Germline bias dictates cross-serotype reactivity in a common dengue virus-specific CD8^{+} T cell response

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ABSTRACT

Adaptive immunity plays a key role in protection from dengue virus (DENV) infection, yet cross-reactivity with distinct serotypes can precipitate life-threatening clinical disease. We report that clonotypes expressing shared *TRBV11-2* gene rearrangements are preferentially activated and mobilized within the immunodominant human leukocyte antigen (HLA)-A*11:01-restricted $NS3_{133}$ epitope-specific $CD8^+$ T cell response to DENV1 and DENV3/4. In contrast, the $NS3_{133}$ DENV2-specific repertoire is largely devoid of such T cell receptors (TCRs). Structural analysis of a representative TRBV11-2$^+$ TCR demonstrated that cross-serotype reactivity is governed by a unique interplay between the variable antigenic determinant and germline-encoded residues in the second β-chain complementarity-determining region (CDR2β). Extensive mutagenesis studies further confirmed that biophysical and functional engagement of three distinct TRBV11-2$^+$ TCRs was highly dependent on key contacts between the serotype-defined peptide and discrete residues in the CDR2β loop. Collectively, these data reveal an innate-like mode of epitope recognition with potential implications for the outcome of sequential exposure to heterologous DENVs.

INTRODUCTION

Dengue is endemic in tropical and sub-tropical regions of the world, causing up to 390 million infections annually with approximately 96 million cases of symptomatic disease and 25,000 deaths[1]. Most clinically apparent infections result in a self-limiting febrile illness termed dengue fever. However, a minority of patients develop dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS), a more serious manifestation characterized by vascular leakage and circulatory shock.

There are four serotypes of dengue virus (DENV1–4), which differ at the amino acid level by 30–35%. As a consequence of this heterogeneity, immunity to one serotype does not necessarily protect against infection with another serotype. Sequential infections are therefore common in dengue-endemic countries, where all four serotypes often circulate at the same time or replace each other in a cyclical manner. Epidemiological studies have further shown that adverse clinical outcomes are more frequent during secondary or recurrent infections. This key observation implicates adaptive immune responses in the pathogenesis of disease[2, 3].

Antibody-dependent enhancement (ADE) is thought to provide the link between severe immunopathology and consecutive infection. The ADE hypothesis posits that antibodies generated in response to the initial serotype will fail to neutralize subsequent viruses, yet still bind with sufficient avidity to enhance viral replication via Fc receptor-mediated uptake into myeloid cells[4]. This process has been demonstrated *in vitro*[5, 6] and in animal models[7, 8, 9]. In addition, higher virus loads driven by ADE correlate with disease severity[10]. However, the vascular leak that occurs during DHF/DSS tends to develop when the fever remits, which coincides with a steep fall in virus load and an inflammatory "cytokine storm". This temporal association between symptomatic disease and viral clearance suggests a pathogenic role for DENV-specific T cells.

Non-structural protein-3 (NS3) is classically described as the immunodominant T cell antigen in DENV[11, 12]. Comprehensive approaches have largely substantiated these initial observations and further enabled the identification of many other targeted epitopes across

the entire viral proteome[13]. Virus-specific CD4^{+} and CD8^{+} T cells are highly activated during acute infection, and the magnitude of the overall response correlates with disease severity[12]. There is also good evidence to support a protective role for DENV-specific T cells in humans[13] and mouse models[14, 15]. Accordingly, the lack of a potent T cell immunogen may explain the relatively poor efficacy of a chimeric vaccine designed to elicit neutralizing antibodies against the precursor membrane and envelope proteins from DENV1–4[16].

To clarify the mechanisms that underpin this dichotomy between pathogenesis and protection, we conducted a detailed investigation of $NS3_{133}$ DENV-specific CD8^{+} T cell responses in a cohort of patients from Thailand. Restricted by human leukocyte antigen (HLA)-A*11:01, which occurs in this population with an allelic frequency of approximately 30%[17], the $NS3_{133}$ epitope is typically immunodominant and can elicit secondary responses comprising up to 10–12% of the circulating CD8^{+} T cell pool[18]. A number of sequence variants have also been described, leading to distinct patterns of cross-serotype reactivity that potentially affect disease outcome[19]. The data presented here reveal that serotype-defined epitope cross-recognition is driven by a previously unknown mode of antigen engagement involving direct interactions between the $NS3_{133}$ peptide and germline-encoded residues in the second β-chain complementarity-determining region (CDR2β) that characterize a highly biased subset of T cell receptors (TCRs). Key components of the adaptive repertoire are therefore inherited at the *TRBV* gene locus, ensuring common and predictable immune reactivity against various circulating strains of DENV.

RESULTS

Cross-serotype TCR bias in NS3$_{133}$ DENV-specific CD8$^+$ T cell populations

To investigate the molecular basis of cross-reactivity within the immunodominant NS3$_{133}$ DENV-specific CD8$^+$ T cell response, we first generated HLA-A*11:01-peptide (HLA-A*11:01p) tetramers corresponding to the most prevalent epitope variant (GTS1, GTS2, or GTS3/4) for each serotype (DENV1–4) among viral isolates from Thailand (Fig. 1a). These reagents were used in conjunction with polychromatic flow cytometry to quantify and sort antigen-specific CD8$^+$ T cell populations directly *ex vivo* from the peripheral blood of patients infected with DENV (Supplementary Table 1). A single HLA-A*11:01p tetramer was used in cases of primary infection, matched to the gene-identified serotype determined by RT-PCR. Two HLA-A*11:01p tetramers with distinct fluorochrome tags were used simultaneously in cases of secondary infection, matched to the recently encountered serotype and the primary serotype determined on the basis of micro-focus reduction neutralization tests (micro-FRNTs). Representative flow cytometry plots are shown in Fig. 1b. An unbiased molecular approach was then used to characterize all expressed *TRB* gene rearrangements in each sorted HLA-A*11:01p tetramer-defined CD8$^+$ T cell population (Supplementary Figs. 1–5).

Preferential usage of the *TRBV11-2* gene was apparent in NS3$_{133}$ DENV-specific CD8$^+$ T cell populations targeting the GTS1 and GTS3/4 variants (Fig. 1c). The *TRBV9* and *TRBV12-3/4* genes were also favoured during infections with DENV1 and DENV3/4. In contrast, these germline transcripts were rarely detected in GTS2-specific CD8$^+$ T cell populations. Further inspection of the biased repertoires associated with HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 revealed centrally located glycines in the somatically rearranged CDR3β loop and preservation of the *TRBV* gene-encoded leucine at position 5. Eight amino acid residue-identical TCRβ sequences were present in more than one patient infected with the corresponding serotypes (DENV1/3/4). Six of these "public" sequences incorporated

TRBV11-2 (Supplementary Table 2), and all were variously encoded across the junctional region (Supplementary Fig. 6).

