Epitope specific T cells in type 1 diabetes: From detection to immunotherapy

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Monitoring autoreactive T cells, directed towards immunodominant epitopes is useful in assessment of disease progression and distinguishing different forms of autoimmune diabetes. In a recent work, the authors reported that individuals with slower progression of autoimmune diabetes, i.e. latent autoimmune diabetes in adults (LADA) harbour lower frequency of preproinsulin (PPI) specific CD8+ T cells compared to those with typical type 1 diabetes (T1D). Further, the PPI specific CD8+ T cells of T1D subjects showed higher proliferation potential and greater proportion of cells with effector phenotype. Besides highlighting the differences in the repertoire of PPI specific peripheral CD8+ T cells between T1D and LADA subjects, this study also demonstrates that autoreactive CD8+ T cells directed towards immunodominant epitopes persist over a period of time in these subjects and hence present as attractive targets for immunotherapy. Generating epitope specific CD4+ regulatory T cells (Tregs) directed towards the same antigen can counter regulate effector CD4+ T cells and further limit the frequency and actions of such autoreactive CD8+ T cells. Recent animal studies have indeed shown that islet antigen specific Tregs can reverse autoimmune diabetes by targeting T cells that infiltrate pancreas. Therefore, combining the approaches of identification of epitope specific T cells using MHC multimers and Treg specific markers, can facilitate the scope of epitope specific immunotherapy in human T1D.

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Introduction

Type-1 diabetes (or Type-1 A or autoimmune diabetes) (T1D) results from destruction of beta cells of the pancreas, leading to insulin deficiency. There are several mechanisms proposed for beta cell death, with autoreactive T cells including both CD4+ and CD8+ T cells playing a major role.

The autoreactive T cells target many beta cell associated antigens such as, preproinsulin (PPI) [1], glutamic acid decarboxylase 65 (GAD65) [2], insulinoma antigen-2 (IA-2) [3], islet cell autoantigens (ICA) [4], heat shock protein (HSP) [5], islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP) [6], imogen-38 [7], zinc transporter-8 (ZnT8) [8], pancreatic duodenal homeobox factor 1 (PDX1) [9], chromogranin A (CHGA) [10] and islet amyloid polypeptide (IAPP) [11]. Of the major epitopes recognized by the autoreactive T cells, PPI derived epitopes are the first ones to be recognized during the initiation of the disease [12]. In particular, it has been recognized that expression of insulin B chain having amino acid sequence, 9–23 (insulin B: 9–23) in pancreatic islets is required for NOD mice to develop anti-islet autoimmunity and elimination of PPI-reactive T cells prevents diabetes in these animals [13-15]. Once the process of autoimmune destruction begins, the T cells begin to recognize more beta cell associated antigens, accelerating the beta cell damage [16].

Monitoring autoreactive T cells, particularly, CD8+ T cells directed towards immunodominant epitopes is useful in
assessment of disease progression and distinguishing different forms of autoimmune diabetes [17]. However, detection of epitope specific CD8+ T cells in peripheral circulation has always been challenging. Initial studies have used ELISpot based assays and progressed to use of MHC multimers, in the form of tetramers, pentamers or dextramers to determine the frequency of such epitope specific CD8+ T cells [18-21]. Initiatives in the form of T cell workshops have also been carried out to standardize these assays with the objectives of validating allele specific epitopes and inter-laboratory harmonization[22, 23]. Recognition of epitope specific CD4+ T cells in T1D is equally important but more difficult, owing to their much lower frequencies, compared to epitope specific CD8+ T cells and challenges associated with generation of stable peptide MHC II complexes [24]. Recent reports have now indicated that persistence of epitope specific CD4+ T cells can be consistently monitored using MHC II tetramers [25]. Amidst such efforts there is growing awareness that immunotherapeutic approaches in T1D must be targeted and hence the importance of antigen or epitope specific T cells is increasingly realized. In the recent report [17] Sachdeva and co-workers have determined the frequency and characteristics of PPI specific autoreactive CD8+ T cells in subjects with T1D and latent autoimmune diabetes in adults (LADA), encompassing individuals with varying HLA alleles, unlike other studies from Europe and US that have investigated T1D subjects with HLA-A*02 allele, the predominant HLA-I allele in the Caucasian population.

