T Cells in the Pathogenesis of Type 1 Diabetes

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T lymphocytes' crucial role in the autoimmune process leading to insulin-dependent type 1 diabetes is now universally recognized. Research focuses on identifying pathogenic and nonpathogenic T cells, understanding how they are primed and expanded, characterizing their antigen specificity, and ultimately on devising strategies to blunt their autoaggressive action. In this review, we focus on recent progress identified in three different areas. Results obtained with transgenic mice acknowledge proinsulin's unique role in triggering autoimmunity and suggest that other β-cell proteins are recognized as a result of epitope spreading, at least in the nonobese diabetic mouse. Progress has also been achieved by developing and validating reliable $CD4^+$ and $CD8^+$ T-cell tests that may prove valuable for diagnostic and prognostic purposes in the near future. Finally, recent results provide novel and important guidance for manipulating autoreactive T-cell responses against β-cell antigens.

Introduction

Type 1 diabetes (T1D) is a T-cell–mediated autoimmune disease targeting the insulin-producing β cells of the pancreas. It has taken time to make this common knowledge.

The emergence of this notion has followed a twisted intellectual journey. First, it was not evident until the 1970s that T1D was an autoimmune disease. The work of Bottazzo and the discovery of islet cell antibodies (ICAs) yielded the concept of an autoimmune pathogenesis [1]. Following the paradigms of other diseases such as myasthenia gravis and Graves' thyroiditis, attention then focused on autoantibodies' pathogenic role. The ensuing studies have been important for advancing research on T1D pathogenesis because essentially all antigens (Ags) targeted by autoreactive T lymphocytes have initially been identified as targets of autoantibodies. However, it is widely accepted today that the anti-islet autoantibodies do not play an essential role, with the possible exception of a modulating effect on the presentation of β-cell Ags [2]. In humans, a case report describing T1D development in a patient affected by X-linked agammaglobulinemia documented the dispensable nature of β-cell autoantibodies, although this does not rule out an adjuvant role of enhanced autoantigen presentation mediated by antibodies in other cases [3].

However, it was the availability of the nonobese diabetic (NOD) mouse model in the 1980s that helped clarify T lymphocytes' central pathogenetic role. Several reports have greatly advanced our understanding of how this T-cell pathogenesis takes place.

$CD4^+$ or $CD8^+$ T Cells?

In light of the strong association between the major histocompatibility complex (MHC) class II locus and T1D, $CD4^+$ T cells have long mesmerized investigators. Frequently, several investigators could isolate from lymphocytic islet infiltrates bulk and cloned T-cell populations causing disease upon transfer into lymphopenic NOD mice or when expressed in transgenic animals. The β-cell Ags recognized by several such clones, including clone BDC2.5, the most widely used and studied diabetogenic $CD4^+$ T cell, remain to be identified [4]. However, data gathered more recently point to a parallel critical role of $CD8^+$ T cells in T1D pathogenesis. NOD mice lacking MHC class I molecules [5–7] or injected with anti-CD8 monoclonal antibodies [8] do not develop insulitis. More importantly, $CD8^+$ T cells specific for insulin $B_{15\text{-}23}$ [9] and for islet-specific glucose-6-phosphatase catalytic subunit-related protein $(IGRP)_{206\text{-}214}$ [10] are early and critical actors in the T1D pathogenesis of NOD mice [11,12].

Overall, $CD4^+$ and $CD8^+$ T cells are required for progression to T1D [13–15], and diabetogenic clones (ie, clones capable of accelerating or inducing T1D upon adoptive transfer in NOD or NOD/SCID recipients) have been described for the $CD4^+$ and $CD8^+$ subset. Three different $CD8^+$ T-cell clones derived from (pre)diabetic mice and specific for insulin $B_{15\text{-}23}$ [9], $IGRP_{206\text{-}214}$ [14] and dystrophia myotonica kinase $(DMK)_{138\text{-}146}$ [16] have been reported (also referred to as clones G9C8, 8.3, and

AI4, respectively). For each of these clones, transgenic T-cell lines expressing the relevant receptors have been produced and represent important tools for studying the role of $CD8^+$ T cells in T1D. Interestingly, the insulin B_{15-23} $CD8^+$ T-cell epitope overlaps with a B_{9-23} $CD4^+$ epitope previously described as the target of a highly diabetogenic $CD4^+$ T-cell clone [17].

Is There an Initiating Autoantigen/Epitope?

