Immunomodulation of Autoimmune Diabetes by Dendritic Cells

Klaus Pechhold, MD, and Kerstin Koczwara, PhD

Corresponding author
Klaus Pechhold, MD
NIDDK-Diabetes Branch, NIH, 10 Center Drive, Building 10-CRC, Room 5W-5888, Bethesda, MD 20892, USA.
E-mail: KlausP@intra.NIDDK.NIH.gov

Current Diabetes Reports 2008, **8:**107–113
Current Medicine Group LLC ISSN 1534-4827

The biology and properties of dendritic cells (DCs) have been intensely studied in the research areas of infectious diseases, tumor immunology, and vaccine development. This unique subset of immune cells has recently also moved to the center of interest for basic and clinical research in autoimmunity, owing not only to the extraordinary importance of DCs in the initiation and sustenance of adaptive immune responses, but also to more recent discoveries about their profound ability to control and downregulate ongoing T-cell responses. We review current progress of using DCs in mice for induction and propagation of autoimmune T-cell responses and their therapeutic potential to dampen or even stop β-cell–specific autoimmunity. Finally, we offer our perspective on how basic research progress in DC technology, mostly from mouse models, may translate into emerging diagnostic and therapeutic applications for human type 1 diabetes.

Introduction

Organ-specific autoimmune diseases, such as type 1 diabetes, occur when self-reactive T cells become persistently activated, leading to a chronic, often slowly progressing organ destruction. At least two safeguards of tolerance must have failed: 1) Central or thymic tolerance normally assures the deletion of T cells whose affinity to endogenous (self) protein is dangerously high. This negative selection is mediated by specialized dendritic cells (DCs) in the thymic medulla. 2) Upon exiting the thymus, autoreactive T cells must evade the regulatory, presumably antigen-specific control mechanisms and become activated in local lymph nodes (LNs).

Interest in the biology and function of DCs is based on overwhelming data that place DCs in the center of T-cell activation and the control of potentially dangerous T-cell responses, reflecting the DCs' unique ability to intimately (ie, antigen specifically) "address" T cells under a variety of very different environmental conditions. For instance, DCs migrating from infectious foci carrying foreign, proteinaceous material to LNs will alert antigen-specific T cells, a process greatly aided by innate immune stimulators, such as toll-like receptor ligands. In contrast, resident DCs in pancreatic islets will take up β-cell antigen but will not be activated in the absence of local innate immune stimulators, so that potentially autoreactive T cells will interpret activation signals differently. As a consequence, T cells will not differentiate into fully armed effectors that could cause autoimmunity, but instead may result in an abortive or even regulatory T-cell response. T-cell activation and proliferation can be demonstrated in the LNs with no further signs of organ-specific autoimmunity or diabetes development (Fig. 1). Therefore, manipulating T cells by using DCs, especially if augmented by autoantigens, has to take into account that the circumstances of DC:T-cell interaction will be critical.

In this article, we summarize approaches involving DC manipulation to affect diabetogenic or diabetes-associated T-cell responses in vivo. After summarizing basic principles recognizing the outstanding role of DCs for T-cell activation, we review approaches that take advantage of DCs' superior T-cell activation properties to identify diabetes-associated T-cell epitopes, with the goal of broadening the availability of autoantigens for diagnostic and therapeutic purposes, and attempts to use DC therapeutically by directly downregulating ongoing autoimmunity.

Induction of T-Cell Responses by DCs

Protective immune responses to invading pathogens initially and quickly invoke a series of mechanisms and properties of the innate immune system that together elicit a process known as inflammation. In contrast, the phylogenetically younger adaptive immune system gathers its full protective strength with some delay primarily due to the need to specifically recruit, activate, and multiply antigen-specific T- and B-effector lymphocytes. T cells can be distinguished in $CD8^+$ and $CD4^+$ subsets whose T-cell receptors interact with major histocompatibility complex (MHC) class I and class II molecules, respectively,

