1(1), pp. 3–18. Available at:

<http://www.ncbi.nlm.nih.gov/pubmed/6101061> (Accessed: 16 January 2020).

Hecht, F. M. *et al.* (2002) 'Use of laboratory tests and clinical symptoms for identification of primary HIV infection', *AIDS*, 16(8), pp. 1119–1129. doi: 10.1097/00002030-200205240-00005.

Hedrick, S. M. *et al.* (1984) 'Isolation of cDNA clones encoding T cell-specific membrane-associated proteins', *Nature*, 308(5955), pp. 149–153. doi: 10.1038/308149a0.

Heemskerk, M. H. M. *et al.* (2007) 'Efficiency of T-cell receptor expression in dual-specific T cells is controlled by the intrinsic qualities of the TCR chains within the TCR-CD3 complex', *Blood*, 109(1), pp. 235–243. doi: 10.1182/blood-2006-03-013318.

Hellman, L. M. *et al.* (2019) 'Improving T Cell Receptor On-Target Specificity via Structure-Guided Design', *Molecular Therapy*. Cell Press, 27(2), pp. 300–313. doi: 10.1016/j.ymthe.2018.12.010.

Hemmer, B. *et al.* (1997) 'Identification of high potency microbial and self ligands for a human autoreactive class II-restricted T cell clone.', *The Journal of experimental medicine*, 185(9), pp. 1651–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9151902> (Accessed: 15 November 2017).

Hemmer, B. *et al.* (1998) 'Probing degeneracy in T-cell recognition using peptide combinatorial libraries', *Immunology Today*. Elsevier Ltd, 19(4), pp. 163–168. doi: 10.1016/S0167-5699(97)01217-6.

Henderson, R. A. *et al.* (1992) 'HLA-A2.1-Associated peptides from a mutant cell line: A second pathway of antigen presentation', *Science*, 255(5049), pp. 1264–1266. doi: 10.1126/science.1546329.

Henkart, P. A. (1994) 'Lymphocyte-mediated cytotoxicity: Two pathways and multiple effector molecules', *Immunity*, pp. 343–346. doi: 10.1016/1074-7613(94)90063-9.

Hennecke, J. and Wiley, D. C. (2002) 'Structure of a complex of the human  $\alpha/\beta$  T cell receptor (TCR) HA1.7, Influenza hemagglutinin peptide, and major histocompatibility complex class II molecule, HLA-DR4 (DRA\*0101 and DRBI\*0401): Insight into TCR cross-restriction and alloreactivity', *Journal of Experimental Medicine*, 195(5), pp. 571–581. doi: 10.1084/jem.20011194.

Herget, M. *et al.* (2011) 'Conformation of peptides bound to the transporter associated with antigen processing (TAP)', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 108(4), pp. 1349–1354. doi: 10.1073/pnas.1012355108.

Hermann, C. *et al.* (2013) 'The Binding of TAPBPR and Tapasin to MHC Class I Is Mutually Exclusive', *The Journal of Immunology*. The American Association of Immunologists, 191(11), pp. 5743–5750. doi: 10.4049/jimmunol.1300929.

Hermann, C. *et al.* (2015) 'TAPBPR alters MHC class I peptide presentation by functioning as a peptide exchange catalyst', *eLife*. eLife Sciences Publications Ltd, 4(OCTOBER2015). doi: 10.7554/eLife.09617.001.

Herzig, E. *et al.* (2019) 'Attacking Latent HIV with convertibleCAR-T Cells, a Highly Adaptable Killing Platform', *Cell*. Cell Press, 179(4), pp. 880–894.e10. doi: 10.1016/j.cell.2019.10.002.

Heusel, J. W. *et al.* (1994) 'Cytotoxic lymphocytes require granzyme B for the rapid induction of DNA fragmentation and apoptosis in allogeneic target cells', *Cell*, 76(6), pp.

977–987. doi: 10.1016/0092-8674(94)90376-X.

Heymann, A. D. *et al.* (2008) 'Diabetes as a risk factor for herpes zoster infection: Results of a population-based study in Israel', *Infection*. *Infection*, 36(3), pp. 226–230. doi: 10.1007/s15010-007-6347-x.

Hiemstra, H. S. *et al.* (1997) 'The identification of CD4+ T cell epitopes with dedicated synthetic peptide libraries', *Proceedings of the National Academy of Sciences of the United States of America*, 94(19), pp. 10313–10318. doi: 10.1073/pnas.94.19.10313.

Hislop, A. D. *et al.* (2002) 'Epitope-specific evolution of human CD8+ T cell responses from primary to persistent phases of Epstein-Barr virus infection', *Journal of Experimental Medicine*. *J Exp Med*, 195(7), pp. 893–905. doi: 10.1084/jem.20011692.

Hislop, A. D. *et al.* (2005) 'Tonsillar homing of Epstein-Barr virus-specific CD8+ T cells and the virus-host balance', *Journal of Clinical Investigation*. *J Clin Invest*, 115(9), pp. 2546–2555. doi: 10.1172/JCI24810.

Hochstenbach, F. *et al.* (1992) 'Endoplasmic reticulum resident protein of 90 kilodaltons associates with the T- And B-cell antigen receptors and major histocompatibility complex antigens during their assembly', *Proceedings of the National Academy of Sciences of the United States of America*. *National Academy of Sciences*, 89(10), pp. 4734–4738. doi: 10.1073/pnas.89.10.4734.

Hodges, E. *et al.* (2003) 'Diagnostic role of tests for T cell receptor (TCR) genes', *Journal of Clinical Pathology*, pp. 1–11. doi: 10.1136/jcp.56.1.1.

Hoffman, W., Lakkis, F. G. and Chalasani, G. (2016) 'B cells, antibodies, and more', *Clinical Journal of the American Society of Nephrology*. *American Society of Nephrology*, 11(1), pp. 137–154. doi: 10.2215/CJN.09430915.

Holler, P. D. and Kranz, D. M. (2003) 'Quantitative analysis of the contribution of TCR/pepMHC affinity and CD8 to T cell activation', *Immunity*. *Cell Press*, 18(2), pp. 255–264. doi: 10.1016/S1074-7613(03)00019-0.

Holling, T. M. *et al.* (2002) 'Activated Human T Cells Accomplish MHC Class II Expression Through T Cell-Specific Occupation of Class II Transactivator Promoter III', *The Journal of Immunology*. *The American Association of Immunologists*, 168(2), pp. 763–770. doi: 10.4049/jimmunol.168.2.763.

Honeyborne, I. *et al.* (2006) 'Motif Inference Reveals Optimal CTL Epitopes Presented by HLA Class I Alleles Highly Prevalent in Southern Africa', *The Journal of Immunology*. *The American Association of Immunologists*, 176(8), pp. 4699–4705. doi: 10.4049/jimmunol.176.8.4699.

Hong, S. C. *et al.* (1992) 'An MHC interaction site maps to the amino-terminal half of the T cell receptor  $\alpha$  chain variable domain', *Cell*. *Cell*, 69(6), pp. 999–1009. doi: 10.1016/0092-8674(92)90618-M.

Hood, L., Steinmetz, M. and Malissen, B. (1983) 'Genes of the Major Histocompatibility Complex of the Mouse', *Annual Review of Immunology*, 1(1), pp. 529–568. doi: 10.1146/annurev.iy.01.040183.002525.

Hoover, D. R. *et al.* (1993) 'Clinical Manifestations of AIDS in the Era of Pneumocystis Prophylaxis', *New England Journal of Medicine*, 329(26), pp. 1922–1926. doi: 10.1056/NEJM199312233292604.

Hopkins, J. R. *et al.* (2020) 'Peptide cargo tunes a network of correlated motions in human leucocyte antigens', *The FEBS Journal*. Blackwell Publishing Ltd, 287(17), pp. 3777–3793. doi: 10.1111/febs.15278.

Horton, B. L. *et al.* (2018) 'Intratumoral CD8+ T-cell apoptosis is a major component of T-cell dysfunction and impedes antitumor immunity', *Cancer Immunology Research*. American Association for Cancer Research Inc., 6(1), pp. 14–24. doi: 10.1158/2326-6066.CIR-17-0249.

Horton, B. L. and Gajewski, T. F. (2018) 'Back from the dead: TIL apoptosis in cancer immune evasion', *British Journal of Cancer*. Nature Publishing Group, pp. 309–311. doi: 10.1038/bjc.2017.483.

Horton, R. *et al.* (2004) 'Gene map of the extended human MHC', *Nature Reviews Genetics*, pp. 889–899. doi: 10.1038/nrg1489.

Horton, R. *et al.* (2008) 'Variation analysis and gene annotation of eight MHC haplotypes: The MHC Haplotype Project', *Immunogenetics*, 60(1), pp. 1–18. doi: 10.1007/s00251-007-0262-2.

Houghten, R. A. (1985) 'General method for the rapid solid-phase synthesis of large numbers of peptides: Specificity of antigen-antibody interaction at the level of individual amino acids', *Proceedings of the National Academy of Sciences of the United States of America*, 82(15), pp. 5131–5135. doi: 10.1073/pnas.82.15.5131.

Houghten, R. A. *et al.* (1991) 'Generation and use of synthetic peptide combinatorial libraries for basic research and drug discovery', *Nature*, 354(6348), pp. 84–86. doi: 10.1038/354084a0.

Howard, T. A., Rochelle, J. M. and Seldin, M. F. (1991) 'Cd28 and Ctlα-4, two related members of the Ig supergene family, are tightly linked on proximal mouse chromosome 1.', *Immunogenetics*, 33(1), pp. 74–6. doi: 10.1007/bf00211698.

Howarth, M. *et al.* (2004) 'Tapasin enhances MHC class I peptide presentation according to peptide half-life', *Proceedings of the National Academy of Sciences of the United States of America*, 101(32), pp. 11737–11742. doi: 10.1073/pnas.0306294101.

Howson, J. M. M. *et al.* (2009) 'Confirmation of HLA class II independent type 1 diabetes associations in the major histocompatibility complex including HLA-B and HLA-A', *Diabetes, Obesity and Metabolism*. Blackwell Publishing Ltd, 11(SUPPL. 1), pp. 31–45. doi: 10.1111/j.1463-1326.2008.01001.x.

Hozumi, N. and Tonegawa, S. (1976) 'Evidence for somatic rearrangement of immunoglobulin genes coding for variable and constant regions', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 73(10), pp. 3628–3632. doi: 10.1073/pnas.73.10.3628.

Hu, S. Y. *et al.* (2016) 'T cell infiltration is associated with kidney injury in patients with anti-glomerular basement membrane disease', *Science China Life Sciences*. Science in China Press, 59(12), pp. 1282–1289. doi: 10.1007/s11427-016-5030-9.

Hu, S. Y. *et al.* (2017) 'The pathogenicity of T cell epitopes on human Goodpasture antigen and its critical amino acid motif', *Journal of Cellular and Molecular Medicine*. Blackwell Publishing Inc., 21(9), pp. 2117–2128. doi: 10.1111/jcmm.13134.

Huang, J., Edwards, L. J., *et al.* (2007) 'Kinetics of MHC-CD8 Interaction at the T Cell Membrane', *The Journal of Immunology*. The American Association of Immunologists,

179(11), pp. 7653–7662. doi: 10.4049/jimmunol.179.11.7653.

Huang, J., Cao, Y., *et al.* (2007) 'Priming with SARS CoV S DNA and boosting with SARS CoV S epitopes specific for CD4+ and CD8+ T cells promote cellular immune responses', *Vaccine*. *Vaccine*, 25(39–40), pp. 6981–6991. doi: 10.1016/j.vaccine.2007.06.047.

Huang, J. and Liang, T. J. (1993) 'A novel hepatitis B virus (HBV) genetic element with Rev response element-like properties that is essential for expression of HBV gene products.', *Molecular and cellular biology*, 13(12), pp. 7476–86. doi: 10.1128/mcb.13.12.7476.

Huber, E. M. *et al.* (2012) 'Immuno- and constitutive proteasome crystal structures reveal differences in substrate and inhibitor specificity', *Cell*. *Cell Press*, 148(4), pp. 727–738. doi: 10.1016/j.cell.2011.12.030.

Hudson, B. G. *et al.* (1989) 'Goodpasture syndrome: molecular architecture and function of basement membrane antigen.', *Laboratory investigation; a journal of technical methods and pathology*, 61(3), pp. 256–69. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2671489> (Accessed: 14 April 2020).

Huey, B. *et al.* (1993) 'Associations of HLA-DR and HLA-DQ types with anti-GBM nephritis by sequence-specific oligonucleotide probe hybridization', *Kidney International*, 44(2), pp. 307–312. doi: 10.1038/ki.1993.245.

Hughes, E. A. *et al.* (1996) 'The protease inhibitor, N-acetyl-L-leucyl-L-leucyl-L-norleucinal, decreases the pool of major histocompatibility complex class I-binding peptides and inhibits peptide trimming in the endoplasmic reticulum', *Journal of Experimental Medicine*. Rockefeller University Press, 183(4), pp. 1569–1578. doi: 10.1084/jem.183.4.1569.

Hughes, E. A. and Cresswell, P. (1998) 'The thiol oxidoreductase ERp57 is a component of the MHC class I peptide-loading complex', *Current Biology*. *Cell Press*, 8(12), pp. 709–713. doi: 10.1016/s0960-9822(98)70278-7.

Hughes, M. S. *et al.* (2005) 'Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions', *Human Gene Therapy*. *Hum Gene Ther*, 16(4), pp. 457–472. doi: 10.1089/hum.2005.16.457.

Hughes, S. J. *et al.* (1991) 'Vaccinia virus encodes an active thymidylate kinase that complements a cdc8 mutant of *Saccharomyces cerevisiae*.' , *The Journal of biological chemistry*, 266(30), pp. 20103–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/1657913> (Accessed: 24 September 2019).

Hulpke, S. and Tampé, R. (2013) 'The MHC I loading complex: A multitasking machinery in adaptive immunity', *Trends in Biochemical Sciences*. Elsevier Current Trends, pp. 412–420. doi: 10.1016/j.tibs.2013.06.003.

Hülsmeier, M. *et al.* (2002) 'HLA-B27 Subtypes Differentially Associated with Disease Exhibit Subtle Structural Alterations', *Journal of Biological Chemistry*, 277(49), pp. 47844–47853. doi: 10.1074/jbc.M206392200.

Hunt, D. F. *et al.* (1992) 'Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry', *Science*, 255(5049), pp. 1261–1263. doi: 10.1126/science.1546328.

Huseby, E. S. *et al.* (2005) 'How the T cell repertoire becomes peptide and MHC specific', *Cell*, 122(2), pp. 247–260. doi: 10.1016/j.cell.2005.05.013.

Ignatowicz, L. *et al.* (1997) 'T cells can be activated by peptides that are unrelated in

- sequence to their selecting peptide', *Immunity*. Cell Press, 7(2), pp. 179–186. doi: 10.1016/S1074-7613(00)80521-X.
- Ignatowicz, L., Kappler, J. and Marrack, P. (1996) 'The repertoire of T cells shaped by a single MHC/peptide ligand', *Cell*. Cell Press, 84(4), pp. 521–529. doi: 10.1016/S0092-8674(00)81028-4.
- Inaba, K. *et al.* (1994) 'The Tissue Distribution of the B7-2 Costimulator in Mice: Abundant Expression on Dendritic Cells In Situ and During Maturation In Vitro', *Journal of Experimental Medicine*, 180(5), pp. 1849–1860. doi: 10.1084/jem.180.5.1849.
- Infante, A. J. *et al.* (1982) 'Definition of T cell idiotypes using anti-idiotypic antisera produced by immunization with T cell clones', *Journal of Experimental Medicine*. The Rockefeller University Press, 155(4), pp. 1100–1107. doi: 10.1084/jem.155.4.1100.
- Ishida, Y. *et al.* (1992) 'Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death.', *The EMBO Journal*. Wiley, 11(11), pp. 3887–3895. doi: 10.1002/j.1460-2075.1992.tb05481.x.
- Ishizuka, J. *et al.* (2009) 'Quantitating T Cell Cross-Reactivity for Unrelated Peptide Antigens', *The Journal of Immunology*. The American Association of Immunologists, 183(7), pp. 4337–4345. doi: 10.4049/jimmunol.0901607.
- Itariu, B. K. and Stulnig, T. M. (2014) 'Autoimmune aspects of type 2 diabetes mellitus - A mini-review', *Gerontology*. S. Karger AG, pp. 189–196. doi: 10.1159/000356747.
- Iversen, A. K. N. *et al.* (2006) 'Conflicting selective forces affect T cell receptor contacts in an immunodominant human immunodeficiency virus epitope', *Nature Immunology*. Nat Immunol, 7(2), pp. 179–189. doi: 10.1038/ni1298.
- Jäger, E. *et al.* (1998) 'Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: Definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes', *Journal of Experimental Medicine*, 187(2), pp. 265–270. doi: 10.1084/jem.187.2.265.
- Jamieson, B. D. *et al.* (2003) 'Epitope Escape Mutation and Decay of Human Immunodeficiency Virus Type 1-Specific CTL Responses', *The Journal of Immunology*. The American Association of Immunologists, 171(10), pp. 5372–5379. doi: 10.4049/jimmunol.171.10.5372.
- Jardetzky, T. S. *et al.* (1991) 'Identification of self peptides bound to purified HLA-B27', *Nature*. Nature Publishing Group, 353(6342), pp. 326–329. doi: 10.1038/353326a0.
- Jardetzky, T. S. *et al.* (1994) 'Three-dimensional structure of a human class II histocompatibility molecule complexed with superantigen', *Nature*, 368(6473), pp. 711–718. doi: 10.1038/368711a0.
- Jean, M. J. *et al.* (2019) 'Current Strategies for Elimination of HIV-1 Latent Reservoirs Using Chemical Compounds Targeting Host and Viral Factors', *AIDS Research and Human Retroviruses*. Mary Ann Liebert Inc., pp. 1–24. doi: 10.1089/aid.2018.0153.
- Jenkins, M. K. and Schwartz, R. H. (1987) 'Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo', *Journal of Experimental Medicine*. The Rockefeller University Press, 165(2), pp. 302–319. doi: 10.1084/jem.165.2.302.
- Jensen, E. R. *et al.* (1998) 'Fas (CD95)-dependent cell-mediated immunity to Listeria



- monocytogenes.', *Infection and immunity*, 66(9), pp. 4143–50. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9712760> (Accessed: 13 January 2020).
- Jerne, N. K. (1955) 'The natural selection theory of antibody formation', *Proceedings of the National Academy of Sciences*. *Proceedings of the National Academy of Sciences*, 41(11), pp. 849–857. doi: 10.1073/pnas.41.11.849.
- Jerne, N. K. (1971) 'The somatic generation of immune recognition', *European Journal of Immunology*, 1(1), pp. 1–9. doi: 10.1002/eji.1830010102.
- Jerome, K. R., Domenech, N. and Finn, O. J. (1993) 'Tumor-specific cytotoxic T cell clones from patients with breast and pancreatic adenocarcinoma recognize EBV-immortalized B cells transfected with polymorphic epithelial mucin complementary DNA.', *Journal of immunology (Baltimore, Md. : 1950)*, 151(3), pp. 1654–62. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8393050> (Accessed: 20 March 2020).
- Ji, Q., Perchellet, A. and Goverman, J. M. (2010) 'Viral infection triggers central nervous system autoimmunity via activation of CD8 + T cells expressing dual TCRs', *Nature Immunology*. NIH Public Access, 11(7), pp. 628–634. doi: 10.1038/ni.1888.
- Jiang, N. *et al.* (2011) 'Two-Stage Cooperative T Cell Receptor-Peptide Major Histocompatibility Complex-CD8 Trimolecular Interactions Amplify Antigen Discrimination', *Immunity*, 34(1), pp. 13–23. doi: 10.1016/j.immuni.2010.12.017.
- Jiménez, E. *et al.* (2002) 'Rat Peripheral CD4 + CD8 + T Lymphocytes Are Partially Immunocompetent Thymus-Derived Cells That Undergo Post-Thymic Maturation to Become Functionally Mature CD4 + T Lymphocytes', *The Journal of Immunology*. The American Association of Immunologists, 168(10), pp. 5005–5013. doi: 10.4049/jimmunol.168.10.5005.
- Jin, X. *et al.* (2002) 'Human Immunodeficiency Virus Type 1 (HIV-1)-Specific CD8+-T-Cell Responses for Groups of HIV-1-Infected Individuals with Different HLA-B\*35 Genotypes', *Journal of Virology*. American Society for Microbiology, 76(24), pp. 12603–12610. doi: 10.1128/jvi.76.24.12603-12610.2002.
- Joglekar, A. V. *et al.* (2019) 'T cell antigen discovery via signaling and antigen-presenting bifunctional receptors', *Nature Methods*. Nature Publishing Group, 16(2), pp. 191–198. doi: 10.1038/s41592-018-0304-8.
- Johnson, L. A. *et al.* (2009) 'Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen', *Blood*. American Society of Hematology, 114(3), pp. 535–546. doi: 10.1182/blood-2009-03-211714.
- Joly, E. and Hudrisier, D. (2003) 'What is trogocytosis and what is its purpose?', *Nature Immunology*. Nature Publishing Group, p. 815. doi: 10.1038/ni0903-815.
- Jordon, R. E. (1982) 'The Complement System and the Skin: An Overview', *Archives of Dermatology*, 118(8), pp. 539–541. doi: 10.1001/archderm.1982.01650200007006.
- Jorgensen, J. L. *et al.* (1992) 'Mapping T-cell receptor-peptide contacts by variant peptide immunization of single-chain transgenics', *Nature*, 355(6357), pp. 224–230. doi: 10.1038/355224a0.
- Julg, B. and Barouch, D. H. (2019) 'Neutralizing antibodies for HIV-1 prevention', *Current Opinion in HIV and AIDS*. Lippincott Williams and Wilkins, pp. 318–324. doi: 10.1097/COH.0000000000000556.