Proinsulin (PI) is the only T1D autoantigen expressed, next to thymic epithelial cells involved in T-cell education, exclusively in β cells. PI's importance as an early target Ag is supported by data on PI knockout NOD mice. Different from humans, rodents express two PI isoforms, referred to as PI1 and PI2. NOD mice defective for the PI2 gene, the prevalent isoform in the thymus, display accelerated T1D, likely related to defective deletion of PI-reactive T cells [18]. Consistent with this hypothesis, NOD mice are protected from diabetes when a PI transgene is inserted that deletes the specific T cells in the thymus [19]. Conversely, NOD mice defective for the PI1 gene, the prevalent isoform in the islets but lacking expression in the thymus, are less susceptible to T1D. However, PI1 knockout islets transplanted into recently diabetic wild-type NOD mice become infiltrated and only transiently reverse T1D, suggesting that PI is an early but not exclusive target [20].

Recent evidence from Nakayama et al. [21••] further suggests that PI may be the initiating β-cell Ag in T1D. These authors produced NOD mice where the endogenous PI1 and PI2 genes have been deleted and replaced by a hormonally active PI transgene carrying a single amino acid mutation at position B16 (tyrosine to alanine). These mice are completely protected from T1D and insulitis [21••]. Intriguingly, the introduced substitution affects PI recognition by $CD4^+$ and $CD8^+$ T cells: position B16 is an anchor for binding to the H-2 K^d in the immunodominant $CD8^+$ epitope PI_{B15-23} and an invariable T-cell contact in the equally immunodominant $CD4^+$ T-cell epitope PI_{B9-23}. These data suggest that recognizing these immunodominant epitopes by $CD4^+$ and/or $CD8^+$ may be a mandatory early event in T1D pathogenesis.

To understand the mechanism of protection from islet autoimmunity by the B16 substitution, Nakayama et al. [22] undertook a series of adoptive transfer and immunization studies. First, an active tolerizing effect of transgene expression (eg, due to promoter leakiness or to increased thymic PI expression) could be ruled out because transgenic expression of a PI molecule with the native Tyr16 restored insulitis fully. Further experimentation suggested that the critical sequence in the PI B chain acts at two different levels: 1) for the initial priming of NOD anti-islet autoimmunity, as transplantation of NOD islets, but not bone marrow, expressing native PI sequences into mice carrying the PI-B16Ala transgene rapidly restored development of insulin autoantibodies and lymphocytic infiltration of recipients' islets carrying mutant PI sequences; and 2) for the effector phase, as splenocytes from B16-mutated mice transplanted with PI wild-type NOD islets induced T1D when transferred into wild-type NOD/SCID or even into B16-mutated NOD/SCID mice. Also, splenocytes from mice immunized with native insulin B_{9-23} peptide induced rapid T1D upon transfer only in recipients expressing the native PI sequence in their pancreata. Additionally, $CD4^+$ T cells from B16-mutant mice immunized with native insulin B_{9-23} peptide promoted insulin autoantibodies in NOD/SCID mice. Therefore, the provision of the native insulin B chain sequence is sufficient to prime anti-insulin autoimmunity, whereas subsequent transfer of T1D after peptide immunization requires native insulin B chain expression in islets [22].

Studies by Krishnamurthy et al. [23•] further corroborated the hypothesis that PI is the initiating Ag in the T1D of the NOD mouse, because mice rendered tolerant to PI by transgenic overexpression of PI2 in Ag-presenting cells do not develop the immunodominant $IGRP_{206-214}$-specific responses and are protected from T1D. Conversely, mice made tolerant to IGRP by the same means are not protected from T1D [23•], suggesting that the IGRP-specific responses lay downstream of PI-specific ones in the pathogenic cascade. The prerequisite of PI-specific responses for T1D to develop is also found in NOD8.3 mice, which are transgenic for a T-cell receptor recognizing the $IGRP_{206-214}$ epitope.

Epitope Spreading and "Secondary" Autoantigens

Despite strong evidence pointing to PI's triggering role, identifying more autoantigens and examining T-cell responses to them remain a high-priority goal of T1D research. Several considerations justify these efforts. First, the evidence supporting PI's critical role has been obtained in the NOD model, and T1D pathogenesis may be different in humans. Second, once autoimmune T-cell (and B-cell) responses to β cells are initiated, the specificity of these responses rapidly enlarges to include more Ags, a phenomenon referred to as epitope spreading. As a result of this process, responses to the presumable triggering Ag can rapidly be "overgrown" by a secondary response, as exemplified by IGRP-specific $CD8^+$ T cells, which rapidly outnumber PI-specific cells in the NOD model [10]. Finally, in the inbred NOD mouse, some variation exists in the specificity of the $CD8^+$ T-cell response between individual mice, a minority of which displays dominant responses to the DMK Ag [24]. Such variation is more extensive in the outbred human population. Because epitope spreading may occur in all individuals displaying clear signs of β-cell autoimmunity and, therefore, eligible for Ag-specific immune intervention strategies that may become available in the future, numerous autoantigens must be studied to cover individual variation in the specificity of autoimmune responses.

