Review article

Obstacles and opportunities for targeting the effector T cell response in type 1 diabetes

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Abstract

Autoreactive lymphocytes display a programmed set of characteristic effector functions and phenotypic markers that, in combination with antigen-specific profiling, provide a detailed picture of the adaptive immune response in Type 1 diabetes (T1D). The CD4+ T cell effector compartment (referred to as “Teff” in this article) has been extensively analyzed, particularly because the HLA genes most strongly associated with T1D are MHC class II alleles that form restriction elements for CD4+ T cell recognition. This “guilt by association” can now be revisited in terms of specific immune mechanisms and specific forms of T cell recognition that are displayed by Teff found in subjects with T1D. In this review, we describe properties of Teff that correlate with T1D, and discuss several characteristics that advance our understanding of disease persistence and progression. Focusing on functional disease-associated immunological pathways within these Teff suggests a rationale for next-generation clinical trials with targeted interventions. Indeed, immune modulation therapies in T1D that do not address these properties of Teff are unlikely to achieve durable clinical response.

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1. T1D Teff display hallmarks of adaptive recognition and expansion

Properties of Teff in T1D closely parallel the types of T cell activation seen in normal immune responses, albeit with some features discussed below that serve to define the autoreactive population. The strong genetic association for T1D with HLA-DQB1*0302 and linked HLA-DRB1*04 genes corresponds to the presence of Teff populations in T1D with specificity for numerous islet-derived proteins and peptides bound and presented in the context of those class II molecules. Although Teff specific for GAD65 and for proinsulin have been the most extensively studied, similar cell populations specific for other islet antigens including ZnT8, IGRP and chromogranin, have also been described [1–5]. In general, there are several features commonly found in these Teff:

1. There are multiple antigens, and a diverse set of islet-derived peptides, recognized by Teff within each individual subject with T1D. In other words, the response is polyclonal with respect to targets, and there is no single dominant antigen recognized by all T1D subjects [6–9].
(ii) Even within a single target specificity there is diverse T cell receptor utilization, indicating a polyclonal response with respect to repertoire selection. However, some particular TCR Vβ genes are found preferentially in GAD65-specific responses in T1D, indicating a bias in the antigen-driven response in many subjects [10,11].

(iii) HLA-matched normal subjects also have islet-responsive T cell repertoires, but the Teff from T1D subjects tend to display higher avidity for the peptide-MHC complex and carry memory (CD45RO) markers, consistent with in vivo activation and exposure to their specific antigens [12–14]. As a consequence of this chronic in vivo activation, T1D Teff also utilize a characteristic Teff potassium channel, Kv1.3 [15].

(iv) Consistent with this diversity of specificities, avidity, and repertoire, the cytokine profile for T1D Teff is also variable, with evidence of Th1-like (IL12, IFNγ), Th17-like (IL17, IL22), and Th- like (IL21) properties in both antigen-specific and antigen-nonspecific Teff derived from peripheral blood, although none of these phenotypes appear to be exclusively present or even always present [16–23]. Reconciling the diversity of this Teff response with the observations of oligo- clone expansion of particular specificities and TCR clonotypes led to the concept of “determinant focusing”, in which an initial highly diverse T cell response is progressively narrowed by the selective expansion of particular sets of dominant Teff during disease progression [24]. The choice of which Teff become expanded may be a random event, or may be driven by factors that are also highly variable within T1D subjects, such as antigen density, antigen processing, additional HLA molecules present, etc. Recent descriptions of Teff that recognize post-translationally modified islet auto- antigens also suggest the possibility that shifting repertoires occur over time, contributing to the variability of particular immunodominance patterns [25–27], as is the possibility that some islet antigens are displayed in more than one class II-binding register [28,29].