To confirm these patterns of epitope-specific bias at the protein level, we used serotype-matched HLA-A*11:01p tetramers and a TRBV11-2-specific mAb to stain peripheral blood mononuclear cells (PBMCs) from 23 additional patients with secondary infection (Supplementary Table 3). Representative flow cytometry plots are shown in Fig. 1d. Antigen-specific usage of TRBV11-2 was significantly more common in CD8$^+$ T cell populations targeting the GTS1 and GTS3/4 epitopes compared with CD8$^+$ T cell populations targeting the GTS2 epitope (Fig. 1e). Cross-staining of TRBV11-2$^+$ clonotypes with the HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 tetramers was also observed during primary infection (data not shown). Collectively, these data show that the serotype-defined epitope sequence dictates germline bias in the NS3$_{133}$ DENV-specific TCR repertoire.

Characterization of representative TRBV11-2$^+$ NS3$_{133}$ DENV-specific CD8$^+$ T cell clones

To examine variant cross-recognition in the context of epitope-dependent TCR bias, we generated a panel of NS3$_{133}$ DENV-specific CD8$^+$ T cell clones. Functionality was assessed in response to HLA-matched B-lymphoblastoid cell lines pulsed separately with exogenous peptides corresponding to GTS1, GTS2, and GTS3/4. Distinct effector readouts were measured independently and simultaneously using polychromatic flow cytometry (Fig. 2a). Clones OX17 2H, 44-173 13, and 44-173 30, all of which express TRBV11-2, preferentially recognized GTS1 and GTS3/4. A similar pattern of activation was observed with the TRBV12-3/4$^+$ clone N15 22. In contrast, the TRBV12-3/4$^+$ clone 44-038 25 and the TRBV7-6$^+$ clone E5 responded maximally to all three peptide variants, whereas the TRBV2$^+$ clone D9 became less polyfunctional according to the hierarchy GTS3/4 > GTS2 > GTS1. These data confirm that TRBV11-2 usage is associated with enhanced recognition of GTS1 and GTS3/4.

Molecular analysis of the rearranged *TRB* gene transcripts from clones OX17 2H, 44-173 13, and 44-173 30 revealed features consistent with the *ex vivo* data, including a

CASSLGXG motif in the CDR3β loop (Fig. 2b). Each TCR β-chain paired with a distinct TCR α-chain. To determine the biophysical correlates of differential epitope recognition, we refolded the corresponding heterodimeric TCRs *in vitro* and undertook surface plasmon resonance (SPR) binding measurements with soluble HLA-A*11:01p complexes (Table 1 and Supplementary Fig. 7). The OX17 2H TCR (termed D2H from hereon) displayed high affinity interactions with HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 (K_{Deq} < 10 μM) and a low affinity interaction with HLA-A*11:01-GTS2 (K_{Deq} > 200 μM). In contrast, the 44-173 13 TCR (termed D13 from hereon) bound very weakly to all three HLA-A*11:01p complexes (K_{Deq} > 400 μM). These are remarkably low affinities for a naturally selected pathogen-specific TCR[20, 21]. Accurate measurements within this range were precluded by technical constraints, but the relative K_{Deq} values presumably mirror the slight functional differences observed at the clonal level. The 44-173 30 TCR (termed D30 from hereon) bound weakly to HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 (K_{Deq} ~ 150 μM) and very weakly to HLA-A*11:01-GTS2 (K_{Deq} > 400 μM). These data are consistent with the activation profiles shown in Fig. 2a.

Serotype-defined NS3$_{133}$ DENV peptides are presented similarly by HLA-A*11:01

To determine how C-terminal amino acid mutations in the bound epitope affect TRBV11-2[+] TCR-mediated antigen recognition, we first used a thermal shift assay to measure the stability of each binary HLA-A*11:01p complex. No significant differences in melting point (T_M) were detected between HLA-A*11:01-GTS1 (T_M = 61.6 ± 0.8 °C), HLA-A*11:01-GTS2 (T_M = 62.7 ± 0.7 °C), and HLA-A*11:01-GTS3/4 (T_M = 62.3 ± 1.0 °C). These data indicate that all three serotype-defined variants of NS3$_{133}$ are readily accommodated by HLA-A*11:01. We then solved the crystal structures of HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 (Fig. 2c–e and Table 2). The structures of HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 were very similar, with a root mean square deviation (r.m.s.d.) of 0.34 Å for the overall complex (Fig. 2e). In each case, the P2-Thr and P10-Arg were buried inside the

antigen-binding cleft, acting as anchor residues, while the central region of the peptide from P4-Gly to P9-Asn was solvent exposed. The shared P5-Ser and P6-Pro were highly mobile. This close structural homology reflects the fact that GTS1 and GTS3/4 differ by a single residue, namely the conservative switch from P8-Val (DENV1) to P8-Ile (DENV3/4). In contrast, GTS2 contains a P9-Asp in place of P9-Asn. Collectively, these data suggest that specific TCR-mediated contacts with the HLA-A*11:01-bound peptide, likely focused on the neutral Asn residue at P9, underpin the preferential deployment of TRBV11-2 in response to $NS3_{133}$ DENV1/3/4.

HLA-A*11:01-GTS1/3/4 recognition is dominated by TCRβ

To define the molecular basis of serotype-restricted bias in the $NS3_{133}$ DENV-specific CD8^{+} T cell response, we solved the ternary structures of HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 in complex with the D30 TCR (Table 2). The electron density at the TCR-HLA-A*11:01p interface was unambiguous in each case, permitting a detailed analysis of the interactions with GTS1 and GTS3/4.

The D30 TCR docked similarly on top of both HLA-A*11:01p complexes (r.m.s.d. of 0.6 Å), with the center of mass towards the N-terminal region of the peptide (Fig. 3a,b). The overall buried surface area (BSA) fell within the normal range at ~ 1850 Å^2 [21]. Contacts between the TCR α-chain and HLA-A*11:01p were limited to a few residues in each ternary complex (31% of the BSA). The CDR1α loop sat above the N-terminal region of the antigen-binding cleft, acting as a "lid" over P1-Gly (Fig. 4a), while interacting solely with Arg163 in the α2-helix of HLA-A*11:01 via a hydrogen bond and van der Waals contacts (Supplementary Table 4). The CDR3α loop also interacted with HLA-A*11:01 via a smattering of van der Waals contacts, together with hydrogen bonds and a salt bridge between Asp109α and residues Gln62 and Arg65, respectively, in the α1-helix (Fig. 4b and Supplementary Table 4). In contrast, the β-chain contributed 69% of the BSA. This interaction was driven predominantly by CDR3β (34% of the BSA), CDR2β (22% of the BSA), and FWβ (11% of the BSA), placing the D30 TCR complexes with HLA-A*11:01-GTS1

and HLA-A*11:01-GTS3/4 among the most β-centric ternary structures reported to date[21, 22, 23].

