




The many faces of islet antigen-specific CD8 T cells: clues to clinical outcome in type 1 diabetes

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Abstract

Immune monitoring enables a better understanding of disease processes and response to therapy, but has been challenging in the setting of chronic autoimmunity because of unknown etiology, variable and protracted kinetics of the disease process, heterogeneity across patients and the complexity of immune interactions. To begin to parse this complexity, we focus here on type 1 diabetes (T1D) and CD8 T cells as a cell type that has features that are associated with different stages of disease, rates of progression and response to therapy. Specifically, we discuss the current understanding of the role of autoreactive CD8 T cells in disease outcome, which implicates particular CD8 functional subsets, rather than unique antigens or total number of autoreactive T cells. Next, we discuss how autoreactive CD8 T-cell features can be reflected in measures of global CD8 T cells, and then pull these concepts together by highlighting immune therapies recently shown to modulate both CD8 T cells and disease progression. We end by discussing outstanding questions about the role of specific subsets of autoreactive CD8 T cells in disease progression and how they may be optimally modulated to treat and prevent T1D.

INTRODUCTION

Genetics and immune monitoring have been used to identify disease state, severity and response to therapy in the context of cancer and infectious diseases, and this monitoring is key to accelerating personalized medicine. However, it has been more challenging to apply these tools to chronic autoimmune disease for which most are polygenic, the etiology is poorly understood, disease manifestations can differ and multiple pathways are implicated in disease. These challenges may be overcome, in part, by focusing on the impact of a single-cell type implicated in disease, and the way that cell type interacts with other cell populations to drive disease progression. Other immune cells including islet antigen-specific B cells and CD4 T cells have been implicated in disease susceptibility and are supported by the association of type 1 diabetes (T1D) with selected human leukocyte antigens. Here, we highlight our understanding of CD8 T cells in T1D, focusing on human autoreactive CD8 T cells and their recently better appreciated role in disease progression and outcomes.

T1D is an autoimmune disease in which the insulin-producing islet beta cells of the pancreas are destroyed, leading to a lifelong dependence on daily insulin therapy. T1D is clinically defined in four stages: stage 1 occurs with the presence of two or more autoantibodies while blood sugar levels remain normal; stage 2 occurs with two or more autoantibodies and impaired glucose tolerance, implicating some decline in beta cell function; stage 3 occurs with acute clinical presentation of loss of glycemic control requiring insulin therapy, commonly referred to as recent onset; and stage 4 includes long-standing T1D when even further loss of beta cells can occur.¹ Almost all individuals with two or more islet autoantibodies will eventually be clinically diagnosed with T1D; however, the rate of disease progression differs within disease stages and between individuals.^{1–5} This heterogeneity is partly explained by age, as pediatric patients tend to progress more rapidly than adults.^{1,5} However, other factors are needed to understand differing rates of progression transitioning from one stage to the next, as well as within the stages of T1D.

Intervening when the disease is most active or in those with the fastest rate of progression may be most efficacious, based on the fact that the majority of successful clinical trials at stage 3 of disease have shown increased responsiveness in pediatric cases, who typically progress more rapidly. Thus, it is key to find biomarkers of disease progression, both as rate of progression and as the transition between stages of disease, and determine association with outcome as it relates to response to therapy, in order to better understand disease mechanisms and target therapies. In this review, we focus on the contribution of human islet antigen-specific CD8 T cells to T1D disease progression. We start by providing arguments for focusing on CD8 T cells, then integrate recent evidence linking the function of islet antigen-specific CD8 T cells to T1D progression and end by addressing the role of CD8 T cells in the outcome of clinical immunotherapy trials.