- June, C. H. *et al.* (1987) 'T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression.', *Molecular and Cellular Biology*. American Society for Microbiology, 7(12), pp. 4472–4481. doi: 10.1128/mcb.7.12.4472.
- June, C. H. *et al.* (1990) 'Inhibition of tyrosine phosphorylation prevents T-cell receptor-mediated signal transduction', *Proceedings of the National Academy of Sciences of the United States of America*, 87(19), pp. 7722–7726. doi: 10.1073/pnas.87.19.7722.
- Jurtz, V. I. *et al.* (2017) 'NetMHCpan-4.0: Improved Peptide–MHC Class I Interaction Predictions Integrating Eluted Ligand and Peptide Binding Affinity Data', *The Journal of Immunology*. doi: 10.4049/jimmunol.1700893.
- Jurtz, V. I. *et al.* (2018) 'NetTCR: sequence-based prediction of TCR binding to peptide-MHC complexes using convolutional neural networks', *bioRxiv*. Cold Spring Harbor Laboratory, p. 433706. doi: 10.1101/433706.
- Kägi, D. *et al.* (1994) 'Cytotoxicity mediated by T cells and natural killer cells is greatly impaired in perforin-deficient mice', *Nature*, 369(6475), pp. 31–37. doi: 10.1038/369031a0.
- Kägi, D. *et al.* (1995) 'The roles of perforin- and Fas-dependent cytotoxicity in protection against cytopathic and noncytopathic viruses', *European Journal of Immunology*, 25(12), pp. 3256–3262. doi: 10.1002/eji.1830251209.
- Kahn, J. O. and Walker, B. D. (1998) 'Acute human immunodeficiency virus type 1 infection', *New England Journal of Medicine*. N Engl J Med, p. 33. doi: 10.1056/NEJM199807023390107.
- Kalergis, A. H. *et al.* (2001) 'Efficient T cell activation requires an optimal dwell-time of interaction between the TCR and the pMHC complex', *Nature Immunology*. Nature Publishing Group, 2(3), pp. 229–234. doi: 10.1038/85286.
- Kamradt, T. and Avriou Mitchison, N. (2001) 'Tolerance and autoimmunity', *New England Journal of Medicine*, pp. 655–664. doi: 10.1056/NEJM200103013440907.
- Kantanen, M. L., Leinikki, P. and Kuismanen, E. (1995) 'Endoproteolytic cleavage of HIV-1 gp160 envelope precursor occurs after exit from the trans-Golgi network (TGN)', *Archives of Virology*. Springer-Verlag, 140(8), pp. 1441–1449. doi: 10.1007/BF01322670.
- Kappler, J. W. *et al.* (1983) 'The major histocompatibility complex-restricted antigen receptor on T cells in mouse and man: Identification of constant and variable peptides', *Cell*. Cell Press, 35(1), pp. 295–302. doi: 10.1016/0092-8674(83)90232-5.
- Kappler, J. W. and Marrack, P. C. (1976) 'Helper T cells recognize antigen and macrophage surface components simultaneously', *Nature*. Nature Publishing Group, 262(5571), pp. 797–799. doi: 10.1038/262797a0.
- Kappler, J. W., Roehm, N. and Marrack, P. (1987) 'T cell tolerance by clonal elimination in the thymus', *Cell*. Cell Press, 49(2), pp. 273–280. doi: 10.1016/0092-8674(87)90568-X.
- Karamanou, M. *et al.* (2014) 'Apollinaire Bouchardat (1806-1886): Founder of modern Diabetology', *Hormones*. Hellenic Endocrine Society, 13(2), pp. 296–300. doi: 10.1007/bf03401345.
- Karamanou, M. (2016) 'Milestones in the history of diabetes mellitus: The main contributors', *World Journal of Diabetes*. Baishideng Publishing Group Inc., 7(1), p. 1. doi: 10.4239/wjd.v7.i1.1.
- Kared, H. *et al.* (2021) 'SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19

individuals.', *The Journal of clinical investigation*. American Society for Clinical Investigation. doi: 10.1172/JCI145476.

Karell, K. *et al.* (2003) 'HLA types in celiac disease patients not carrying the DQA1 \*05-DQB1 \*02 (DQ2) heterodimer: Results from the European genetics cluster on celiac disease', *Human Immunology*. Elsevier Inc., 64(4), pp. 469–477. doi: 10.1016/S0198-8859(03)00027-2.

Kass, I., Buckle, A. M. and Borg, N. A. (2014) 'Understanding the structural dynamics of TCR-pMHC interactions', *Trends in Immunology*, 35(12), pp. 604–612. doi: 10.1016/j.it.2014.10.005.

Katsarou, A. *et al.* (2017) 'Type 1 diabetes mellitus', *Nature Reviews Disease Primers*. Nature Publishing Group, 3(1), pp. 1–17. doi: 10.1038/nrdp.2017.16.

Katz, D. H. and Armerding, D. (1976) 'The role of histocompatibility gene products in lymphocyte triggering and differentiation.', *Federation proceedings*, 35(9), pp. 2053–60. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/776706> (Accessed: 17 March 2020).

Katz, D. H., Dixon, F. J. and Kunkel, H. G. (1977) *Lymphocyte Differentiation, Recognition, and Regulation*. Elsevier Science.

Kawakami, Y. *et al.* (1994) 'Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes', *Journal of Experimental Medicine*. The Rockefeller University Press, 180(1), pp. 347–352. doi: 10.1084/jem.180.1.347.

Kawasaki, E. (2012) 'ZnT8 and type 1 diabetes', *Endocrine Journal*, pp. 531–537. doi: 10.1507/endocrj.EJ12-0069.

Kaye, J. *et al.* (1983) 'Both a monoclonal antibody and antisera specific for determinants unique to individual cloned helper T cell lines can substitute for antigen and antigen-presenting cells in the activation of T cells', *Journal of Experimental Medicine*. The Rockefeller University Press, 158(3), pp. 836–856. doi: 10.1084/jem.158.3.836.

Kelleher, A. D. *et al.* (2001) 'Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses', *Journal of Experimental Medicine*. The Rockefeller University Press, 193(3), pp. 375–385. doi: 10.1084/jem.193.3.375.

Kelly, A. *et al.* (1991) 'Second proteasome-related gene in the human MHC class II region', *Nature*. Nature Publishing Group, 353(6345), pp. 667–668. doi: 10.1038/353667a0.

Kelly, A. *et al.* (1992) 'Assembly and function of the two ABC transporter proteins encoded in the human major histocompatibility complex', *Nature*. Nature Publishing Group, 355(6361), pp. 641–644. doi: 10.1038/355641a0.

Kemming, J. *et al.* (2019) 'ERAP1 allotypes shape the epitope repertoire of virus-specific CD8+ T cell responses in acute hepatitis C virus infection', *Journal of Hepatology*. Elsevier B.V., 70(6), pp. 1072–1081. doi: 10.1016/j.jhep.2019.01.034.

Kenway-Lynch, C. S. *et al.* (2014) 'Cytokine/Chemokine Responses in Activated CD4+ and CD8+ T Cells Isolated from Peripheral Blood, Bone Marrow, and Axillary Lymph Nodes during Acute Simian Immunodeficiency Virus Infection', *Journal of Virology*. American Society for Microbiology, 88(16), pp. 9442–9457. doi: 10.1128/jvi.00774-14.

Kersh, G. J. *et al.* (1998) 'High- and low-potency ligands with similar affinities for the TCR:

- The importance of kinetic in TCR signaling', *Immunity*. Cell Press, 9(6), pp. 817–826. doi: 10.1016/S1074-7613(00)80647-0.
- Kersh, G. J. and Allen, P. M. (1996) 'Structural basis for T cell recognition of altered peptide ligands: A single T cell receptor can productively recognize a large continuum of related ligands', *Journal of Experimental Medicine*, 184(4), pp. 1259–1268. doi: 10.1084/jem.184.4.1259.
- Khan, U. and Ghazanfar, H. (2018) 'T Lymphocytes and Autoimmunity', *International Review of Cell and Molecular Biology*. Elsevier Inc., 341, pp. 125–168. doi: 10.1016/bs.ircmb.2018.05.008.
- Kim, J. *et al.* (2018) 'CD8+ cytotoxic T lymphocyte responses and viral epitope escape in acute HIV-1 infection', *Viral Immunology*. Mary Ann Liebert Inc., 31(7), pp. 525–536. doi: 10.1089/vim.2018.0040.
- Kim, J. H. *et al.* (2011) 'High Cleavage Efficiency of a 2A Peptide Derived from Porcine Teschovirus-1 in Human Cell Lines, Zebrafish and Mice', *PLoS ONE*. Edited by V. Thiel, 6(4), p. e18556. doi: 10.1371/journal.pone.0018556.
- King, C. and Sarvetnick, N. (1997) 'Organ-specific autoimmunity', *Current Opinion in Immunology*. Elsevier Ltd, 9(6), pp. 863–871. doi: 10.1016/S0952-7915(97)80191-4.
- Kingsbury, D. J., Griffin, T. A. and Colbert, R. A. (2000) 'Novel Propeptide Function in 20 S Proteasome Assembly Influences Subunit Composition\*', *ASBMB*. doi: 10.1074/jbc.M001742200.
- Kitze, B. *et al.* (1988) 'Myelin-specific T lymphocytes in multiple sclerosis patients and healthy individuals', *Journal of Neuroimmunology*, 20(2–3), p. 237. doi: 10.1016/0165-5728(88)90166-x.
- Klebanoff, C. A. *et al.* (2004) 'IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8 + T Cells', *Proceedings of the National Academy of Sciences of the United States of America*. Proc Natl Acad Sci U S A, 101(7), pp. 1969–1974. doi: 10.1073/pnas.0307298101.
- Kleijmeer, M. J. *et al.* (1992) 'Location of MHC-encoded transporters in the endoplasmic reticulum and cis-Golgi', *Nature*. Nature Publishing Group, 357(6376), pp. 342–344. doi: 10.1038/357342a0.
- Klein, J. (1986) 'Seeds of time: Fifty years ago Peter A. Gorer discovered the H-2 complex', *Immunogenetics*. Springer-Verlag, 24(6), pp. 331–338. doi: 10.1007/BF00377947.
- Klein, L. *et al.* (2000) 'Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells', *Nature Medicine*, 6(1), pp. 56–61. doi: 10.1038/71540.
- Klein, L. *et al.* (2014) 'Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see)', *Nature Publishing Group*. doi: 10.1038/nri3667.
- Klein, R. *et al.* (2006) 'WPRE-mediated enhancement of gene expression is promoter and cell line specific', *Gene*, 372, pp. 153–161. doi: 10.1016/j.gene.2005.12.018.
- Kløverpris, H. N. *et al.* (2012) 'HLA-B\*57 Micropolymorphism Shapes HLA Allele-Specific Epitope Immunogenicity, Selection Pressure, and HIV Immune Control', *Journal of Virology*. American Society for Microbiology, 86(2), pp. 919–929. doi: 10.1128/jvi.06150-11.
- Kløverpris, H. N., Cole, D. K., *et al.* (2015) 'A molecular switch in immunodominant HIV-1-specific CD8 T-cell epitopes shapes differential HLA-restricted escape', *Retrovirology*.

BioMed Central Ltd., 12(1), p. 20. doi: 10.1186/s12977-015-0149-5.

Kløverpris, H. N., McGregor, R., *et al.* (2015) 'CD8 + TCR Bias and Immunodominance in HIV-1 Infection', *The Journal of Immunology*. The American Association of Immunologists, 194(11), pp. 5329–5345. doi: 10.4049/jimmunol.1400854.

Klump, H. *et al.* (2001) 'Retroviral vector-mediated expression of HoxB4 in hematopoietic cells using a novel coexpression strategy', *Gene Therapy*. *Gene Ther*, 8(10), pp. 811–817. doi: 10.1038/sj.gt.3301447.

Knittler, M. R. *et al.* (1999) 'Nucleotide binding by TAP mediates association with peptide and release of assembled MHC class I molecules', *Current Biology*. Current Biology Ltd, 9(18), pp. 999–1008. doi: 10.1016/S0960-9822(99)80448-5.

Koble, C. and Kyewski, B. (2009) 'The thymic medulla: A unique microenvironment for intercellular self-antigen transfer', *Journal of Experimental Medicine*, 206(7), pp. 1505–1513. doi: 10.1084/jem.20082449.

Koch, J. *et al.* (2004) 'Functional Dissection of the Transmembrane Domains of the Transporter Associated with Antigen Processing (TAP)', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 279(11), pp. 10142–10147. doi: 10.1074/jbc.M312816200.

Kolb, W. P. and Granger, G. A. (1968) 'Lymphocyte in vitro cytotoxicity: characterization of human lymphotoxin.', *Proceedings of the National Academy of Sciences of the United States of America*, 61(4), pp. 1250–1255. doi: 10.1073/pnas.61.4.1250.

Koopmann, J. O. *et al.* (1996) 'Translocation of long peptides by transporters associated with antigen processing (TAP)', *European Journal of Immunology*. Wiley-VCH Verlag, 26(8), pp. 1720–1728. doi: 10.1002/eji.1830260809.

Koszinowski, U. and Ertl, H. (1975) 'Lysis mediated by T cells and restricted by H-2 antigen of target cells infected with vaccinia virus', *Nature*. Nature Publishing Group, 255(5509), pp. 552–554. doi: 10.1038/255552a0.

Koup, R. A. (1994) 'Virus escape from CTL recognition', *Journal of Experimental Medicine*. *J Exp Med*, 180(3), pp. 779–782. doi: 10.1084/jem.180.3.779.

Kozak, M. (1984) 'Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs.', *Nucleic acids research*. Oxford University Press, 12(2), pp. 857–72. doi: 10.1093/nar/12.2.857.

Kozak, M. (1987) 'An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs', *Nucleic Acids Research*, 15(20), pp. 8125–8148. doi: 10.1093/nar/15.20.8125.

Krensky, A. M. *et al.* (1982) 'Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4+', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 79(7 I), pp. 2365–2369. doi: 10.1073/pnas.79.7.2365.

Kronenberg, D. *et al.* (2012) 'Circulating Preproinsulin Signal Peptide-Specific CD8 T Cells Restricted by the Susceptibility Molecule HLA-A24 Are Expanded at Onset of Type 1 Diabetes and Kill b-Cells', *Diabetes*, 61, pp. 1752–1759. doi: 10.2337/db11-1520.

Krummel, M. F. and Allison, J. P. (1995) 'CD28 and CTLA-4 have opposing effects on the response of T ceils to stimulation', *Journal of Experimental Medicine*, 182(2), pp. 459–465. doi: 10.1084/jem.182.2.459.

- Kuball, J. *et al.* (2007) 'Facilitating matched pairing and expression of TCR chains introduced into human T cells', *Blood*. The American Society of Hematology, 109(6), pp. 2331–2338. doi: 10.1182/blood-2006-05-023069.
- Kuida, K. *et al.* (1991) 'Post-translational attainment of allelic exclusion of the T cell receptor  $\alpha$  chain in a T cell clone', *International Immunology*, 3(1), pp. 75–82. Available at: <https://academic.oup.com/intimm/article-abstract/3/1/75/662958> (Accessed: 16 April 2020).
- Kula, T. *et al.* (2019) 'T-Scan: A Genome-wide Method for the Systematic Discovery of T Cell Epitopes', *Cell*. Cell Press, 178(4), pp. 1016–1028.e13. doi: 10.1016/j.cell.2019.07.009.
- Kulig, K. *et al.* (1998) 'Physical and functional association of the major histocompatibility complex class I heavy chain  $\alpha$ 3 domain with the transporter associated with antigen processing', *Journal of Experimental Medicine*. The Rockefeller University Press, 187(6), pp. 865–874. doi: 10.1084/jem.187.6.865.
- Kumánovics, A., Takada, T. and Lindahl, K. F. (2003) 'Genomic reorganisation of the mammalian MHC', *Annual Review of Immunology*. Annual Reviews, 21(1), pp. 629–657. doi: 10.1146/annurev.immunol.21.090501.080116.
- Kwan, A. H. *et al.* (2011) 'Macromolecular NMR spectroscopy for the non-spectroscopist', *FEBS Journal*, 278(5), pp. 687–703. doi: 10.1111/j.1742-4658.2011.08004.x.
- Van Laethem, F. *et al.* (2007) 'Deletion of CD4 and CD8 Coreceptors Permits Generation of  $\alpha\beta$ T Cells that Recognize Antigens Independently of the MHC', *Immunity*, 27(5), pp. 735–750. doi: 10.1016/j.immuni.2007.10.007.
- Van Laethem, F. *et al.* (2013) 'XLck availability during thymic selection determines the recognition specificity of the T cell repertoire', *Cell*. Cell Press, 154(6), p. 1326. doi: 10.1016/j.cell.2013.08.009.
- Van Laethem, F., Tikhonova, A. N. and Singer, A. (2012) 'MHC restriction is imposed on a diverse T cell receptor repertoire by CD4 and CD8 co-receptors during thymic selection', *Trends in Immunology*, pp. 437–441. doi: 10.1016/j.it.2012.05.006.
- Lafferty, K. J., Misko, I. S. and Cooley, M. A. (1974) 'Allogeneic stimulation modulates the in vitro response of t cells to transplantation antigen', *Nature*, 249(5454), pp. 275–276. doi: 10.1038/249275a0.
- Lafferty, K. J. and Woolnough, J. (1977) 'The origin and mechanism of the allograft reaction.', *Immunological reviews*, 35, pp. 231–62. doi: 10.1111/j.1600-065x.1977.tb00241.x.
- Lai, S. W., Lin, C. L. and Liao, K. F. (2019) 'Real-world database investigating the association between diabetes mellitus and herpes zoster in Taiwan', *Medicine (United States)*. Lippincott Williams and Wilkins, 98(18). doi: 10.1097/MD.00000000000015463.
- Laine, M., Luukkainen, R. and Toivanen, A. (2004) 'Sindbis viruses and other alphaviruses as cause of human arthritic disease', *Journal of Internal Medicine*, pp. 457–471. doi: 10.1111/j.1365-2796.2004.01413.x.
- Lambotte, O. *et al.* (2005) 'HIV Controllers: A Homogeneous Group of HIV-1--Infected Patients with Spontaneous Control of Viral Replication', *Clinical Infectious Diseases*. Oxford University Press (OUP), 41(7), pp. 1053–1056. doi: 10.1086/433188.
- Lang, H. L. E. *et al.* (2002) 'A functional and structural basis for TCR cross-reactivity in

- multiple sclerosis', *Nature Immunology*. Nat Immunol, 3(10), pp. 940–943. doi: 10.1038/ni835.
- Lanzavecchia, A. (1995) 'How can cryptic epitopes trigger autoimmunity?', *Journal of Experimental Medicine*, 181(6), pp. 1945–1948. doi: 10.1084/jem.181.6.1945.
- Larsen, C. E. and Alper, C. A. (2004) 'The genetics of HLA-associated disease', *Current Opinion in Immunology*, pp. 660–667. doi: 10.1016/j.coi.2004.07.014.
- Lasky, L. A. *et al.* (1987) 'Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor', *Cell*. Cell, 50(6), pp. 975–985. doi: 10.1016/0092-8674(87)90524-1.
- Latchman, Y. *et al.* (2001) 'PD-L2 is a second ligand for PD-1 and inhibits T cell activation', *Nature Immunology*, 2(3), pp. 261–268. doi: 10.1038/85330.
- Laugel, B. *et al.* (2007) 'Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 282(33), pp. 23799–23810. doi: 10.1074/jbc.M700976200.
- Leach, D. R., Krummel, M. F. and Allison, J. P. (1996) 'Enhancement of antitumor immunity by CTLA-4 blockade', *Science*. American Association for the Advancement of Science, 271(5256), pp. 1734–1736. doi: 10.1126/science.271.5256.1734.
- Leahy, D. J., Axel, R. and Hendrickson, W. A. (1992) 'Crystal structure of a soluble form of the human T cell coreceptor CD8 at 2.6 Å resolution', *Cell*. Cell Press, 68(6), pp. 1145–1162. doi: 10.1016/0092-8674(92)90085-Q.
- Lebien, T. W. and Tedder, T. F. (2008) 'B lymphocytes: How they develop and function', *Blood*, 112(5), pp. 1570–1580. doi: 10.1182/blood-2008-02-078071.
- Lee, C. H. *et al.* (2020) 'Predicting Cross-Reactivity and Antigen Specificity of T Cell Receptors', *Frontiers in Immunology*. Frontiers Media S.A., p. 565096. doi: 10.3389/fimmu.2020.565096.
- Lee, J. K. *et al.* (2004) 'T cell cross-reactivity and conformational changes during TCR engagement', *Journal of Experimental Medicine*. J Exp Med, 200(11), pp. 1455–1466. doi: 10.1084/jem.20041251.
- Lee, K. P. *et al.* (1990) 'The genomic organization of the CD28 gene. Implications for the regulation of CD28 mRNA expression and heterogeneity.', *Journal of immunology (Baltimore, Md. : 1950)*, 145(1), pp. 344–52. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2162892> (Accessed: 15 April 2020).
- Lee, N. A., Loh, D. Y. and Lacy, E. (1992) 'CD8 surface levels alter the fate of  $\alpha/\beta$  T cell receptor-expressing thymocytes in transgenic mice', *Journal of Experimental Medicine*, 175(4), pp. 1013–1025. doi: 10.1084/jem.175.4.1013.
- Legut, M. *et al.* (2018) 'CRISPR-mediated TCR replacement generates superior anticancer transgenic t cells', *Blood*. American Society of Hematology, 131(3), pp. 311–322. doi: 10.1182/blood-2017-05-787598.
- Lehmann, P. V. *et al.* (1992) 'Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen', *Nature*, 358(6382), pp. 155–157. doi: 10.1038/358155a0.
- Leisegang, M. *et al.* (2008) 'Enhanced functionality of T cell receptor-redirected T cells is defined by the transgene cassette', *Journal of Molecular Medicine*. J Mol Med (Berl), 86(5),

pp. 573–583. doi: 10.1007/s00109-008-0317-3.