The CDR2β loop and the downstream framework residues Val66β and Asp67β interacted with residues 65–76 in the α1-helix of HLA-A*11:01, while the β-hairpin shape of the CDR2β-FWβ segment sat parallel above the α1-helix, maximizing contact with the HLA-A*11:01 molecule (Fig. 4c). These interactions were mediated largely by van der Waals contacts (Supplementary Table 4). The CDR3β loop interacted with HLA-A*11:01 primarily via the α2-helix, while also stretching across the antigen-binding cleft to contact Ala69 in the α1-helix (Fig. 4d). Of particular note, the aromatic group of Tyr114β was inserted between the peptide and the hinge of the α2-helix, forming hydrogen bonds with Ala150 and Gln155. Accordingly, the D30 TCR interacts with HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 mainly via the β-chain, with dominant contributions from CDR2β-FWβ and CDR3β.

TRBV11-2 bias is driven by serotype-defined NS3$_{133}$ DENV peptides

The GTS1 and GTS3/4 peptides both contributed 24% of the BSA, which falls within the normal range established by previous studies[21]. Each peptide also interacted similarly with the D30 TCR (Supplementary Table 4). Interactions with the α-chain were limited to a few contacts between the CDR3α loop and residues P4-Gly and P5-Ser. In contrast, the CDR3β loop sat above the central hydrophobic region of the peptide (^{4}GSPIV/I^8), dominating the overall interaction between the TCR and GTS1/3/4 (Fig. 4e and Supplementary Fig. 8).

The central region of each peptide showed a degree of mobility in the binary HLA-A*11:01p structure, but adopted a more stable conformation after TCR engagement to sit deeper in the antigen-binding cleft (Supplementary Fig. 9). In the ternary structures, the P5-Ser Cα and the P6-Pro Cα of the GTS1 peptide shifted by 2.3 Å and 1.2 Å, respectively (peptide r.m.s.d. of 0.78 Å), while the P5-Ser Cα and the P6-Pro Cα of the GTS3/4 peptide shifted by 1.5 Å and 1 Å, respectively (peptide r.m.s.d. of 0.59 Å). This conformational change was due to extensive peptide interactions with the CDR3β loop, the majority of which were main chain-main chain contacts (Fig. 4e, Supplementary Fig. 8, and Supplementary

Table 4). In contrast, most of the CDR3β side chains were pushed outside the antigen-binding cleft to mediate specific contacts with the HLA-A*11:01 molecule. These findings are somewhat reminiscent of a previous report in which TCR recognition was dependent on the length rather than the amino acid sequence of the CDR3β loop[24].

The CDR2β loop engaged GTS1 and GTS3/4 via side chain-mediated interactions, providing a structural explanation for the observed peptide-dependent TRBV11-2 bias (Fig. 4f and Supplementary Fig. 8). Specifically, Gln57β hydrogen bonded with the backbone of P7-Ile, while Asn58β formed a double hydrogen bond with the side chain of P9-Asn (Fig. 4f and Supplementary Table 4). The importance of Asn58β was reinforced by further analysis of the *ex vivo* NS3$_{133}$ DENV-specific TCRβ sequences, which revealed substantially higher proportions of Asn58β[+] clonotypes in the HLA-A*11:01-GTS1/3/4-specific repertoires compared with the HLA-A*11:01-GTS2-specific repertoires (Fig. 5a,b). In addition, Asn58β[+] TRBV segments with a charged amino acid at position 59 were poorly represented in the overall dataset, barring TRBV7-8. These observations link conceptually with the finding that antigen recognition can be impaired by single-residue allelic polymorphisms in the vicinity of the CDR2β loop[25].

Peptide interactions with the CDR2β loop are required for TRBV11-2[+] TCR-mediated recognition of HLA-A*11:01-GTS1/3/4

To define the key residues that underpin antigen-specific usage of TRBV11-2, we conducted alanine/glycine scanning mutagenesis experiments to probe "energetic hotspots" at the D30 TCR-HLA-A*11:01-GTS1 interface. Single point substitutions were designed using the ternary crystal structure as a guide, and each alanine/glycine-substituted mutant was expressed, refolded, and purified in a standardized manner. Thirteen individual substitutions were introduced into the D30 TCR β-chain (Supplementary Table 5). Of these mutants, only the Tyr114βAla TCR failed to refold, precluding further analysis. The control Leu81βAla mutation did not affect TCR binding to HLA-A*11:01-GTS1 (Fig. 6a). Similarly,

residue substitutions in the CDR1β loop were tolerated with minimal changes in the overall interaction affinity, as determined using SPR (Fig. 6a, Supplementary Fig. 10, and Supplementary Table 5). In contrast, mutations at positions 108–112 in the CDR3β loop, aligned with the central footprint on the surface of the HLA-A*11:01p complex, and mutations at Gln57β and Asn58β, which interacted predominantly with the peptide residues P7-Ile and P9-Asn, respectively, either abrogated or substantially diminished TCR binding to HLA-A*11:01-GTS1 (Fig. 6a, Supplementary Fig. 10, and Supplementary Table 5). These results identify a continuous energetic hotspot that runs across the central axis of the D30 TCR (Fig. 6a).