Although most T1D Teff studies have utilized cells derived from peripheral blood, there is also strong evidence for the presence of these cells in the islet lesions themselves. Immunohistochemistry identifies CD4 T cells within islets in pancreatic specimens from T1D subjects [30,31], and T cell clones derived from islets or lymph nodes mirror the properties of similar cells found in blood [32–35]. In a longitudinal study of pancreas organ transplantation in T1D, activated and expanded islet-specific Teff were identified in peripheral blood of subjects who had experienced a return of their hyperglycemia, consistent with a recurrence of T1D, correlating with staining for CD4 T cells in pancreatic biopsy specimens [36]. Interestingly, in some of these cases, tetramer and TCR repertoire studies documented the presence of the same T cell clonotypes in specimens collected years apart, indicating a persistent and dominant Teff memory response in association with disease. Indeed, simply transplanting islets into T1D recipients may be sufficient to boost Teff numbers, directly demonstrating the capacity for recalling memory responses [37].

The Teff properties summarized above in T1D indicate a major role for these cells in disease pathogenesis, consistent with an active immunological process of T cell recognition, activation, expansion, and effector function. While the overall framework for this immune response displays typical features of repertoire heterogeneity and avidity maturation, there are a number of distinct disease-promoting elements, which lead to a response that deviates from the homeostatic norm. These include properties that support unregulated Teff expansion, signals that favor the deviation of Teff toward proinflammatory lineages, and failure of effector functions to be controlled by other regulatory cell populations (Fig. 1). These are reviewed in the next section, followed by a discussion of the implications for immunotherapy in T1D.

2. CD4 effector T cells are programmed to be pathogenic in T1D

As noted in Section 1 the strong genetic association for T1D with HLA-DQB1*0302 and linked HLA-DRB1*04 genes support a role for islet specific CD4 T cells in disease that is further supported by identification of such cells in the peripheral blood and islets of individuals with T1D. However, additional studies support the concept that there are global alterations in CD4 T cell function in T1D, which promote the development of pathogenic T cell responses. These properties support unregulated Teff expansion, a skewing of lineage commitment, and failure of effector T cells to be controlled by other regulatory cell populations.

A disturbance in the CD4 T cell pool has been consistently observed in T1D subjects. This includes the observation that there is an expanded number of CD4 T cells in the peripheral blood [38], but more consistently investigators have shown that the CD4 memory compartment is expanded in T1D as compared to healthy subjects. In several of these studies the increase was seen among the recently activated T cells based on CD27 expression and the CD45RA + RO + subset of cells which are thought to be chronically activated [38–40]. This observation has been extended to the pancreatic draining lymph nodes (PLN) [33]. Further evidence of altered homeostasis within the CD4 memory compartment is the observation that in vivo the turnover of CD4 memory T cells is increased in T1D subjects as compared to healthy controls [41]. A recent study evaluated transition from central memory to effector memory in CD 4 T cells of T1D subjects and their relatives and found enhanced activation of central memory T cell in these individuals compared to healthy controls resulting in the transition to effector memory T cells that are relatively short lived [42]. These studies argue that the Teff cells of T1D subjects are influenced by factors including genetic, environmental or the immune milieu present in T1D that promotes CD4 T cells activation and expansion of memory T cells.

Among the CD4 T cells there is further evidence that cell fate decisions are altered in T1D. Studies of peripheral blood T cells have been done examining the function and lineage of effector T cells through assessment of cytokines and chemokine expression on CD4 T cells isolated from peripheral blood. This is an evolving area as the variety and plasticity of the CD4 T cell lineages has become better understood. Studies of murine models of diabetes have implicated Th1 (IFNγ producing) cells in disease, and studies in humans show an increase in IFNγ expression by CD4 T cells in new onset T1D [43] and more recently it has been shown that the effector memory T cells of T1D subjects display an elevated cytokine signature that is predominantly IFNγ [42]. More recently...
Th17 cells have been described and their role in human T1D examined, in this process several groups have found an increase in the frequency of IL-17 producing CD4 T cells in the peripheral blood of new onset T1D subjects [16,44]. Studies of the Th1 and Th17 cells in PLN of new onset T1D subjects, demonstrated a more prominent increase in IL-17 producing T cells compared to control PLN and this increase is much greater than seen between IFNγ producing cells of T1D and controls. This study was also informative as the peripheral blood from these same individuals demonstrated an increase in IFNγ+ T cells in T1D subjects as compared to controls, but a very modest difference between groups when IL-17 + T cells were examined [33]. This suggests that tissue specific differences in the distribution of these T cell subsets may be an important aspect of our understanding of their role in disease.