Leko, V. *et al.* (2019) 'Identification of Neoantigen-Reactive Tumor-Infiltrating Lymphocytes in Primary Bladder Cancer', *The Journal of Immunology*. The American Association of Immunologists, 202(12), pp. 3458–3467. doi: 10.4049/jimmunol.1801022.

Leslie, A. *et al.* (2005) 'Transmission and accumulation of CTL escape variants drive negative associations between HIV polymorphisms and HLA', *Journal of Experimental Medicine*. J Exp Med, 201(6), pp. 891–902. doi: 10.1084/jem.20041455.

Lesslauer, W. *et al.* (1986) 'T90/44 (9.3 antigen). A cell surface molecule with a function in human T cell activation', *European Journal of Immunology*, 16(10), pp. 1289–1296. doi: 10.1002/eji.1830161017.

Levin, L. I. *et al.* (2003) 'Multiple Sclerosis and Epstein-Barr Virus', *Journal of the American Medical Association*. American Medical Association, 289(12), pp. 1533–1536. doi: 10.1001/jama.289.12.1533.

Levine, B. L. *et al.* (2006) 'Gene transfer in humans using a conditionally replicating lentiviral vector', *Proceedings of the National Academy of Sciences of the United States of America*. Proc Natl Acad Sci U S A, 103(46), pp. 17372–17377. doi: 10.1073/pnas.0608138103.

Lewandowski, L. J., Gerhard, W. U. and Palmer, J. C. (1976) 'Cell-mediated cytotoxicity against murine cells infected with 6/94 virus, a parainfluenza type 1 isolate from multiple sclerosis brain tissue.', *Infection and Immunity*, 13(3).

Lewis, J. W. *et al.* (1996) 'Point mutations in the  $\alpha 2$  domain of HLA-A2.1 define a functionally relevant Interaction with TAP', *Current Biology*. Cell Press, 6(7), pp. 873–883. doi: 10.1016/S0960-9822(02)00611-5.

Lewis, J. W. and Elliott, T. (1998) 'Evidence for successive peptide binding and quality control stages during MHC class I assembly', *Current Biology*. Cell Press, 8(12), pp. 717–721. doi: 10.1016/s0960-9822(98)70280-5.

Lewis, S. M. (1994) 'The mechanism of V(D)J joining: Lessons from molecular, immunological, and comparative analyses', *Advances in Immunology*. Academic Press Inc., pp. 27–150. doi: 10.1016/s0065-2776(08)60450-2.

Li, D. *et al.* (2019) 'Genetically engineered t cells for cancer immunotherapy', *Signal Transduction and Targeted Therapy*. Springer Nature, pp. 1–17. doi: 10.1038/s41392-019-0070-9.

Li, G. *et al.* (2019) 'T cell antigen discovery via trogocytosis', *Nature Methods*. Nature Publishing Group, 16(2), pp. 183–190. doi: 10.1038/s41592-018-0305-7.

Li, J. Z. *et al.* (2016) 'The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption', *AIDS*. Lippincott Williams and Wilkins, 30(3), pp. 343–353. doi: 10.1097/QAD.0000000000000953.

Li, N., Wang, T. and Han, D. (2012) 'Structural, cellular and molecular aspects of immune privilege in the testis', *Frontiers in Immunology*. Frontiers, p. 152. doi: 10.3389/fimmu.2012.00152.

Li, S. W. *et al.* (2010) 'Pancreatic duodenal homeobox 1 protein is a novel B-cell-specific autoantigen for type i diabetes', *Laboratory Investigation*, 90(1), pp. 31–39. doi: 10.1038/labinvest.2009.116.



- Li, Yi *et al.* (2005) 'Directed evolution of human T-cell receptors with picomolar affinities by phage display', *Nature Biotechnology*. Nature Publishing Group, 23(3), pp. 349–354. doi: 10.1038/nbt1070.
- Li, Yili *et al.* (2005) 'Structure of a human autoimmune TCR bound to a myelin basic protein self-peptide and a multiple sclerosis-associated MHC class II molecule', *EMBO Journal*, 24(17), pp. 2968–2979. doi: 10.1038/sj.emboj.7600771.
- Li, Y., Yin, Y. and Mariuzza, R. A. (2013) 'Structural and Biophysical Insights into the Role of CD4 and CD8 in T Cell Activation', *Frontiers in Immunology*. Frontiers, 4(JUL), p. 206. doi: 10.3389/fimmu.2013.00206.
- Liénard, D. *et al.* (2004) 'Ex vivo detectable activation of Melan-A-specific T cells correlating with inflammatory skin reactions in melanoma patients vaccinated with peptides in IFA', *Cancer Immunity*. Academy of Cancer Immunology, 4, pp. 4–4. Available at: <https://europepmc.org/article/med/15149168> (Accessed: 13 December 2020).
- Lindbäck, S. *et al.* (2000) 'Diagnosis of primary HIV-1 infection and duration of follow-up after HIV exposure', *AIDS*, 14(15), pp. 2333–2339. doi: 10.1097/00002030-200010200-00014.
- Linette, G. P. *et al.* (2013) 'Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma', *Blood*, 122(6), pp. 863–871. doi: 10.1182/blood-2013-03-490565.
- Linsley, P. S., Brady, W., Grosmaire, L., *et al.* (1991) 'Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation', *Journal of Experimental Medicine*, 173(3), pp. 721–730. doi: 10.1084/jem.173.3.721.
- Linsley, P. S., Brady, W., Urnes, M., *et al.* (1991) 'CTLA4 is a second receptor for the B cell activation antigen B7', *Journal of Experimental Medicine*, 174(3), pp. 561–569. doi: 10.1084/jem.174.3.561.
- Linsley, P. S., Clark, E. A. and Ledbetter, J. A. (1990) 'T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 87(13), pp. 5031–5035. doi: 10.1073/pnas.87.13.5031.
- Lipham, W. J. *et al.* (1991) 'Recognition of peptides that are immunopathogenic but cryptic. Mechanisms that allow lymphocytes sensitized against cryptic peptides to initiate pathogenic autoimmune processes.', *Journal of immunology (Baltimore, Md. : 1950)*, 146(11), pp. 3757–62. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/1709661> (Accessed: 16 April 2020).
- Lissina, A. *et al.* (2009) 'Protein kinase inhibitors substantially improve the physical detection of T-cells with peptide-MHC tetramers.', *Journal of immunological methods*, 340(1), pp. 11–24. doi: 10.1016/j.jim.2008.09.014.
- Little, S. J. *et al.* (1999) 'Viral dynamics of acute HIV-1 infection', *Journal of Experimental Medicine*. J Exp Med, 190(6), pp. 841–850. doi: 10.1084/jem.190.6.841.
- Liu, J. *et al.* (1999) 'Major DQ8-restricted T-cell epitopes for human GAD65 mapped using human CD4, DQA1\*0301, DQB1\*0302 transgenic IA(null) NOD mice', *Diabetes*. American Diabetes Association Inc., 48(3), pp. 469–477. doi: 10.2337/diabetes.48.3.469.
- Liu, J. Q. *et al.* (1998) 'Inhibition of experimental autoimmune encephalomyelitis in Lewis rats by nasal administration of encephalitogenic MBP peptides: Synergistic effects of MBP

- 68-86 and 87-99', *International Immunology*. *Int Immunol*, 10(8), pp. 1139–1148. doi: 10.1093/intimm/10.8.1139.
- Liu, Z. *et al.* (2017) 'Systematic comparison of 2A peptides for cloning multi-genes in a polycistronic vector', *Scientific Reports*. Nature Publishing Group, 7(1), p. 2193. doi: 10.1038/s41598-017-02460-2.
- Van Loenen, M. M. *et al.* (2010) 'Mixed T cell receptor dimers harbor potentially harmful neoreactivity', *Proceedings of the National Academy of Sciences of the United States of America*. *Proc Natl Acad Sci U S A*, 107(24), pp. 10972–10977. doi: 10.1073/pnas.1005802107.
- Loh, E. Y. *et al.* (1987) 'Identification and sequence of a fourth human T cell antigen receptor chain', *Nature*, 330(6148), pp. 569–572. doi: 10.1038/330569a0.
- Lohmann, T. *et al.* (1994) 'Immunodominant epitopes of glutamic acid decarboxylase 65 and 67 in insulin-dependent diabetes mellitus', *The Lancet*. Elsevier, 343(8913), pp. 1607–1608. doi: 10.1016/S0140-6736(94)93061-9.
- Londei, M. *et al.* (1989) 'Persistence of collagen type II-specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis', *Proceedings of the National Academy of Sciences of the United States of America*, 86(2), pp. 636–640. doi: 10.1073/pnas.86.2.636.
- Luz, J. G. *et al.* (2002) 'Structural comparison of allogeneic and syngeneic T cell receptor-peptide-major histocompatibility complex complexes: A buried alloreactive mutation subtly alters peptide presentation substantially increasing V $\beta$  interactions', *Journal of Experimental Medicine*, 195(9), pp. 1175–1186. doi: 10.1084/jem.20011644.
- Lv, Y. *et al.* (2009) 'Identification of a novel conserved HLA-A 0201-restricted epitope from the spike protein of SARS-CoV', *BMC Immunology*. *BMC Immunol*, 10. doi: 10.1186/1471-2172-10-61.
- MacDonald, H. R., Thiernesse, N. and Cerottini, J. C. (1981) 'Inhibition of T cell-mediated cytolysis by monoclonal antibodies directed against Lyt-2: heterogeneity of inhibition at the clonal level.', *The Journal of Immunology*. American Association of Immunologists, 126(5), pp. 1671–1675.
- MacLachlan, B. J. *et al.* (2017) 'Using X-ray crystallography, biophysics, and functional assays to determine the mechanisms governing T-cell receptor recognition of cancer antigens', *Journal of Visualized Experiments*. *Journal of Visualized Experiments*, 2017(120). doi: 10.3791/54991.
- MacLachlan, B. J. *et al.* (2019) 'Human leukocyte antigen (HLA) class II peptide flanking residues tune the immunogenicity of a human tumor-derived epitope', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology Inc., 294(52), pp. 20246–20258. doi: 10.1074/jbc.RA119.009437.
- Madden, D. R. *et al.* (1991) 'The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation', *Nature*. Nature Publishing Group, 353(6342), pp. 321–325. doi: 10.1038/353321a0.
- Madden, D. R. *et al.* (1992) 'The three-dimensional structure of HLA-B27 at 2.1 Å resolution suggests a general mechanism for tight peptide binding to MHC', *Cell*, 70(6), pp. 1035–1048. doi: 10.1016/0092-8674(92)90252-8.
- Madden, D. R., Garboczi, D. N. and Wiley, D. C. (1993) 'The antigenic identity of peptide-MHC complexes: A comparison of the conformations of five viral peptides presented by

HLA-A2', *Cell*, 75(4), pp. 693–708. doi: 10.1016/0092-8674(93)90490-H.

Madura, F. *et al.* (2015) 'Structural basis for ineffective T-cell responses to MHC anchor residue-improved "heteroclitic" peptides', *Eur. J. Immunol*, 45, pp. 584–591. doi: 10.1002/eji.201445114.

Madura, F. *et al.* (2019) 'TCR-induced alteration of primary MHC peptide anchor residue', *European Journal of Immunology*. Wiley-VCH Verlag, 49(7), pp. 1052–1066. doi: 10.1002/eji.201948085.

Magarian-Blander, J. *et al.* (1998) 'Intercellular and intracellular events following the MHC-unrestricted TCR recognition of a tumor-specific peptide epitope on the epithelial antigen MUC1.', *Journal of immunology (Baltimore, Md. : 1950)*, 160(7), pp. 3111–20. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9531265> (Accessed: 20 March 2020).

Marcilla, M., Villasevil, E. M. and López de Castro, J. A. (2008) 'Tripeptidyl peptidase II is dispensable for the generation of both proteasome-dependent and proteasome-independent ligands of HLA-B27 and other class I molecules', *European Journal of Immunology*, 38(3), pp. 631–639. doi: 10.1002/eji.200737444.

Marker, O. and Volkert, M. (1973) 'Studies on cell-mediated immunity to lymphocytic choriomeningitis virus in mice', *Journal of Experimental Medicine*. The Rockefeller University Press, 137(6), pp. 1511–1525. doi: 10.1084/jem.137.6.1511.

Market, E. and Papavasiliou, F. N. (2003) 'V(D)J Recombination and the Evolution of the Adaptive Immune System', *PLoS Biology*, 1(1), p. e16. doi: 10.1371/journal.pbio.0000016.

Marrack, P. *et al.* (2008) 'Evolutionarily Conserved Amino Acids That Control TCR-MHC Interaction', *Annual Review of Immunology*. Annual Reviews, 26(1), pp. 171–203. doi: 10.1146/annurev.immunol.26.021607.090421.

Marron, M. P. *et al.* (2002) 'Functional evidence for the mediation of diabetogenic T cell responses by HLA-A2.1 MHC class I molecules through transgenic expression in NOD mice', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 99(21), pp. 13753–13758. doi: 10.1073/pnas.212221199.

Martin, W. *et al.* (1995) 'Lens-specific expression of a major histocompatibility complex class I molecule disrupts normal lens development and induces cataracts in transgenic mice.', *Investigative Ophthalmology & Visual Science*, 36(6), pp. 1144–1154.

Martinez-Hackert, E. *et al.* (2006) 'Structural basis for degenerate recognition of natural HIV peptide variants by cytotoxic lymphocytes', *Journal of Biological Chemistry*. J Biol Chem, 281(29), pp. 20205–20212. doi: 10.1074/jbc.M601934200.

Martinez, C. K. and Monaco, J. J. (1991) 'Homology of proteasome subunits to a major histocompatibility complex-linked LMP gene', *Nature*. Nature Publishing Group, 353(6345), pp. 664–667. doi: 10.1038/353664a0.

Mason, D. (1998) 'A very high level of crossreactivity is an essential feature of the T- cell receptor', *Immunology Today*. Elsevier Ltd, 19(9), pp. 395–404. doi: 10.1016/S0167-5699(98)01299-7.

Mason, D. and Fowell, D. (1992) 'T-cell subsets in autoimmunity', *Current Opinion in Immunology*. Elsevier Current Trends, 4(6), pp. 728–732. doi: 10.1016/0952-7915(92)90053-H.

Mathers, C. D. and Loncar, D. (2006) 'Projections of global mortality and burden of disease

- from 2002 to 2030', *PLoS Medicine*. Public Library of Science, 3(11), pp. 2011–2030. doi: 10.1371/journal.pmed.0030442.
- Matis, L. A., Ezquerra, A. and Coligan, J. E. (1988) 'Expression of two distinct T cell receptor  $\alpha/\beta$  heterodimers by an antigen-specific T cell clone', *Journal of Experimental Medicine*. The Rockefeller University Press, 168(6), pp. 2379–2384. doi: 10.1084/jem.168.6.2379.
- Matsumura, M. *et al.* (1992) 'Emerging principles for the recognition of peptide antigens by MHC class I molecules', *Science*, 257(5072), pp. 927–934. doi: 10.1126/science.1323878.
- Mauri, C. and Bosma, A. (2012) 'Immune Regulatory Function of B Cells', *Annual Review of Immunology*, 30(1), pp. 221–241. doi: 10.1146/annurev-immunol-020711-074934.
- Mayer, K. H. *et al.* (2017) 'Safety, pharmacokinetics, and immunological activities of multiple intravenous or subcutaneous doses of an anti-HIV monoclonal antibody, VRC01, administered to HIV-uninfected adults: Results of a phase 1 randomized trial', *PLoS Medicine*. Public Library of Science, 14(11). doi: 10.1371/journal.pmed.1002435.
- Mazanet, M. M. and Hughes, C. C. W. (2002) 'B7-H1 Is Expressed by Human Endothelial Cells and Suppresses T Cell Cytokine Synthesis', *The Journal of Immunology*. The American Association of Immunologists, 169(7), pp. 3581–3588. doi: 10.4049/jimmunol.169.7.3581.
- McGarrity, G. J. *et al.* (2013) 'Patient monitoring and follow-up in lentiviral clinical trials', *Journal of Gene Medicine*. J Gene Med, 15(2), pp. 78–82. doi: 10.1002/jgm.2691.
- McIntyre, B. W. and Allison, J. P. (1983) 'The mouse T cell receptor: Structural heterogeneity of molecules of normal T cells defined by Xenoantiserum', *Cell*. Cell Press, 34(3), pp. 739–746. doi: 10.1016/0092-8674(83)90530-5.
- McMichael, A. J. *et al.* (1979) 'A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody', *European Journal of Immunology*, 9(3), pp. 205–210. doi: 10.1002/eji.1830090307.
- McMichael, A. J. *et al.* (1983) 'Cytotoxic T-Cell Immunity to Influenza', *New England Journal of Medicine*. N Engl J Med, 309(1), pp. 13–17. doi: 10.1056/NEJM198307073090103.
- Medzhitov, R. and Janeway, C. (2000) 'Innate Immunity', *New England Journal of Medicine*. Edited by I. R. Mackay and F. S. Rosen, 343(5), pp. 338–344. doi: 10.1056/NEJM200008033430506.
- Melbye, M. *et al.* (1986) 'Long-term seropositivity for human T-lymphotropic virus type III in homosexual men without the acquired immunodeficiency syndrome: Development of immunologic and clinical abnormalities. A longitudinal study', *Annals of Internal Medicine*. Ann Intern Med, 104(4), pp. 496–500. doi: 10.7326/0003-4819-104-4-496.
- Mellors, J. W. *et al.* (1995) 'Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion', *Annals of Internal Medicine*. American College of Physicians, 122(8), pp. 573–579. doi: 10.7326/0003-4819-122-8-199504150-00003.
- Menéndez-Arias, L. (2002) 'Targeting HIV: Antiretroviral therapy and development of drug resistance', *Trends in Pharmacological Sciences*. Trends Pharmacol Sci, pp. 381–388. doi: 10.1016/S0165-6147(02)02054-0.
- Mering, J. and Minkowski, O. (1890) 'Diabetes mellitus nach Pankreasexstirpation', *Archiv für Experimentelle Pathologie und Pharmakologie*. Springer-Verlag, 26(5–6), pp. 371–387. doi: 10.1007/BF01831214.
- Merkel, F. *et al.* (1996) 'Autoreactive T-cells in Goodpasture's syndrome recognize the N-

terminal NC1 domain on  $\alpha 3$  type IV collagen', in *Kidney International*. Nature Publishing Group, pp. 1127–1133. doi: 10.1038/ki.1996.163.

Merkenschlager, M. *et al.* (1997) 'How many thymocytes audition for selection?', *Journal of Experimental Medicine*, 186(7), pp. 1149–1158. doi: 10.1084/jem.186.7.1149.

Merrifield, R. B. (1963) 'Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide', *Journal of the American Chemical Society*, 85(14), pp. 2149–2154. doi: 10.1021/ja00897a025.

van der Merwe, P. A. *et al.* (1994) 'Human cell-adhesion molecule CD2 binds CD58 (LFA-3) with a very low affinity and an extremely fast dissociation rate but does not bind CD48 or CD59.', *Biochemistry*, 33(33), pp. 10149–60. doi: 10.1021/bi00199a043.

Van Der Merwe, P. A. *et al.* (2000) 'Cytoskeletal polarization and redistribution of cell-surface molecules during T cell antigen recognition', *Seminars in Immunology*. Academic Press, 12(1), pp. 5–21. doi: 10.1006/smim.2000.0203.

van der Merwe, P. A. and Davis, S. J. (2003) 'Molecular interactions mediating T cell antigen recognition', *Annual Review of Immunology*. Annual Reviews 4139 El Camino Way, P.O. Box 10139, Palo Alto, CA 94303-0139, USA, 21(1), pp. 659–684. doi: 10.1146/annurev.immunol.21.120601.141036.

Mestan, J. *et al.* (1986) 'Antiviral effects of recombinant tumour necrosis factor in vitro', *Nature*, 323(6091), pp. 816–819. doi: 10.1038/323816a0.