In reciprocal experiments, we generated single alanine-substituted mutants of HLA-A*11:01-GTS1 to define the corresponding energetic landscape and determine the extent to which interaction hotspots are conserved across different TRBV11-2[+] TCRs (Supplementary Fig. 11 and Supplementary Table 6). Four residues were mutated in the α1-helix of HLA-A*11:01 (Arg65, Lys68, Gln72, and Val76), and two residues were mutated in the α2-helix of HLA-A*11:01 (Arg145 and Gln155). A third residue in the α2-helix was mutated as a control (Arg154). In addition, we investigated the effect of amino acid substitutions at P5-Ser and P9-Asn in the GTS1 peptide. All of these mutant proteins were stable (Supplementary Table 7). Common hotspots on the HLA-A*11:01-GTS1 complex were apparent for the D2H, D13, and D30 TCRs (Fig. 6b–d). For example, mutations at Arg65 and Lys68 in the α1-helix of HLA-A*11:01 negatively impacted binding of the D13 and D30 TCRs, while mutations at Arg65 in α1-helix and Gln155 in the α2-helix of HLA-A*11:01 similarly impacted binding of the D2H and D30 TCRs. The corresponding mutant HLA-A*11:01-GTS1 tetramers displayed analogous staining patterns in titration experiments with the parental NS3$_{133}$ DENV-specific CD8^{+} T cell clones (Supplementary Fig. 12a). These findings indicate that a consensus footprint underpins TRBV11-2^{+} TCR-mediated recognition of HLA-A*11:01-GTS1. Of note, the mutation at Gln72 enhanced binding of the D13 and D30 TCRs to HLA-A*11:01-GTS1 (Fig. 6b,c, Supplementary Fig. 11, and Supplementary Table 6). A similar effect was

observed with the Asn59βAla mutant D30 TCR (Supplementary Fig. 10 and Supplementary Table 5). In the corresponding ternary structure, Gln72 interacted with Asn59β in the CDR2β loop (Fig. 6e). It therefore seems likely that the respective alanine substitutions allow more intimate contacts between the CDR2β loop and HLA-A*11:01-GTS1.

Alanine substitution of P9-Asn dramatically reduced binding of the D2H, D13, and D30 TCRs to HLA-A*11:01-GTS1 (Fig. 6b–e, Supplementary Fig. 11, and Supplementary Table 6). Similarly, replacement of P9-Asp with aspartic acid, a negatively charged residue that occurs naturally in the GTS2 peptide, abrogated binding of the D2H, D13, and D30 TCRs to HLA-A*11:01-GTS1. Analogous effects were observed in functional assays with the parental NS3$_{133}$ DENV-specific CD8$^+$ T cell clones (Fig. 6f and Supplementary Fig. 12b,c). Collectively, these data show that P9-Asn acts as a common hotspot for TRBV11-2$^+$ TCRs within a conserved footprint on the surface of HLA-A*11:01-GTS1.

DISCUSSION

In this study, we investigated the phenomenon of cross-serotype reactivity within the immunodominant HLA-A*11:01-restricted $NS3_{133}$ DENV-specific $CD8^+$ T cell response. The key findings were: (i) $TRBV11-2^+$ clonotypes are preferentially recruited during infections with DENV1 and DENV3/4; (ii) $CD8^+$ T cell clones expressing $TRBV11-2^+$ TCRs recognize the corresponding HLA-A*11:01-restricted peptides GTS1 and GTS3/4 more efficiently compared with GTS2; (iii) $TRBV11-2^+$ TCRs engage HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 with higher affinities relative to HLA-A*11:01-GTS2; and (iv) TCR bias in the $NS3_{133}$ DENV-specific $CD8^+$ T cell repertoire is governed by specific contacts between the germline-encoded CDR2β loop and the serotype-defined peptides GTS1 and GTS3/4.

The ternary structures of HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4 in complex with the $TRBV11-2^+$ D30 TCR revealed that Asn58β in the CDR2β loop hydrogen bonds with the peptide residue P9-Asn. In addition, the TRBV11-2 segment perfectly complements these serotype-defined peptides, because the CDR2β loop provides a neutral surface that facilitates docking with the neutral C-terminus of GTS1 and GTS3/4. The key P9-Asn residue is not present in GTS2, which instead contains a negatively charged P9-Asp. Alanine scanning mutagenesis experiments with the D30 TCR confirmed the importance of Asn58β in the overall interaction with HLA-A*11:01-GTS1. Reciprocal studies with mutant HLA-A*11:01-GTS1 molecules further demonstrated a common footprint for three distinct $TRBV11-2^+$ TCRs, all of which exhibited an absolute dependence on CDR2β-mediated contacts with P9-Asn. Accordingly, TRBV11-2 is preferentially incorporated in the $NS3_{133}$ DENV1/3/4-specific $CD8^+$ T cell repertoire as a function of peptide-driven contacts with germline-encoded regions of the TCR.

It is notable that other TRBV segments were commonly represented in the HLA-A*11:01-restricted $CD8^+$ T cell populations specific for GTS1 (TRBV9, TRBV12-3/4, and TRBV27) and GTS3/4 (TRBV4-1, TRBV9, and TRBV12-3/4). Indeed, TRBV11-2 and TRBV12-3/4 were expressed by > 50% of all clonotypes mobilized in response to GTS1 and GTS3/4. The CDR2β loops of TRBV11-2 (^{56}FQNNGV^{61}) and TRBV12-3/4 (^{56}FNNNVP^{61}) are

similar and share the key Asn58β residue that interacts directly with P9-Asn. Remarkably, Asn58β is also present in TRBV9 ([56]YYNGEE[61]) and TRBV27 ([56]SMNVEV[61]). A peptide-specific "mosaicism" therefore exists within the selected germline-encoded CDR2β loops. Such mosaics have only been observed previously for somatically rearranged components of the TCR[26].

Asn58β is also present in TRBV segments that are rarely selected in response to GTS1 and GTS3/4, including TRBV7-9, TRBV11-3, TRBV12-5, and TRBV23-1. All of these "neglected genes" encode CDR2β loops with a charged residue at position 59 (Arg or Glu). This observation is likely explained by the neutral surface around P9-Asn and adjacent residues in the HLA-A*11:01 molecule (Thr73–Val76). The exquisite specificity of TCR recognition focused on this area of the HLA-A*11:01p complex is further substantiated by the relative paucity of Asn58β[+] clonotypes in the corresponding GTS2-specific repertoires, which were dominated individually by TRBV4-1[+] ([57]YSYEKL[61]) and TRBV5-1[+] ([57]YFSETQ[61]) TCRs.

It should be noted that our findings do not imply a universal "peptide sensing" role for the *TRBV11-2* gene-encoded CDR2β loop. Indeed, a number of studies have reported that the CDR2β loop can adopt different configurations to engage other HLA class I-restricted peptides recognized by TRBV11-2[+] TCRs[21]. Similarly, descriptions of "interaction codons" in mice that pair certain major histocompatibility complex (MHC) allotypes with Vα3-3 or Vβ8-2[27, 28] do not predicate fixed rules of engagement for germline-encoded regions of the TCR[29]. Diverse recognition modes can also be anticipated on philosophical grounds, considering the limited number of *TRAV/TRBV* genes and the vast array of potential antigens and MHC class I/II molecules.