CD8 T CELLS PLAY A KEY ROLE IN T1D DISEASE PROGRESSION

The non-obese diabetic mouse model of T1D has been a key tool in understanding mechanisms of autoimmune disease susceptibility, progression and treatment as one of the few spontaneous animal models of autoimmunity, and reliably had those findings confirmed in humans. CD8 T cells are one of the major cell types infiltrating the pancreatic islets of non-obese diabetic mice as disease progresses.⁶ In humans, CD8 T cells are also a major component of immune cell infiltration in pancreatic islets of cadaveric donors who died with T1D.^{7,8} In humans, as disease progresses, the number of islet antigen-specific cells increases and focuses to the islet beta cells,⁹ and increased numbers of islet antigen-specific cells correlate with islet graft rejection,¹⁰ suggesting an antigen-driven process. However, the simple presence of autoreactive CD8 T cells is not indicative of autoimmunity, as it has been shown that islet antigen-specific CD8 T cells are also present in the circulation and throughout the pancreas of healthy individuals.^{9,11} Additional support of CD8 T cells playing a role in T1D includes Class I human leukocyte antigen B39 and A24 association with T1D, and more rapid disease onset.¹² CD8 T cells have been shown to cluster in regions where Class I expression is increased in the pancreas of T1D patients.^{13,14} In addition, several single-nucleotide polymorphisms that are associated with T1D are implicated in CD8 T-cell biology including *PTPN22*, *IL2RA* and *PTPN2*.¹² Together, these data strongly support a role for CD8 T cells in T1D progression, whether it is driving the transition to the next stage of disease or influencing the rate of progression.

IDENTIFYING AND QUANTIFYING ISLET ANTIGEN-SPECIFIC CD8 T CELLS IN T1D

The specificity of the immune response toward the pancreas, and thus the identification of specific pancreatic antigens, makes T1D a model disease in which to study autoreactive cellular immune responses. In 1974, insulin was confirmed as an autoantigen in T1D by the discovery of insulin-specific autoantibodies,¹⁵ but it was not until decades later that the presence of insulin antigen-specific CD8 T cells was confirmed in T1D.¹⁶ Since that time, multiple pancreatic targets of CD8 T cells in T1D have been reported, including well-characterized unmodified islet antigens, but also a growing list of neoantigens including proteins with post-translational modification, hybrid proteins and defective translational products termed defective ribosome products or DRiPs.¹⁷ Of those reported, approximately seven unmodified islet antigens are solidified as targets of CD8 T cells in T1D. These include insulin/preproinsulin, glutamic acid decarboxylase (GAD65), islet tyrosinase phosphatase and zinc transporter (ZnT8) corresponding to the autoantibody targets typically included in natural history studies, as well as islet-specific glucose-6-phosphatase catalytic subunit-related protein, chromogranin A and islet amyloid polypeptide. Insulin-specific CD8 T cells can also be activated by exogenous insulin used as therapy in stages 3 and 4 of T1D, so understanding whether cells are reacting to a common region of preprocessed and secreted insulin is an important variable to consider. Identification of specific CD8 T-cell epitopes is a rapidly evolving field, and have been reviewed in detail elsewhere,^{18,19} and thus are not specifically addressed here.

Technologies to identify and quantify autoreactive T cells directly *ex vivo* broadly include protein or peptide stimulation of T cells, and binding of labeled major histocompatibility complex (MHC)–peptide multimers.²⁰ The benefit of the stimulation assay is that reactivity to multiple different antigens or epitopes may be tested simultaneously and the assay does not necessarily require the individuals to have a particular human leukocyte antigen; however, detection depends on the ability of the T cells to respond to the stimuli by proliferation, cytotoxicity, expression of activation markers (i.e. CD137) or cytokine production, and most assays (e.g. ELISpot) do not allow for further characterization of the antigen-specific cells. Typically the function of islet antigen-specific CD8 T cells is then confirmed by generating T-cell lines and clones,²¹ but this method inherently selects for and enhances the effector properties of the cells. By contrast, peptide–MHC multimers are highly specific for a single epitope and human leukocyte

antigen, limiting their scope, but can identify cells regardless of functional response, and also allow for isolation of unmanipulated multimer-specific cells for subsequent phenotypic and functional analyses.

Because of the technical difficulty and safety risk of pancreatic biopsy,²² studies of autoreactive T cells in T1D have primarily utilized circulating cells. Peripheral blood CD8 T cells responding to pancreatic antigens have been reported in both T1D patients and healthy controls. With the use of stimulation assays, the quantity of islet antigen-specific CD8 T cells is nearly universally reported to be greater in T1D patients than in healthy controls,^{16,23–34} and this extends to at-risk autoantibody-positive first-degree relatives^{23,26} (Table 1). By contrast, by peptide–MHC multimer assays, which do not require functionality of a cell for its detection, the results are mixed. In some cases, greater frequency of autoreactive cells is observed in T1D than in healthy controls,^{35–40} whereas for others there are no significant differences^{11,41–45} across multiple specificities. The lack of consistency in these findings from peptide–MHC multimer assays suggests that other biological factors are contributing to the detection of islet antigen-specific CD8 T cells. However, the fact that autoreactive CD8 T cells are found in healthy controls without apparent harm, and that the result of stimulation assays differs from peptide–MHC multimer assays, suggests that their function or phenotype, rather than strictly their quantity, may be a more important feature driving disease progression.