Metzler, B. and Wraith, D. C. (1993) 'Inhibition of experimental autoimmune encephalomyelitis by inhalation but not oral administration of the encephalitogenic peptide: Influence of MHC binding affinity', *International Immunology*. Int Immunol, 5(9), pp. 1159–1165. doi: 10.1093/intimm/5.9.1159.

Metzler and Wraith (1999) 'Inhibition of T-cell responsiveness by nasal peptide administration: influence of the thymus and differential recovery of T-cell-dependent functions', *Immunology*. John Wiley & Sons, Ltd, 97(2), pp. 257–263. doi: 10.1046/j.1365-2567.1999.00795.x.

Meuer, S. C. *et al.* (1983) 'Evidence for the T3-associated 90K heterodimer as the T-cell antigen receptor', *Nature*. Nature, 303(5920), pp. 808–810. doi: 10.1038/303808a0.

Meuer, S. C., Schlossman, S. F. and Reinherz, E. L. (1982) 'Clonal analysis of human cytotoxic T lymphocytes: T4+ and T8+ effector T cells recognize products of different major histocompatibility complex regions', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 79(14 I), pp. 4395–4399. doi: 10.1073/pnas.79.14.4395.

Miceli, M. C., Von Hoegen, P. and Parnes, J. R. (1991) 'Adhesion versus coreceptor function of CD4 and CD8: Role of the cytoplasmic tail in coreceptor activity', *Proceedings of the National Academy of Sciences of the United States of America*, 88(7), pp. 2623–2627. doi: 10.1073/pnas.88.7.2623.

Michalek, M. T. *et al.* (1993) 'A role for the ubiquitin-dependent proteolytic pathway in MHC class I-restricted antigen presentation', *Nature*. Nature Publishing Group, 363(6429), pp. 552–554. doi: 10.1038/363552a0.

Mier, J. W. and Gallo, R. C. (1980) 'Purification and some characteristics of human T-cell growth factor from phytohemagglutinin-stimulated lymphocyte-conditioned media (lymphokines/blast transformation/cultured T cells/colony-stimulating activity)', *Medical*

*Sciences*, 77(10), pp. 6134–6138.

Mignot, E. *et al.* (1997) 'Extensive HLA class II studies in 58 non-DRB 1\*15 (DR2) narcoleptic patients with cataplexy', *Tissue Antigens*. Blackwell Publishing Ltd, 49(4), pp. 329–341. doi: 10.1111/j.1399-0039.1997.tb02761.x.

Migueles, S. A. *et al.* (2002) 'HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors', *Nature Immunology*. Nat Immunol, 3(11), pp. 1061–1068. doi: 10.1038/ni845.

Mijušković, M. *et al.* (2015) 'Off-Target V(D)J Recombination Drives Lymphomagenesis and Is Escalated by Loss of the Rag2 C Terminus', *Cell Reports*. Elsevier, 12(11), pp. 1842–1852. doi: 10.1016/j.celrep.2015.08.034.

Mildvan, D. *et al.* (1982) 'Opportunistic infections and immune deficiency in homosexual men', *Annals of Internal Medicine*. Ann Intern Med, 96(6 l), pp. 700–704. doi: 10.7326/0003-4819-96-6-700.

Miles, J. J. *et al.* (2018) 'Peptide mimic for influenza vaccination using nonnatural combinatorial chemistry', *Journal of Clinical Investigation*. American Society for Clinical Investigation, 128(4), pp. 1569–1580. doi: 10.1172/JCI91512.

Milone, M. C. and O'Doherty, U. (2018) 'Clinical use of lentiviral vectors', *Leukemia*. Nature Publishing Group, pp. 1529–1541. doi: 10.1038/s41375-018-0106-0.

Mishra, P. and Jha, S. K. (2019) 'Slow Motion Protein Dance Visualized Using Red-Edge Excitation Shift of a Buried Fluorophore', *The Journal of Physical Chemistry B*. American Chemical Society, 123(6), pp. 1256–1264. doi: 10.1021/acs.jpcc.8b11151.

Misko, I. S. *et al.* (1999) 'Crossreactive recognition of viral, self, and bacterial peptide ligands by human class I-restricted cytotoxic T lymphocyte clonotypes: Implications for molecular mimicry in autoimmune disease', *Proceedings of the National Academy of Sciences of the United States of America*, 96(5), pp. 2279–2284. doi: 10.1073/pnas.96.5.2279.

Mizuguchi, H. *et al.* (2000) 'IRES-Dependent Second Gene Expression Is Significantly Lower Than Cap-Dependent First Gene Expression in a Bicistronic Vector', *Molecular Therapy*. Mol Ther, 1(4), pp. 376–382. doi: 10.1006/mthe.2000.0050.

Mo, F. *et al.* (2020) 'Engineered off-the-shelf therapeutic T cells resist host immune rejection', *Nature Biotechnology*. Nature Research, pp. 1–8. doi: 10.1038/s41587-020-0601-5.

Mo, X. Y. *et al.* (1999) 'Distinct proteolytic processes generate the C and N termini of MHC class I-binding peptides.', *Journal of immunology (Baltimore, Md. : 1950)*, 163(11), pp. 5851–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10570269> (Accessed: 3 March 2020).

Modigliani, Y. *et al.* (1995) 'Lymphocytes selected in allogeneic thymic epithelium mediate dominant tolerance toward tissue grafts of the thymic epithelium haplotype', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 92(16), pp. 7555–7559. doi: 10.1073/pnas.92.16.7555.

Moebius, U. *et al.* (1991) 'Expression of different CD8 isoforms on distinct human lymphocyte subpopulations', *European Journal of Immunology*, 21(8), pp. 1793–1800. doi: 10.1002/eji.1830210803.

- Molloy, P. E., Sewell, A. K. and Jakobsen, B. K. (2005) 'Soluble T cell receptors: Novel immunotherapies', *Current Opinion in Pharmacology*. Elsevier BV, pp. 438–443. doi: 10.1016/j.coph.2005.02.004.
- Monaco, J. J., Cho, S. and Attaya, M. (1990) 'Transport protein genes in the marine MHC: Possible implications for antigen processing', *Science*. American Association for the Advancement of Science, 250(4988), pp. 1723–1726. doi: 10.1126/science.2270487.
- Moon, C. P. and Fleming, K. G. (2011) 'Using tryptophan fluorescence to measure the stability of membrane proteins folded in liposomes', in *Methods in Enzymology*. Academic Press Inc., pp. 189–211. doi: 10.1016/B978-0-12-381268-1.00018-5.
- Moore, C. B. *et al.* (2002) 'Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level', *Science*. Science, 296(5572), pp. 1439–1443. doi: 10.1126/science.1069660.
- Moore, M. W., Carbone, F. R. and Bevan, M. J. (1988) 'Introduction of soluble protein into the class I pathway of antigen processing and presentation', *Cell*, 54(6), pp. 777–785. doi: 10.1016/S0092-8674(88)91043-4.
- Morgan, R. A. *et al.* (2003) 'High Efficiency TCR Gene Transfer into Primary Human Lymphocytes Affords Avid Recognition of Melanoma Tumor Antigen Glycoprotein 100 and Does Not Alter the Recognition of Autologous Melanoma Antigens', *The Journal of Immunology*. The American Association of Immunologists, 171(6), pp. 3287–3295. doi: 10.4049/jimmunol.171.6.3287.
- Morgan, R. A. *et al.* (2006) 'Cancer regression in patients after transfer of genetically engineered lymphocytes', *Science*. American Association for the Advancement of Science, 314(5796), pp. 126–129. doi: 10.1126/science.1129003.
- Morgan, R. A. *et al.* (2013) 'Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy', *Journal of Immunotherapy*. NIH Public Access, 36(2), pp. 133–151. doi: 10.1097/CJI.0b013e3182829903.
- Morikawa, Y. *et al.* (1998) 'Detection of a Trimeric Human Immunodeficiency Virus Type 1 Gag Intermediate Is Dependent on Sequences in the Matrix Protein, p17', *Journal of Virology*. American Society for Microbiology, 72(9), pp. 7659–7663. doi: 10.1128/jvi.72.9.7659-7663.1998.
- Moriyama, M. *et al.* (2017) 'Induction of lung CD8+ T cell responses by consecutive inoculations of a poly(I:C) influenza vaccine', *Vaccine*. Elsevier Ltd, 35(48), pp. 6620–6626. doi: 10.1016/j.vaccine.2017.10.038.
- Morrison, L. A. *et al.* (1986) 'Differences in antigen presentation to MHC class I- and class II-restricted influenza virus-specific cytolytic T lymphocyte clones', *Journal of Experimental Medicine*. The Rockefeller University Press, 163(4), pp. 903–921. doi: 10.1084/jem.163.4.903.
- Moskophidis, D. *et al.* (1987) 'The immune response of the mouse to lymphocytic choriomeningitis virus V. High numbers of cytolytic T lymphocytes are generated in the spleen during acute infection', *European Journal of Immunology*, 17(7), pp. 937–942. doi: 10.1002/eji.1830170707.
- Motozono, C. *et al.* (2013) 'CD8+ T Cell Cross-Reactivity Profiles and HIV-1 Immune Escape towards an HLA-B35-Restricted Immunodominant Nef Epitope', *PLoS ONE*. Public Library of Science, 8(6). doi: 10.1371/journal.pone.0066152.

- Motozono, C., Bridgeman, J. S., *et al.* (2015) 'Clonotypically similar hybrid  $\alpha\beta$  T cell receptors can exhibit markedly different surface expression, antigen specificity and cross-reactivity', *Clinical and Experimental Immunology*. Blackwell Publishing Ltd, 180(3), pp. 560–570. doi: 10.1111/cei.12610.
- Motozono, C., Pearson, J. A., *et al.* (2015) 'Distortion of the major histocompatibility complex class I binding groove to accommodate an insulin-derived 10-mer peptide', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology Inc., 290(31), pp. 18924–18933. doi: 10.1074/jbc.M114.622522.
- Moudgil, K. D. and Sercarz, E. E. (1994) 'The T cell repertoire against cryptic self determinants and its involvement in autoimmunity and cancer', *Clinical Immunology and Immunopathology*, pp. 283–289. doi: 10.1006/clin.1994.1200.
- Mueller, D. L., Jenkins, M. K. and Schwartz, R. H. (1989) 'Clonal expansion versus functional clonal inactivation: A costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy', *Annual Review of Immunology*, pp. 445–480. doi: 10.1146/annurev.iy.07.040189.002305.
- Mullazehi, M. *et al.* (2012) 'Anti-type II collagen antibodies are associated with early radiographic destruction in rheumatoid arthritis', *Arthritis Research and Therapy*, 14(3). doi: 10.1186/ar3825.
- Mungall, A. J. *et al.* (2003) 'The DNA sequence and analysis of human chromosome 6', *Nature*, 425(6960), pp. 805–811. doi: 10.1038/nature02055.
- Muñoz-Quiles, C. *et al.* (2017) 'Risk and impact of herpes zoster on patients with diabetes: A population-based study, 2009–2014', *Human Vaccines and Immunotherapeutics*. Taylor and Francis Inc., 13(11), pp. 2606–2611. doi: 10.1080/21645515.2017.1368600.
- Münz, C. *et al.* (2009) 'Antiviral immune responses: Triggers of or triggered by autoimmunity?', *Nature Reviews Immunology*. Nature Publishing Group, pp. 246–258. doi: 10.1038/nri2527.
- Nakatsugawa, M. *et al.* (2009) 'Novel spliced form of a lens protein as a novel lung cancer antigen, Lengsin splicing variant 4', *Cancer Science*. Cancer Sci, 100(8), pp. 1485–1493. doi: 10.1111/j.1349-7006.2009.01187.x.
- Nakatsugawa, M. *et al.* (2011) 'Identification of an HLA-A\*0201-restricted cytotoxic T lymphocyte epitope from the lung carcinoma antigen, Lengsin', *International Journal of Oncology*. Int J Oncol, 39(4), pp. 1041–1049. doi: 10.3892/ijo.2011.1089.
- Nakayama, E. *et al.* (1979) 'Cytotoxic T cells: Lyt phenotype and blocking of killing activity by Lyt antisera', *Proceedings of the National Academy of Sciences of the United States of America*, 76(4), pp. 1977–1981. doi: 10.1073/pnas.76.4.1977.
- Nakayama, K. I. *et al.* (1994) 'Requirement for CD8  $\beta$  chain in positive selection of CD8-lineage T cells', *Science*, 263(5150), pp. 1131–1133. doi: 10.1126/science.8108731.
- Naluai, Å. T. *et al.* (2000) 'The CTLA4/CD28 gene region on chromosome 2q33 confers susceptibility to celiac disease in a way possibly distinct from that of type 1 diabetes and other chronic inflammatory disorders', *Tissue Antigens*, 56(4), pp. 350–355. doi: 10.1034/j.1399-0039.2000.560407.x.
- Naquet, P. *et al.* (1988) 'T cell autoreactivity to insulin in diabetic and related non-diabetic individuals.', *Journal of immunology (Baltimore, Md. : 1950)*, 140(8), pp. 2569–78. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2451692> (Accessed: 8 April 2020).



- Nduati, E. *et al.* (2011) 'The plasma concentration of the B cell activating factor is increased in children with acute malaria', *Journal of Infectious Diseases*, 204(6), pp. 962–970. doi: 10.1093/infdis/jir438.
- Neefjes, J. J., Momburg, F. and Hämmerling, G. J. (1993) 'Selective and ATP-dependent translocation of peptides by the MHC-encoded transporter', *Science*. American Association for the Advancement of Science, 261(5122), pp. 769–771. doi: 10.1126/science.8342042.
- Nejentsev, S. *et al.* (2007) 'Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A', *Nature*. Nature Publishing Group, 450(7171), pp. 887–892. doi: 10.1038/nature06406.
- Nguyen, A. W., Le, K. C. and Maynard, J. A. (2018) 'Identification of high affinity HER2 binding antibodies using CHO Fab surface display', *Protein Engineering, Design and Selection*. Oxford University Press, 31(3), p. 91. doi: 10.1093/PROTEIN/GZY004.
- Niederberger, N. *et al.* (2003) 'Allelic Exclusion of the TCR  $\alpha$ -Chain Is an Active Process Requiring TCR-Mediated Signaling and c-Cbl', *The Journal of Immunology*. The American Association of Immunologists, 170(9), pp. 4557–4563. doi: 10.4049/jimmunol.170.9.4557.
- Nietfield, W. *et al.* (1995) 'Sequence constraints and recognition by CTL of an HLA-B27-restricted HIV-1 gag epitope.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 154(5), pp. 2189–97. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7868892> (Accessed: 30 March 2020).
- Nijenhuis, M. and Hämmerling, G. J. (1996) 'Multiple regions of the transporter associated with antigen processing (TAP) contribute to its peptide binding site.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 157(12), pp. 5467–77. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8955196> (Accessed: 4 March 2020).
- Nikoopour, E. *et al.* (2011) 'Cutting Edge: Vasostatin-1-Derived Peptide ChgA29–42 Is an Antigenic Epitope of Diabetogenic BDC2.5 T Cells in Nonobese Diabetic Mice', *The Journal of Immunology*. The American Association of Immunologists, 186(7), pp. 3831–3835. doi: 10.4049/jimmunol.1003617.
- Ninovasquez, J. (2004) 'A powerful combination: the use of positional scanning libraries and biometrical analysis to identify cross-reactive T cell epitopes', *Molecular Immunology*, 40(14–15), pp. 1063–1074. doi: 10.1016/j.molimm.2003.11.005.
- Nishimura, H. *et al.* (1999) 'Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor', *Immunity*. Cell Press, 11(2), pp. 141–151. doi: 10.1016/S1074-7613(00)80089-8.
- Nishimura, H. *et al.* (2001) 'Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice', *Science*. American Association for the Advancement of Science, 291(5502), pp. 319–322. doi: 10.1126/science.291.5502.319.
- Nixon, D. F. *et al.* (1988) 'HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides', *Nature*. Nature, 336(6198), pp. 484–487. doi: 10.1038/336484a0.
- Noble, J. A. *et al.* (2002) 'The HLA class I A locus affects susceptibility to type 1 diabetes', *Human Immunology*, 63(8), pp. 657–664. doi: 10.1016/S0198-8859(02)00421-4.
- Noble, J. A. and Valdes, A. M. (2011) 'Genetics of the HLA region in the prediction of type 1 diabetes', *Current Diabetes Reports*. NIH Public Access, 11(6), pp. 533–542. doi:

10.1007/s11892-011-0223-x.

Norment, A. M. *et al.* (1988) 'Cell-cell adhesion mediated by CD8 and MHC class I molecules', *Nature*. Nature Publishing Group, 336(6194), pp. 79–81. doi: 10.1038/336079a0.

Nunes, J. M. *et al.* (2014) 'The HLA-net GENE[RATE] pipeline for effective HLA data analysis and its application to 145 population samples from Europe and neighbouring areas', *Tissue Antigens*. Blackwell Publishing Ltd, 83(5), pp. 307–323. doi: 10.1111/tan.12356.

O'Brien, T. R. (1996) 'Serum HIV-1 RNA Levels and Time to Development of AIDS in the Multicenter Hemophilia Cohort Study', *JAMA: The Journal of the American Medical Association*. American Medical Association, 276(2), p. 105. doi: 10.1001/jama.1996.03540020027025.

O'Brien, T. R. *et al.* (1997) 'HIV-1 infection in a man homozygous for CCR5 $\Delta$ 32', *Lancet*. Lancet Publishing Group, 349(9060), p. 1219. doi: 10.1016/S0140-6736(97)24017-1.

Obstet, M. B. (1920) 'The relation of the islets of langerhans to diabetes with apecial reference to cases of pancreatic lithiasis', *Surgery, gynecology & obstetrics*, 31, pp. 437–448. Available at: <https://ci.nii.ac.jp/naid/10005566560/> (Accessed: 18 April 2020).

Oettinger, M. A. *et al.* (1990) 'RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination', *Science*, 248(4962), pp. 1517–1523. doi: 10.1126/science.2360047.

Okamoto, S. *et al.* (2009) 'Improved expression and reactivity of transduced tumor-specific TCRs in human lymphocytes by specific silencing of endogenous TCR', *Cancer Research*. Cancer Res, 69(23), pp. 9003–9011. doi: 10.1158/0008-5472.CAN-09-1450.

Ólafsdóttir, G. *et al.* (2008) 'In vitro analysis of expression vectors for DNA vaccination of horses: the effect of a Kozak sequence', *Acta Veterinaria Scandinavica*, 50(1), p. 44. doi: 10.1186/1751-0147-50-44.

Oldham, R. A. A., Berinstein, E. M. and Medin, J. A. (2015) 'Lentiviral vectors in cancer immunotherapy', *Immunotherapy*. Future Medicine Ltd., pp. 271–284. doi: 10.2217/imt.14.108.

Oliver, J. D. *et al.* (1999) 'ERp57 functions as a subunit of specific complexes formed with the ER lectins calreticulin and calnexin', *Molecular Biology of the Cell*. American Society for Cell Biology, 10(8), pp. 2573–2582. doi: 10.1091/mbc.10.8.2573.

Olson, J. K. *et al.* (2001) 'A virus-induced molecular mimicry model of multiple sclerosis.', *The Journal of clinical investigation*, 108(2), pp. 311–8. doi: 10.1172/JCI13032.

Ono, A., Orenstein, J. M. and Freed, E. O. (2000) 'Role of the Gag Matrix Domain in Targeting Human Immunodeficiency Virus Type 1 Assembly', *Journal of Virology*. American Society for Microbiology, 74(6), pp. 2855–2866. doi: 10.1128/jvi.74.6.2855-2866.2000.

Ortiz-Navarrete, V. *et al.* (1991) 'Subunit of the "20S" proteasome (multicatalytic proteinase) encoded by the major histocompatibility complex', *Nature*. Nature Publishing Group, 353(6345), pp. 662–664. doi: 10.1038/353662a0.

Ortmann, B. *et al.* (1997) 'A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes', *Science*, 277(5330), pp. 1306–1309. doi: 10.1126/science.277.5330.1306.