Beyond the standard lineage distinctions, further studies have demonstrated an increase in dual IFNγ and IL-17 producing cells in children with beta cell dysfunction who have not yet developed T1D, indicating that this may be a biomarker for early disease progression and a contributor to disease pathology [45]. Recent studies have also examined the production of IL-21 by CD4 T cells in T1D as well as the frequency of the Th cells that are principal producers of IL-21. Two studies have identified an increase in IL-21 producing CD4 T cells in T1D as compared to controls and a third study has shown that IL-21 message is increased in CD4 T cells of T1D subjects [21,46,47]. IL-21’s central role in B cell maturation, the promotion of T cell survival and ability to confer resistance to Treg mediated suppression to CD4 T cells, each factors that may be at play in T1D development [48].

2.1. Genetic variants associated with T1D indicate a genetic basis for altered effector function in T1D

The alterations that are seen in the CD4 T cell compartment of individuals with T1D suggest that the mechanisms involved impact the entire CD4 T cell population. One clear source is genetic variants that are linked to T1D and immune function. Studies of genetic risk for T1D have revealed well over 40 loci that are associated with T1D development [49]. Many of the genes are implicated in pathways involved in immune function and many are expressed in T cells. As noted above the greatest genetic risk is conferred by MHC Class II, including HLA DR3, DR4 and DQ0302. The role of Class II in the binding and presentation of peptides to CD4 T cells indicates the importance of CD4 T cells and antigen specificity in T1D. Further support for the argument that specificity of the response is important in T1D is found in the second strongest genetic association, the INS VNTR, a variant that is thought to alter expression of insulin in the thymus resulting in the escape of pro-insulin specific T cells from the thymus into the periphery [50]. Yet many of the genes implicated by GWAS impact intracellular signaling pathways. These include protein tyrosine phosphatases such as PTPN22, PTPN2, SH2B3, while others are molecules involved in cell surface interactions, including CTLA4, the IL-2, IL-6 and IL-7 receptors [49,51], all of which have potential to impact the effector T cell response.

Among the protein tyrosine phosphatases the PTPN22 C1858T risk variant has been most extensively studied for its role in altering T cell function. This variant results in a change in a single amino acid at position 620 from an R to a W within the proline rich SH3 binding domain of the protein [52]. Alterations in this binding domain have implications for proximal TCR signals. When modeled in KI mice, naïve T cells display enhanced positive selection in the thymus, enhanced response to TCR stimulation including increased proliferation of CD4 T cells and production of IL-2 and an expansion of the memory T cell population [53]. In human cell lines a similar hyper-responsive phenotype is observed when the PTPN22 risk variant is expressed in Jurkat [54]. However primary human T cells display a blunted TCR response upon activation, but like the KI mice they have an expanded memory compartment. These findings are mirrored in T1D—suggesting common mechanisms, that may initially be driven by hyper-responsiveness, that the T cells compensated for with time, but which ultimately results in an expanded memory compartment. In addition to the expanded CD4 T cell memory compartment seen in healthy subjects who carry the PTPN22 risk variant [55], Vang et al. reported increased production of IFNγ, and decreased production of IL-17, in PMA/ionomycin-stimulated CD4+ memory T cells from healthy individuals homozygous for the risk variant (T/T) that paralleled enhanced activation of Akt [56]. These findings implied altered lineage commitment within Lyp620W-expressing CD4+ T cells. This phenotype may be seen in T1D with the risk variant, but also potentially in other T1D subjects due to additional mechanisms that lead to alterations in the PI3K-AKT pathway (Habib unpublished). Thus the PTPN22 risk variant may favor pathogenic CD4 T cells through altered thymic selection allowing the escape of autoreactive T cells, enhanced early activation followed by the expansion of a memory population, skewed toward the proinflammatory Th1 lineage.