In outbred human populations, a complex interplay between host genetics and viral diversity, overlaid by the phenomenon of sequential infection with distinct serotypes, convolutes associative studies of disease outcome. Several correlates have nonetheless been identified in different parts of the world. For example, in South-East Asia, severe disease has been linked with *HLA-A*0:07, HLA-A*24, HLA-A*31, and HLA-B*53*, while protection has been linked with *HLA-A*03*, *HLA-B*18*, and the *HLA-B*44* supertype[30, 31, 32, 33,]

[34, 35]. There is also evidence that sequential patterns of infection can predispose to serious clinical manifestations. For example, in Cuba, severe disease has been observed in secondary infections with DENV2 or DENV3 following primary infection with DENV1[3, 36, 37]. More complicated scenarios have been reported in Nicaragua, where secondary infection with DENV2 clade NI-1, but not DENV2 clade NI-2B, causes severe disease in individuals with pre-existing immunity to DENV1[38]. However, it remains to be determined to what extent cross-serotype reactivity in the memory T cell pool is associated with enhanced protection or a greater risk of immunopathology in the setting of recurrent DENV infection[12, 13, 19, 39].

In summary, the data presented here reveal a novel mechanism of TCR bias, whereby serotype-defined epitope recognition is "hard-wired" into the human genome. This key finding demonstrates that germline-encoded residues can dictate the cross-reactivity profile of individual clonotypes targeting a variable antigenic determinant and potentially explains the lack of a reproducible correlation between clinical outcome and the magnitude of the $NS3_{133}$ DENV-specific $CD8^+$ T cell response[18, 19, 40, 41].

METHODS

Study population

Samples of venous blood were collected from DENV-infected patients at serial time points during acute infection and convalescence. Approval was granted by the Ethics Committees at Khon Kaen, Songkhla, and Siriraj Hospitals (Thailand), and by the Riverside Research Ethics Committee (UK). Informed consent was obtained in all cases. PBMCs and serum were isolated using density gradient centrifugation and cryopreserved according to standard protocols. The infecting serotype was identified using a DENV gene-based RT-PCR[42]. Secondary infection was defined as a DENV-specific IgM:IgG ratio < 1.8 in capture ELISAs[43]. The infection history of each patient was determined using micro-FRNTs[44]. Disease severity was classified according to World Health Organization criteria[45].

Identification of antigen-specific CD8$^+$ T cells

Antigen-specific CD8$^+$ T cells were labeled with optimal concentrations of the indicated HLA-A*11:01p tetramers (PE or APC) for 20 min at 37 °C, and then stained with the following monoclonal antibodies (mAbs) for 30 min at 4 °C: (i) αCD8-BV711 or αCD8-PerCP (BioLegend); (ii) αCD3-APC-H7, αCD14-V500, and αCD19-V500 (BD Biosciences); and, in some experiments, (iii) αTRBV11-2-FITC (Beckman Coulter). Non-viable events were excluded using Fixable Aqua or Violet Dead Cell Stain Kits (Thermo Fisher Scientific).

TCR clonotype analysis

Live CD3$^+$CD8$^+$CD14$^-$CD19$^-$ HLA-A*11:01p tetramer$^+$ cells were sorted at > 98% purity directly into O-ring microtubes (Sarstedt) containing 100 µL RNAlater (Applied Biosystems) using a custom-modified FACSAria II flow cytometer (BD Biosciences). Unbiased amplification of all expressed *TRB* gene products was conducted using a template-switch anchored RT-PCR as described previously[46]. At least 50 functional sequences were

analyzed per sample. Gene usage was determined according to the ImMunoGeneTics (IMGT) nomenclature[47].

Generation and maintenance of CD8^{+} T cell clones

NS3$_{133}$ DENV-specific CD8^{+} T cell clones were derived from parental lines stained with serotype-matched HLA-A*11:01p tetramers. Single tetramer^{+} events were sorted by flow cytometry and amplified in RPMI-1640 containing 10% human serum, 100 U/mL penicillin G, 100 μg/mL streptomycin, and 2 mM L-glutamine, together with mixed irradiated PBMCs, 40 μg/mL phytohaemagglutinin, and 200 U/mL IL-2. Cells were restimulated every 2–3 weeks using the same protocol. Clonally expressed *TRA* and *TRB* gene rearrangements were analyzed as described previously[46].

Intracellular cytokine staining

Clonal CD8^{+} T cells were incubated with HLA-matched B-lymphoblastoid cells at a ratio of 1:1 for 5 hr in the absence or presence of 1 μg/mL peptide (as indicated), αCD107a-FITC (BD Biosciences), brefeldin A (10 μg/mL; Sigma-Aldrich), and GolgiStop (0.7 μL/mL; BD Biosciences). Non-viable events were excluded using a Fixable Violet Dead Cell Stain Kit (Thermo Fisher Scientific) prior to surface staining with αCD8-PerCP (BD Biosciences). Cells were then fixed/permeabilized with BD Cytofix/Cytoperm (BD Biosciences), washed with BD Perm/Wash Buffer (BD Biosciences), and stained intracellularly with the following mAbs: (i) αIFN-γ-APC-Cy7 and αTNF-α-PE-Cy7 (BioLegend); and (ii) αIL-2-APC and αMIP-1β-PE (BD Biosciences). Staphylococcal enterotoxin B (1 μg/mL; Sigma-Aldrich) was used as a positive control. Data were acquired using a FACSVerse flow cytometer (BD Biosciences) and analyzed with FlowJo software version 7.6.5 (Tree Star Inc.).

IFN-γ ELISpot assay

Clonal CD8^{+} T cells were incubated in duplicate with mock-treated or peptide-pulsed HLA-matched B-lymphoblastoid cells at a ratio of 1:8 for 12–16 hr in 96-well polyvinylidene difluoride-backed plates (Millipore) pre-coated with an αIFN-γ capture mAb (1-D1K; Mabtech). IFN-γ spots were revealed using an ELISpot Kit (Mabtech) and counted using an automated ELISpot plate reader (Autoimmun Diagnostika GmbH).

Tetramer dilution assay

Clonal CD8^{+} T cells were incubated with various concentrations of the indicated wild-type or mutant HLA-A*11:01p tetramers for 15 min at 37°C. Non-viable events were excluded using a Fixable Violet Dead Cell Stain Kit (Thermo Fisher Scientific) prior to surface staining with αCD8-APC (BD Biosciences). Data were acquired using an LSR Fortessa flow cytometer (BD Biosciences) and analyzed with FlowJo software version 7.6.5 (Tree Star Inc.).