Ortmann, B., Androlewicz, M. J. and Cresswell, P. (1994) 'MHC class I/ $\beta$ 2-microglobulin complexes associate with TAP transporters before peptide binding', *Nature*. Nature

- Publishing Group, 368(6474), pp. 864–867. doi: 10.1038/368864a0.
- Osti, D. *et al.* (2006) 'Comparative analysis of molecular strategies attenuating positional effects in lentiviral vectors carrying multiple genes', *Journal of Virological Methods*. *J Virol Methods*, 136(1–2), pp. 93–101. doi: 10.1016/j.jviromet.2006.04.003.
- Ott, M. *et al.* (2017) 'T-cell Responses to HSV-1 in Persons Who Have Survived Childhood Herpes Simplex Encephalitis', *Pediatric Infectious Disease Journal*. Lippincott Williams and Wilkins, 36(8), pp. 741–744. doi: 10.1097/INF.0000000000001631.
- Overwijk, W. W. *et al.* (2003) 'Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells', *Journal of Experimental Medicine*. The Rockefeller University Press, 198(4), pp. 569–580. doi: 10.1084/jem.20030590.
- Owen, R. D. (1945) 'Immunogenetic consequences of vascular anastomoses between bovine twins', *Science*, 102(2651), pp. 400–401. doi: 10.1126/science.102.2651.400.
- Oxenius, A. *et al.* (2002) 'Functional discrepancies in HIV-specific CD8+ T-lymphocyte populations are related to plasma virus load', *Journal of Clinical Immunology*. *J Clin Immunol*, 22(6), pp. 363–374. doi: 10.1023/A:1020656300027.
- Pachter, J. S., De Vries, H. E. and Fabry, Z. (2003) 'The blood-brain barrier and its role in immune privilege in the central nervous system', *Journal of Neuropathology and Experimental Neurology*. American Association of Neuropathologists Inc., pp. 593–604. doi: 10.1093/jnen/62.6.593.
- Padovan, E. *et al.* (1993) 'Expression of two T cell receptor  $\alpha$  chains: Dual receptor T cells', *Science*. American Association for the Advancement of Science, 262(5132), pp. 422–424. doi: 10.1126/science.8211163.
- Padovan, E. *et al.* (1995) 'Dual Receptor T-Cells: Implications for Alloreactivity and Autoimmunity', *Annals of the New York Academy of Sciences*, 756(1), pp. 66–70. doi: 10.1111/j.1749-6632.1995.tb44482.x.
- Pan, D. *et al.* (2019) 'Lack of T-cell-mediated IL-2 and TNF $\alpha$  production is linked to decreased CD58 expression in intestinal tissue during acute simian immunodeficiency virus infection', *Journal of General Virology*. Microbiology Society, 100(1), pp. 26–34. doi: 10.1099/jgv.0.001181.
- Pancer, Z. and Cooper, M. D. (2006) 'The evolution of adaptive immunity', *Annual Review of Immunology*, 24(1), pp. 497–518. doi: 10.1146/annurev.immunol.24.021605.090542.
- Panina-Bordignon, P. *et al.* (1995) 'Cytotoxic T cells specific for glutamic acid decarboxylase in autoimmune diabetes', *Journal of Experimental Medicine*. The Rockefeller University Press, 181(5), pp. 1923–192. doi: 10.1084/jem.181.5.1923.
- Papadopoulos, E. B. *et al.* (1994) 'Infusions of Donor Leukocytes to Treat Epstein-Barr Virus-Associated Lymphoproliferative Disorders after Allogeneic Bone Marrow Transplantation', *New England Journal of Medicine*. Massachusetts Medical Society, 330(17), pp. 1185–1191. doi: 10.1056/NEJM199404283301703.
- Parham, P. *et al.* (1977) 'Carbohydrate moiety of HLA antigens. Antigenic properties and amino acid sequences around the site of glycosylation.', *The Journal of biological chemistry*, 252(21), pp. 7555–67. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/72068> (Accessed: 16 January 2020).
- Parkhurst, M. R. *et al.* (2011) 'T cells targeting carcinoembryonic antigen can mediate

- regression of metastatic colorectal cancer but induce severe transient colitis', *Molecular Therapy*. Nature Publishing Group, 19(3), pp. 620–626. doi: 10.1038/mt.2010.272.
- Parkin, J. and Cohen, B. (2001) 'An Overview of the Immune System', *Nursing standard (Royal College of Nursing (Great Britain) : 1987)*, pp. 1777–1789. doi: 10.1016/S0140-6736(00)04904-7.
- Parnes, J. R. (1989) 'Molecular Biology and Function of CD4 and CD8', *Advances in Immunology*. Academic Press, 44(C), pp. 265–311. doi: 10.1016/S0065-2776(08)60644-6.
- Peace-Brewer, A. L. *et al.* (1996) 'A point mutation in HLA-A\*0201 results in failure to bind the TAP complex and to present virus-derived peptides to CTL.', *Immunity*, 4(5), pp. 505–14. doi: 10.1016/s1074-7613(00)80416-1.
- Peiris, J. S. M. *et al.* (2003) 'Coronavirus as a possible cause of severe acute respiratory syndrome', *Lancet*. Elsevier Limited, 361(9366), pp. 1319–1325. doi: 10.1016/S0140-6736(03)13077-2.
- Peralta-Zaragoza, O., Recillas-Targa, F. and Madrid-Marina, V. (2004) 'Terminal deoxynucleotidyl transferase is down-regulated by AP-1-like regulatory elements in human lymphoid cells', *Immunology*, 111(2), pp. 195–203. doi: 10.1111/j.0019-2805.2003.01791.x.
- Perez, C., Gruber, I. and Arber, C. (2020) 'Off-the-Shelf Allogeneic T Cell Therapies for Cancer: Opportunities and Challenges Using Naturally Occurring "Universal" Donor T Cells', *Frontiers in Immunology*. Frontiers Media S.A., p. 583716. doi: 10.3389/fimmu.2020.583716.
- Petrova, G. V., Naumova, E. N. and Gorski, J. (2011) 'The Polyclonal CD8 T Cell Response to Influenza M1 58–66 Generates a Fully Connected Network of Cross-Reactive Clonotypes to Structurally Related Peptides: A Paradigm for Memory Repertoire Coverage of Novel Epitopes or Escape Mutants', *The Journal of Immunology*. The American Association of Immunologists, 186(11), pp. 6390–6397. doi: 10.4049/jimmunol.1004031.
- Phillips, R. E. *et al.* (1991) 'Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition', *Nature*. Nature, 354(6353), pp. 453–459. doi: 10.1038/354453a0.
- Pinilla, C. *et al.* (1992) 'Rapid identification of high affinity peptide ligands using positional scanning synthetic peptide combinatorial libraries', *BioTechniques*, 13(6), pp. 901-902+904.
- Pinkse, G. G. M. *et al.* (2005) 'Autoreactive CD8 T cells associated with  $\beta$  cell destruction in type 1 diabetes', *Proceedings of the National Academy of Sciences of the United States of America*, 102(51), pp. 18425–18430. doi: 10.1073/pnas.0508621102.
- Pipkin, M. E. *et al.* (2010) 'Interleukin-2 and Inflammation Induce Distinct Transcriptional Programs that Promote the Differentiation of Effector Cytolytic T Cells'. doi: 10.1016/j.immuni.2009.11.012.
- Piskurich, J. F. *et al.* (1998) 'Identification of distinct regions of 5' flanking DNA that mediate constitutive, IFN-gamma, STAT1, and TGF-beta-regulated expression of the class II transactivator gene.', *Journal of immunology (Baltimore, Md. : 1950)*, 160(1), pp. 233–40. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9551976> (Accessed: 16 January 2020).
- Podack, E. R., Hengartner, H. and Lichtenheld, M. G. (1991) *A central role of perforin in cytotoxicity?*, *Annu. Rev. Immunol.* Available at: [www.annualreviews.org](http://www.annualreviews.org) (Accessed: 13 January 2020).

- Pöhlmann, T. *et al.* (2004) 'Differential peptide dynamics is linked to major histocompatibility complex polymorphism.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 279(27), pp. 28197–201. doi: 10.1074/jbc.C400128200.
- Powrie, F. and Mason, D. (1990) 'OX-22high CD4+ T cells induce wasting disease with multiple organ pathology: Prevention by the ox-22low subset', *Journal of Experimental Medicine*, 172(6), pp. 1701–1708. doi: 10.1084/jem.172.6.1701.
- Praveen, P. V. K. *et al.* (2010) 'Tapasin edits peptides on MHC class I molecules by accelerating peptide exchange', *European Journal of Immunology*, 40(1), pp. 214–224. doi: 10.1002/eji.200939342.
- Price, D. A. *et al.* (1997) 'Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 94(5), pp. 1890–1895. doi: 10.1073/pnas.94.5.1890.
- Price, D. A. *et al.* (1998) 'Antigen-specific release of  $\beta$ -chemokines by anti-HIV-1 cytotoxic T lymphocytes', *Current Biology*. Cell Press, 8(6), pp. 355–358. doi: 10.1016/s0960-9822(98)70138-1.
- Price, P. *et al.* (1999) 'The genetic basis for the association of the 8.1 ancestral haplotype (A1, B8, DR3) with multiple immunopathological diseases', *Immunological Reviews*. Blackwell Munksgaard, pp. 257–274. doi: 10.1111/j.1600-065X.1999.tb01398.x.
- Protein Atlas, T. (2020) *Expression of LGSN in cancer - Summary - The Human Protein Atlas*. Available at: <https://www.proteinatlas.org/ENSG00000146166-LGSN/pathology> (Accessed: 24 December 2020).
- Pudney, V. A. *et al.* (2005) 'CD8+ immunodominance among Epstein-Barr virus lytic cycle antigens directly reflects the efficiency of antigen presentation in lytically infected cells', *Journal of Experimental Medicine*. J Exp Med, 201(3), pp. 349–360. doi: 10.1084/jem.20041542.
- Pugliese, A. *et al.* (1997) 'The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes', *Nature Genetics*, 15(3), pp. 293–297. doi: 10.1038/ng0397-293.
- Purbhoo, M. A. *et al.* (1998) 'Copresentation of natural HIV-1 agonist and antagonist ligands fails to induce the T cell receptor signaling cascade', *Proceedings of the National Academy of Sciences of the United States of America*. Proc Natl Acad Sci U S A, 95(8), pp. 4527–4532. doi: 10.1073/pnas.95.8.4527.
- Purbhoo, M. A. *et al.* (2001) 'The Human CD8 Coreceptor Effects Cytotoxic T Cell Activation and Antigen Sensitivity Primarily by Mediating Complete Phosphorylation of the T Cell Receptor  $\eta$  Chain', *Journal of Biological Chemistry*. J Biol Chem, 276(35), pp. 32786–32792. doi: 10.1074/jbc.M102498200.
- Puri, J. and Factorovich, Y. (1988) 'Selective inhibition of antigen presentation to cloned T cells by protease inhibitors.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 141(10), pp. 3313–7. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/3263420> (Accessed: 20 February 2020).
- Pymm, P. *et al.* (2017) 'MHC-I peptides get out of the groove and enable a novel mechanism of HIV-1 escape', *Nature Structural and Molecular Biology*. Nature Publishing

Group, 24(4), pp. 387–394. doi: 10.1038/nsmb.3381.

Queenan, J. A. *et al.* (2018) 'The Prevalence and Risk for Herpes Zoster Infection in Adult Patients With Diabetes Mellitus in the Canadian Primary Care Sentinel Surveillance Network', *Canadian Journal of Diabetes*. Elsevier B.V., 42(5), pp. 465–469. doi: 10.1016/j.jcjd.2017.10.060.

Ragazzo, J. L. (2001) 'Costimulation via lymphocyte function-associated antigen 1 in the absence of CD28 ligation promotes anergy of naive CD4+ T cells', *Proceedings of the National Academy of Sciences*. Proceedings of the National Academy of Sciences, 98(1), pp. 241–246. doi: 10.1073/pnas.011397798.

Raman, M. C. C. *et al.* (2016) 'Direct molecular mimicry enables off-target cardiovascular toxicity by an enhanced affinity TCR designed for cancer immunotherapy', *Scientific Reports*. Nature Publishing Group, 6, p. 18851. doi: 10.1038/srep18851.

Ramasamy, M. N. *et al.* (2020) 'Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial', *The Lancet*. Lancet Publishing Group, 396(10267), pp. 1979–1993. doi: 10.1016/S0140-6736(20)32466-1.

Rangarajan, S. and Mariuzza, R. A. (2014) 'T cell receptor bias for MHC: Co-evolution or co-receptors?', *Cellular and Molecular Life Sciences*. Birkhauser Verlag AG, pp. 3059–3068. doi: 10.1007/s00018-014-1600-9.

Rapoport, A. *et al.* (2015) 'Abstract 4701: NY-ESO T cells administered post ASCT for MM exhibit extended functionality without exhaustion in a natural pattern of effector and memory programming', in *Cancer Research*. American Association for Cancer Research (AACR), pp. 4701–4701. doi: 10.1158/1538-7445.am2015-4701.

Rashin, A. A. and Jernigan, R. L. (2016) 'Clusters of Structurally Similar MHC I HLA-A2 Molecules, Found with a New Method, Suggest Mechanisms of T-Cell Receptor Avidity', *Biochemistry*, 55(1), pp. 167–185. doi: 10.1021/acs.biochem.5b01077.

Reay, P. A., Kantor, R. M. and Davis, M. M. (1994) 'Use of global amino acid replacements to define the requirements for MHC binding and T cell recognition of moth cytochrome c (93-103).', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 152(8), pp. 3946–57. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7511662> (Accessed: 23 March 2020).

Reid, S. W. *et al.* (1996) 'Production and crystallization of MHC class I B allele single peptide complexes', *FEBS Letters*, 383(1–2), pp. 119–123. doi: 10.1016/0014-5793(96)00226-8.

Reinherz, E. L. *et al.* (1979) 'Separation of functional subsets of human T cells by a monoclonal antibody', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 76(8), pp. 4061–4065. doi: 10.1073/pnas.76.8.4061.

Reinherz, E. L. *et al.* (1980) 'Discrete stages of human intrathymic differentiation: Analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 77(3 I), pp. 1588–1592. doi: 10.1073/pnas.77.3.1588.

Reiser, J. B. *et al.* (2002) 'A T cell receptor CDR3beta loop undergoes conformational changes of unprecedented magnitude upon binding to a peptide/MHC class I complex.', *Immunity*, 16(3), pp. 345–54. Available at:

- <http://www.ncbi.nlm.nih.gov/pubmed/11911820> (Accessed: 14 November 2017).
- Reiser, J. B. *et al.* (2003) 'CDR3 loop flexibility contributes to the degeneracy of TCR recognition', *Nature Immunology*, 4(3), pp. 241–247. doi: 10.1038/ni891.
- Reits, E. *et al.* (2004) 'A major role for TPP1 in trimming proteasomal degradation products for MHC class I antigen presentation', *Immunity*, 20(4), pp. 495–506. doi: 10.1016/S1074-7613(04)00074-3.
- Ren, L. *et al.* (2019) 'Identification of neoantigen-specific T cells and their targets: implications for immunotherapy of head and neck squamous cell carcinoma', *Oncotarget*. Taylor and Francis Inc., 8(4). doi: 10.1080/2162402X.2019.1568813.
- Reth, M. (1989) 'Antigen receptor tail clue [5]', *Nature*, pp. 383–384. doi: 10.1038/338383b0.
- Reynolds, J. *et al.* (2000) 'CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis', *Journal of Clinical Investigation*. The American Society for Clinical Investigation, 105(5), pp. 643–651. doi: 10.1172/JCI6710.
- Reynolds, J. *et al.* (2002) 'Anti-CD8 monoclonal antibody therapy is effective in the prevention and treatment of experimental autoimmune glomerulonephritis', *Journal of the American Society of Nephrology*, 13(2), pp. 359–369.
- Reynolds, J. *et al.* (2004) 'Blockade of the CD154-CD40 costimulatory pathway prevents the development of experimental autoimmune glomerulonephritis', *Kidney International*. Blackwell Publishing Inc., 66(4), pp. 1444–1452. doi: 10.1111/j.1523-1755.2004.00907.x.
- Van Rhijn, I. *et al.* (2015) 'Lipid and small-molecule display by CD1 and MR1', *Nature Reviews Immunology*. Nature Publishing Group, pp. 643–654. doi: 10.1038/nri3889.
- Riddell, S. R. *et al.* (1992) 'Restoration of Viral Immunity in Immunodeficient Humans by the Adoptive Transfer of T Cell Clones', *Science*, 257, pp. 238–241. Available at: <http://science.sciencemag.org/> (Accessed: 21 January 2021).
- Riddell, S. R. *et al.* (1996) 'T-cell mediated rejection of gene-modified HIV-specific cytotoxic T lymphocytes in HIV-infected patients', *Nature Medicine*. Nat Med, 2(2), pp. 216–223. doi: 10.1038/nm0296-216.
- Riera Romo, M., Pérez-Martínez, D. and Castillo Ferrer, C. (2016) 'Innate immunity in vertebrates: An overview', *Immunology*. Blackwell Publishing Ltd, 148(2), pp. 125–139. doi: 10.1111/imm.12597.
- Rigney, E. *et al.* (1998) 'A soluble major histocompatibility complex class I peptide-binding platform undergoes a conformational change in response to peptide epitopes', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 273(23), pp. 14200–14204. doi: 10.1074/jbc.273.23.14200.
- Riha, P. and Rudd, C. E. (2010) 'CD28 co-signaling in the adaptive immune response', *Self/Nonself - Immune Recognition and Signaling*. Taylor & Francis, pp. 231–240. doi: 10.4161/self.1.3.12968.
- Rimmelzwaan, G. F. *et al.* (2004) 'Sequence variation in the influenza A virus nucleoprotein associated with escape from cytotoxic T lymphocytes', in *Virus Research*. Virus Res, pp. 97–100. doi: 10.1016/j.virusres.2004.02.020.
- Rist, M. J. *et al.* (2013) 'HLA Peptide Length Preferences Control CD8 + T Cell Responses', *The Journal of Immunology*. The American Association of Immunologists, 191(2), pp. 561–

571. doi: 10.4049/jimmunol.1300292.

Roberts, J. D., Bebenek, K. and Kunkel, T. A. (1988) 'The accuracy of reverse transcriptase from HIV-1', *Science*. *Science*, 242(4882), pp. 1171–1173. doi: 10.1126/science.2460925.

Rock, K. L. *et al.* (1994) 'Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules', *Cell*. Elsevier, 78(5), pp. 761–771. doi: 10.1016/S0092-8674(94)90462-6.

Rodés, B. *et al.* (2004) 'Differences in disease progression in a cohort of long-term non-progressors after more than 16 years of HIV-1 infection', *AIDS*. *AIDS*, 18(8), pp. 1109–1116. doi: 10.1097/00002030-200405210-00004.

Rodig, N. *et al.* (2003) 'Endothelial expression of PD-L1 and PD-L2 down-regulates CD8+ T cell activation and cytolysis', *European Journal of Immunology*, 33(11), pp. 3117–3126. doi: 10.1002/eji.200324270.

Romero, P. *et al.* (1991) 'H-2Kd-restricted Antigenic Peptides Share a Simple Binding Motif', *Journal of Experimental Medicine*, 174(3), pp. 603–612. Available at: <https://rupress.org/jem/article-pdf/174/3/603/491400/603.pdf> (Accessed: 3 March 2020).

Roomp, K. and Domingues, F. S. (2011) 'Predicting interactions between T cell receptors and MHC-peptide complexes', *Molecular Immunology*, 48(4), pp. 553–562. doi: 10.1016/j.molimm.2010.10.014.

Rosenberg, A. H. *et al.* (1987) 'Vectors for selective expression of cloned DNAs by T7 RNA polymerase', *Gene*. Elsevier, 56(1), pp. 125–135. doi: 10.1016/0378-1119(87)90165-X.

Rosenberg, Y. J., Anderson, A. O. and Pabst, R. (1998) 'HIV-induced decline in blood CD4/CD8 ratios: Viral killing or altered lymphocyte trafficking?', *Immunology Today*. Elsevier Ltd, pp. 10–17. doi: 10.1016/S0167-5699(97)01183-3.

Rosenstein, Y. *et al.* (1989) 'Direct evidence for binding of CD8 to HLA class I antigens', *Journal of Experimental Medicine*, 169(1), pp. 149–160. doi: 10.1084/jem.169.1.149.

Rosenthal, A. S. and Shevach, E. M. (1973) 'Function of macrophages in antigen recognition by guinea pig t lymphocytes: Requirement for histocompatible macroptages and lymphocytes', *Journal of Experimental Medicine*. The Rockefeller University Press, 138(5), pp. 1194–1212. doi: 10.1084/jem.138.5.1194.

Roska, A. K. and Lipsky, P. E. (1985) 'Dissection of the functions of antigen-presenting cells in the induction of T cell activation.', *Journal of immunology (Baltimore, Md. : 1950)*, 135(5), pp. 2953–61. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/3876370> (Accessed: 9 April 2020).

Rossjohn, J. *et al.* (2012) 'Recognition of CD1d-restricted antigens by natural killer T cells', *Nature Reviews Immunology*, pp. 845–857. doi: 10.1038/nri3328.

Roth, T. L. *et al.* (2018) 'Reprogramming human T cell function and specificity with non-viral genome targeting', *Nature*. Nature Publishing Group, 559(7714), pp. 405–409. doi: 10.1038/s41586-018-0326-5.

Rowen, L., Koop, B. F. and Hood, L. (1996) 'The Complete 685-kilobase DNA Sequence of the Human Beta T Cell Receptor Locus', *Science*, 272(5269), pp. 1755–1762. Available at: <http://science.sciencemag.org/> (Accessed: 16 December 2019).

Rowland-Jones, S. *et al.* (1995) 'HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women', *Nature Medicine*. Nature Publishing Group, 1(1), pp. 59–64. doi:



10.1038/nm0195-59.

Ru, H. *et al.* (2015) 'Molecular Mechanism of V(D)J Recombination from Synaptic RAG1-RAG2 Complex Structures', *Cell*. Cell Press, 163(5), pp. 1138–1152. doi: 10.1016/j.cell.2015.10.055.

Rubio, V. *et al.* (2003) 'Ex vivo identification, isolation and analysis of tumor-cytolytic T cells.', *Nature medicine*, 9(11), pp. 1377–82. doi: 10.1038/nm942.

