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REVIEW

The primacy of CD8 T lymphocytes in type 1 diabetes and implications for therapies

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Abstract Type I diabetes (TID) is an autoimmune disease in which insulin-secreting beta cells of the pancreatic islets are destroyed by T lymphocytes. Until the 1990s, the prevailing dogma was that the attack was attributable to rogue T lymphocytes bearing CD4 markers on their surface (CD4 T helper lymphocytes). Today, the prevailing view is that rogue T cells bearing CD8 markers or cytotoxic CD8 T lymphocytes are also important and perhaps the foremost contributors to beta-cell death. Recognizing CD8 T-cell subsets as the prime culprits has helped to trace the disease's pathogenesis to abnormal T-cell education. Defective education can occur when antigen-presenting cells fail to assemble and present self-antigens to naïve T cells. The failure in that process, normally designed to prevent T cells' attack on the body's own antigens, enables self-reactive T cells to escape into the circulation. Once released, the self-reactive CD8 T cells kill specific self-antigens, which, in the case of TID, include insulin and other key proteins associated with beta cell functions. Abnormalities during T-cell education have been mapped in part to genetic defects in specific gene-encoding regions of the major histocompatibility complex class I region and to proteins that assemble self-peptides into the MHC class I structure that map within the MHC class II region. Two decades of research have led to understanding of genetic and functional defects in the immune system, placing us at the threshold of finding new therapeutic strategies aimed at eliminating autoreactive CD8 T cells, while preserving healthy immune cells.

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Introduction

Beta cells of the pancreas normally secrete insulin, an essential hormone that converts glucose into its storage form, glycogen, which in turn lowers blood glucose levels. In type I diabetes (TID), insulin-secreting beta cells are targets of autoimmune destruction by T lymphocytes and subsequent humoral response, resulting in insulin deficiency and dysregulation of glucose metabolism. Better understanding of the details of the pathogenesis of TID—especially what type(s) of T lymphocytes destroy the insulin secreting beta cells of the islets—are essential for finding treatments for a chronic condition whose onset in childhood carries lifelong morbidity and early mortality [1, 2]. In the last 20 years, much progress has been made in identifying rogue CD8 T cells specifically targeted at destroying self-tissues such as the insulin-secreting islets essential for normoglycemia. That progress underpins hope that new therapies can specifically and selectively kill only disease-inducing subsets of autoreactive CD8 T cells.

Role of CD4 T cells in pathogenesis of type I diabetes

For decades prior to the 1990s, the cause of TID was largely ascribed to T lymphocytes (T cells) bearing the cell surface marker CD4, known as T-helper cells [3]. As helper cells, they do not directly kill their targets, but they do so indirectly by summoning and cooperating with other immune cells, in this case, to launch the autoimmune response that annihilates the beta cells. Before the 1990s, a

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range of evidence favored a sole role of CD4 T cells in TID: (1) CD4 T cells in animal models could be identified in abundance in islet cell infiltrates; (2) transfer studies showed that the isolation of purified populations of CD4 T cells from NOD mice (non-obese diabetic mice) or clones of CD4 T cells, an animal model of TID, could transfer diabetes into still disease-free young mice without autoimmune disease [4]; 3) the genetic region to which the defective genes mapped, in mice and humans, was the major histocompatibility complex for expression of class II polypeptides that interact specifically with CD4 T cells. This genetic region carried the highest statistical risk for conferring TID. Taken together, the evidence amply suggested that TID, at least in mice, was caused by rouge CD4 T cell clone. The immune pathology was thought to be from the autoreactive CD4 T cells escaping into the circulation, as a result of faulty positive T selection, consistent with MHC class II structures presenting the wrong diabetogenic peptide. A role for CD8 T cells in TID was not considered because these types of cells, or subsets thereof, were thought to be restricted to combating foreign antigens, such as viruses or viral fragments and CD4 T cells seems to be able alone to cause disease.

But over time, evidence began to mount and implicate CD8 T cells in TID. The evidence came from research pointing to specific abnormalities in T-cell education. To understand the evidence, it is key to describe the normal process of T-cell education and its role in averting autoimmunity.