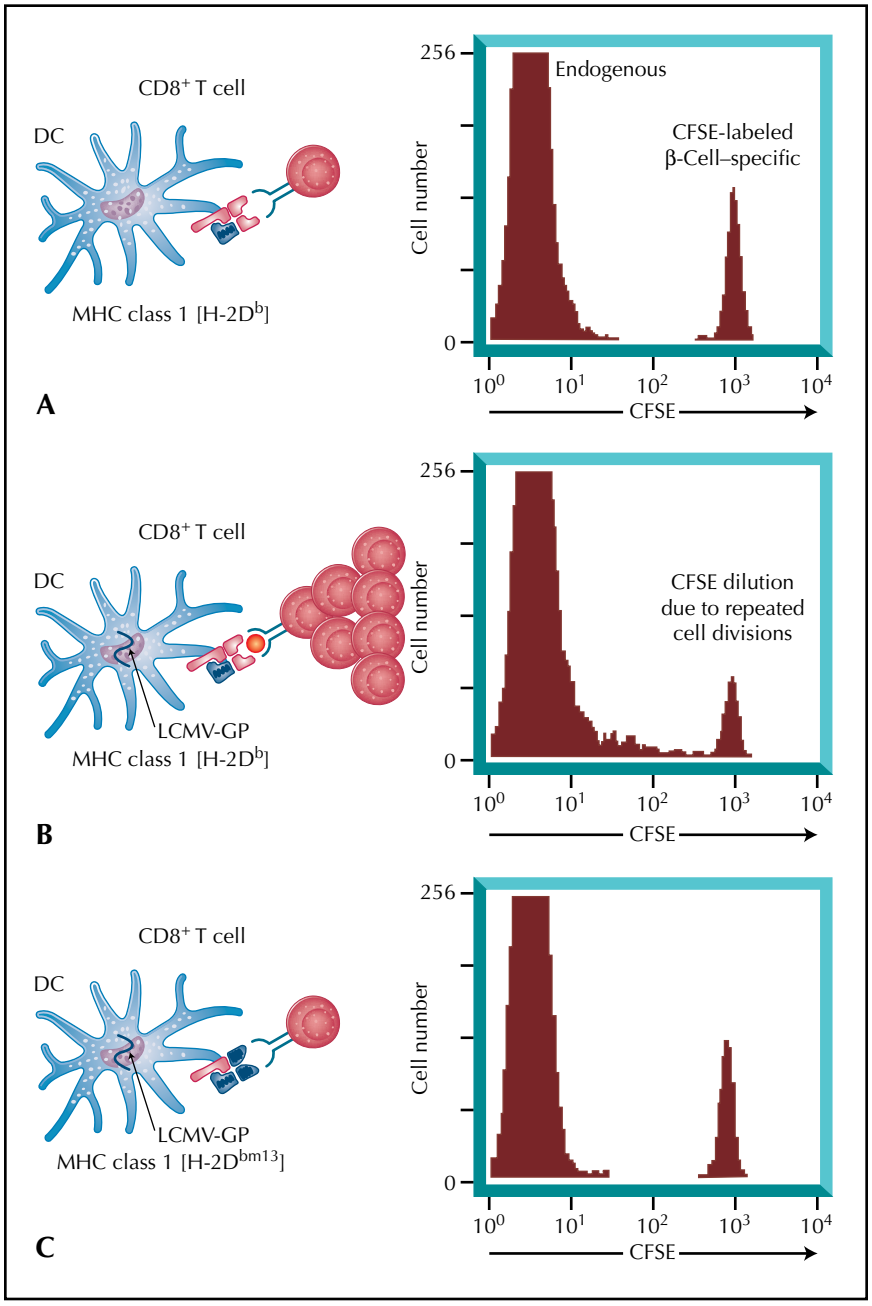

Figure 1. Constitutive cross-presentation of islet-derived antigen in pancreatic lymph nodes by bone marrow–derived antigen-presenting cells. In the absence of cognate antigen (no transgenic expression of lymphocytic choriomeningitis virus glycoprotein [LCMV-GP]), naive, LCMV-GP–specific $CD8^+$ T cells are not activated and do not undergo cell division (**A**). In the presence of transgenic β-cell LCMV-GP antigen and the correct major histocompatibility complex (MHC) class 1 restriction molecule, $CD8^+$ T cells proliferate in pancreatic lymph nodes by a mechanism involving cross-presentation (**B**), resulting in halving of carboxyfluorescein succinimidyl ester (CFSE) fluorescence with each cell division. Proliferation did not occur in the presence of antigen after exchange of MHC class 1 molecules on hematopoietic cells by allogeneic bone marrow transplantation due to the prevention of cross-presentation (**C**).