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF ISLET ANTIGEN-SPECIFIC CD8 T CELLS IN T1D

CD8 T cells exhibit a broad range of functional and developmental states, including effector, regulatory and hypofunctional states across stages of memory differentiation.^{46–48} Circulating islet antigen-specific CD8 T cells are well established as phenotypically heterogeneous,^{11,35,37,40,43–45,49–52} and encompass a spectrum of functional and developmental states. This is exemplified between two individuals with established T1D that were assayed for islet antigen-specific CD8 T cells as compared with chronic virus-specific cells (Figure 1a). To avoid confounding results with insulin therapy, insulin specificities were detected separately from a pool of other islet antigens. Notably, while both islet antigen- and insulin-specific T-cell phenotypes are heterogeneous, the cells are more represented in particular phenotypic states: overall, both specificities have a substantial proportion in the exhausted subset that is also shared by chronic virus-specific cells, but islet antigen-specific cells are more

abundant in early memory phenotypes, whereas insulin-specific cells are abundant in CD57⁺ terminal effector subsets, reflecting repeated exposure to antigen.⁴³

Stimulation assays, which rely on an activation response to detect islet antigen-specific CD8 T cells have, by definition, characterized those cells as effector cells. Consistent with this, ELISpot assays demonstrate that the effector response to islet antigens is much greater in T1D patients than controls (Table 1), and islet antigen-specific CD8 T cells from T1D patients have demonstrated enhanced expansion in response to antigen stimulation.^{41,53} Peptide–MHC multimer detection, however, has revealed a preponderance of other phenotypes of islet antigen-specific CD8 T cells, including functional states that may not be activated or proliferate to the same degree as effector CD8 T cells. Interestingly, these include a full range of differentiation states from early differentiation states characterized as naïve,^{40,44} stem cell memory^{45,50,54} and transitional memory,⁴³ to later differentiation states including terminal effector memory expressing CD57^{21,52} and exhausted⁵⁴ subsets, both of which are hyporesponsive functional states. This breadth and variability in phenotypic and functional properties of islet antigen-specific CD8 T cells raise the question of whether such characteristics are associated with current disease activity and/or rate of progression through stages of T1D (Figure 1b). Two pieces of evidence support phenotypic association with disease activity and rate of progression; whereas naïve cells are abundant in islet antigen-specific cells of both T1D patients and controls, there is skewing toward early memory at stage 3, during the progression to clinical disease.^{35,45,50} Furthermore, some cross-sectional and longitudinal studies of T1D patients indicate that the number of peripheral islet antigen-specific CD8 T cells detected by ELISpot assays (presumably effector cells) wanes with disease duration (Table 1). However, because this was not found in all studies and has not been addressed using multimer assays, further investigation is needed.

The finding that islet antigen-specific CD8 T cells in the pancreas become more differentiated with disease progression supports observations of CD8 T-cell differentiation in the periphery described above. Indeed, islet antigen-specific CD8 T cells are found at greater frequency in the insulitic islets of T1D pancreas donors,^{7,9,11,44,55} and are enriched in a memory phenotype.⁹ By contrast, islet antigen-specific cells are also found in the pancreas of healthy controls, but they are not enriched in islets and maintain a naïve phenotype.^{9,11,44} As a highly vascularized organ, cells in the pancreas likely also circulate into the periphery,⁵⁶ particularly after destruction of beta cells and therefore loss of the driving autoantigen. This would account for

