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STRAPLINE: DIABETES

A predictive CD8$^+$ T cell phenotype for T1DM progression

In a cross-sectional study of individuals with type 1 diabetes mellitus, those who were designated to be slow disease progressors had an increased proportion of autoreactive islet-specific CD8$^+$ T cells expressing an 'exhausted' phenotype. By contrast, rapid progressors had increased numbers of islet-specific CD8$^+$ T cells with a transitional memory phenotype.

Refers to Wiedeman, A.E. et al. Autoreactive CD8$^+$ T cell exhaustion distinguishes subjects with slow type 1 diabetes progression. J. Clin. Invest. https://doi.org/10.1172/JCI126595 (2019).

At the time of type 1 diabetes mellitus (T1DM) onset, individuals have different levels of β-cell loss, detected by measuring serum C-peptide (a by-product of insulin synthesis), which contributes to heterogeneity of T1DM. Furthermore, a study examining pancreatic islets by histology found evidence of increased loss of β-cells, increased loss of insulin staining and increased immune infiltration with increased numbers of intra-islet CD8$^+$ T cells and B cells in individuals who developed T1DM aged <7 years[1]. Understanding the immunological

factors that contribute to these differences is important to stratify different progression phenotypes and could inform future phenotype-dependent immunotherapies.

In the present study, Alice Wiedeman and colleagues[2] studied cryopreserved peripheral blood mononuclear cells from HLA-A2$^+$ individuals (20 healthy control participants and 46 individuals with T1DM). The people with T1DM were stratified into two groups based on the serum level of C-peptide — slow and fast progression of disease at 5 years after onset. The authors categorized individuals with <0.05 ng/ml C-peptide 5 years after diagnosis as rapidly progressing and individuals with >0.1 ng/ml C-peptide as slow progressing, regardless of the age of onset of the participants studied[2].

Mass cytometry with readout by time-of-flight (CyTOF) enables scientists to analyse a vast range of cell markers not hitherto possible with conventional flow cytometry. Combined with MHC–peptide-tetramer technology, the authors of the present study examined the very small subsets of antigen-specific T cells and phenotypically characterized the rare islet-specific CD8$^+$ T cells. Using the analytical tool, DISCOV-R, Wiedeman and co-authors showed that the overall frequency of islet-specific CD8$^+$ T cells did not differ between the rapid and slow progressors[2]. However, within the islet-specific CD8$^+$ T cell populations, the authors demonstrated the presence of a number of subsets based on the expression of previously identified cell surface and intracellular transcription markers for T cells. In particular, slow progressors had increased numbers of islet-specific CD8$^+$ T cells with an exhausted phenotype (**Figure 1**). These antigen-specific exhausted CD8$^+$ T cells expressed high levels of the chemokine receptor CXCR3 and the transcription factor EOMES. By contrast, the rapid progressors had increased numbers of islet-specific CD8$^+$ T cells that also expressed CXCR3 and the regulatory HELIOS+ transitional memory phenotype (**Figure 1**) [2]. Interestingly, these changes were not related to T1DM duration or age of the patients.

Previous studies have documented a transcriptional signature indicating that exhausted $CD8^+$ T cells occur as a result of a balance between stimulatory and inhibitory signals from other subsets of T cells, and have suggested that strategies to increase numbers of exhausted $CD8^+$ T cells could be beneficial in autoimmune disease[3]. Interestingly, the present study suggests that the increased presence of exhausted islet-specific $CD8^+$ T cells could slow down the progression of β-cell loss caused by autoimmune destruction[2]. $CD8^+$ T cells with the exhausted phenotype are also found in tumours, where these exhausted cells are unable to kill the tumour cells. Furthermore, exhausted cells are seen in chronic viral infections, where the host is unable to mount an effective immune response [4].

Given the emerging understanding of events leading to exhausted cells in both tumours and chronic infection, it is important to investigate what is occurring to other subsets of cells that interact with the $CD8^+$ T cells, leading to exhaustion[5]. Findings from studies in the past few years indicate that the help of $CD4^+$ T cells is a mechanism by which exhaustion is overcome. Understanding the biology of $CD4^+$ T cells in conditions with prominent $CD8^+$ T cell exhaustion could be of specific importance in relation to the current finding in T1DM, and $CD4^+$ T cells might also provide a therapeutic target[6].

The observations from the careful study by Wiedeman and colleagues[2] are an important contribution to our understanding of immunological events in the pancreas which is an organ that is very inaccessible. Of note, some overlap in phenotypes occurred between slow and rapid progressors; some slow progressors had lower numbers of exhausted islet-specific $CD8^+$ T cells and higher numbers of transitional HELIOS+ cells, which is similar to the rapid phenotype. Thus, combining phenotype studies with some functional studies is important, as the authors of the present study attempted to do in a subset of study participants[2].