which are displayed on the surface of antigen-presenting cells (APCs). Whereas MHC class I molecules generally display 8 to 10 amino acid residue long-protein fragments (peptides) of endogenous proteins, MHC class II molecules bind slightly longer peptides generated by the phagosomal/lysosomal processing of exogenous (ie, endocytosed) proteins. While remaining receptive to regulation by the innate immune system, DCs serve the primary link between both immune systems by simultaneously controlling the antigen uptake in the peripheral organ and its processing for subsequent T-cell activation. In addition to presenting endocytosed protein fragments via MHC class II molecules to DC4+ T cells, DCs are capable of antigenic "cross-presentation," indicating the ability to present endocytic protein fragments to $CD8^+$ T cells via MHC class I molecules, to allow for activation of both major T-cell subsets. DCs also modulate their responsiveness to inflammatory environments, leading to transformation into highly potent APCs, and their directed migration to the regional LNs. LNs constitute an integral component of the secondary lymphoid organ by attracting nonspecifically antigen-inexperienced (ie, naïve) T cells from the bloodstream, providing a critical location of likely encounter between DCs (from tissue) and naïve T cells from the blood.

T cells accumulate in the T-cell zone of LNs, where they constantly contact DCs by a process termed *scanning*. It indicates the frequent sampling (ie, attempted

binding) of DCs' MHC/peptide complexes by their antigen-specific T-cell receptors (TCRs). Depending on the "fit" (ie, whether the structural constraints of individual MHC-bound peptides allow stable TCR binding), the T cell receives an activation signal through its TCR complex (signal 1). Mature DCs are particularly well equipped to strengthen the MHC:TCR interaction by providing multiple accessory signals, collectively called signal 2, including monomorphic receptor:ligand binding and soluble factors, which all facilitate or enable full T-cell activation. The overall stability of DC:T-cell interaction, called avidity, determines the differentiation into effector T cells. Activated T cells undergo a series of changes that result in proliferation, cytokine secretion, and elaboration of regulatory or suppressive properties, and for $CD8^+$ T cells, the acquisition of cytolytic activity. In addition, activated T cells profoundly alter their responsiveness to chemokines and adhesion molecules that together allow them to efficiently access peripheral inflamed tissue. Finally, the quality of T-cell activation determines short-term survival and probably affects the chances of long-term persistence, also known as memory cell development.

Role of DCs in Autoimmune Diseases

Self-reactive B and T cells undergo fine-regulated selection and deletion procedures during development in the thymus and bone marrow, respectively, but some autoreactive B and T cells evade deletion and reach the periphery. Peripheral tolerance mechanisms, including anergy, regulation or suppression, antigen sequestration, and clonal deletion, assure that these autoreactive T cells remain under control. The exacerbation of autoimmune diseases is often associated with some influence by susceptibility genes and environmental triggers that synergistically facilitate autoreactive T-cell activation and expansion. Environmental triggers accused of derailing self-tolerance and causing autoimmunity include innate immune stimulators during infectious diseases, the exposure of otherwise ignored (ie, cryptic) autoantigens, or mimics of host antigens introduced by the invading pathogen (molecular mimicry). It has been proposed that a reduction in the number or function of regulatory T cells (Tregs) [1], alterations of the DCs' physiology, and changes in the immunologic synapse occurring at the DC:T-cell interface [2] could precipitate the breakdown of self-tolerance and induce autoimmunity. Several studies have attempted to identify individual pathomechanisms and the contribution by DCs to the etiology of autoimmune diseases.

DCs involvement in autoimmune pathomechanisms has been described for rheumatoid syndromes, including rheumatoid arthritis and systemic lupus erythematosus, and for neuroinflammatory disorders, such as multiple sclerosis and Alzheimer disease [3]. Evaluating the current literature on DC involvement in the innate immune system is beyond the scope of this review, such that we focus on the current knowledge of DCs' role in autoimmune diabetes pathogenesis.