The protein tyrosine phosphatase N2, is also associated with T1D and studies have clearly linked this variant with impaired responses to IL-2 and IL-15 among CD4 effector T cells [57]. An impaired response to IL-2 is also seen in the memory T cells of subjects with T1D [58]. These cytokines are central to CD4 T cell growth and survival, and therefore a decrease in the ability to respond to these cytokines would be expected to negatively impact the pathogenic CD4 T cells in carriers of the risk variant. However, several factors are at play in this setting: IL-2 plays a central role in the development and survival of Treg, thus this variant, in addition to those in the high affinity IL-2 receptor may have a detrimental effect on regulatory T cells allowing effectors to escape suppression. In addition, lineage commitment is in part determined by exposure to cytokines. In humans Th17 cells are induced by TGFβ and IL-6, then further amplified and stabilized by IL-21 and IL-23 [59]. In contrast to its role in supporting development of Treg, IL-2 through phosphorylation of STAT5 has a negative regulatory effect on RORγt, the expression of IL-17 and opposes Th17 development [60,61]. Thus in low IL-2 environments, Th17 cell differentiation is enhanced [62] suggesting that diminished response to IL-2 may facilitate Th17 development in T1D. More recently a link between low IL-2 and the enhanced development of Thε cells has also been described [21] which would allow for enhanced B cell development through Thε derived help and the potential to increase autoantibody development.

Another risk variant that highlights the global alterations in T cells function in T1D is the risk variant associated with CTLA4. CTLA4 is a negative regulator of T cell activation and alterations in the CTLA4 gene region have been linked to risk for diabetes in the NOD model (Idd5.1) [63] and in human disease [49]. In humans, although the causative snp in the CTLA4 locus is still unclear, (rs3087243) CT60A/G and a snp in the 3’UTR in linkage disequilibrium with CT60A/G have been implicated in T1D risk. Studies of healthy subjects who carry the risk variant show a decrease in CTLA4 mRNA and that of its soluble isoform sCTLA-4 [64]. A finding that has been extended to islet specific T cells in T1D [65]. The modest alterations found in association with this risk variant also contribute to a dysregulated effector T cell population.

2.2. Response to regulation is altered in T1D-effector cells are resistant to suppression

Regulatory T cells are required to protect an individual from autoimmunity and specifically T1D. Animal models of diabetes...
have demonstrated the requirement for Treg to protect from disease and in children with IPEX, who have a mutation in FOXP3 resulting in a lack of Treg, T1D develops very early in life [66]. Treg based regulation is indisputably impaired in T1D, but studies have indicated that the source of this defect is that the effector CD4 T cells are resistant to the suppression by Treg rather than due to decreased function of Treg [67,68]. This is a finding that has been described in many autoimmune diseases including lupus erythematosus (SLE) [69,70], psoriasis [71], RRMS [72,73] in T1DMS [67,68]. The resistance of effector T cells to Treg has been observed in both the NOD [74—76] and DO11.10 RIP-mOVA [77] model of diabetes. In these models, inflammation and tissue destruction progress despite the presence of functional Treg at the site of inflammation. In the NOD, Treg resistance appears to be intrinsic to the NOD T effector cell itself [76].

The mechanism or mechanisms that lead to Treg resistance in T1D are still unknown. However multiple factors have been linked to Treg resistance in model systems that have translated to human autoimmune disease and likely play a role in T1D. The maturation state and lineage of the effector T cells leads to differences in suppression, most notably Th17 T cells are resistant to suppression [78]. Cytokines including those for which T1D effector T cells are exposed such as IL-6 [71], IL-15 [79,80] and IL-21 [77,81] have been shown to lead to a failure of suppression. Additionally the Treg resistance is mediated through phosphorylation of STAT3 and PKB/c-AKT activation [73]. There is evidence that each of these pathways may be altered in T1D: 1) A skewing of the T cell toward Th17 and Th1 lineages is described (see above), 2) IL-6 levels are increased in T1D [83], a variant in the IL-6R gene is associated with T1D further implicating this pathway, and ongoing studies indicate that the IL-6 signaling pathway may be enhanced in T1D (unpublished observations JHB) and 3) Genetic risk variants including PTPN22 impact the PKB/c-AKT pathway and may contribute to Treg resistance. In this respect the PTPN22 risk variant associated with T1D could play a role as it has been shown to lead to enhanced phosphorylation of AKT [56], (unpublished JHB). Defining the mechanisms through which effector T cells evade Treg in T1D will allow directed therapies to reinstate tolerance.