Rudd, C. E. *et al.* (1988) 'The CD4 receptor is complexed in detergent lysates to a protein-tyrosine kinase (pp58) from human T lymphocytes.', *Proceedings of the National Academy of Sciences of the United States of America*, 85(14), pp. 5190–5194. doi: 10.1073/pnas.85.14.5190.

Rudolph, M. G., Stanfield, R. L. and Wilson, I. A. (2006) 'How TCRs bind MHCs, peptides, and co-receptors', *Annual Review of Immunology*, 24(1), pp. 419–466. doi: 10.1146/annurev.immunol.23.021704.115658.

Rudolph, M. G. and Wilson, I. A. (2002) 'The specificity of TCR/pMHC interaction.', *Current opinion in immunology*, 14(1), pp. 52–65. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11790533> (Accessed: 14 November 2017).

Ruff, W. E. *et al.* (2019) 'Pathogenic Autoreactive T and B Cells Cross-React with Mimotopes Expressed by a Common Human Gut Commensal to Trigger Autoimmunity', *Cell Host and Microbe*. Cell Press, 26(1), pp. 100-113.e8. doi: 10.1016/j.chom.2019.05.003.

Rulli, N. E. *et al.* (2007) 'The molecular and cellular aspects of arthritis due to alphavirus infections: Lesson learned from Ross River virus', in *Annals of the New York Academy of Sciences*. Blackwell Publishing Inc., pp. 96–108. doi: 10.1196/annals.1408.007.

Russell, T. A. *et al.* (2018) 'Increasing antigen presentation on HSV-1-infected cells increases lesion size but does not alter neural infection or latency', *Journal of General Virology*. Microbiology Society, 99(5), pp. 682–692. doi: 10.1099/jgv.0.001059.

Sadasivan, B. *et al.* (1996) 'Roles for calreticulin and a novel glycoprotein, tapasin, in the interaction of MHC class I molecules with TAP', *Immunity*. Cell Press, 5(2), pp. 103–114. doi: 10.1016/S1074-7613(00)80487-2.

Sakaguchi, S. *et al.* (1995) 'Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases.', *Journal of immunology (Baltimore, Md. : 1950)*, 155(3), pp. 1151–64. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7636184> (Accessed: 8 April 2020).

Sakano, H. *et al.* (1979) 'Sequences at the somatic recombination sites of immunoglobulin light-chain genes', *Nature*, 280(5720), pp. 288–294. doi: 10.1038/280288a0.

Salemi, S. *et al.* (1995) 'HIVgp120 activates autoreactive CD4-specific T cell responses by unveiling of hidden CD4 peptides during processing', *Journal of Experimental Medicine*, 181(6), pp. 2253–2257. doi: 10.1084/jem.181.6.2253.

Salter, R. D. *et al.* (1990) 'A binding site for the T-cell co-receptor CD8 on the  $\alpha$ 3 domain of HLA-A2', *Nature*, 345(6270), pp. 41–46. doi: 10.1038/345041a0.

Samelson, L. E., Germain, R. N. and Schwartz, R. H. (1983) 'Monoclonal antibodies against the antigen receptor on a cloned T-cell hybrid', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 80(22 I), pp. 6972–

6976. doi: 10.1073/pnas.80.22.6972.

Sanderson, J. P. *et al.* (2020) 'Preclinical evaluation of an affinity-enhanced MAGE-A4-specific T-cell receptor for adoptive T-cell therapy', *Oncolimmunology*. Taylor and Francis Inc., 9(1). doi: 10.1080/2162402X.2019.1682381.

Sandrin, M. S. *et al.* (1993) 'CD48 is a low affinity ligand for human CD2.', *Journal of immunology (Baltimore, Md. : 1950)*, 151(9), pp. 4606–13. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7691954> (Accessed: 11 October 2019).

Sant'Angelo, D. B. *et al.* (1996) 'The specificity and orientation of a TCR to its peptide-MHC class II ligands', *Immunity*. Cell Press, 4(4), pp. 367–376. doi: 10.1016/S1074-7613(00)80250-2.

Sant'Angelo, D. B. *et al.* (2002) 'Recognition of core and flanking amino acids of MHC class II-bound peptides by the T cell receptor.', *European journal of immunology*, 32(9), pp. 2510–20. doi: 10.1002/1521-4141(200209)32:9<2510::AID-IMMU2510>3.0.CO;2-Q.

Santos, S. G. *et al.* (2007) 'Major histocompatibility complex class I-ERp57-tapasin interactions within the peptide-loading complex', *Journal of Biological Chemistry*, 282(24), pp. 17587–17593. doi: 10.1074/jbc.M702212200.

Saper, M. A., Bjorkman, P. J. and Wiley, D. C. (1991) 'Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution', *Journal of Molecular Biology*. Academic Press, 219(2), pp. 277–319. doi: 10.1016/0022-2836(91)90567-P.

Sarafianos, S. G. *et al.* (2009) 'Structure and Function of HIV-1 Reverse Transcriptase: Molecular Mechanisms of Polymerization and Inhibition', *Journal of Molecular Biology*. Academic Press, pp. 693–713. doi: 10.1016/j.jmb.2008.10.071.

Saric, T. *et al.* (2002) 'An IFN- $\gamma$ -induced aminopeptidase in the ER, ERAP I, trims precursors to MHC class I-presented peptides', *Nature Immunology*. Nature Publishing Group, 3(12), pp. 1169–1176. doi: 10.1038/ni859.

Saveanu, L. *et al.* (2005) 'Concerted peptide trimming by human ERAP1 and ERAP2 aminopeptidase complexes in the endoplasmic reticulum', *Nature Immunology*. Nature Publishing Group, 6(7), pp. 689–697. doi: 10.1038/ni1208.

Savoldo, B., Heslop, H. E. and Rooney, C. M. (2000) 'The Use of Cytotoxic T Cells for the Prevention and Treatment of Epstein-Barr Virus Induced Lymphoma in Transplant Recipients', *Leukemia & Lymphoma*. Harwood Academic Publishers GmbH, 39(5–6), pp. 455–464. doi: 10.3109/10428190009113376.

Schatz, D. G., Oettinger, M. A. and Baltimore, D. (1989) 'The V(D)J recombination activating gene, RAG-1', *Cell*, 59(6), pp. 1035–1048. doi: 10.1016/0092-8674(89)90760-5.

Scheper, W. *et al.* (2019) 'Low and variable tumor reactivity of the intratumoral TCR repertoire in human cancers', *Nature Medicine*. Nature Publishing Group, 25(1), pp. 89–94. doi: 10.1038/s41591-018-0266-5.

Schild, H. *et al.* (1990) 'Limit of T cell tolerance to self proteins by peptide presentation', *Science*. American Association for the Advancement of Science (AAAS), 247(4950), pp. 1587–1589. doi: 10.1126/science.2321019.

Schimpl, A. and Wecker, E. (1971) 'Reconstitution of a thymus cell-deprived immune system by syngeneic and allogeneic thymocytes *in vitro*', *European Journal of Immunology*. John Wiley & Sons, Ltd, 1(4), pp. 304–306. doi: 10.1002/eji.1830010419.

- Schimpl, A. and Wecker, E. (1972) 'Replacement of t-cell function by a t-cell product', *Nature New Biology*. Nature Publishing Group, 237(70), pp. 15–17. doi: 10.1038/newbio237015a0.
- Scholten, K. B. J. *et al.* (2006) 'Codon modification of T cell receptors allows enhanced functional expression in transgenic human T cells', *Clinical Immunology*. Academic Press, 119(2), pp. 135–145. doi: 10.1016/j.clim.2005.12.009.
- Schroff, R. W. *et al.* (1983) 'Immunological studies of homosexual men with immunodeficiency and Kaposi's sarcoma', *Clinical Immunology and Immunopathology*. Clin Immunol Immunopathol, 27(3), pp. 300–314. doi: 10.1016/0090-1229(83)90083-1.
- Schuldt, N. J. and Binstadt, B. A. (2019) 'Dual TCR T Cells: Identity Crisis or Multitaskers?', *The Journal of Immunology*. The American Association of Immunologists, 202(3), pp. 637–644. doi: 10.4049/jimmunol.1800904.
- Schwartz, R. H. (2012) 'Historical overview of immunological tolerance', *Cold Spring Harbor Perspectives in Biology*, 4(4), p. a006908. doi: 10.1101/cshperspect.a006908.
- Schwenter, F., Déglon, N. and Aebischer, P. (2003) 'Optimization of human erythropoietin secretion from MLV-infected human primary fibroblasts used for encapsulated cell therapy', *The Journal of Gene Medicine*, 5(3), pp. 246–257. doi: 10.1002/jgm.338.
- Scott-Browne, J. P. *et al.* (2009) 'Germline-encoded amino acids in the  $\alpha\beta$  T-cell receptor control thymic selection', *Nature*, 458(7241), pp. 1043–1046. doi: 10.1038/nature07812.
- Scott-Browne, J. P. *et al.* (2011) 'Evolutionarily Conserved Features Contribute to  $\alpha\beta$  T Cell Receptor Specificity', *Immunity*, 35(4), pp. 526–535. doi: 10.1016/j.immuni.2011.09.005.
- Scott, B. *et al.* (1989) 'The generation of mature T cells requires interaction of the  $\alpha\beta$  T-cell receptor with major histocompatibility antigens', *Nature*, 338, pp. 591–593.
- Sebzda, E. *et al.* (1994) 'Positive and negative thymocyte selection induced by different concentrations of a single peptide', *Science*, 263(5153), pp. 1615–1618. doi: 10.1126/science.8128249.
- Selvaraj, P. *et al.* (1987) 'The T lymphocyte glycoprotein CD2 binds the cell surface ligand LFA-3', *Nature*, 326(6111), pp. 400–403. doi: 10.1038/326400a0.
- Seo, S. H. and Webster, R. G. (2002) 'Tumor Necrosis Factor Alpha Exerts Powerful Anti-Influenza Virus Effects in Lung Epithelial Cells', *Journal of Virology*. American Society for Microbiology, 76(3), pp. 1071–1076. doi: 10.1128/jvi.76.3.1071-1076.2002.
- Serwold, T. *et al.* (2002) 'ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum', *Nature*. Nature Publishing Group, 419(6906), pp. 480–483. doi: 10.1038/nature01074.
- Sette, A. *et al.* (1994) 'The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 153(12), pp. 5586–92. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/7527444> (Accessed: 31 March 2020).
- Sewell, A. K. *et al.* (1997) 'Antagonism of cytotoxic T lymphocyte-mediated lysis by natural HIV-1 altered peptide ligands requires simultaneous presentation of agonist and antagonist peptides', *Eur. J. Immunol*, 27, pp. 2323–2329.
- Sewell, A. K. (2012) 'Why must T cells be cross-reactive?', *Nature Reviews Immunology*. Nature Publishing Group, pp. 669–677. doi: 10.1038/nri3279.

- Sha, W. C. *et al.* (1988) 'Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice', *Nature*, 335(6187), pp. 271–274. doi: 10.1038/335271a0.
- Shahinian, A. *et al.* (1993) 'Differential T cell costimulatory requirements in CD28-deficient mice', *Science*, 261(5121), pp. 609–612. doi: 10.1126/science.7688139.
- Sharma, P. *et al.* (2019) 'T-cell receptors engineered de novo for peptide specificity can mediate optimal t-cell activity without self cross-reactivity', *Cancer Immunology Research*. American Association for Cancer Research Inc., 7(12), pp. 2025–2035. doi: 10.1158/2326-6066.CIR-19-0035.
- Shearer, G. M. (1974) 'Cell-mediated cytotoxicity to trinitrophenyl-modified syngeneic lymphocytes', *European Journal of Immunology*, 4(8), pp. 527–533. doi: 10.1002/eji.1830040802.
- Shepherd, J. C. *et al.* (1993) 'TAP1-dependent peptide translocation in vitro is ATP dependent and peptide selective', *Cell*. Cell Press, 74(3), pp. 577–584. doi: 10.1016/0092-8674(93)80058-M.
- Sheppard, K. A. *et al.* (2004) 'PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3 $\zeta$  signalosome and downstream signaling to PKC $\theta$ ', *FEBS Letters*. No longer published by Elsevier, 574(1–3), pp. 37–41. doi: 10.1016/j.febslet.2004.07.083.
- Shin, H. and Wherry, E. J. (2007) 'CD8 T cell dysfunction during chronic viral infection', *Current Opinion in Immunology*. Curr Opin Immunol, pp. 408–415. doi: 10.1016/j.coi.2007.06.004.
- Shresta, S. *et al.* (1998) 'How do cytotoxic lymphocytes kill their targets?', *Current Opinion in Immunology*. Current Biology Ltd, 10(5), pp. 581–587. doi: 10.1016/S0952-7915(98)80227-6.
- Shugay, M. *et al.* (2018) 'VDJdb: A curated database of T-cell receptor sequences with known antigen specificity', *Nucleic Acids Research*. Oxford University Press, 46(D1), pp. D419–D427. doi: 10.1093/nar/gkx760.
- Siegal, F. P. *et al.* (1981) 'Severe Acquired Immunodeficiency in Male Homosexuals, Manifested by Chronic Perianal Ulcerative Herpes Simplex Lesions', *New England Journal of Medicine*. N Engl J Med, 305(24), pp. 1439–1444. doi: 10.1056/NEJM198112103052403.
- Sieh, P. and Chen, J. (2001) 'Distinct Control of the Frequency and Allelic Exclusion of the V $\beta$  Gene Rearrangement at the TCR $\beta$  Locus', *The Journal of Immunology*. The American Association of Immunologists, 167(4), pp. 2121–2129. doi: 10.4049/jimmunol.167.4.2121.
- Silver, M. L. *et al.* (1992) 'Atomic structure of a human MHC molecule presenting an influenza virus peptide', *Nature*, 360(6402), pp. 367–369. doi: 10.1038/360367a0.
- Simitsek, P. D. *et al.* (1995) 'Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants', *Journal of Experimental Medicine*, 181(6), pp. 1957–1963. doi: 10.1084/jem.181.6.1957.
- Skibinski, D. A. G. *et al.* (2018) 'Induction of Human T-cell and Cytokine Responses Following Vaccination with a Novel Influenza Vaccine', *Scientific Reports*. Nature Publishing Group, 8(1). doi: 10.1038/s41598-018-36703-7.
- Skowera, A. *et al.* (2008) 'CTLs are targeted to kill  $\beta$  cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope', *Journal of Clinical*

*Investigation*, 118(10), pp. 3390–402. doi: 10.1172/JCI35449.

Slifka, M. K. *et al.* (2003) 'Preferential Escape of Subdominant CD8 + T Cells During Negative Selection Results in an Altered Antiviral T Cell Hierarchy', *The Journal of Immunology*. The American Association of Immunologists, 170(3), pp. 1231–1239. doi: 10.4049/jimmunol.170.3.1231.

Smith, K. A. (1988) 'Interleukin-2 : Inception, Impact, and Implications Downloaded from', *Science*, 240(4856), pp. 1169–1176. Available at: <http://science.sciencemag.org/> (Accessed: 13 January 2020).

Smith, S. N. *et al.* (2013) 'Plasticity in the contribution of T cell receptor variable region residues to binding of peptide-HLA-A2 complexes', *Journal of Molecular Biology*. Academic Press, 425(22), pp. 4496–4507. doi: 10.1016/j.jmb.2013.08.007.

Smith, S. N. *et al.* (2014) 'Changing the peptide specificity of a human T-cell receptor by directed evolution', *Nature Communications*. Nature Publishing Group, 5(1), pp. 1–13. doi: 10.1038/ncomms6223.

Snyder, G. H. *et al.* (1975) 'Complete Tyrosine Assignments in the High Field 1H Nuclear Magnetic Resonance Spectrum of the Bovine Pancreatic Trypsin Inhibitor', *Biochemistry*. ^ Present address, 14(17), pp. 3765–3777. doi: 10.1021/bi00688a008.

Snyder, H. L., Yewdell, J. W. and Bennink, J. R. (1994) 'Trimming of antigenic peptides in an early secretory compartment', *Journal of Experimental Medicine*. The Rockefeller University Press, 180(6), pp. 2389–2394. doi: 10.1084/jem.180.6.2389.

Sok, D. *et al.* (2016) 'A Prominent Site of Antibody Vulnerability on HIV Envelope Incorporates a Motif Associated with CCR5 Binding and Its Camouflaging Glycans', *Immunity*. Cell Press, 45(1), pp. 31–45. doi: 10.1016/j.immuni.2016.06.026.

Sourav, H. and Chattopadhyay, A. (2007) 'Dipolar Relaxation within the Protein Matrix of the Green Fluorescent Protein: A Red Edge Excitation Shift Study'. American Chemical Society. doi: 10.1021/JP076797Z.

Speir, J. A. *et al.* (2001) 'Two different, highly exposed, bulged structures for an unusually long peptide bound to rat MHC class I RT1-Aa', *Immunity*. Cell Press, 14(1), pp. 81–92. doi: 10.1016/S1074-7613(01)00091-7.

Speiser, D. E. *et al.* (2008) 'Unmodified self antigen triggers human CD8 T cells with stronger tumor reactivity than altered antigen', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 105(10), pp. 3849–3854. doi: 10.1073/pnas.0800080105.

Spies, T. *et al.* (1990) 'A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway', *Nature*. Nature Publishing Group, 348(6303), pp. 744–747. doi: 10.1038/348744a0.

Spies, T. *et al.* (1992) 'Presentation of viral antigen by MHC class I molecules is dependent on a putative peptide transporter heterodimer', *Nature*. Nature Publishing Group, 355(6361), pp. 644–646. doi: 10.1038/355644a0.

Spies, T. and DeMars, R. (1991) 'Restored expression of major histocompatibility class I molecules by gene transfer of a putative peptide transporter', *Nature*. Nature Publishing Group, 351(6324), pp. 323–324. doi: 10.1038/351323a0.

Spits, H. *et al.* (1982) 'The role of T cell differentiation markers in antigen-specific and

lectin-dependent cellular cytotoxicity mediated by T8+ and T4+ human cytotoxic T cell clones directed at class I and class II MHC antigens.', *Journal of immunology (Baltimore, Md. : 1950)*, 129(4), pp. 1563–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/6980945> (Accessed: 16 January 2020).

Sridhar, S. *et al.* (2013) 'Cellular immune correlates of protection against symptomatic pandemic influenza', *Nature Medicine*. Nat Med, 19(10), pp. 1305–1312. doi: 10.1038/nm.3350.

Stanislowski, T. *et al.* (2001) 'Circumventing tolerance to a human MDM2-derived tumor antigen by TCR gene transfer', *Nature Immunology*. Nature Publishing Group, 2(10), pp. 962–970. doi: 10.1038/ni1001-962.

Starr, T. K., Jameson, S. C. and Hogquist, K. A. (2003) 'Positive and negative selection of T cells', *Annual Review of Immunology*. Annual Reviews, 21(1), pp. 139–176. doi: 10.1146/annurev.immunol.21.120601.141107.

Steinman, R. M., Hawiger, D. and Nussenzweig, M. C. (2003) 'Tolerogenic dendritic cells', *Annual Review of Immunology*. Annual Reviews, 21(1), pp. 685–711. doi: 10.1146/annurev.immunol.21.120601.141040.

Stepanek, O. *et al.* (2014) 'Coreceptor scanning by the T cell receptor provides a mechanism for T cell tolerance', *Cell*. Cell Press, 159(2), pp. 333–345. doi: 10.1016/j.cell.2014.08.042.

Stern, L. J. *et al.* (1994) 'Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide', *Nature*, 368(6468), pp. 215–221. doi: 10.1038/368215a0.

Stern, L. J. and Wiley, D. C. (1994) 'Antigenic peptide binding by class I and class II histocompatibility proteins', *Structure*, 2(4), pp. 245–251. doi: 10.1016/S0969-2126(00)00026-5.

Steven, N. M. *et al.* (1997) 'Immediate early and early lytic cycle proteins are frequent targets of the Epstein-Barr virus-induced cytotoxic T cell response', *Journal of Experimental Medicine*. J Exp Med, 185(9), pp. 1605–1617. doi: 10.1084/jem.185.9.1605.

Stranford, S. A. *et al.* (1999) 'Lack of infection in HIV-exposed individuals is associated with a strong CD8+ cell noncytotoxic anti-HIV response', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 96(3), pp. 1030–1035. doi: 10.1073/pnas.96.3.1030.

Straus, D. B. and Weiss, A. (1993) 'The CD3 chains of the T cell antigen receptor associate with the ZAP-70 tyrosine kinase and are tyrosine phosphorylated after receptor stimulation', *Journal of Experimental Medicine*, 178(5), pp. 1523–1530. doi: 10.1084/jem.178.5.1523.

Streeter, H. B. *et al.* (2015) 'Preclinical development and first-in-human study of ATX-MS-1467 for immunotherapy of MS', *Neurology: Neuroimmunology and NeuroInflammation*. Lippincott Williams and Wilkins, p. e93. doi: 10.1212/NXI.0000000000000093.

Streilein, J. W. (2003) 'Ocular immune privilege: Therapeutic opportunities from an experiment of nature', *Nature Reviews Immunology*. European Association for Cardio-Thoracic Surgery, pp. 879–889. doi: 10.1038/nri1224.