Table 1. Islet-specific CD8 T-cell frequencies in T1D

Relationship	Method	Antigen	References	
T1D > HC	Stimulation	PPI ^a	16 ^c , 25,27,29 ^d , 31 ^{c,e} , 76,77	
		Insulin	16 ^{c,e} , 25,27,29 ^d	
		GAD65	23 ^b , 27–29 ^d , 77 ^d	
		IA-2	25,28,29,78	
		IAPP	24 ^e ,25,26 ^b ,30	
		IGRP	25,30,27,29 ^d ,32,26	
		ZnT8	34	
		ChgA	32	
		S100	33 ^c	
		pMHC Mmr	PPI ^a	36,37,38 ^{c,e} ,39,40 ^{c,e} ,42 ^c ,79
			Insulin	11 ^c ,36,38 ^c ,40 ^c ,42,79
			GAD65	35 ^b ,36,38,41
	IA-2		36,38 ^{c,e}	
	IAPP		36,38 ^{c,e}	
	IGRP		36,38 ^{c,e}	
	ZnT8		21 ^c	
	T1D = HC	Stimulation	Insulin–DRiP	80 ^c
		pMHC Mmr	GFAP	26 ^b
			PPI ^a	11 ^c ,40 ^c ,44,45
Insulin			11 ^c ,45	
GAD65			11 ^c ,44,45	
IA-2			11 ^c ,45	
IAPP			44	
IGRP			11 ^c ,45	
ZnT8			11 ^c	
Pooled antigens			43 ^c	

ChgA, chromogranin A; DRiP, defective ribosome product; GAD, glutamic acid decarboxylase; GFAP, glial fibrillary acidic protein; HC, healthy control; IA-2, islet tyrosinase phosphatase; IAPP, islet amyloid polypeptide; IGRP, islet-specific glucose-6-phosphatase catalytic subunit-related protein; pMHC Mmr, peptide–major histocompatibility complex multimer; PPI, preproinsulin; S100, neurotrophic factor S100-β; T1D, type 1 diabetes; ZnT8, zinc transporter 8.

Studies compare new-onset T1D (stage 3) patients with healthy controls, unless otherwise indicated.

^aEpitopes of preproinsulin (PPI) that are not contained in insulin therapy.

^bThe study also supports the relationship between autoantibody-positive individuals (stage 1 or 2) and healthy controls.

^cThe study also supports the relationship between long-standing T1D individuals (stages 4) and healthy controls.

^dThe study also supports a loss of autoreactive CD8 T cells over time in T1D in longitudinal studies of the same individual after onset.

^eThe study also supports a loss of autoreactive CD8 T cells over time in T1D in cross-sectional studies comparing recent-onset (stage 3) with long-standing T1D patients (stages 4).

the apparent phenotypic and functional features that distinguish peripheral islet antigen-specific cells of T1D patients from controls, even if their frequencies do not differ. In addition, the presence of islet antigen-specific CD8 T cells in the healthy pancreas raises the question of their role in both health and disease. In health, autoreactive CD8 T cells may survey the pancreas and kill dysfunctional cells.⁵⁷ In the disease state, increased availability of islet antigens or enhanced T-cell activation may augment this killing, thereby initiating or driving disease.⁵⁸ Together, beta cell health and T-cell state may be reflected in the phenotype and function of peripheral CD8 T cells, whether driven by intrinsic beta cell dysfunction or extrinsic inflammation.

ISLET ANTIGEN-SPECIFIC CD8 T-CELL PHENOTYPES ARE ASSOCIATED WITH DISEASE PROGRESSION

Not all individuals who develop autoimmune diabetes follow the same disease course. While some individuals progress quite rapidly, others maintain a detectable level of insulin production decades after clinical diagnosis.^{59,60} Understanding the factors that influence these divergent outcomes could point to important modulators of disease for prevention and treatment. One of the most utilized measures of beta cell function is C-peptide, a cleavage product generated 1:1 with active insulin during endogenous insulin secretion. Within the spectrum of