3. Targeting T effector with T1D immunotherapy

The T effector properties described above create a challenge for therapies in T1D that utilize immune intervention. T effector that are long-lived, resistant to regulation and deletion, and have a diverse set of antigenic specificities present a ‘perfect storm’, threatening to be refractory to immunomodulatory drugs that may be effective in other autoimmune diseases. Although numerous immune-based clinical trials have been conducted in T1D, success has been limited to a few cases, which provide important insights into the necessity for addressing the prevalent refractory T effector population. In these intervention trials, strategies to target T effector are either designed as depletion agents, specifically deleting T effector populations, or designed to deviate immune responses, diverting the phenotypic and functional properties of T effector towards nonpathogenic pathways (Fig. 2).

Depletion of T effector can potentially be accomplished by non-selective agents that delete all lymphocytes, by agents that target T effector with partial selectivity, or with antigen-based approaches that exploit activation-induced death pathways. In the START trial conducted by the Immune Tolerance Network (ITN), subjects recently diagnosed with T1D were treated with anti-thymocyte globulin (ATG) in an attempt to delete effector cells and preserve residual beta cell function, measured as persistence of stimulated c-peptide levels [84]. Flow cytometric analysis of the peripheral blood documented major depletion of multiple lymphocyte populations, as expected, including the majority of circulating CD4 T cells. Detailed phenotypic characterization of T cell subpopulations, however, indicated that regulatory CD4 T cells were almost completely depleted as well; furthermore, effector memory T cells were relatively refractory to deletion. Thus, paradoxically, treated subjects displayed a potentially adverse shift of subpopulations in the CD4+ T cell compartment, without significant decreases in T effector. This phenotypic result paralleled a disappointing clinical result, in that subjects in the ATG treatment arm showed no differences in their c-peptide outcomes compared to control T1D individuals.

The negative outcome of the START trial suggests that depletion therapies for T effector in T1D should more specifically target the populations of interest. Fortunately, there are many potential...
targets that may satisfy this need: As described in Section 1 above, Teff in T1D display markers associated with chronic activation, recent in vivo activation, and lineage commitment, each of which provide potential targets for novel immune therapy. A large number of therapeutic candidates are currently under development, and some are already well advanced, including an anti-CD30 immunotoxin (brentuximab vedotin) that is currently used to treat some forms of lymphoma, an anti-Kv1.3 inhibitor (dalazatide) that blocks the potassium channel used by some Teff for chronic activation, and LFA3-lg (alefacept), a fusion protein that targets CD2, previously approved for use in psoriasis.

Of these therapies, only alefacept has been tested in T1D clinical trials. The T1DAL trial, conducted by the ITN, recently reported highly successful outcomes, in which the majority of subjects receiving two short courses of treatment with alefacept were found to retain most or all of their capacity for insulin production over two years, measured as persistence of c-peptide release [83]. Flow cytometry measurements of cells from the subjects at the time of entry into the study demonstrated high levels of CD2 expression on Teff, with much lower levels on naïve and regulatory T cells [86]. This differential in CD2 level correlated with the depletional effect of alefacept, which elicited the rapid disappearance of peripheral T eff, but had no discernable effect on regulatory T cell compartments. This study validates the concept of depletional therapies directed against Teff, and should be a foundation for expanded studies that correlate clinical outcomes with detailed analysis of the phenotype and function of residual and recurrent T cell populations following therapy. Although there were no significant adverse infectious events with alefacept treatment, longer follow-up and larger studies, as well as comparison with other similar Teff selective targets, should be evaluated.