Strelkauskas, A. J. *et al.* (1978) 'Direct evidence for loss of human suppressor cells during active autoimmune disease', *Proceedings of the National Academy of Sciences of the United*

- States of America*, 75(10), pp. 5150–5154. doi: 10.1073/pnas.75.10.5150.
- Suaya, J. A. *et al.* (2014) 'Incidence of herpes zoster and persistent post-zoster pain in adults with or without diabetes in the United States', *Open Forum Infectious Diseases*. Oxford University Press, 1(2). doi: 10.1093/ofid/ofu049.
- Suh, W. K. *et al.* (1994) 'Interaction of MHC class I molecules with the transporter associated with antigen processing', *Science*. Science, 264(5163), pp. 1322–1326. doi: 10.1126/science.8191286.
- Suh, W. K. *et al.* (1999) 'Interaction of murine MHC class I molecules with tapasin and TAP enhances peptide loading and involves the heavy chain alpha3 domain.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 162(3), pp. 1530–40. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9973410> (Accessed: 6 March 2020).
- Sun, R. *et al.* (1995) 'Evidence that the antigen receptors of cytotoxic T lymphocytes interact with a common recognition pattern on the H-2Kb molecule', *Immunity*. Immunity, 3(5), pp. 573–582. doi: 10.1016/1074-7613(95)90128-0.
- Sung, J. A. *et al.* (2018) 'HIV-Specific, Ex Vivo Expanded T Cell Therapy: Feasibility, Safety, and Efficacy in ART-Suppressed HIV-Infected Individuals', *Molecular Therapy*. Cell Press, 26(10), pp. 2496–2506. doi: 10.1016/j.ymthe.2018.08.015.
- Surh, C. D. and Sprent, J. (1994) 'T-cell apoptosis detected in situ during positive and negative selection in the thymus', *Nature*, 372(6501), pp. 100–103. doi: 10.1038/372100a0.
- Swain, S. L. (1980) 'Association of Ly phenotypes, T cell function and MHC recognition.', *Federation proceedings*, 39(13), pp. 3110–3. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/6968693> (Accessed: 18 March 2020).
- Swain, S. L. (1981) 'Significance of Lyt phenotypes: Lyt2 antibodies block activities of T cells that recognize class 1 major histocompatibility complex antigens regardless of their function.', *Proceedings of the National Academy of Sciences of the United States of America*, 78(11), pp. 7101–7105. doi: 10.1073/pnas.78.11.7101.
- Swain, S. L. (1983) 'T Cell Subsets and the Recognition of MHC Class', *Immunological Reviews*. John Wiley & Sons, Ltd, 74(1), pp. 129–142. doi: 10.1111/j.1600-065X.1983.tb01087.x.
- Swain, S. L. *et al.* (1984) 'Monoclonal antibody to L3T4 blocks the function of T cells specific for class 2 major histocompatibility complex antigens.', *Journal of immunology (Baltimore, Md. : 1950)*, 132(3), pp. 1118–23. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/6198377> (Accessed: 16 January 2020).
- Swain, S. L. and Panfili, P. (1979) 'Helper cells activated by allogeneic H-2K or H-2D differences have a Ly phenotype distinct from those responsive to I differences', *Journal of Immunology*, 122(2), pp. 383–397. Available at: <https://www.jimmunol.org/content/122/2/383.short> (Accessed: 18 March 2020).
- Sykulev, Y. *et al.* (1994) 'Kinetics and affinity of reactions between an antigen-specific T cell receptor and peptide-MHC complexes', *Immunity*. Cell Press, 1(1), pp. 15–22. doi: 10.1016/1074-7613(94)90005-1.
- Szomolay, B. *et al.* (2016) 'Identification of human viral protein-derived ligands recognized by individual MHCI-restricted T-cell receptors', *Immunology and Cell Biology*. Nature Publishing Group, 94(6), pp. 573–582. doi: 10.1038/icb.2016.12.

Szymczak, A. L. *et al.* (2004) 'Correction of multi-gene deficiency in vivo using a single "self-cleaving" 2A peptide-based retroviral vector', *Nature Biotechnology*. Nature Publishing Group, 22(5), pp. 589–594. doi: 10.1038/nbt957.

T-Cell Epitope Database (2020) *Search CTL/CD8+ T-Cell Epitope Database*. Available at: [https://www.hiv.lanl.gov/content/immunology/ctl\\_search?](https://www.hiv.lanl.gov/content/immunology/ctl_search?) (Accessed: 2 January 2021).

Takada, K., Kondo, K. and Takahama, Y. (2017) 'Generation of Peptides That Promote Positive Selection in the Thymus', *The Journal of Immunology*, 198, pp. 2215–2222. doi: 10.4049/jimmunol.1601862.

Talmage, D. W. (1957) 'Diversity of antibodies', *Journal of Cellular and Comparative Physiology*. John Wiley & Sons, Ltd, 50(S1), pp. 229–246. doi: 10.1002/jcp.1030500415.

Tan, M. P. *et al.* (2015) 'T cell receptor binding affinity governs the functional profile of cancer-specific CD8 + T cells', *Clinical & Experimental Immunology*, 180(2), pp. 255–270. doi: 10.1111/cei.12570.

Tan, Q. *et al.* (2019) 'Isolation of T cell receptor specifically reactive with autologous tumour cells from tumour-infiltrating lymphocytes and construction of T cell receptor engineered T cells for esophageal squamous cell carcinoma', *Journal for ImmunoTherapy of Cancer*. BioMed Central Ltd., 7(1), p. 232. doi: 10.1186/s40425-019-0709-7.

Taub, D. *et al.* (1993) 'Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 alpha and MIP-1 beta', *Science*, 260(5106), pp. 355–358. doi: 10.1126/science.7682337.

Taubenberger, J. K. and Kash, J. C. (2010) 'Influenza virus evolution, host adaptation, and pandemic formation', *Cell Host and Microbe*. Cell Press, pp. 440–451. doi: 10.1016/j.chom.2010.05.009.

Teng, M. K. *et al.* (1998) 'Identification of a common docking topology with substantial variation among different TCR-peptide-MHC complexes', *Current Biology*. Cell Press, 8(7), pp. 409–414. doi: 10.1016/s0960-9822(98)70160-5.

Tenzer, S. *et al.* (2009) 'Antigen processing influences HIV-specific cytotoxic T lymphocyte immunodominance', *Nature Immunology*. Nat Immunol, 10(6), pp. 636–646. doi: 10.1038/ni.1728.

Teschendorf, C. *et al.* (2002) 'Comparison of the EF-1 alpha and the CMV promoter for engineering stable tumor cell lines using recombinant adeno-associated virus.', *Anticancer research*, 22(6A), pp. 3325–30. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12530082> (Accessed: 11 October 2019).

Theaker, S. M. *et al.* (2016) 'T-cell libraries allow simple parallel generation of multiple peptide-specific human T-cell clones', *Journal of Immunological Methods*. Elsevier B.V., 430, pp. 43–50. doi: 10.1016/j.jim.2016.01.014.

Ting, J. P. Y. and Baldwin, A. S. (1993) 'Regulation of MHC gene expression', *Current Opinion in Immunology*, 5(1), pp. 8–16. doi: 10.1016/0952-7915(93)90074-3.

Todd, J. A., Bell, J. I. and McDevitt, H. O. (1987) 'HLA-DQ $\beta$  gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus', *Nature*, 329(6140), pp. 599–604. doi: 10.1038/329599a0.

Toivanen, A. (2008) 'Alphaviruses: An emerging cause of arthritis?', *Current Opinion in Rheumatology*, pp. 486–490. doi: 10.1097/BOR.0b013e328303220b.



- Tonegawa, S. (1983) 'Somatic generation of antibody diversity', *Nature*. *Nature*, 302(5909), pp. 575–581. doi: 10.1038/302575a0.
- Townsend, A. R. M., Bastin, J., *et al.* (1986) 'Cytotoxic T lymphocytes recognize influenza haemagglutinin that lacks a signal sequence', *Nature*, 324(6097), pp. 575–577. doi: 10.1038/324575a0.
- Townsend, A. R. M., Rothbard, J., *et al.* (1986) 'The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides', *Cell*, 44(6), pp. 959–968. doi: 10.1016/0092-8674(86)90019-X.
- Townsend, A. R. M., Gotch, F. M. and Davey, J. (1985) 'Cytotoxic T cells recognize fragments of the influenza nucleoprotein', *Cell*, 42(2), pp. 457–467. doi: 10.1016/0092-8674(85)90103-5.
- Townsend, S. E. and Allison, J. P. (1993) 'Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells', *Science*, 259(5093), pp. 368–370. doi: 10.1126/science.7678351.
- Treat, B. R. *et al.* (2017) 'Influence of an immunodominant herpes simplex virus type 1 CD8+T cell epitope on the target hierarchy and function of subdominant CD8+T cells', *PLoS Pathogens*. Public Library of Science, 13(12). doi: 10.1371/journal.ppat.1006732.
- Treiner, E. *et al.* (2003) 'Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1', *Nature*, 422(6928), pp. 164–169. doi: 10.1038/nature01433.
- Triebel, F. *et al.* (1988) 'Evidence for expression of two distinct T cell receptor beta-chain transcripts in a human diphtheria toxoid-specific T cell clone.', *The Journal of Immunology*. American Association of Immunologists, 140(1), pp. 300–304.
- Trolle, T. *et al.* (2016) 'The Length Distribution of Class I–Restricted T Cell Epitopes Is Determined by Both Peptide Supply and MHC Allele–Specific Binding Preference', *The Journal of Immunology*. The American Association of Immunologists, 196(4), pp. 1480–1487. doi: 10.4049/jimmunol.1501721.
- Trowsdale, J. (2005) 'HLA genomics in the third millennium', *Current Opinion in Immunology*, pp. 498–504. doi: 10.1016/j.coi.2005.07.015.
- Trowsdale, J. *et al.* (2008) 'Sequences encoded in the class II region of the MHC related to the "ABC" superfamily of transporters. 1990.', *Journal of immunology (Baltimore, Md. : 1950)*, 180(5), pp. 2733–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/18292489> (Accessed: 4 March 2020).
- Trowsdale, J. and Knight, J. C. (2013) 'Major Histocompatibility Complex Genomics and Human Disease', *Annual Review of Genomics and Human Genetics*, 14(1), pp. 301–323. doi: 10.1146/annurev-genom-091212-153455.
- Trudeau, J. D. *et al.* (2003) 'Prediction of spontaneous autoimmune diabetes in NOD mice by quantification of autoreactive T cells in peripheral blood', *Journal of Clinical Investigation*. American Society for Clinical Investigation, 111(2), pp. 217–223. doi: 10.1172/jci16409.
- Tsao, Y. P. *et al.* (2006) 'HLA-A\*0201 T-cell epitopes in severe acute respiratory syndrome (SARS) coronavirus nucleocapsid and spike proteins', *Biochemical and Biophysical Research Communications*. Academic Press, 344(1), pp. 63–71. doi: 10.1016/j.bbrc.2006.03.152.
- Tse, H. Y. and Dutton, R. W. (1977) 'Separation of helper and suppressor T lymphocytes: II.

Ly phenotypes and lack of DNA synthesis requirement for the generation of concanavalin A helper and suppressor cells\*', *Journal of Experimental Medicine*, 146(3), pp. 747–758. doi: 10.1084/jem.146.3.747.

Tsuruha, J. *et al.* (2001) 'Implication of cartilage intermediate layer protein in cartilage destruction in subsets of patients with osteoarthritis and rheumatoid arthritis.', *Arthritis and rheumatism*, 44(4), pp. 838–45. doi: 10.1002/1529-0131(200104)44:4<838::AID-ANR140>3.0.CO;2-C.

Tungatt, K. *et al.* (2015) 'Antibody stabilization of peptide-MHC multimers reveals functional T cells bearing extremely low-affinity TCRs.', *Journal of immunology (Baltimore, Md. : 1950)*, 194(1), pp. 463–74. doi: 10.4049/jimmunol.1401785.

Turner, J. M. *et al.* (1990) 'Interaction of the unique N-terminal region of tyrosine kinase p56lck with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs', *Cell*, 60(5), pp. 755–765. doi: 10.1016/0092-8674(90)90090-2.

Turner, S. J. *et al.* (2006) 'Structural determinants of T-cell receptor bias in immunity', *Nature Reviews Immunology*, pp. 883–894. doi: 10.1038/nri1977.

Tynan, F. E., Borg, N. A., *et al.* (2005) 'High Resolution Structures of Highly Bulged Viral Epitopes Bound to Major Histocompatibility Complex Class I', *Journal of Biological Chemistry*, 280(25), pp. 23900–23909. doi: 10.1074/jbc.M503060200.

Tynan, F. E., Burrows, S. R., *et al.* (2005) 'T cell receptor recognition of a "super-bulged" major histocompatibility complex class I-bound peptide', *Nature Immunology*, 6(11), pp. 1114–1122. doi: 10.1038/ni1257.

Tynan, F. E. *et al.* (2007) 'A T cell receptor flattens a bulged antigenic peptide presented by a major histocompatibility complex class I molecule', *Nature Immunology*. Nature Publishing Group, 8(3), pp. 268–276. doi: 10.1038/ni1432.

Tysoe-Calnon, V. A., Grundy, J. E. and Perkins, S. J. (1991) 'Molecular comparisons of the  $\beta$ 2-microglobulin-binding site in Class I major-histocompatibility-complex  $\alpha$ -chains and proteins of related sequences', *Biochemical Journal*. Portland Press Ltd, 277(2), pp. 359–369. doi: 10.1042/bj2770359.

Udyavar, A. *et al.* (2009) 'Subtle Affinity-Enhancing Mutations in a Myelin Oligodendrocyte Glycoprotein-Specific TCR Alter Specificity and Generate New Self-Reactivity', *The Journal of Immunology*. The American Association of Immunologists, 182(7), pp. 4439–4447. doi: 10.4049/jimmunol.0804377.

Uebel, S. *et al.* (1995) 'Requirements for peptide binding to the human transporter associated with antigen processing revealed by peptide scans and complex peptide libraries', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 270(31), pp. 18512–18516. doi: 10.1074/jbc.270.31.18512.

Uebel, S. *et al.* (1997) 'Recognition principle of the TAP transporter disclosed by combinatorial peptide libraries', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 94(17), pp. 8976–8981. doi: 10.1073/pnas.94.17.8976.

Unger, W. W. J. *et al.* (2012) 'Islet-Specific CTL Cloned from a Type 1 Diabetes Patient Cause Beta-Cell Destruction after Engraftment into HLA-A2 Transgenic NOD/SCID/IL2RG Null Mice', *PLoS ONE*, 7(11), p. e49213. doi: 10.1371/journal.pone.0049213.

Valitutti, S. *et al.* (1995) 'Serial triggering of many T-cell receptors by a few peptide-MHC

- complexes', *Nature*. Nature Publishing Group, pp. 148–151. doi: 10.1038/375148a0.
- Valkenburg, S. A. *et al.* (2016) 'Molecular basis for universal HLA-A\*0201-restricted CD8+ T-cell immunity against influenza viruses', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 113(16), pp. 4440–4445. doi: 10.1073/pnas.1603106113.
- Valmori, D. *et al.* (1998) 'Enhanced generation of specific tumor-reactive CTL in vitro by selected Melan-A/MART-1 immunodominant peptide analogues.', *Journal of immunology (Baltimore, Md. : 1950)*. *J Immunol*, 160(4), pp. 1750–8. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9469433> (Accessed: 10 February 2020).
- Varela-Rohena, A. *et al.* (2008) 'Control of HIV-1 immune escape by CD8 T cells expressing enhanced T-cell receptor', *Nature Medicine*. Europe PMC Funders, 14(12), pp. 1390–1395. doi: 10.1038/nm.1779.
- Veillette, A. *et al.* (1988) 'The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase p56lck', *Cell*, 55(2), pp. 301–308. doi: 10.1016/0092-8674(88)90053-0.
- Veillette, A. *et al.* (1989) 'Engagement of CD4 and CD8 expressed on immature thymocytes induces activation of intracellular tyrosine phosphorylation pathways', *Journal of Experimental Medicine*, 170(5), pp. 1671–1680. doi: 10.1084/jem.170.5.1671.
- Velthuis, J. H. *et al.* (2010) 'Simultaneous detection of circulating autoreactive CD8+ T-cells specific for different islet cell-associated epitopes using combinatorial MHC multimers', *Diabetes*. American Diabetes Association, 59(7), pp. 1721–1730. doi: 10.2337/db09-1486.
- Venigalla, S. S. K., Premakumar, S. and Janakiraman, V. (2020) 'A possible role for autoimmunity through molecular mimicry in alphavirus mediated arthritis', *Scientific Reports*. Nature Research, 10(1), p. 938. doi: 10.1038/s41598-019-55730-6.
- Veronese, F. D. *et al.* (1987) 'Immunological and chemical analysis of P6, the carboxyl-terminal fragment of HIV P15.', *AIDS research and human retroviruses*, 3(3), pp. 253–64. doi: 10.1089/aid.1987.3.253.
- Vibhakar, R. *et al.* (1997) 'Activation-induced expression of human programmed death-1 gene in T- lymphocytes', *Experimental Cell Research*. Academic Press Inc., 232(1), pp. 25–28. doi: 10.1006/excr.1997.3493.
- Vickers, M. A. *et al.* (2014) 'Establishment and operation of a Good Manufacturing Practice-compliant allogeneic Epstein-Barr virus (EBV)-specific cytotoxic cell bank for the treatment of EBV-associated lymphoproliferative disease', *British Journal of Haematology*, 167, pp. 402–410. doi: 10.1111/bjh.13051.
- Visseaux, B. *et al.* (2016) 'Hiv-2 molecular epidemiology', *Infection, Genetics and Evolution*. Elsevier B.V., 46, pp. 233–240. doi: 10.1016/j.meegid.2016.08.010.
- Voeten, J. T. M. *et al.* (2000) 'Antigenic Drift in the Influenza A Virus (H3N2) Nucleoprotein and Escape from Recognition by Cytotoxic T Lymphocytes', *Journal of Virology*. American Society for Microbiology, 74(15), pp. 6800–6807. doi: 10.1128/jvi.74.15.6800-6807.2000.
- Wagner, G. *et al.* (1999) 'Structure, specificity and CDR mobility of a class II restricted single-chain T-cell receptor.', *Nature Structural Biology*, 6(6), pp. 574–581. doi: 10.1038/9359.
- Wagner, T. A. (2018) 'Quarter Century of Anti-HIV CAR T Cells', *Current HIV/AIDS Reports*.

Current Medicine Group LLC 1, 15(2), pp. 147–154. doi: 10.1007/s11904-018-0388-x.

Wainberg, M. A. *et al.* (1974) 'Cellular immunity against Rous sarcomas of chickens. Preferential reactivity against autochthonous target cells as determined by lymphocyte adherence and cytotoxicity tests in vitro', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 71(9), pp. 3565–3569. doi: 10.1073/pnas.71.9.3565.

Walker, D. M. (2004) 'Oral mucosal immunology: an overview.', *Annals of the Academy of Medicine, Singapore*, 33(4 Suppl), pp. 27–30. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/15389303> (Accessed: 5 November 2019).

Walter, E. A. *et al.* (1995) 'Reconstitution of Cellular Immunity against Cytomegalovirus in Recipients of Allogeneic Bone Marrow by Transfer of T-Cell Clones from the Donor', *New England Journal of Medicine*. Massachusetts Medical Society, 333(16), pp. 1038–1044. doi: 10.1056/nejm199510193331603.

Walunas, T. L. *et al.* (1994) 'CTLA-4 can function as a negative regulator of T cell activation', *Immunity*, 1(5), pp. 405–413. doi: 10.1016/1074-7613(94)90071-X.

Wang, B. *et al.* (2004) 'Identification of an HLA-A\*0201-restricted CD8+ T-cell epitope SSp-1 of SARS-CoV spike protein', *Blood*. Blood, 104(1), pp. 200–206. doi: 10.1182/blood-2003-11-4072.

Wang, J. H. *et al.* (2001) 'Crystal structure of the human CD4 N-terminal two-domain fragment complexed to a class II MHC molecule', *Proceedings of the National Academy of Sciences of the United States of America*, 98(19), pp. 10799–10804. doi: 10.1073/pnas.191124098.

Wang, Q. *et al.* (2017) 'Genome modification of CXCR4 by Staphylococcus aureus Cas9 renders cells resistance to HIV-1 infection', *Retrovirology*. BioMed Central Ltd., 14(1). doi: 10.1186/s12977-017-0375-0.

Wang, R. F. *et al.* (1996) 'Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen', *Journal of Experimental Medicine*, 183(3), pp. 1137–1140. doi: 10.1084/jem.183.3.1131.

Wang, R., Natarajan, K. and Margulies, D. H. (2009) 'Structural Basis of the CD8 $\alpha\beta$ /MHC Class I Interaction: Focused Recognition Orients CD8 $\beta$  to a T Cell Proximal Position', *The Journal of Immunology*. The American Association of Immunologists, 183(4), pp. 2554–2564. doi: 10.4049/jimmunol.0901276.

Wang, X. *et al.* (2017) 'The EF-1 $\alpha$  promoter maintains high-level transgene expression from episomal vectors in transfected CHO-K1 cells', *Journal of Cellular and Molecular Medicine*, 21(11), pp. 3044–3054. doi: 10.1111/jcmm.13216.