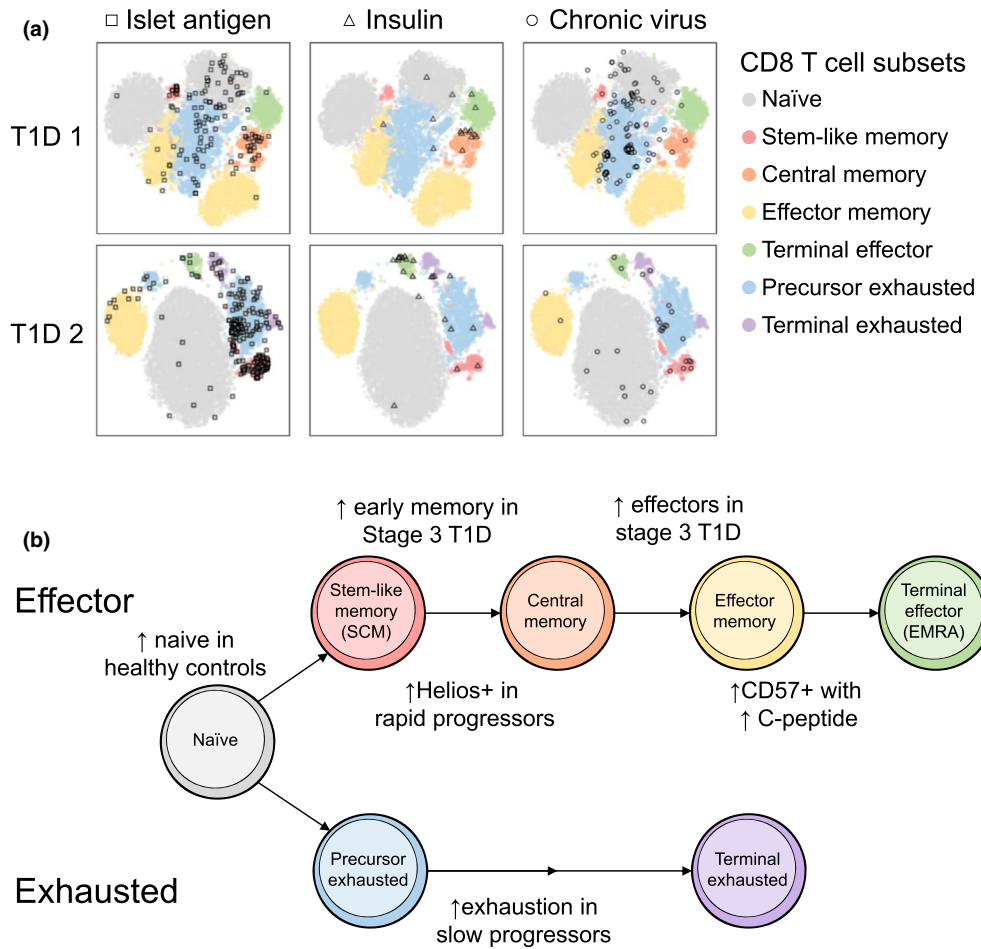


Figure 1. Islet antigen-specific CD8 T cells are phenotypically heterogeneous and differ by disease stage and rate of progression in type 1 diabetes (T1D). **(a)** Total and antigen-specific CD8 T cells of two representative established T1D patients (T1D 1 and T1D 2, 1.8 and 10.1 years since diagnosis, respectively) were identified using a cytometry by time of flight panel in combination with peptide-loaded major histocompatibility complex tetramer staining and then analyzed using DISCOV-R as in the study by Wiedeman *et al.*,⁴³ showing the distribution of antigen-specific T cells in the total CD8 landscape. Clusters are annotated by color and related differentiation state. **(b)** Islet antigen-specific CD8 T cells are present across the spectrum of CD8 T-cell differentiation states, including effector and exhausted pathways. Enrichment of particular phenotypes among islet antigen-specific CD8 T cells that are associated with transition to disease and rate of progression are noted. The naïve phenotype is present in T1D patients,^{40,44} but is more prevalent in healthy controls.^{35,45,50} Early memory subsets including stem-like memory,^{45,50,54} transitional memory⁴³ and effector cells are increased in stage 3 (Table 1). Terminal effector expressing CD57^{21,52} and exhausted⁵⁴ subsets have also been found. Importantly, prevalence of a Helios⁺ early memory phenotype is associated with more rapid progression of disease after onset, while a more terminal CD57⁺ effector memory⁵² or exhausted phenotype⁴³ is associated with beta cell health and preservation of C-peptide or slow progression.

reduced beta cell function in T1D, relatively higher levels and longer-term maintenance of insulin secretion are indicative of improved beta cell mass and/or function and better disease outcome. Two studies of distinct cohorts offer some initial clues as to the relationship between rate of disease progression and quantity of islet antigen-specific CD8 T cells. In one study, Hanna *et al.*³⁸ examined a unique group of T1D patients that retained C-peptide long after diagnosis (slow progressors). By peptide–MHC tetramer, they found that slow progressors had very low frequencies of

peripheral islet antigen-specific CD8 T cells, lower than new-onset or long-standing T1D cohorts, and comparable to healthy controls. This is consistent with a separate finding that identified lower frequencies of memory-like peripheral preproinsulin-specific cells in latent autoimmune diabetes in adults than in T1D cases.³⁷ Together, these studies support a role for the quantity of autoreactive CD8 T cells in defining disease progression.