Immune deviation strategies are based on the concept that T cells exhibit plasticity in terms of function and lineage commitment, and therefore it may be possible to change the nature of Teff populations using specific immune modulators. Several of these strategies have been attempted in the context of T1D clinical trials, with inconclusive outcomes requiring additional investigation. Examples include the use of anti-CD3 monoclonal antibodies (teplizumab and oxeлизumab), which act as partial T cell agonists and may lead to a redirection of T cell activation towards regulatory phenotypes [87–91], CTLA-4lg fusion protein (abatacept), which predominantly acts by blocking B7-dependent costimulatory T cell pathways [92], and two anti-cytokine monoclonal antibodies – anti-IL12/23 (ustekinumab) and anti-IL6R (tocilizumab), each designed to block Teff activation and commitment pathways and therefore potentially favor immune regulation. These latter two trials are currently underway, with investigations planned for detailed analysis of Teff modulation that should inform future therapeutic use, either as single therapeutics or potentially in combination with specific depletional agents such as alefacept.

The effects of anti-CD3 therapies on T cell compartments have been extensively studied, showing increases in the CD8/CD4 ratio in peripheral blood, likely due to a partial agonist effect of therapy resulting in expansion of particular CD8 subsets. The effect on CD4 Teff is less dramatic, although in the AbATE clinical trial – an intervention study conducted by the ITN in recent-onset T1D subjects – a significant decrease in Teff was observed at the completion of the study in subjects with a favorable clinical response [93]. A similar type of modest immune modulation was seen with CTLA-4-lg therapy in a comparable trial conducted by TrialNet, although in this analysis the reduction in Teff was limited to the central memory compartment, and it appeared to precede clinical response [94].

The choice of agent to use in these clinical trials has been guided in the past by inference from phenotypic studies of immune cells in T1D, by extrapolation from use in other autoimmune diseases, or by positive experience in the NOD murine model of autoimmune diabetes. However, as summarized in Section 2 above, there is now an evolving understanding of specific immune response pathways in T1D Teff that are associated with disease incidence and disease progression. This offers a new opportunity to identify and test novel agents that more specifically target these specific pathways, attempting to reverse the immunologic defects that represent Teff profiles in T1D. For example, if the resistance of Teff to regulatory control is partially due to hyper-responsiveness to IL6, there is therapeutic potential to reverse or block this pathway not only by using anti-IL6R (tocilizumab) but also a variety of anti-IL6 monoclonal antibodies and/or JAK inhibitors that are currently under development. Similar strategies may identify opportunities for agents that reverse the block in activation-induced Teff cell death, potentially as adjunct therapy with specific islet antigens, which may be necessary to achieve therapeutic benefit from therapies with GAD and/or proinsulin antigens, each of which has already entered clinical trials. Indeed, interventions that fail to address these fundamental disease-associated mechanisms of Teff function are likely to have only transient this regard, there is a compelling rationale for combination therapy in T1D, in which Teff depletion is used as an induction strategy, followed by Teff deviation using immunomodulators.

One of the questions raised by studies of Teff pathway defects in T1D is the issue of individual variation, and whether targeted therapies should be tailored for different subjects based on their genetic, phenotypic, and functional profiles. Although there is a conceptual logic to such personalized forms of therapy, there is also a strong rationale for choosing therapies that can be applied broadly, based on the notion that targeting disease-associated pathways will enhance homeostatic and regulatory mechanisms that favor beneficial clinical outcomes. In other words, the pathways that are defective in T1D Teff identify logical targets for therapy based on their role in promoting or maintaining autoreactive Teff, so there are strong prospects for clinical benefit in all subjects, irrespective of which pathway is dominant in any particular individual.

Disease pathogenesis in T1D is characterized by multiple immunological components, in addition to Teff autoreactivity. Some of the targeted immune therapies mentioned in this section also have potential efficacy towards these other components, such as CD8, NK, and B cells, making them even more attractive as therapeutic candidates. The clear associations between Teff phenotype and disease progression, however, strongly suggests that therapies that fail to delete and/or deviate Teff function are unlikely to have durable efficacy. Targeting specific pathways that characterize autoreactive Teff – in particular those that reflect chronic activation, resistance to apoptosis and regulation, and persistence with standard T1D therapy – will be an important advance towards a favorable clinical outcome.

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