Role of DCs in Autoimmune Diabetes

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease caused by T-cell–mediated destruction of the insulin-producing β cells in the pancreatic islets of Langerhans [4]. Very early in the development of insulitis (inflammation of pancreatic islets caused by infiltrating leucocytes), DCs migrate into the islets, secondary to infectious (viral infection) or noninfectious causes (abnormal remodeling of islet architecture during development). Imagawa et al. [5] found predominantly $CD8^+$ T cells, but also $CD4^+$ T cells, B lymphocytes, and macrophages in islet infiltrates of pancreatic biopsies of subjects with prediabetes or recent-onset T1DM. Moreover, inflamed islet cells expressed increased levels of MHC class I molecules.

The spontaneous nonobese diabetic (NOD) mouse model is genetically diabetes prone, and its immunopathology resembles that occurring in humans. Peri-islet infiltration in female NOD mice becomes evident by 4 weeks of age, followed by slowly progressing islet destruction, with most β cells destroyed by 4 to 6 months of age. This process was accompanied throughout by elevated numbers of DCs and macrophages in the inflamed islets [6]. Charre et al. [7] demonstrated elevated levels of DCs already in the neonatal pancreas, followed by a further accumulation around islets at about 4 weeks of age [6]. DCs and macrophages acquire antigen and are very likely to present it to T cells in the pancreatic LNs. The transient absence of DCs and macrophages in NOD mice (by depletion using clodronate-loaded liposomes) resulted in the disappearance of islet-infiltrating lymphocytes, (transient) resolution of insulitis, and a substantial delay in diabetes recurrence, supporting the pivotal role of DCs (and macrophages) during the islet destructive process [8]. Alterations in the islet microenvironment can precipitate islet inflammation and diabetes development, demonstrated by β-cell–specific, transgenic tumor necrosis factor-α (TNF-α) secretion in NOD islets (rat insulin promoter [RIP]-TNF-α-NOD mice [9]). Neonatal expression of the inflammatory cytokine TNF-α could lead to increased β-cell apoptosis and in turn to recruitment and activation of DCs and macrophages. It is conceivable that the resulting enhanced presentation of autoantigens to islet-specific T cells and differentiation into effector T cells could exacerbate autoimmunity [9]. Even without a genetic mutation (transgene), DCs accumulating in pancreatic lesions can produce TNF-α [10].

Because phenotypic and functional differences between distinct DC subsets in NOD mice exist [11], several studies aimed to more precisely determine the relevance of DCs in the initiation and maintenance of autoimmune β-cell destruction. All of them support a central role of DCs for initiating the autoimmune cascade that culminates

in diabetes [12]. Major advances in imaging techniques using two-photon laser-scanning microscopy in vivo led to the observation that T-cell activation in NOD mice resulting from persistent DC:T-cell interaction was essential for full activation and differentiation of autoreactive, islet antigen–specific T cells. Using the same technology, Tang et al. [13••] demonstrated an islet antigen–dependent interaction between DCs and Tregs. To date, the precise factors that predict DC-induced proinflammatory (prodiabetic) and regulatory (protective) T-cell responses remain poorly understood.

Several studies examined whether the phenotype (and function) of DCs in the context of autoimmune diabetes was abnormal. Constitutive functional abnormalities of DCs have been reported in spontaneous diabetes models, indicating hyperactivation [14–16] or reduced functionality [17,18]. Marleau and Singh [14] suggested that DCs from NOD mice had excessive costimulatory capacity leading to pronounced pathogenic Th1 responses, which are thought to facilitate the activation and persistence of autoreactive T cells. Also, NOD DCs exhibited increased interleukin (IL)-12 secretion upon introduction of various stimuli, favoring Th1-type immune responses during the diabetogenic process [15].

Although impaired responses to infection, vaccination, or altered hypersensitivity reactions have not been observed in patients, some reports noted potential abnormalities of DCs in T1DM [19,20]. Most recently, it has been reported that absolute numbers of peripheral blood DC subsets were decreased in new-onset and in established T1DM, although the phenotypic maturation state of those DCs was indistinguishable between patients and healthy controls [21].