Normal T-cell education

Before T cells become fully differentiated, they are subject to T-cell education, a normal process by which naive T cells learn to become tolerant to the body's self-antigens, thereby preventing autoimmunity. Orchestrated by the immune system's antigen-presenting cells (APCs), T-cell education eradicates T cells that are reactive to self-antigens. The process, which takes place in the thymus, bone marrow, or in the periphery, serves the purpose of distinguishing self-antigens versus foreign antigens (Fig. 1a). Normal APCs express on their surface two major classes of antigen presenting structures, i.e., MHC classes I and II. The genes expressing these two classes of cell surface markers possess such abundant polymorphisms that they confer to any person a unique identity of the structures as well as a unique identity of the 100s of peptides presented in the exterior facing grooves of the diverse MHC proteins. Each class is encoded by adjoining segments of the genome known as the major histocompatibility complex (MHC). Found on chromosome 6, the MHC, also known as the HLA region in the human, encodes at least 200 proteins controlling the immune response. After the completion of T-cell education, T cells can be activated to mount an attack on a foreign antigen (e.g., a virus) when APCs present T cells with the virus (or viral fragment) bound to an MHC-encoded class I or foreign antigen (e.g., a bacteria) bound to an MHC-encoded class II cell surface antigen. If foreign antigens are not bound to an MHC-encoded class I or class II markers, they will not be recognized as foreign. Failure to recognize a foreign antigen leaves the host to succumb to infection.

Class I polypeptides on the surface of APCs only bind to CD8 T cells, whereas class II polypeptides only bind to CD4 T cells. During normal T-cell education, the binding between an APC (displaying class I or II polypeptides on its surface) and a T cell (CD4 or CD8) occurs only when an intracellular antigen fits into a physical groove on the APC's external-facing surface of a class I or II structures. In other words, the APC and the T cell can only bind through the displayed peptide, which is exposed in the groove of the class I or II polypeptide on the APC surface. Once adequate binding occurs between the APC and the T cell during the normal education process, the T cell subsequently becomes tolerant (i.e., unreactive) to the specific self-antigens, a process termed positive selection and the T cell is released into the circulation. During normal negative T-cell selection, a developing T cells bind very strongly with "self"-peptides presented by MHC class I or II structures and these strongly self-reactive cells receive an apoptotic signal and should die during normal T-cell development and prior to release into the circulation. Any type of defect in the education process can induce autoimmunity by enabling naïve, poorly educated T cell to escape into the circulation. There, the rogue T cell is activated by exposure to specific self-antigens, which it has never seen before if the immune defect is in negative selection, and the T cell matures into an autoreactive T cell. Those autoreactive T cells “see” self-antigens as foreign, not as self. The consequence is an inflammatory process, unleashed by autoreactive T cells, leading to death of certain self-antigens and, ultimately, the body's cells that display them on their cell membrane.

Two distinct processes take place during normal T-cell education. One is "positive selection," during which the body's self-antigens (e.g., insulin) are properly displayed within the groove of the class I or class II polypeptide. The T cell thereby learns to become tolerant to—that is, avoid killing of—the self-antigen whenever encountered again on the target organ. A defect, in positive selection, i.e., by an incorrect antigen in the Class II groove rather than the correct self-antigen, was considered the cause of CD4 T cell-mediated TID. In the second type of defect, a defect can occur in negative selection, i.e., APCs bear deficient self-antigen in the groove of class I structures, an observational difference we uncovered in discordant identical twins with diabetes (Fig. 1b). The event of self-peptide