Protein expression and purification

The D2H, D13, and D30 TCRs were expressed, refolded, and purified using an engineered disulfide linkage between the α and β constant domains[48]. Soluble HLA-A*11:01 complexes incorporating the GTS1 (wild-type or mutant), GTS2, and GTS3/4 peptides were prepared as described previously[49, 50]. Site-directed mutagenesis was used to introduce single residue substitutions (alanine or glycine) into the D30 TCR β-chain (n = 13) and the HLA-A*11:01 heavy chain (n = 7).

Thermal stability assay

HLA-A*11:01p stability was determined using a thermal shift assay with a real time detection system (Rotor-Gene 3000; Corbett Life Science). Two concentrations of each HLA-A*11:01p complex (5 and 10 μM) were heated from 30 to 95 °C at a rate of 1 °C/min in 10 mM Tris-HCl pH 8 and 150 mM NaCl. Protein unfolding was monitored using the fluorescent dye Sypro Orange (Sigma-Aldrich). Fluorescence intensity was measured with excitation at 530

nm and emission at 555 nm. All experiments were performed in duplicate. The thermal melt point (T_M) represents the temperature at which 50% of the protein is unfolded.

Crystallization and structure determination

Crystals of HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4, either free or in complex with the D30 TCR, were grown by the hanging-drop, vapour-diffusion method. The proteins were incubated at a concentration of 5 mg/mL in 10 mM Tris-HCl pH 8 and 150 mM NaCl at a temperature of 20 °C with a protein/reservoir drop ratio of 1:1. The HLA-A*11:01p complexes were crystallized in 1.4–1.8 M NH_4SO_4, 0.2 M NaCl, 0.1 M Na cacodylate pH 6.5, and 2% ethylene glycol (EG). The TCR-HLA-A*11:01p complexes were crystallized in 17% polyethylene glycol (PEG) 8000, 2% EG, and 0.1 M HEPES pH 7.5. All crystals were soaked in a cryoprotectant solution containing mother liquor with the EG or PEG concentration increased to 30% (w/v), and then flash frozen in liquid nitrogen. Data were collected on the MX1 and MX2 beamlines at the Australian Synchrotron using the ADSC-Quantum 210 and 315r CCD detectors, respectively (at 100 K). Data were processed using XDS software[51] and scaled using SCALA software[52] from the CCP4 suite. Structures were determined by molecular replacement using the Phaser program[53], with the CF34 TCR as the search model for the D30 TCR (Protein Data Bank accession code, 3FFC)[48] and HLA-A*02:01 as the search model for peptide-free HLA-A*11:01 (Protein Data Bank accession code, 3GSO)[54]. Manual model building was conducted using Coot software[55] followed by maximum-likelihood refinement with the Buster program (http://www.globalphasing.com). The TCR was numbered according to the IMGT unique numbering system[47], whereby the CDR1 loops start at residue number 27, the CDR2 loops start at residue number 56, and the CDR3 loops start at residue number 105. The final model was validated using Protein Data Bank software, and the final refinement statistics are summarized in Table 2. All molecular graphics representations were created using PyMol (http://www.pymol.org).

Surface plasmon resonance

TCR binding to HLA-A*11:01p complexes was quantified using surface plasmon resonance. All experiments were conducted at 25 °C on a BIAcore 3000 or T200 instrument (GE Healthcare) with TBS/BSA buffer (10 mM Tris-HCl pH 8, 150 mM NaCl, 0.005% surfactant P20, and 1% bovine serum albumin) as described previously[48]. The D2H, D13, and D30 (wild-type and mutant) TCRs were immobilized separately on research-grade CM5 chips via a specific amine-coupled mAb[56]. Each HLA-A*11:01p complex was then tested in duplicate over a fluid phase concentration range of 0.78–200 µM (for the D2H TCR) or 1.56–400 µM (for the D13, wild-type D30, and mutant D30 TCRs). Data were analyzed with BIAevaluation software version 3.1 using the 1:1 Langmuir binding model.

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Figure 1 TRBV bias in $NS3_{133}$ DENV-specific $CD8^+$ T cell populations. (**a**) The most common serotype-defined variants of the $NS3_{133}$ DENV epitope in viral isolates from Thailand. Amino acid mutations are underlined. The epitope sequences from DENV3 and DENV4 are identical (GTS3/4). *Frequency as reported by the National Center for Biotechnology Information. (**b**) Representative flow cytometry data showing HLA-A*11:01p tetramer reactivity patterns in venous blood samples from patients with primary or secondary DENV infection. Plots are gated on $CD3^+CD14^-CD19^-$ events (left) or $CD3^+CD8^+CD14^-CD19^-$ events (middle and right). (**c**) Pie charts depicting concatenated TRBV transcript frequencies across all patients for the indicated $CD3^+CD8^+CD14^-CD19^-$ HLA-A*11:01p $tetramer^+$ populations. SP, single positive; DP, double positive; 3, 3/4 (pie legends). (**d**) Representative flow cytometry data showing TRBV11-2 expression versus HLA-A*11:01p tetramer reactivity in venous blood samples from patients with secondary DENV infection. Plots are gated on $CD8^+$ events. (**e**) TRBV11-2 expression frequency (%) within the indicated $CD8^+$ HLA-A*11:01p $tetramer^+$ populations. Each symbol represents one patient with secondary DENV infection. * $P < 0.05$ (Mann-Whitney U test).

Figure 2 Characterization of $NS3_{133}$ DENV-specific $CD8^+$ T cell clones and the binary structures of HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4. (**a**) Effector function profiles for the indicated $NS3_{133}$ DENV-specific $CD8^+$ T cell clones stimulated with GTS1, GTS2, or GTS3/4. The negative control is shown in each case (medium alone). Five readouts were measured by flow cytometry (CD107a, MIP-1β, TNF-α, IFN-γ, and IL-2). Data represent two independent experiments. Pie chart segments depict the fraction of cells expressing the number of functions indicated in the key. (**b**) TCRα and TCRβ sequences expressed by clones OX17H 2H (D2H), 44-173 13 (D13), and 44-173 30 (D30). (**c**) The binary structure of HLA-A*11:01 (white cartoon) in complex with GTS1 (blue stick). (**d**) The binary structure of

HLA-A*11:01 (white cartoon) in complex with GTS3/4 (purple stick). (**e**) Superposition of the structures in (c) and (d).