The significance of cross-presentation has been shown for the immune defense against viruses, intracellular bacteria and tumors, and tolerogenic and proinflammatory roles of cross-presented autoantigens in autoimmune diseases have been reported. DCs, and to a lesser extent macrophages, activated B lymphocytes, and pancreatic endothelial cells [22], have the ability to cross-present endocytosed antigen to cytolytic T lymphocytes. Early evidence that tissue-associated "self" antigens can be presented in the context of MHC class I to $CD8^+$ T cells via an exogenous processing pathway came from the adoptive transfer of ovalbumin (OVA)-specific $CD8^+$ T cells (OT-I) into RIP-mOVA transgenic mice, whose islet β cells expressed a membrane-bound form of OVA. OT-I cells became activated in the draining LNs necessarily due to MHC class I–restricted cross-presentation of OVA by a bone marrow–derived APC population, presumably DCs. This finding introduced a mechanism to explain how $CD8^+$ T cells can be primed to antigens of nonlymphoid tissues, which are not normally surveyed by recirculating naïve T cells [23]. Cross-presentation plays an important role in organ-specific autoimmunity because simultaneous priming and expansion of $CD4^+$ and $CD8^+$ T cells in LNs is known to be an essential process in the initiation and progression of autoimmune diseases [24].

Tolerance and Regulatory T-Cell Induction by DCs

Although DCs are fundamentally important in autoimmune diabetes, it is necessary to point out that autoantigen presentation in lymphoid organs is not exclusively mediated via DCs. Recently, Lee et al. [25•] have shown that LN stromal cells are also capable of antigen presentation and promotion of deletional T-cell tolerance, possibly by a mechanism similar to thymic negative selection.

The observation that DCs and a diabetes-protective Th2 cytokine environment were present during early, nondestructive insulitis raised the possibility that under certain conditions, DCs have a protective role in autoimmune diabetes. Papaccio et al. [26] reported the disappearance of DCs from peri-islet infiltrate coincided with an increase in systemic proinflammatory Th1 cytokine levels. Using the RIP-mOVA transgenic mouse model, Kurts et al. [27] have shown that OT-I cells activated by cross-presentation are subsequently deleted from the peripheral pool of recirculating lymphocytes, a process for deletion of antigen-specific $CD8^+$ T cells that depended on DCs with exquisite tolerogenic properties [27].

Tremendous progress has been made in characterizing DC-induced Tregs, a specialized yet diverse subset of T cells with the potential to mediate protection against autoimmunity. It is likely that various lineages and distinct differentiation states of DCs profoundly affect the properties and function of Tregs and its antigen-dependent control of autoimmunity [28]. Current concepts of Treg function in general, and in autoimmune diseases in particular, include cytokine-mediated effects (transforming-growth factor-β, IL-10) and cell contact-dependent mechanisms and are reviewed in detail elsewhere [29].

Intervention with DCs to Modulate Autoimmune Diabetes

DCs have been used as a vehicle to directly (exogenous administration of antigen-loaded cells) and indirectly (antigen that requires uptake, processing, and presentation by endogenous DCs upon administration in vivo) sensitize T cells to candidate autoantigens. These autoantigens typically constitute β-cell–expressed proteins that could represent a target antigen for autoreactive T cells and stimulate β-cell–specific immune responses, leading to overt diabetes in susceptible mice. These studies aimed to characterize the significance of individual β-cell autoantigens, identifying critical T-cell antigenic determinants (peptides), or to study the pathophysiology of immune-mediated islet cell destruction. Immunization with plasmid DNA (DNA vaccination) encoding β-cell

autoantigens can induce robust T-cell responses by a mechanism that involves DCs and antigen cross-presentation. Intramuscular preproinsulin-2 plasmid DNA injections led to enhanced diabetes development in NOD mice and caused overt diabetes in most RIP-CD80 transgenic mice [30,31]. In contrast, intramuscular injection with glutamic acid decarboxylase (GAD65)–expressing plasmids conferred partial diabetes protection in NOD mice and failed completely in RIP-CD80 mice [30]. In agreement with such a protective role of GAD65 but not preproinsulin-2, Tisch et al. [32] prevented diabetes in the NOD mouse using an intramuscular injected plasmid encoding a GAD65/IgG-Fc fusion protein. In this model, IL-4 was critical to prevent a Th1-response because GAD65-specific Th1-cell reactivity was significantly enhanced in animals immunized with DNA encoding GAD65-IgGFc alone. Unlike GAD65, plasmids encoding insulin B/IgG-Fc (but not insulin A/IgG-FC) exhibited accelerated, IL-4–independent diabetes in NOD mice [33].