Two key studies have addressed the phenotype of islet antigen-specific CD8 T cells in relation to the rate of disease

progression within T1D. Yeo *et al.*⁵² assayed longitudinal samples from adults and children newly diagnosed with T1D and correlated change in C-peptide with subsets of T-cell differentiation among islet antigen-specific CD8 T cells. They found that an increase in C-peptide among children (<12 years old at diagnosis), but not adults, was associated with an increase of CD57⁺ effector memory cells, which displayed enhanced cytotoxic potential. In a larger cross-sectional study, Wiedeman *et al.*⁴³ compared adult T1D patients with rapid or slow C-peptide loss after onset, and found that an exhausted-like phenotype among islet antigen-specific CD8 T cells was associated with slower progression, and a memory Helios⁺ subset was most dominant among rapid progressors, regardless of age (Figure 1b). Both studies demonstrate the importance of the phenotype of islet antigen-specific cells to disease progression after onset, and suggest that a terminally differentiated or exhausted state is beneficial. In neither study did islet antigen-specific CD8 T-cell phenotypes associate with absolute levels of C-peptide, but instead, they correlated with degree or rate of change in beta cell function. Furthermore, healthy control and long-standing T1D patients exhibited similar frequencies and phenotypes of islet antigen-specific CD8 T cells.⁴³ This suggests that the quality of autoreactive CD8 T cells in the absence of disease may predispose individuals to a particular outcome should they progress to disease.

Considered together, studies of islet antigen-specific CD8 T cells indicate that the transition to T1D is associated with a change in phenotype or function rather than frequency, both in the periphery and in the pancreas, and that CD8 T-cell phenotype is linked to the rate of disease progression after onset.

CONNECTING ISLET ANTIGEN-SPECIFIC AND GLOBAL CD8 T-CELL RESPONSES

While the number and function of islet antigen-specific CD8 T cells directly link specific adaptive responses to islet beta cell antigens, autoreactive CD8 T cells are rare and thus challenging to study and use as a robust biomarker.⁶¹ Evidence in other diseases strongly suggests that global CD8 T-cell signatures can predict the antigen-specific immune response.⁴⁶ In T1D, the same adult T1D slow progressors in which exhausted islet antigen-specific cells were increased display a parallel increase in global CD8 T-cell exhaustion.⁴³ This is consistent with a transcriptional signature of CD8 T-cell exhaustion that is associated with better outcome across multiple autoimmune diseases⁶² and worse outcome in infectious diseases where inflammation and cytolytic activity are advantageous.⁶³ However, not all islet antigen-specific CD8 T-cell phenotypes are well reflected in the global

CD8 T-cell population. Transitional memory Helios⁺ CD8 T cells are found primarily among islet antigen-specific CD8 T cells, with far fewer cells of this phenotype being found among the global CD8 T-cell population. Understanding the ontogeny, activation and differentiation state of exhausted and transitional memory cells along with their function is currently under investigation and will help determine how these particular CD8 T-cell subsets, regardless of islet peptide specificity, are contributing to faster disease progression.

Additional global CD8 T-cell phenotypes have been associated with disease state or progression. Increased CD8 effector T cells correlated with higher basal levels of C-peptide in adult stage 4 T1D.⁶¹ In addition, CD8 T-cell features represent one-third of a composite signature of C-peptide decline in recent-onset patients.⁶⁴ However, how each of these CD8 T-cell subsets relate to each other and the mechanisms underlying the association between islet antigen-specific and global CD8 T-cell responses are not well understood. In fact, bystander CD8 T-cell activation can prevent T1D progression in a mouse model.⁶⁵ However, it is not yet clear whether bystander cells are directly regulatory or whether they modulate the overall immune environment. Together, these findings suggest that expansion of non-islet antigen-specific CD8 T-cell subsets can both reflect and contribute to the context of the autoantigen (islet and insulin)-specific immune response, and that they may both play a role in disease outcome.