Wang, X. X. *et al.* (2011) 'Affinity maturation of human CD4 by yeast surface display and crystal structure of a CD4-HLA-DR1 complex', *Proceedings of the National Academy of Sciences of the United States of America*, 108(38), pp. 15960–15965. doi: 10.1073/pnas.1109438108.

Wang, Z. *et al.* (2015) 'Recovery from severe H7N9 disease is associated with diverse response mechanisms dominated by CD8+ T cells', *Nature Communications*. Nature Publishing Group, 6. doi: 10.1038/ncomms7833.

Wearsch, P. A. and Cresswell, P. (2007) 'Selective loading of high-affinity peptides onto major histocompatibility complex class I molecules by the tapasin-ERp57 heterodimer',

*Nature Immunology*, 8(8), pp. 873–881. doi: 10.1038/ni1485.

Weber, G. and Shinitzky, M. (1970) 'Failure of Energy Transfer between Identical Aromatic Molecules on Excitation at the Long Wave Edge of the Absorption Spectrum.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 65(4), pp. 823–30. doi: 10.1073/PNAS.65.4.823.

Weber, S. *et al.* (1992) 'Specific low-affinity recognition of major histocompatibility complex plus peptide by soluble T-cell receptor', *Nature*, 356(6372), pp. 793–796. doi: 10.1038/356793a0.

Weigert, M. *et al.* (1980) 'The joining of V and J gene segments creates antibody diversity [24]', *Nature*, pp. 497–499. doi: 10.1038/283497a0.

Weiss, A. *et al.* (1984) 'Role of T3 surface molecules in human T-cell activation: T3-dependent activation results in an increase in cytoplasmic free calcium', *Proceedings of the National Academy of Sciences of the United States of America*, 81(13 I), pp. 4169–4173. doi: 10.1073/pnas.81.13.4169.

Weiss, A. (1993) 'T cell antigen receptor signal transduction: A tale of tails and cytoplasmic protein-tyrosine kinases', *Cell*, pp. 209–212. doi: 10.1016/0092-8674(93)90221-B.

Weiss, A. and Littman, D. R. (1994) 'Signal transduction by lymphocyte antigen receptors', *Cell*, pp. 263–274. doi: 10.1016/0092-8674(94)90334-4.

Wen, J. *et al.* (2017) 'Dengue virus-reactive CD8+ T cells mediate cross-protection against subsequent Zika virus challenge', *Nature Communications*. Nature Publishing Group, 8(1), pp. 1–11. doi: 10.1038/s41467-017-01669-z.

Wenzlau, J. M. *et al.* (2007) 'The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 104(43), pp. 17040–17045. doi: 10.1073/pnas.0705894104.

Wettstein, P. J. *et al.* (1978) 'T-lymphocyte response to H-2 mutants: I. Proliferation is dependent on Ly 1+2+ cells\*', *Journal of Experimental Medicine*, 147(5), pp. 1395–1404. doi: 10.1084/jem.147.5.1395.

Whalley, T. *et al.* (2020) 'GPU-Accelerated Discovery of Pathogen-Derived Molecular Mimics of a T-Cell Insulin Epitope', *Frontiers in Immunology*. Frontiers Media S.A., 11. doi: 10.3389/fimmu.2020.00296.

Wheelock, E. F. (1965) 'Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by Phytohemagglutinin', *Science*. American Association for the Advancement of Science (AAAS), 149(3681), pp. 310–311. doi: 10.1126/science.149.3681.310.

Wieczorek, M. *et al.* (2017) 'Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins: Conformational Plasticity in Antigen Presentation.', *Frontiers in Immunology*. Frontiers Media SA, 8, p. 292. doi: 10.3389/fimmu.2017.00292.

Wilde, D. B. *et al.* (1983) 'Evidence implicating L3T4 in class II MHC antigen reactivity; monoclonal antibody GK1.5 (anti-L3T4a) blocks class II MHC antigen-specific proliferation, release of lymphokines, and binding by cloned murine helper T lymphocyte lines.', *Journal of Immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 131(5), pp. 2178–83. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/6195255> (Accessed: 20 March 2020).

- Wilens, C. B., Tilton, J. C. and Doms, R. W. (2012) 'HIV: Cell binding and entry', *Cold Spring Harbor Perspectives in Medicine*. Cold Spring Harbor Laboratory Press, 2(8). doi: 10.1101/cshperspect.a006866.
- Willcox, A. *et al.* (2009) 'Analysis of islet inflammation in human type 1 diabetes', *Clinical and Experimental Immunology*, 155(2), pp. 173–181. doi: 10.1111/j.1365-2249.2008.03860.x.
- Willcox, B. E. *et al.* (1999) 'TCR binding to peptide-MHC stabilizes a flexible recognition interface.', *Immunity*, 10(3), pp. 357–65. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10204491> (Accessed: 15 November 2017).
- William Studier, F. *et al.* (1990) 'Use of T7 RNA polymerase to direct expression of cloned genes', *Methods in Enzymology*. Academic Press, 185, pp. 60–89. doi: 10.1016/0076-6879(90)85008-C.
- Williams, A. P. *et al.* (2002) 'Optimization of the MHC class I peptide cargo is dependent on tapasin', *Immunity*. Cell Press, 16(4), pp. 509–520. doi: 10.1016/S1074-7613(02)00304-7.
- Williams, C. B. *et al.* (1999) 'A kinetic threshold between negative and positive selection based on the longevity of the T cell receptor-ligand complex', *Journal of Experimental Medicine*, 189(10), pp. 1531–1544. doi: 10.1084/jem.189.10.1531.
- Williams, M. A., Tyznik, A. J. and Bevan, M. J. (2006) 'Interleukin-2 signals during priming are required for secondary expansion of CD8 1 memory T cells'. doi: 10.1038/nature04790.
- Wilson, I. A. and Fremont, D. H. (1993) 'Structural analysis of MHC class I molecules with bound peptide antigens', *Seminars in Immunology*, 5(2), pp. 75–80. doi: 10.1006/smim.1993.1011.
- Wilson, J. D. K. *et al.* (1998) 'Oligoclonal expansions of CD8+ T cells in chronic HIV infection are antigen specific', *Journal of Experimental Medicine*. The Rockefeller University Press, 188(4), pp. 785–790. doi: 10.1084/jem.188.4.785.
- Wistow, G. *et al.* (2002) 'Expressed sequence tag analysis of human retina for the NEIBank project: Retbindin, an abundant, novel, retinal cDNA and alternative splicing of other retina-preferred gene transcripts', *Molecular Vision*, 8, pp. 196–204.
- Wistow, G. (2006) 'The NEIBank project for ocular genomics: Data-mining gene expression in human and rodent eye tissues', *Progress in Retinal and Eye Research*, pp. 43–77. doi: 10.1016/j.preteyeres.2005.05.003.
- Wong, C. P. *et al.* (2007) 'Identical  $\beta$  Cell-Specific CD8 + T Cell Clonotypes Typically Reside in Both Peripheral Blood Lymphocyte and Pancreatic Islets', *The Journal of Immunology*. The American Association of Immunologists, 178(3), pp. 1388–1395. doi: 10.4049/jimmunol.178.3.1388.
- Wood, P. and Elliott, T. (1998) 'Glycan-regulated antigen processing of a protein in the endoplasmic reticulum can uncover cryptic cytotoxic T cell epitopes', *Journal of Experimental Medicine*. The Rockefeller University Press, 188(4), pp. 773–778. doi: 10.1084/jem.188.4.773.
- Wooldridge, L. *et al.* (2005) 'Interaction between the CD8 Coreceptor and Major Histocompatibility Complex Class I Stabilizes T Cell Receptor-Antigen Complexes at the Cell Surface', *The Journal of biological chemistry*. NIH Public Access, 280(30), p. 27491. doi: 10.1074/JBC.M500555200.

- Wooldridge, L. *et al.* (2009) 'Tricks with tetramers: how to get the most from multimeric peptide-MHC.', *Immunology*, 126(2), pp. 147–64. doi: 10.1111/j.1365-2567.2008.02848.x.
- Wooldridge, L., Laugel, B., *et al.* (2010) 'CD8 Controls T Cell Cross-Reactivity', *The Journal of Immunology*, 185(8), pp. 4625–4632. doi: 10.4049/jimmunol.1001480.
- Wooldridge, L., Clement, M., *et al.* (2010) 'MHC Class I Molecules with Superenhanced CD8 Binding Properties Bypass the Requirement for Cognate TCR Recognition and Nonspecifically Activate CTLs', *The Journal of Immunology*. The American Association of Immunologists, 184(7), pp. 3357–3366. doi: 10.4049/jimmunol.0902398.
- Wooldridge, L. *et al.* (2012) 'A single autoimmune T cell receptor recognizes more than a million different peptides', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology, 287(2), pp. 1168–1177. doi: 10.1074/jbc.M111.289488.
- Wooldridge, L. (2013) 'Individual MHCI-restricted T-cell receptors are characterized by a unique peptide recognition signature', *Frontiers in Immunology*. Frontiers Research Foundation, 4(JUL). doi: 10.3389/fimmu.2013.00199.
- Wright, J. K. *et al.* (2012) 'Impact of HLA-B\*81-Associated Mutations in HIV-1 Gag on Viral Replication Capacity', *Journal of Virology*. American Society for Microbiology, 86(6), pp. 3193–3199. doi: 10.1128/jvi.06682-11.
- Wu, C. *et al.* (2011) 'Systematic identification of immunodominant CD8+ T-cell responses to influenza A virus in HLA-A2 individuals', *Proceedings of the National Academy of Sciences of the United States of America*. Proc Natl Acad Sci U S A, 108(22), pp. 9178–9183. doi: 10.1073/pnas.1105624108.
- Wu, J. *et al.* (2003) 'T-cell epitope of  $\alpha 3$  chain of type IV collagen induces severe glomerulonephritis', *Kidney International*. Blackwell Publishing Inc., 64(4), pp. 1292–1301. doi: 10.1046/j.1523-1755.2003.00227.x.
- Wu, L. C. *et al.* (2002) 'Two-step binding mechanism for T-cell receptor recognition of peptide-MHC', *Nature*. Nature, 418(6897), pp. 552–556. doi: 10.1038/nature00920.
- Wucherpfennig, K. W. and Strominger, J. L. (1995) 'Molecular mimicry in T cell-mediated autoimmunity: Viral peptides activate human T cell clones specific for myelin basic protein', *Cell*. Cell, 80(5), pp. 695–705. doi: 10.1016/0092-8674(95)90348-8.
- Wüthrich, K. and Wagner, G. (1978) 'Internal motion in globular proteins', *Trends in Biochemical Sciences*. Elsevier Current Trends, pp. 227–230. doi: 10.1016/S0968-0004(78)94607-8.
- Wyatt, K. *et al.* (2006) 'Lengsin Is a Survivor of an Ancient Family of Class I Glutamine Synthetases Re-engineered by Evolution for a Role in the Vertebrate Lens', *Structure*. Structure, 14(12), pp. 1823–1834. doi: 10.1016/j.str.2006.10.008.
- Wyatt, K. *et al.* (2008) 'A role for lengsin, a recruited enzyme, in terminal differentiation in the vertebrate lens', *Journal of Biological Chemistry*. NIH Public Access, 283(10), pp. 6607–6615. doi: 10.1074/jbc.M709144200.
- Wyer, J. R. *et al.* (1999) 'T cell receptor and coreceptor CD8 $\alpha\alpha$  bind peptide-MHC independently and with distinct kinetics', *Immunity*. Cell Press, 10(2), pp. 219–225. doi: 10.1016/S1074-7613(00)80022-9.
- Xiong, N. and Raulet, D. H. (2007) 'Development and selection of  $\gamma\delta$  T cells', *Immunological*

Reviews. John Wiley & Sons, Ltd, pp. 15–31. doi: 10.1111/j.1600-065X.2006.00478.x.

Xu, X. N. *et al.* (2001) 'A novel approach to antigen-specific deletion of CTL with minimal cellular activation using  $\alpha 3$  domain mutants of MHC class I/peptide complex', *Immunity*. *Immunity*, 14(5), pp. 591–602. doi: 10.1016/S1074-7613(01)00133-9.

Yanagi, Y. *et al.* (1984) 'A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains', *Nature*. Nature Publishing Group, 308(5955), pp. 145–149. doi: 10.1038/308145a0.

Yanaka, S. *et al.* (2014) 'Peptide-dependent conformational fluctuation determines the stability of the human leukocyte antigen class I complex.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 289(35), pp. 24680–90. doi: 10.1074/jbc.M114.566174.

Yang, J. *et al.* (2011) 'Molecular features of the complementarity determining region 3 motif of the T cell population and subsets in the blood of patients with chronic severe hepatitis B', *Journal of Translational Medicine*. *J Transl Med*, 9(1). doi: 10.1186/1479-5876-9-210.

Yang, L. *et al.* (2007) 'Persistent memory CD4+ and CD8+ T-cell responses in recovered severe acute respiratory syndrome (SARS) patients to SARS coronavirus M antigen', *Journal of General Virology*. NIH Public Access, 88(10), pp. 2740–2748. doi: 10.1099/vir.0.82839-0.

Yang, S. *et al.* (2008) 'Development of optimal bicistronic lentiviral vectors facilitates high-level TCR gene expression and robust tumor cell recognition', *Gene Therapy*. Nature Publishing Group, 15(21), pp. 1411–1423. doi: 10.1038/gt.2008.90.

Yannelli, J. R. *et al.* (1996) 'Growth of tumor-infiltrating lymphocytes from human solid cancers: Summary of a 5-year experience.', 65, pp. 413–421.

Yazdanifar, M. *et al.* (2020) ' $\gamma\delta$  T Cells: The Ideal Tool for Cancer Immunotherapy', *Cells*. NLM (Medline), 9(5), p. 1305. doi: 10.3390/cells9051305.

Yeo, T. W. *et al.* (2007) 'A second major histocompatibility complex susceptibility locus for multiple sclerosis', *Annals of Neurology*. John Wiley and Sons Inc., 61(3), pp. 228–236. doi: 10.1002/ana.21063.

Yewdell, J. W. *et al.* (1994) 'MHC-encoded proteasome subunits LMP2 and LMP7 are not required for efficient antigen presentation.', *Journal of immunology (Baltimore, Md. : 1950)*. American Association of Immunologists, 152(3), pp. 1163–70. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8301122> (Accessed: 3 March 2020).

Yewdell, J. W. (2011) 'DRiPs solidify: Progress in understanding endogenous MHC class I antigen processing', *Trends in Immunology*. NIH Public Access, pp. 548–558. doi: 10.1016/j.it.2011.08.001.

Yewdell, J. W., Antón, L. C. and Bennink, J. R. (1996) 'Defective ribosomal products (DRiPs): a major source of antigenic peptides for MHC class I molecules?', *Journal of immunology (Baltimore, Md. : 1950)*, 157(5), pp. 1823–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8757297> (Accessed: 3 March 2020).

Yewdell, J. W., Bennink, J. R. and Hosaka, Y. (1988) 'Cells process exogenous proteins for recognition by cytotoxic T lymphocytes', *Science*. American Association for the Advancement of Science, 239(4840), pp. 637–640. doi: 10.1126/science.3257585.

Yin, L. *et al.* (2011) 'A single T cell receptor bound to major histocompatibility complex class



I and class II glycoproteins reveals switchable TCR conformers', *Immunity*. Cell Press, 35(1), pp. 23–33. doi: 10.1016/j.immuni.2011.04.017.

Yin, Y., Li, Y. and Mariuzza, R. A. (2012) 'Structural basis for self-recognition by autoimmune T-cell receptors', *Immunological Reviews*, 250(1), pp. 32–48. doi: 10.1111/imr.12002.

Yin, Y., Wang, X. X. and Mariuzza, R. A. (2012) 'Crystal structure of a complete ternary complex of T-cell receptor, peptide-MHC, and CD4', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 109(14), pp. 5405–5410. doi: 10.1073/pnas.1118801109.

Yokota, S., Geppert, T. D. and Lipsky, P. E. (1988) 'Enhancement of antigen- and mitogen-induced human T lymphocyte proliferation by tumor necrosis factor-alpha.', *Journal of immunology (Baltimore, Md. : 1950)*, 140(2), pp. 531–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/3257243> (Accessed: 13 January 2020).

York, I. A. *et al.* (2002) 'The Er aminopeptidase ERAP I enhances or limits antigen presentation by trimming epitopes to 8-9 residues', *Nature Immunology*. Nature Publishing Group, 3(12), pp. 1177–1184. doi: 10.1038/ni860.

York, I. A. *et al.* (2006) 'Tripeptidyl Peptidase II Is the Major Peptidase Needed to Trim Long Antigenic Precursors, but Is Not Required for Most MHC Class I Antigen Presentation', *The Journal of Immunology*. The American Association of Immunologists, 177(3), pp. 1434–1443. doi: 10.4049/jimmunol.177.3.1434.

Zamvil, S. S. and Steinman, L. (1990) 'The T lymphocyte in experimental allergic encephalomyelitis', *Annual Review of Immunology*. Annu Rev Immunol, pp. 579–621. doi: 10.1146/annurev.iy.08.040190.003051.

Zavala-Ruiz, Z. *et al.* (2004) 'A hairpin turn in a II MHC-bound peptide orients outside the binding groove for T cell recognition', *Proceedings of the National Academy of Sciences of the United States of America*, 101(36), pp. 13279–13284. doi: 10.1073/pnas.0403371101.

Zerrahn, J., Held, W. and Raulet, D. H. (1997) 'The MHC reactivity of the T cell repertoire prior to positive and negative selection.', *Cell*, 88(5), pp. 627–36. doi: 10.1016/s0092-8674(00)81905-4.

Zhang, N. and Bevan, M. J. (2011) 'CD8+ T Cells: Foot Soldiers of the Immune System', *Immunity*, pp. 161–168. doi: 10.1016/j.immuni.2011.07.010.

Zhang, Y., Baig, E. and Williams, D. B. (2006) 'Functions of ERp57 in the folding and assembly of major histocompatibility complex class I molecules', *Journal of Biological Chemistry*, 281(21), pp. 14622–14631. doi: 10.1074/jbc.M512073200.

Zhao, K. *et al.* (2010) 'CD8+ T cell response in HLA-A\*0201 transgenic mice is elicited by epitopes from SARS-CoV S protein', *Vaccine*. Elsevier, 28(41), pp. 6666–6674. doi: 10.1016/j.vaccine.2010.08.013.

Zhao, Y. *et al.* (2005) 'Primary Human Lymphocytes Transduced with NY-ESO-1 Antigen-Specific TCR Genes Recognize and Kill Diverse Human Tumor Cell Lines', *The Journal of Immunology*. The American Association of Immunologists, 174(7), pp. 4415–4423. doi: 10.4049/jimmunol.174.7.4415.

Zhao, Z. S. *et al.* (1998) 'Molecular mimicry by herpes simplex virus-type 1: Autoimmune disease after viral infection', *Science*. Academic Press, 279(5355), pp. 1344–1347. doi: 10.1126/science.279.5355.1344.

Zheng, X. *et al.* (2019) 'Mucosal CD8+ T cell responses induced by an MCMV based vaccine vector confer protection against influenza challenge', *PLoS Pathogens*. Public Library of Science, 15(9). doi: 10.1371/journal.ppat.1008036.

Zhong, T. *et al.* (2020) 'The remission phase in type 1 diabetes: Changing epidemiology, definitions, and emerging immuno-metabolic mechanisms', *Diabetes/Metabolism Research and Reviews*. John Wiley and Sons Ltd, 36(2). doi: 10.1002/dmrr.3207.

Zhou, C. Y. *et al.* (2017) 'Alanine mutagenesis in the complementarity determining region 3 of the MTB and hiV-1 peptide-bispecific T cell receptor beta chain affects ligand recognition', *Frontiers in Immunology*. Frontiers Media S.A., 8(AUG). doi: 10.3389/fimmu.2017.00983.

Zhu, J. *et al.* (2017) 'Resistance to cancer immunotherapy mediated by apoptosis of tumor-infiltrating lymphocytes', *Nature Communications*. Nature Publishing Group, 8(1), pp. 1–15. doi: 10.1038/s41467-017-00784-1.

Zinkernagel, R. M., Callahan, G. N., *et al.* (1978) 'Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus', *Nature*. Nature Publishing Group, 271(5642), pp. 251–253. doi: 10.1038/271251a0.

Zinkernagel, R. M., Callahan, G., *et al.* (1978) 'On the thymus in the differentiation of "H-2 self-recognition" by T cells: Evidence for dual recognition?', *Journal of Experimental Medicine*, 147(3), pp. 882–896. doi: 10.1084/jem.147.3.882.

Zinkernagel, R. M. and Doherty, P. C. (1974) 'Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system', *Nature*, 248(5450), pp. 701–702. doi: 10.1038/248701a0.

Zoete, V. *et al.* (2013) 'Structure-based, rational design of T cell receptors', *Frontiers in Immunology*. Frontiers Media SA. doi: 10.3389/fimmu.2013.00268.

Zou, C. *et al.* (2017) 'γδ T cells in cancer immunotherapy', *Oncotarget*. Impact Journals, LLC, 8(5). doi: 10.18632/oncotarget.13051.