Identifying T-cell epitopes has huge practical implications for diabetes research. Various strategies to find diabetes-related T-cell epitopes have been used. These include prediction algorithms from (viral) peptide-recognition databases, soluble MHC multimer peptide libraries for binding and overlapping peptide screens for functional responsiveness of diabetogenic T-cell clones, respectively, and DC-facilitated autoantigen immunization regimens. Specific epitope targets for islet-specific cytotoxic T lymphocyte could be detected in spontaneously diabetic NOD mice [34]. An alternative model using peptide-loaded DC immunizations exploited the extraordinary susceptibility to antigen-induced diabetes conferred by transgenically expressed CD80 by pancreatic β cells (RIP-CD80) [30,31,35]. Here, even a modest sensitization (achieved by low-affinity epitopes presented by potent mature DCs) can induce diabetes [36]. The recent generation of several "humanized" mouse autoimmune diabetes models, which are characterized by the expression of chimeric human/mouse HLA molecules [37–41], will continue to accelerate the transition of identification of mouse-specific epitopes to human HLA-restricted T-cell epitopes. Overall, diabetes-relevant T-cell epitopes, especially those detectable in patients, are extremely useful to track diabetogenic T-cell responses during disease development. They allow the monitoring of disease activity during therapeutic intervention, and hold promise to facilitate the development of peptide-based immunomodulatory intervention strategies.

DC-Dependent Therapeutic Interventions in Autoimmune Diabetes

The understanding that DCs orchestrate innate and adaptive immune responses has stimulated research on harnessing DCs to create more effective vaccines, and early clinical trials have begun. For instance, autologous DCs with a proinflammatory phenotype have been loaded with tumor-derived antigens ex vivo. Administering those cells to cancer patients led to antitumor T-cell responses [42]. Counterintuitively, it seems DCs have also been used for autoantigen-specific prevention of autoimmune diabetes. Clare-Salzler et al. [43] prevented the development of diabetes in NOD mice using autoantigen-presenting DCs, which were isolated from pancreatic LNs (ie, carrying endogenously sampled pancreatic proteins) or pulsed with islet cell lysates. The long-term culture of splenocytes resulted in the generation of a cell line, termed NOD-DC1, which has the phenotype of myeloid DCs. Vaccination with insulin-pulsed NOD-DC1 cells resulted in antigen-specific prevention of diabetes [44]. The transfer of interferon (IFN)-γ–stimulated DCs into the NOD mouse afforded long-lasting protection against clinical and histologic signs of autoimmune diabetes in recipient NOD mice. The anti-diabetogenic ability depended on IFN-γ stimulation of DCs [45]. Another study reported that treatment with human γ globulin–pulsed DCs was associated with diabetes protection and increased levels of IL-4, IL-10, and IFN-γ and diminished levels of TNF-α in the supernatants of islets from NOD mice [46]. More recent advances led to techniques to direct soluble antigens to DC surface receptors in vivo, thereby replacing demanding ex vivo culturing of cells and allowing large-scale applications of DC-based vaccination therapies [47].

The ability to actively suppress an immune response makes Tregs an attractive candidate for novel therapeutic agents with specificity for and potency of treating autoimmune diseases effectively [1]. Some have investigated DC-mediated activation and expansion of Tregs as a potential therapeutic strategy to halt autoimmunity and restore self-tolerance in overt autoimmune diabetes [28,48]. Tarbell et al. [28] demonstrated that $CD4^+CD25^+$ Tregs generated by DC stimulation remain responsive to a single autoantigen and can inhibit diabetes induced or sustained by multiple specificities in NOD mice.

Conclusions

Basic research has provided tremendous insights into the biology and functional properties of DCs and their pivotal role in the adaptive immune system. This knowledge has fueled a host of refined therapeutic efforts using DC technology in areas such as vaccine development and tumor immunology and has energized attempts to advance two major challenges in autoimmune diseases: 1) our lack of knowledge about the set of autoantigenic target epitopes, which are recognized spontaneously by autoreactive T cells, and function to maintain this same T-cell population in an activated state long term, the ultimate cause of chronic debilitating organ destruction in autoimmune diseases, and 2) the failure, in most instances, to achieve or re-instate tolerance therapeutically. The latter is particularly frustrating because advances in organ regeneration

and stem cell therapy offer the vested prospect of restoring organ function in diseases such as T1DM once autoimmunity and continuing organ destruction have been stopped.