CD8 T CELLS ARE ASSOCIATED WITH DISEASE OUTCOME UPON IMMUNE MODULATION

Understanding islet antigen-specific CD8 T-cell changes after immunotherapy will help to clarify the role of CD8 T-cell subsets in health and disease. Immune therapies may directly target autoreactive CD8 T cells with antigen-specific approaches, or may directly or indirectly alter global T-cell responses. CD4 T cells have primarily been studied in patients treated with antigen-specific therapies, but CD8 T cells can also be affected. Islet antigen-specific CD8 T-cell multimer data have not been reported so far, but stimulation assays with whole antigen, which may include CD8 T-cell responses in addition to CD4 T cells, suggest a reduction in functional cells with therapy. Autoreactive CD8 T cells are tangentially associated with response to antigen-specific therapy and immune modulation: maintenance of disease with peptide therapy specific for Class II DR4 epitopes is associated with lower levels of islet antigen-specific CD8 T cells,⁶⁶ and expansion and activation of insulin-specific CD8 T cells can be driven by microbiota cross-reactivity.⁶⁷ Thus,

beyond a correlation with disease state or progression, modulation of islet antigen-specific CD8 T cells is linked to changes in immunity and outcome, whether the effects are direct or indirect.

T-cell targeted, but not antigen-specific, immune modulatory therapies have resulted in changes in global CD8 T-cell subsets. A key example is non-FcR-binding anti-CD3 (teplizumab) treatment which leads to increased frequencies of partially exhausted CD8 T cells with reduced, but not absent, proliferation and inflammatory cytokine production.⁶⁸ Importantly, this is a reproducible result. The same partially exhausted CD8 T-cell population increased to a similar degree and with similar kinetics in patients with better outcome in two independent trials of T1D patients; one in recent-onset patients^{68,69} and the other in stage 2 at-risk patients with abnormal glucose tolerance.⁷⁰ In addition, a related anti-CD3 treatment (otelixizumab) resulted in an increase of partially exhausted CD8 T cells in responders.⁷¹ Within the partially exhausted CD8 T-cell population, T-cell receptor sequences were identified that matched CD8 T cells with reactivity to autoantigens, alloantigens and viral antigens,⁶⁸ suggesting that expanded islet antigen-specific CD8 T cells may also be partially exhausted. In fact, in a handful of patients studied so far, islet antigen-reactive T cells increased following anti-CD3 treatment and acquired a terminally differentiated state.⁷² Additional studies are ongoing to directly characterize islet antigen-specific CD8 T cells across a wide range of anti-CD3-treated patients. The fact that CD8 T-cell phenotypes are linked to outcome upon immune modulation, in addition to correlating with stage of disease and rate of progression, strongly suggests that they are involved in T1D disease progression and outcome.

FUTURE DIRECTIONS

Through systematic review of studies addressing features of insulin- and other islet antigen-specific CD8 T cells, including peptide–MHC specificities, quantity, functionality and phenotypes, collective evidence strongly suggests that disease progression and the outcome with therapy are linked to the phenotype or function of the islet antigen-specific cells, and not their quantity or peptide/protein specificity. This implies that the microenvironment where T cells first encounter antigen, regardless of the particular islet antigen, likely plays a dominant role in determining the phenotype of islet antigen-specific CD8 T cells. However, because T-cell differentiation is an antigen-driven process, one would also expect the type of antigen to play a role. Thus, understanding the range of islet antigens that drive

differentiation and expansion of CD8 T cells will advance the field and may reveal subtle differences in T-cell phenotypes determined by specificity. With newer single-cell technologies that enable definition of the phenotypic and functional states and clonal expansion of these rare cells, researchers can more fully address the range of phenotypes across multiple islet antigen specificities and their association with disease onset, disease progression and outcome with immune modulation.