In this work, the authors reported that individuals with slower progression of autoimmune diabetes, i.e. LADA, harbor, lower frequency of PPI specific CD8+ T cells compared to those demonstrating faster disease progression, i.e T1D. This difference in frequency of epitope specific CD8+ T cells was observed independent of HLA allelic variation. Besides frequency, the proportion of PPI specific CD8+ T cells with effector phenotype in LADA subjects was also lower than T1D subjects. The first part of this study highlights that rate of disease progression in autoimmune diabetes is associated with frequency and differentiation stages of CD8+ T cells specific for the immunodominant epitope (PPI). In the second part, the authors assessed the proliferation potential of PPI specific CD8+ T cells in these subjects, by isolating their peripheral lymphocytes and subjecting them to in-vitro stimulation with PPI. Interestingly, the PPI specific cells from T1D subjects showed greater proliferation than their LADA counterparts, revealing another aspect of PPI specific CD8+ T cells in faster disease progression in T1D subjects. To investigate, whether the proliferation of PPI specific CD8+ T cells was indeed due to antigenic stimulation, subsets of proliferated PPI specific CD8+ T cells were analyzed, whereby the central memory subset showed significant increase in T1D subjects compared to the LADA subjects. In terms of perforin and granzyme-B expression by the PPI specific CD8+ T cells, no differences were observed between T1DM and LADA groups, either in peripheral blood or in-vitro stimulated lymphocytes, suggesting that the main difference in PPI specific CD8+ T cells between the two forms of autoimmune diabetes was in terms of frequency and proliferation potential. These observations were independent of age, as PPI specific CD8+ T cells of adult onset T1D subjects showed similar characteristics as those of juvenile T1D subjects.

Besides highlighting the differences in the repertoire of PPI specific peripheral CD8+ T cells between T1D and LADA subjects, the study also demonstrates that autoreactive CD8+ T cells directed towards immunodominant epitopes persist over a period of time in subjects with autoimmune diabetes. Therefore, epitope specific CD8+ T cells are not only useful for monitoring disease progression but also present as attractive targets for immunotherapy. Since, CD8+ T cells are primed by CD4+ T cells, particularly during the initial stages of pathogenesis of autoimmune diabetes, therefore targeting effector CD4+ T cells directed towards immunodominant epitopes is a suitable option to curb autoimmune diabetes, without the need for generalized immunosuppression. One such approach is generating epitope specific CD4+ regulatory T cells (Tregs) that counter-regulate effector CD4+ T cells directed towards the same antigen and further limit the frequency and actions of autoreactive CD8+ T cells. Though the concept of epitope specific Tregs has been around for more than a decade [26, 27], it is only in the recent years that extensive research in this field has picked up with improvements in methods of generation, isolation, characterization and in-vitro expansion of stable and efficacious Tregs. For immunotherapy, usefulness of both natural (CD4+CD25high CD127low and FoxP3+) and induced (IL-10+, derived from CD4+CD25+/− T cells) Tregs have been described in the literature [28]. Adoptive transfer studies suggest antigen specificity is required by T cells for trafficking and maintenance in inflammatory tissues such as the pancreas in NOD mice [29, 30]. Recently, Mahne et al [31] have indeed shown that islet antigen specific Tregs can reverse autoimmune diabetes by targeting effector CD4+ T and CD8+ T cells that infiltrate pancreas in NOD mice. Such epitope specific Tregs have a multifaceted role in suppressing the effector T cells, including priming by dendritic cells, proliferation, activation, migration and function of autoreactive effector T cells. And, compared to polyclonal Tregs, much smaller numbers of epitope specific Tregs are sufficient to reverse T1D [27, 32]. Just as in case of detection, finding the immunodominant epitope in the antigen, for generation of epitope specific Tregs is a major challenge. There are improved, epitope prediction softwares and assays [33] that have come up recently, however such
assays require careful validation in animal models, such as humanized mice for choosing the right epitope for immunoprotection\textsuperscript{[34]}.

Conclusions

Advances in polychromatic flow cytometry and availability of stable peptide MHC multimer complexes have improved the detection of epitope specific T cells even at lower frequencies along with characterization including detection of intracellular cytokines and transcription factors. Simultaneously, identification of newer and stable markers of Treg differentiation has enabled better classification of Treg subtypes. Therefore, combining the approaches of identification of epitope specific T cells using MHC multimers and Treg specific markers, can facilitate the scope of epitope specific immunotherapy in human T1D. These technologies can also assess whether these Tregs maintain their phenotypic and functional characteristics and not revert to a pathogenic phenotype, a major concern associated with immunotherapy with antigen specific Tregs\textsuperscript{[35]}.

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