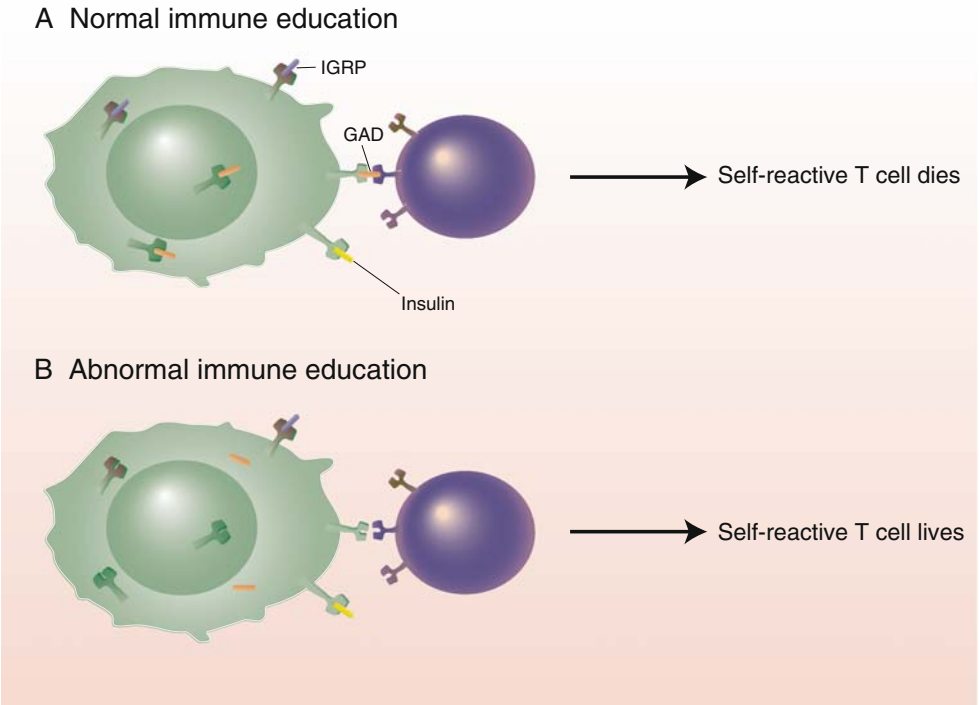

Fig. 1 Normal and abnormal education of T cells. Normal antigen presenting cells such as macrophages, dendritic cells, or B cells efficiently display many self-proteins, especially in the MHC class I groove to developing CD8 T cells. During normal T cell selection, this presentation of self-proteins results in the death of highly auto reactive CD8 T cells that bind tightly and this leads to the death of the autoreactive T cells before release into the blood (**a**). In the case of autoimmunity, we identified a primary defect in the antigen presenting cells and these cells have gaps in the ability to present many self-proteins (**b**) [6]. As a result of interrupted antigen presenation of self-peptides, these autoreactive T cells are not killed, i.e., negatively selected and live to circulate after release into the circulation

presentation should trigger, during T-cell education, apoptosis of the tightly bound potentially autoreactive T cells, according to our research if the system functioned normally. Although self-antigens are correctly presented to T cells, in autoimmunity, the full repertoire is not presented. These defects in negative T-cell selection certainly exist in MHC class I presentation for humans, and there is even evidence of similar defects in negative selection in MHC class II as well [5, 6]. Negative selection, rather than positive selection, turns out to be a common defect in the pathogenesis of TID and may be controlled by some of the MHC class I presentation genes within the MHC class II region such as the transporter for antigen presentation (TAP) and low-molecular-weight protein (LMP) proteasome related genes [6, 7]. TAP genes are members of the ATP-binding cassette transporter family that specializes in delivering proteasome cleaved cytosolic proteins into the endoplasmic reticulum for MHC class I assembly; genetic diversity of TAP proteins can contribute to the selection of peptides. LMP genes, which include LMP2 and LMP7, are two MHC class II-encoded genes whose proteins contribute immune cells to some of the subunits of the large proteasomes. Allelic variability in the LMP subunits contributes to the activity of the proteomes to produce fragments of self-peptides. Crucial to uncovering the T cell's role is by pinpointing the class and identity of T cells capable of escaping death during their education and the genetic and functional defects they possess.

Roles of CD8 T cells in pathogenesis

CD4 T cells were long implicated in the etiology and pathogenesis of TID, with the underlying hypothesis that autoreactive CD4 T cells were generated by a defect in positive selection during T-cell education. The hypothesis generated a large body of favorable evidence, noted previously, which implicated an exclusive role for CD4 T cells. CD8 T cells, or subsets thereof, were not considered because of the long-standing view that they only could become cytotoxic by exposure to viral antigens or viral fragments, not to self-antigens.

The view among immunologists was that CD8 T cells had a well-established role in viral defense but no relationship to autoimmunity. Viruses or viral fragments were shepherded in the cytoplasm into tiny polypeptide fragments via the protasome, transported in the endoplasmic reticulum by the TAP transporters, assembled with

MHC class I in the endoplasmic reticulum, and then transported to the cell surface of APCs. The cell surface viral MHC class I complex could then bind to CD8 T cells. The binding CD8 T cells are activated, proliferate, and attack viral antigens. Crystallography studies identified separate proteins within the MHC class I structures of APCs and had repeatedly found viral peptides being displayed together with class I peptides [8–10].

The primacy of CD8 T cells in autoimmunity, especially as it relates to diabetes, came into focus with a study of human monozygotic twins and NOD mice [6, 11]. It was found that the diabetic twin, as compared with the normal one, expressed a lower density of certain types of class I proteins on the surface of APCs. The density of MHC class I structures was the same but the affinity of selected MHC class I antibodies was different. The antibodies with lowered binding were all known to be specific for peptide-filled MHC class I structures. Furthermore, decreased expression of class I proteins was also found in prediabetic individuals, as well as in prediabetic NOD mice. We found that fewer antibodies were able to bind with high affinity to conformationally specific class I structures, .e., antigen-filled class I structures. This finding suggested that although class I molecules could reach the cell surface of APCs, there was a deficiency in peptide filling into the class I groove. One explanation was that viral antigen presentation was interrupted in the diabetic twins conferring disease. Another interpretation, since the difference was between identical twins, was that the MHC class I structures also presented self-peptides and the diseased twin was not presenting the full repertoire of self-peptides i.e. a negative selection defect. We interpreted this as being secondary to a deficiency in assembling internal self-proteins for display on MHC class I structures leading to faulty CD8 T-cell education [6, 7]. Remarkably similar studies in the NOD mouse also confirmed that the APC of this spontaneous autoimmune animal model also had defects in MHC class I presentation [11].