Although several autoantigens recognized by diabetogenic CD4+ T cells remain to be identified [4], recent data allow better assessment of two major autoantigens targeted by β-cell autoimmunity in NOD mice and humans: 65-kDa glutamic acid decarboxylase (GAD65) and tyrosine phosphatase-like molecule 512/insulinoma-associated protein 2 (IA-2). Knockout of the latter Ag in NOD mice did not prevent cyclophosphamide-induced diabetes, suggesting that IA-2, although required for normal glucose-stimulated insulin secretion, is dispensable for T1D development [25]. Using an even more conclusive approach, Jaeckel et al. [26] demonstrated that efficient tolerization of GAD65-specific CD4+ T cells by transgenic GAD expression in professional Ag-presenting cells of NOD mice did not affect T1D incidence or time of onset, suggesting that autoimmunity to this protein emerges downstream of earlier triggering events involving other Ags. Conversely, when the same authors used a similar approach to induce PI tolerance, T1D incidence was reduced. That some mice still developed T1D was interpreted as evidence that autoimmunity to PI is not an absolute requirement for T1D development. However, the fate of PI-specific CD8+ T cells was not addressed, and experimental evidence for tolerization of CD4+ T cells was more difficult to obtain in this study than in the previous one concerning GAD65. Consequently, PI tolerance may not have been complete in PI-transgenic mice [27]. Thus, considering current published evidence, only PI appears to be a plausible candidate for the role of a triggering autoantigen in the NOD mouse.

What Is the Mechanism of β-Cell Destruction?

Han et al. [28••] proposed an avidity maturation model in which the autoimmune evolution toward full-blown T1D requires the gradual selection of β-cell–specific CD8+ clonotypes of higher avidity [10]. Blunting of this avidity maturation process by peptide treatment effectively prevents disease. The corollary to this model is that peptide therapy should target immunodominant epitopes with peptides of limited affinity and/or in limited amounts. As shown for the $IGRP_{206\text{-}214}$ immunodominant epitope of NOD mice, this is important to spare low-avidity clonotypes, which can thus expand to occupy the intra-islet space left free by their high-avidity counterparts [28••]. Otherwise, near complete deletion of the epitope-reactive T-cell pool enhances the recruitment of subdominant (yet pathogenic) specificities, which then can cause disease. This model may explain why previous attempts to silence human autoimmunity by peptide injection were unsuccessful [29] but seemed to work better at lower doses [30]. Peptide therapy in autoimmunity may be most effective under conditions that foster occupation of the target organ lymphocyte niche by nonpathogenic, low-avidity immunodominant clonotypes than by pathogenic subdominant ones. It remains to be seen whether protection from T1D by low-affinity clones is a passive phenomenon, based on simple occupation by such clones of lymphoid "space," or involves an active regulatory mechanism.

The mechanisms responsible for the final damage to the β cells remain uncertain. The fact that CD4+ and CD8+ T-cell clones can mediate T1D development under certain conditions may suggest that cytokine- and cytotoxicity-mediated mechanisms are taking place. Besides a direct toxic effect on β cells, the role of Th1 cytokines may also be to "sensitize" β cells for killing by inducing Fas expression [31,32]. The cytotoxic activity may also be shared by β-cell–specific CD4+ T cells, as CD4+ T cells can also differentiate into cytotoxic T lymphocytes (CTLs) [33]. The lack of class II expression on β cells makes the Fas pathway more likely for CD4+ CTLs after their Ag-specific activation [34]. Perforin- and Fas-mediated killing mechanisms may instead be at play for CD8+ T cells [35,36].

What Is the Evidence in Human T1D?

In contrast to all evidence in the NOD mouse, formal demonstration for T cells' pathogenic role in human T1D is lacking. We know that CD4+ and CD8+ T cells are abundant in the T1D insulitis infiltrates [37] and that detecting CD4+ and CD8+ T-cell responses may offer new autoimmune markers for clinical applications [38,39,40•]. However, assessing whether β-cell–specific T cells are pathogenic would require demonstrating cytotoxic activity in vitro and/or of pathogenic potential in adoptive transfer experiments in humanized mouse models.

Another open issue is whether a similar Ag/epitope hierarchy can be established for human T1D, as observed in the NOD mouse. Is PI also the initiating Ag for human disease? Only indirect evidence suggests that this could be the case. The genomic locus conferring T1D susceptibility ranking second after the HLA class II region is a variable number of tandem repeats (VNTR) upstream of the PI gene promoter. Although the precise mechanism underlying increased T1D susceptibility is unknown, protective (class III) VNTR alleles are associated with higher PI mRNA and protein expression in the thymus and, likely, more efficient deletion of PI-reactive T cells [41]. Even though a recent clinical trial with subcutaneous insulin in at-risk subjects was unsuccessful [42], other regimens formulated from more recent data on Ag-based therapy [28••] may bring different results. Longitudinal analysis of the evolving β-cell–specific T-cell responses throughout the healthy prediabetic and diabetic period in selected high-risk subjects may also help to clarify this point.