Figure 3 Structures of the D30 TCR bound to HLA-A*11:01-GTS1 and HLA-A*11:01-GTS3/4. (**a**) The overall D30 TCR-HLA-A*11:01-GTS1 complex (top) with atomic footprints on the surface of HLA-A*11:01-GTS1 (bottom; HLA-A*11:01, white surface; GTS1, gray surface). (**b**) The overall D30 TCR-HLA-A*11:01-GTS3/4 complex (top) with atomic footprints on the surface of HLA-A*11:01-GTS3/4 (bottom; HLA-A*11:01, white surface; GTS3/4, gray surface). The pie charts depict the relative contributions of each D30 TCR segment to the interaction with each HLA-A*11:01p complex. Top: TCRα, light pink; TCRβ, pale blue; HLA-A*11:01, gray; GTS1, black stick; GTS3/4, magenta stick. Bottom: CDR1α, teal; CDR2α, green; CDR3α, purple; CDR1β, red; CDR2β, orange; CDR3β, yellow; FWβ, pale blue. Black spheres represent the mass center of Vα and Vβ.

Figure 4 Interactions between the D30 TCR and HLA-A*11:01-GTS1. (**a**) Interaction between the CDR1α loop (teal) and HLA-A*11:01 (gray). The peptide is depicted in black, with the Cα of P1-Gly as a sphere. (**b**) Interaction between the CDR3α loop (purple) and residues in the α1-helix of HLA-A*11:01 (gray). (**c**) The CDR2β loop (orange) and FWβ (pale blue) are positioned above a long stretch of the HLA-A*11:01 α1-helix (gray). (**d**) The CDR3β loop (yellow) bridges the antigen-binding cleft to contact both helices of HLA-A*11:01 (gray). (**e**, **f**) The GTS1 peptide (black) is mainly contacted by the CDR3β loop (yellow) and the CDR2β loop (orange). Red dashed lines represent hydrogen bonds, and blue dashed lines represent van der Waals contacts.

Figure 5 Asn58β usage in $NS3_{133}$ DENV-specific $CD8^+$ T cell populations. (**a**) Pie charts depicting concatenated frequencies of $Asn58β^+$ (purple and cyan) and $Asn58β^-$ (orange) TRBV segments across all patients for the indicated $CD3^+CD8^+CD14^-CD19^-$ HLA-A*11:01p

tetramer[+] populations. The Asn58β[+] TRBV segments are subdivided according to the absence (purple) or presence (cyan) of a charged residue at position 59. (**b**) Individual TRBV frequencies and associated CDR2β sequences in the same dataset. The highest frequencies are shown in blue for each CD3^{+}CD8^{+}CD14^{−}CD19^{−} HLA-A*11:01p tetramer^{+} population (GTS1, GTS2, and GTS3/4). The conserved Asn58β residue is highlighted in red, and the charged residues at position 59 are highlighted in cyan (CDR2β).

Figure 6 Energetic hotspot analysis of TRBV11-2[+] TCR interactions with HLA-A*11:01-GTS1. (**a**) Energetic footprint on the D30 TCR. Mutated residues are colored to indicate effects on the D30 TCR interaction with HLA-A*11:01-GTS1: yellow, minimal impact (< 3-fold change in affinity); orange, moderate impact (3–5-fold decrease in affinity); red, critical impact (> 5-fold decrease in affinity); blue, positive impact (> 3-fold increase in affinity). TCRα, pale pink; TCRβ, pale blue. (**b**) Energetic footprint on HLA-A*11:01 (white surface) complexed with GTS1 (gray surface) for the D30 TCR. Mutated residues are colored as in panel (a). (**c**) Energetic footprint on HLA-A*11:01 (white surface) complexed with GTS1 (gray surface) for the D13 TCR. Mutated residues are colored as in panel (a). (**d**) Energetic footprint on HLA-A*11:01 (white surface) complexed with GTS1 (gray surface) for the D2H TCR. Mutated residues are colored as in panel (a). (**e**) Detail of the critical interaction between Asn58β (CDR2β) and P9-Asn (GTS1). Residues are colored as in panel (a). (**f**) Functional sensitivity of the NS3$_{133}$ DENV-specific CD8^{+} T cell clones 44-173 13 (D13) and 44-173 30 (D30) for the indicated GTS1 mutant peptides. The TRBV7-6^{+} NS3$_{133}$ DENV-specific CD8^{+} T cell clone E5 was included as a control. EC$_{50}$ values represent the peptide concentration required to elicit a half-maximal response in IFN-γ ELISpot assays (μg/mL). Data represent three independent experiments. Yellow, minimal impact (< 5-fold change in EC$_{50}$); red, critical impact (> 10-fold increase in EC$_{50}$).

TCR	GTS1	GTS2	GTS3/4
D2H	7.35 ± 0.90 μM	> 200 μM	9.75 ± 0.13 μM
D13	> 400 μM	> 400 μM	> 400 μM
D30	136.0 ± 7.0 μM	> 400 μM	142.5 ± 2.5 μM

Representative surface plasmon resonance data are shown in Supplementary Figure 7. The listed K_{Deq} values were averaged over three independent experiments carried out in duplicate (mean ± SEM).

Table 1 Equilibrium binding affinities of soluble HLA-A*11:01-GTS1, HLA-A*11:01-GTS2, and HLA-A*11:01-GTS3/4 complexes to the D2H, D13, and D30 TCRs.

	TCR-HLA-A*11:01-GTS1	TCR-HLA-A*11:01-GTS3/4	HLA-A*11:01-GTS1	HLA-A*11:01-GTS3/4
Data collection				
Space group	$P\,2_1 2_1 2_1$	$P\,2_1$	$C\,2$	$C\,2$
Cell dimensions				
a, b, c (Å)	88.06, 146.38, 167.56	77.39, 170.54, 85.69	155.50, 150.20, 105.83	158.32, 145.52, 106.30
α, β, γ (°)		β = 113.89	β = 120.53	β = 121.14
Resolution (Å)	47.17 – 2.95	46.01 – 3.20	49.09 – 3.15	48.97 – 2.85
	(3.05 – 2.95)	(3.37 – 3.20)	(3.32 – 3.15)	(3.00 – 2.85)
Total no. of observations	396406	122531	154836	179270
	(39145)	(17850)	(22722)	(27072)
No. of unique observations	46399	33473	36207	47263
	(4499)	(4865)	(2578)	(7023)
Multiplicity	8.5 (8.7)	3.7 (3.7)	4.3 (4.3)	6.7 (2.1)
Data completeness (%)	100.0 (100.0)	99.8 (99.9)	99.9 (99.9)	98.2 (100.0)
I/σ_I	7.2 (2.0)	6.7 (2.7)	14.8 (1.9)	6.7 (2.1)
R_{pim}[a] (%)	11.3 (48.2)	13.1 (36.4)	5.4 (44.6)	8.8 (35.3)
Refinement				
Non-hydrogen atoms				
Protein	13216	13217	9426	9439
Water	364	2	91	339
R_{factor}[b] (%)	18.9	17.5	17.70	20.05
R_{free}[b] (%)	25.4	24.6	23.07	22.99
Rmsd from ideality				
Bond lengths (Å)	0.007	0.008	0.010	0.007
Bond angles (°)	1.02	1.09	1.224	0.99
Ramachandran plot (%)				
Favored	95.1	92.4	90.8	96.4
Allowed	5.0	6.5	7.9	3.1
Disallowed	0.9	1.1	1.3	0.5