The influence of T-cell responses by DCs may well turn out to become the tool necessary to accomplish these goals. However, because DCs can be extremely potent at stimulating inflammatory T-cell responses, genuine efforts to dampen autoimmune activity by DCs can backfire and worsen or exacerbate autoimmune diseases. There is a growing consensus that autoimmune diseases such as T1DM can be treated effectively and safely only by using autoantigen-specific approaches. Such modality could selectively alter disease-associated T-cell specificities without affecting the immune systems as a whole. It remains to be seen if DC/antigen combinations can be established that are safe and effective. In this respect, it was of interest that that T cell with low affinity to autoantigenic epitopes [49•], or "ignored" determinants [50••], not used during the progressive autoimmune response might be particularly useful to slow the immune-mediated islet destruction and/or propagate Treg numbers and function in vivo. Because there is currently no way to know, other than testing individual autoantigens or peptides, which epitopes work therapeutically and why, it is imperative to identify more T-cell epitopes. Humanized mouse diabetes models have begun to greatly facilitate the discovery of human HLA-specific epitopes. Most importantly, however, we will have to learn more about the precise mechanisms that operate in healthy subjects and why and how they have failed in patients.

Disclosures

This work was supported by the Intramural Research Program of the National Institutes of Health and the National Institute of Diabetes and Digestive and Kidney Diseases.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Tang Q, Bluestone JA: **Regulatory T-cell physiology and application to treat autoimmunity.** *Immunol Rev* 2006, **212:**217–237.
2. Iruretagoyena MI, Wiesendanger M, Kalergis AM: **The dendritic cell-T cell synapse as a determinant of autoimmune pathogenesis.** *Curr Pharm Des* 2006, **12:**131–147.
3. Manuel SL, Rahman S, Wigdahl B, et al.: **Dendritic cells in autoimmune diseases and neuroinflammatory disorders.** *Front Biosci* 2007, **12:**4315–4335.
4. Atkinson MA, Eisenbarth GS: **Type 1 diabetes: new perspectives on disease pathogenesis and treatment.** *Lancet* 2001, **358:**221–229.
5. Imagawa A, Hanafusa T, Itoh N, et al.: **Immunological abnormalities in islets at diagnosis paralleled further deterioration of glycaemic control in patients with recent-onset type 1 (insulin-dependent) diabetes mellitus.** *Diabetologia* 1999, **42:**574–578.
6. Jansen A, Homo-Delarche F, Hooijkaas H, et al.: **Immunohistochemical characterization of monocytes-macrophages and dendritic cells involved in the initiation of the insulitis and beta-cell destruction in NOD mice.** *Diabetes* 1994, **43:**667–675.
7. Charre S, Rosmalen JG, Pelegri C, et al.: **Abnormalities in dendritic cell and macrophage accumulation in the pancreas of nonobese diabetic (NOD) mice during the early neonatal period.** *Histol Histopathol* 2002, **17:**393–401.
8. Nikolic T, Geutskens SB, van Rooijen N, et al.: **Dendritic cells and macrophages are essential for the retention of lymphocytes in (peri)-insulitis of the nonobese diabetic mouse: a phagocyte depletion study.** *Lab Invest* 2005, **85:**487–501.
9. Green EA, Eynon EE, Flavell RA: **Local expression of TNF-alpha in neonatal NOD mice promotes diabetes by enhancing presentation of islet antigens.** *Immunity* 1998, **9:**733–743.
10. Dahlen E, Dawe K, Ohlsson L, Hedlung G: **Dendritic cells and macrophages are the first and major producers of TNF-alpha in pancreatic islets in the nonobese diabetic mouse.** *J Immunol* 1998, **160:**3585–3593.
11. Morel PA, Vasquez AC, Feili–Hariri M: **Immunobiology of DC in NOD mice.** *J Leukoc Biol* 1999, **66:**276–280.
12. Yoon JW, Jun HS: **Autoimmune destruction of pancreatic beta cells.** *Am J Ther* 2005, **12:**580–591.
13.•• Tang Q, Adams JY, Tooley AJ, et al.: **Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice.** *Nat Immunol* 2006, **7:**83–92.