T-Cell Assays in Humans

Although direct evidence for a pathogenic or otherwise disease-modifying role of specific T cells will be difficult to obtain in humans, assays allowing for quantitative detec-

tion and phenotypic and functional characterization of T cells recognizing β-cell Ags can provide information with diagnostic, prognostic, and therapeutic relevance in managing patients with T1D or at high risk of developing it. Such assays might help to assess the disease risk in concert with, or independently of, autoantibody measurements. Moreover, they might provide guidance for immunointervention strategies (eg, by helping to choose the time point for intervention and/or the Ag targeted by it). Another possible application of such tests might be monitoring of immune intervention that if successful would be expected to alter the frequency and/or phenotype of autoantigen-specific T cells. Although developing reliable T-cell assays has been a research goal for many years, substantial progress has only been seen in recent years.

Assays measuring $CD4^+$ T-cell responses, long plagued by poor specificity and/or sensitivity, have recently been evaluated in a blinded fashion in a study sponsored by the Immune Tolerance Network. Whereas a standard proliferation assay using soluble Ags and interleukin-2 addition showed excellent specificity (94%) but poor sensitivity (58%), an assay measuring proliferation to human pancreatic proteins added to cultures after blotting on nitrocellulose particles displayed excellent specificity (83%) and sensitivity (91%) [40•]. Thus, an assay using many of the identified autoantigens (PI, GAD, IA-2, and others) showed much lower sensitivity than one using bulk β-cell Ags, which may suggest that Ags remaining to be identified account for a significant proportion of β-cell targeted autoimmunity at T1D onset. Although the source of antigenic material used in the latter assay (islets prepared from cadaveric human pancreata) may not conveniently be obtainable for more widespread use of the assay, it is encouraging that at least one group can distinguish T1D patients and healthy individuals reliably in a $CD4^+$ T-cell assay. It is hoped that additional and less cumbersome assays (eg, ones reported to detect secretion of different cytokines by $CD4^+$ T cells from patients and controls in response to IA-2 and PI peptides [38]) will be validated in a similar fashion in the near future.

Impressive progress has recently been achieved by several groups with $CD8^+$ T-cell responses against β-cell Ags. We initially used an enhanced "reverse immunology strategy" to identify six naturally processed, HLA-A2–presented epitopes derived from PI [43•]. More epitopes derived from GAD65 and IA-2 were identified upon DNA vaccination of HLA-A2 transgenic mice, followed by screening with splenocytes from immunized mice of candidate epitopes selected by a prediction algorithm [44]. Using the resulting panel of 20 epitopes, we found that an Elispot assay measuring interferon-γ secretion by peripheral blood mononuclear cells from $HLA\text{-}A2^+$ donors could discriminate patients at T1D onset and controls, with sensitivity (86%) and specificity (91%) similar to that seen with the immunoblot test discussed above [39•]. Although these results remain to be validated with a larger number of patients and in blinded fashion, they suggest that $CD4^+$ and $CD8^+$ T-cell tests capable of reliable and specific detection of T1D-associated autoimmunity may be at hand. Once tests are validated, it will be a priority to analyze T cells from individuals at high risk for T1D and to include pediatric patients in screening. The latter will be facilitated by the fact that the performance of our Elispot assay is identical when a restricted panel of five epitopes is used.

Other groups also identified HLA class I (mainly HLA-A2) presented autoantigenic epitopes recognized by patient CTLs [45–48]. Even though most of these studies did not provide statistically validated information on the suitability of the discovered epitopes for distinguishing patients from controls, covering a wide array of Ags and epitopes will be helpful for developing tests with wide population coverage. An interesting study published very recently found that tetramers of HLA-A2 with peptide $GAD_{114\text{-}122}$, an epitope also immunodominant in our study, detect the same number of specific $CD8^+$ T cells in patients and controls; however, in patients (and not controls), many of the specific cells were found in the activated/memory $CD45RO^+$ fraction [49•]. Reconciling this report with our finding of exclusive responses to this and other epitopes by patient CTLs, naïve-specific cells from healthy donors would not be expected to secrete interferon-γ.

Conclusions

Although the notion of T1D as an autoimmune disease first stemmed from the description of ICAs in patients, evidence gathered to date about the T-cell–mediated T1D pathogenetic mechanisms comes from the NOD mouse model. Validating and transferring this knowledge to human T1D will further deepen our understanding of the disease and may provide new inspiration for therapeutic interventions.

Disclosures

No potential conflicts of interest relevant to this article were reported.

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