Of note, the techniques to examine very small subsets of cells used in the present study[2] require highly sophisticated analytical tools, which are potentially not accessible for many investigators and clinicians in smaller centres and/or clinics. Thus, this approach might not be used routinely in the near future to stratify individual patients. The finding from the current study is focused on individuals with an HLA-A2 haplotype, who constitute up to 50% of patients with T1DM. With time, more information will become available about other HLA types when antigenic epitopes are identified — currently only limited epitopes are available for HLA-A24 and B39[7]. It will be interesting to understand if antigen-specific $CD8^+$ T cells interact with other subsets of cells, including B cells and $CD4^+$ T cells, which have also been found in islets[1].

In addition to the differences in disease progression rate, individual responses to immunotherapy at the onset of clinical T1DM are heterogeneous, which correlates with immune cell signatures in peripheral blood[8]. Thus, understanding the phenotype and functional processes of the underlying immune cell subsets, particularly of antigen-specific T cells in the individuals presenting with different speed of progression, might have an important bearing on the response to immunotherapy. The different rates of loss of endogenous β-cell function make it imperative that immunotherapeutic treatment is started as early as possible, particularly in rapid progressors.

Thus far, several immunotherapeutic strategies have shown transitory effects in reducing the rate of loss of endogenous β-cell function after diagnosis; however, differential effects occur in subsets of treated individuals, which might relate to heterogeneous immune cell profiles. In 2019, the anti-CD3 monoclonal antibody teplizumab was found to delay disease onset in high risk relatives of people with T1DM[9]. Interestingly, response to the treatment correlated with increases in the number of $CD8^+$ T cells with an exhausted

phenotype[9]. Understanding the functional correlates of heterogeneous immune cell profiles will aid in the design of targeted therapy and in this context, promoting antigen-specific immune exhaustion could be of value. Moreover, the current finding[2] might help to shed light on why anti-checkpoint therapy for cancer, which reverses an exhausted $CD8^+$ T cell phenotype, has in some cases led to the development of autoimmune diabetes mellitus. This finding could also have important implications for testing individuals for autoimmune propensity and preparing them for possible autoimmunity when these cancer immunotherapies are used.

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Competing interests

The authors declare no competing interests.

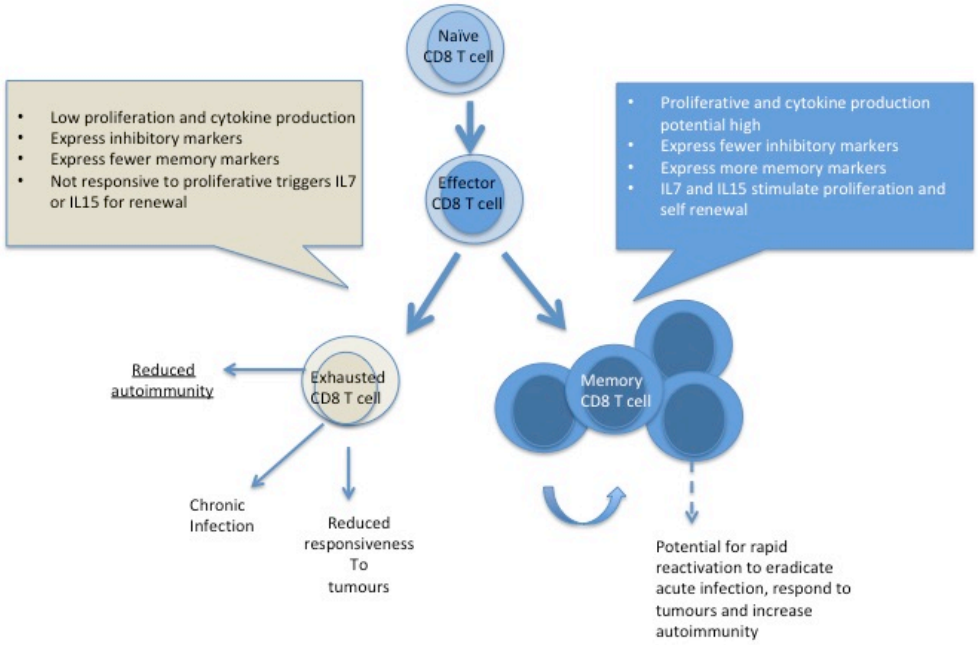

Figure 1. Characteristics of exhausted and memory T cells. Naive $CD8^+$ T cells differentiate to effector cells and a subset of these effectors differentiate to memory cells. In addition, if there is chronic antigen stimulation and an imbalance between stimulatory and inhibitory stimuli, then $CD8^+$ T cells might become exhausted[4, 5] The main types of memory cells are designated effector memory T_{EM} and central memory T_{CM}, distinguished by their surface markers, cytokine production and their effector functions. T_{EM} are able to respond with rapid effector function, while T_{CM} self renew and are maintained as a long-lived memory population [10]. However, there are other subsets, including transitional memory cells (intermediate between T_{EM} and T_{CM}) and those referred to in this article also express the regulatory marker HELIOS.