[a]$R_{pim} = \Sigma_{hkl} [1/(N-1)]^{1/2} \Sigma_i \mid I_{hkl,\,i} - \langle I_{hkl}\rangle \mid / \Sigma_{hkl} \langle I_{hkl}\rangle$. [b]$R_{factor} = \Sigma_{hkl} \mid\mid F_o \mid - \mid F_c \mid\mid / \Sigma_{hkl} \mid F_o \mid$ for all data except ~ 5%, which were used for R_{free} calculation. Values in parentheses refer to the highest resolution bin for each dataset.

Table 2 Data collection and refinement statistics for the structure of HLA-A*11:01 presenting GTS1 or GTS3/4, either free or bound to the D30 TCR.

a

Serotype	Sequence	Frequency*
DENV1	GTSGSPIVNR	35/35
DENV2	GTSGSPIIDK	61/62
DENV3	GTSGSPIINR	32/32
DENV4	GTSGSPIINR	14/17

b

Primary
DENV1

Secondary
DENV1/DENV4

DENV3/DENV2

c

Primary   Secondary →

DENV1   DENV1/DENV2   DENV1/DENV3 or 4   DENV2/DENV3 or 4

1SP   1SP   1SP   3SP   1/3DP   2SP   3SP   2/3DP

3-1 5-1 6-1 6-6 7-8 10-2 11-3 15 20-1 27
4-1 5-4 6-2/3 6-5 9 10-3 12-3/4 18 23-1 28
4-2 5-5 6-5 7-3 11-2 12-5 19 24-1 29-1
7-2
7-9

d

GTS1   GTS2

e

Figure 1

a

	TRBV11-2			TRBV12-3/4		TRBV7-6	TRBV2
	44-173 13	44-173 30	OX17 2H	N15 22	44-038 25	E5	D9

Legend: 0, 1, 2, 3, 4, 5

Rows: Medium, GTS1, GTS2, GTS3/4

b

OX17 2H

TRBV	CDR3β	TRBJ
11-2	CASTTGGGGYEQY	2-7

TRAV	CDR3α	TRAJ
9-2	CALDSGNTGKLI	37

44-173 13

TRBV	CDR3β	TRBJ
11-2	CASSLGQGSYEQY	2-7

TRAV	CDR3α	TRAJ
8-2	CWSPFGNEKLT	48

44-173 30

TRBV	CDR3β	TRBJ
11-2	CASSLGQGLLYGYT	1-2

TRAV	CDR3α	TRAJ
30	CGLGDAGNMLT	39

c

GTS1

P5-Ser, P8-Val, P9-Asn, P4-Gly, P7-Ile, P2-Thr, P10-Arg, P1-Gly

d

GTS3/4

P5-Ser, P8-Ile, P9-Asn, P4-Gly, P7-Ile, P2-Thr, P10-Arg, P1-Gly

e

GTS1 and GTS3/4

P5-Ser, P8-Val/Ile, P9-Asn, P4-Gly, P7-Ile, P2-Thr, P10-Arg, P1-Gly

Figure 2

a

β-D30

α-D30

GTS1

HLA-A*11:01

β2m

1α
2α
3α
FWβ
1β
2β
3β

α2-helix

α1-helix

Vβ

Vα

GTS1

Figure 3

b

β-D30

α-D30

GTS3/4

HLA-A*11:01

β2m

1α
2α
3α
FWβ
1β
2β
3β

α2-helix

α1-helix

Vβ

Vα

GTS3/4

Figure 4

a

■ Asn58β	■ Asn58β/59β	■ x58β

b

	CDR2β	TRBV	GTS1	GTS2	GTS3/4
Asn58β	YYNGEE	9	12.11	10.83	7.08
	FQNNGV	11-2	38.76	5.41	45.50
	FNNNVP	12-3/4	28.38	0	8.40
	SMNVEV	27	7.32	0	4.92
Asn58β/59β	YNNKEL	3-1	0.41	0	0.36
	FQNEAQ	7-8	5.09	23.36	16.21
	FQNEAQ	7-9	0	2.28	1.44
	YENEEA	11-3	0.20	0	0.24
	FRNRAP	12-5	0.31	0.28	0
	FQNEQV	23-1	0.10	0	0
x58β	YSYEKL	4-1	2.64	24.79	3.36
	YNFKEQ	4-2	0	0	0.12
	YFSETQ	5-1	0	5.41	0
	YYREEE	5-4	0.20	0	0
	YYEKEE	5-5	0.41	0	0
	SASEGT	6-1	0.10	1.42	0.60
	SVGEGT	6-2	0	2.85	0
	SVGAGI	6-5	0.20	0.57	0.48
	SVGAGI	6-6	0	0	0.24
	FQGNSA	7-2	0	0.85	0
	FQGTGA	7-3	1.02	3.99	3.24
	SAAADI	10-2	0	10.82	4.56
	SYGVKD	10-3	0.31	2.85	0.36
	YYDKDF	15	0.81	2.56	0.48
	LQKENI	18	0.10	0.28	0.12
	SQIVND	19	0.41	1.42	0
	SNEGSKA	20-1	0.92	0	0.48
	SFDVKD	24-1	0	0	0.48
	SYDVKM	28	0.20	0	1.20
	ANQGSEA	29-1	0	0	0.12

a D30 TCR

L81β
N58β
N59β
Q57β
G28β
Q110β
A30β
G111β
L108β
L112β
G109β
L113β

b HLA-A*11:01-GTS1
(D30 TCR)

K68
Q72
R65
V76
N9
S5
Q155
R145
E154

c HLA-A*11:01-GTS1
(D13 TCR)

K68
Q72
R65
V76
N9
S5
Q155
R145
E154

d HLA-A*11:01-GTS1
(D2H TCR)

K68
Q72
R65
V76
N9
S5
Q155
R145
E154

e

L112β
L108β
G28β
Q57β
N58β
N59β
Q72
N9
V76
GTS1
R145

f

EC$_{50}$	GTS1	S5A	N9A	N9D
D13	0.11	0.064	0.414	3.00
D30	0.085	3.94	0.231	4.65
E5	0.003	0.007	0.01	0.02

Figure 6