Many questions remain as to how and when CD8 T-cell phenotypes are determined. In T1D, partial exhaustion of CD8 T cells is the most consistently reported phenotype associated with better outcome in both the islet antigen-specific⁴³ and global CD8 T-cell compartments.^{43,63,68,70} However, CD8 T-cell exhaustion alone is not sufficient to identify a slow progressor or better responder to therapy, suggesting that other factors are involved. Antigen and homeostatic cytokines including interleukin (IL)-7 and IL-15 are known to influence effector memory T-cell differentiation in T1D^{48,73} and may counterbalance differentiation to exhaustion. Based on the association of earlier differentiation states with more rapid disease progression,^{43,50,54} we suggest that there may be a balance of CD8 T cells with opposing functions that contributes to disease progression (i.e. an exhausted to effector ratio). Factors influencing CD8 T-cell exhaustion in T1D have been reviewed elsewhere,⁷⁴ highlighting the role of antigen, costimulation and cytokines. Understanding the factors in T1D that contribute to the potential balance of exhaustion and effector cells (Figure 2), including disease-associated alterations in antigen receptor signaling (i.e. PTPN22),⁷⁵ costimulation (i.e. CTLA-4 and inhibitory receptors)^{12,64} and cytokine pathways (i.e. IL-2, IL-21, IL-7),^{50,73} may lead to selection of targeted therapies to modulate the function of specific CD8 T-cell subsets. Likewise, understanding when in the disease course islet antigen-specific CD8 T-cell phenotypes change, and whether these changes are stable, will inform timing and duration of future therapies.

T1D presents a unique opportunity to better dissect the role of autoimmune CD8 T cells in disease, because peripheral autoreactive T cells (1) mirror the specificity and phenotype of those in the pancreas, (2) can be identified and characterized and (3) are associated with clinical outcome. Determination of the phenotype of islet antigen-specific CD8 T cells that distinguishes individuals with rapid or slow disease progression, or that identifies responders to immune-modulating therapeutics, will help establish biomarkers to support precision medicine. Furthermore, a better understanding of the phenotype and function of islet antigen-specific CD8 T cells that

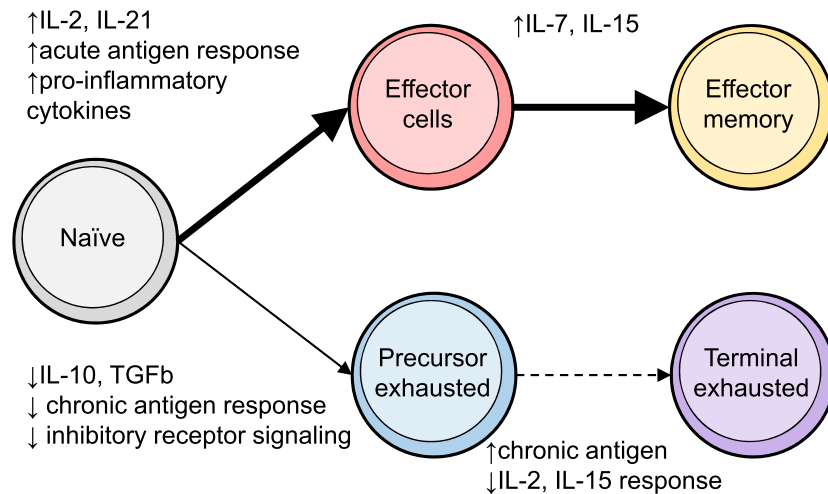


Figure 2. Proposed factors in type 1 diabetes (T1D) that may contribute to the imbalance between effector and exhaustion differentiation paths. Antigen receptor signaling, costimulation and cytokines that are altered in T1D as compared with controls or across stages of disease are annotated in this simplified differentiation map. IL, interleukin; TGF- β , transforming growth factor beta.

associate with stage of disease, rate of progression and clinical outcome after immune modulation will provide insight into the mechanism by which autoreactive CD8 T cells participate in driving or restraining disease. More broadly, a better understanding of T-cell differentiation in the context of T1D is needed to define the ontogeny, function and stability of early memory and more terminal phenotypes known to be influential in T1D disease outcome. Determining whether T1D phenotypes are also present in other autoimmune and disease settings will expand and solidify lessons learned in this model disease. Overall, determining the phenotype and function of islet antigen-specific CD8 T cells may be key to guiding development and selection of new therapies to treat and prevent T1D.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

Alice Emily Wiedeman: Writing-original draft; Writing-review & editing. **Cate Speake:** Writing-original draft; Writing-review & editing. **S Alice Long:** Writing-original draft; Writing-review & editing.

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