A related line of structural biology studies of normal APC found evidence that not only foreign viral antigens, but also endogenous self antigens that start out in the cytoplasm, could fit into the groove of class I molecules [12–15]. The process of endogenous antigen delivery into the endoplasmic reticulum and into the class I groove is controlled in part by two genes, TAP1 and TAP2, which encode peptide transporter proteins essential for antigen presentation. Another set of genes was also implicated, LMP2 and LMP7, closely linked catalytic subunits of a proteasome associated with the cytoplasmic cleavage of peptides for transportation into the endoplasmic reticulum by the Tap transporters. MHC class I binding to self-peptides occurs within the endoplasmic reticulum. Support for the concept that MHC class I could also present self-peptides came from confirmatory crystallography studies that class I molecules have self-peptides in their grooves [12, 13]. this buttressed the concept that MHC class I structures perhaps had two job assignments i.e. presentation of viral fragments for the generation of cytotoxic T cells and presentation of self-peptides during T-cell education to assist in the class I-driven negative selection process to prevent autoreactivity.

We extended these findings to the NOD mouse and TID in humans. We and others have found defects in expression levels and polymorphims of TAP1 and TAP2 genes, as well as the LMP2 gene [16–24]. Data also suggested polymorphisms of these genes could either directly or indirectly through splice variants change the self-peptide repertoire [16, 17]. These findings provided the first evidence of defects in loading of self-antigens into class I polypeptides in the pathogenesis of TID. Human studies also implicated defects in class I genetic loci itself with the many allelic variants could itself also confer risk factors for TID [21–23]. Indeed, subsequent research demonstrated that the LMP2 deficiency in the NOD mouse was the same as that identified in patients with 100% Sjogren's syndrome, an autoimmune disease in humans of the salivary and lachrymal glands [24]. Overall, defects in class I assembly and loading implied that TID as well as other autoimmune diseases was a result of a negative selection defect.

The body of mouse and human evidence broadened the long-established role of class I polypeptides from solely providing protection against viral antigens to protecting against both viral and self-antigens [6, 7, 11, 17–25]. Other investigators confirmed our findings by revealing a direct way for poorly educated cytotoxic CD8 T cells to kill beta cells expressing self-peptides in the class I groove in murine models [26–28]. Taken as a whole, these studies suggest that CD8 cells exert a strong role in the etiology of TID.

Treatment implications

Today, the foremost treatment for TID is insulin replacement in accordance with blood glucose monitoring. Insulin is the predominant autoantigen targeted by autoreactive T cells, although there are several other self-antigens. Insulin replacement therapy is still far from ideal, considering that patients are beset by considerable morbidity and mortality [1, 2]. Insulin replacement does not attack TID's underlying pathology, poorly educated autoreactive CD8 T lymphocytes, nor does the other mainstay of treatment, immunosuppression, which is too nonspecific, leaving patients highly vulnerable to infection and other adverse effects. Worldwide effort has been focused on targeted immunotherapies that capitalize on better understanding of TID's pathogenesis.

In animal models, one immune approach has been to reselect naïve CD8 T cells while killing autoreactive CD8 T cells by administering matched MHC class I self-peptide complexes [29, 30]. Another successful attempt to induce tolerance was by specifically destroying autoreactive T cells through administration of the immunoregulatory cytokine tumor necrosis factor (TNF) or a TNF inducer [31]. Autoreactive T cells in TID, as compared with normal T cells, are expressly vulnerable to TNF-induced death as a result of an error in signaling by an intracellular transcription factor. NOD mice treated with a TNF inducer succeeded in reversing TID, returning hyperglycemic animals to normoglycemia [29, 30]. Our laboratory has recently shown that, in fresh blood drawn from patients with TID as compared with healthy controls, a subpopulation of CD8, but not CD4, T cells died with exposure to TNF, or an inducer of TNF [31]. The subpopulation was traced to at least one subset of autoreactive T cells for insulin. The potential for TNF as a highly targeted treatment is dampened, however, because it is systemically toxic. Most cells in the body have TNFR1 receptors and many believe the TNFR1 receptor pathway is the exclusive pathway for toxicity. But there is another TNF receptor, TNFR2, which is less ubiquitously expressed. The same study showed that an agonist or agonist antibody targeted specifically at the second type of TNF receptor was effective in eradicating autoreactive CD8 T cells in human blood samples, while protecting normal T cells from death [31]. The findings underscore the value of unraveling pathogenesis to drive new opportunities for targeted immunotherapies.

Conflict of interest The authors declare that they have no conflict of interests.

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