Using two-photon laser scanning microscopy of T-cell:DC interactions of NOD mice in vivo, the authors revealed that autoantigen-specific Tregs in the pancreatic LNs interacted specifically with DCs that had processed islet antigens, and that this interaction could abolish critical activation phases of autoaggressive $CD4^+$ Th cells. Thus, the immunoregulatory effect of Tregs likely proceeds via DC modification rather than by direct Treg:Th interaction.

14. Marleau AM, Singh B: **Myeloid dendritic cells in non-obese diabetic mice have elevated costimulatory and T helper-1-inducing abilities.** *J Autoimmun* 2002, **19:**23–35.
15. Weaver DJ, Jr, Poligone B, Bui T, et al.: **Dendritic cells from nonobese diabetic mice exhibit a defect in NF-kappaB regulation due to a hyperactive I kappa B kinase.** *J Immunol* 2001, **167:**1461–1468.
16. Wheat W, Kupfer R, Gutches DG, et al.: **Increased NF-kappaB activity in B cells and bone marrow-derived dendritic cells from NOD mice.** *Eur J Immunol* 2004, **34:**1395–1404.
17. Boudaly S, Morin J, Berthier R, et al.: **Altered DCs (DC) might be responsible for regulatory T cell imbalance and autoimmunity in nonobese diabetic (NOD) mice.** *Eur Cytokine Netw* 2002, **13:**29–37.
18. Strid J, Lopes L, Marcinkiewicz J, et al.: **A defect in bone marrow derived dendritic cell maturation in the nonobese diabetic mouse.** *Clin Exp Immunol* 2001, **123:**375–381.
19. Peng R, Li Y, Brezner K, et al.: **Abnormal peripheral blood DC populations in type 1 diabetes.** *Ann N Y Acad Sci* 2003, **1005:**222–225.
20. Summers KL, Marleau AM, Mahon JL, et al.: **Reduced IFN-alpha secretion by blood dendritic cells in human diabetes.** *Clin Immunol* 2006, **121:**81–89.
21. Vuckovic S, Withers G, Harris M, et al.: **Decreased blood dendritic cell counts in type 1 diabetic children.** *Clin Immunol* 2007, **123:**281–288.
22. Savinov AY, Wong FS, Stonebraker AC, Chervonsky AV: **Presentation of antigen by endothelial cells and chemoattraction are required for homing of insulin-specific CD8+ T cells.** *J Exp Med* 2003, **197:**643–656.
23. Kurts C, Heath WR, Carbone FR, et al.: **Constitutive class I-restricted exogenous presentation of self antigens in vivo.** *J Exp Med* 1996, **184:**923–930.
24. Kurts C: **Cross-presentation: inducing CD8 T cell immunity and tolerance.** *J Mol Med* 2000, **78:**326–332.

25.• Lee JW, Epardaud M, Sun J, et al.: **Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self.** *Nat Immunol* 2007, **8:**181–190.
Using transgenic expression of a model autoantigen (OVA) in the small intestine, the authors found that CD8+ T cells, which were specific for antigen, could be activated by nonhematopoietic LN stroma cells, a property previously thought to be unique to DCs. Apparently, this was possible because LN stroma cells expressed low but significant levels of autoantigens, which mediated deletional tolerance by a mechanism reminiscent of thymic negative selection by medullary thymic epithelial cells.
26. Papaccio G, De Luca A, De Luca B, et al.: **Detection of dendritic cells in the non-obese diabetic (NOD) mouse islet pancreas infiltrate is correlated with Th2-cytokine production.** *J Cell Biochem* 1999, **74:**447–457.
27. Kurts C, Kosaka H, Carbone FR, et al.: **Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells.** *J Exp Med* 1997, **186:**239–245.
28. Tarbell KV, Yamazaki S, Olson K, et al.: **CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes.** *J Exp Med* 2004, **199:**1467–1477.
29. Shevach EM: **Regulatory T cells in autoimmmunity*.** *Annu Rev Immunol* 2000, **18:**423–449.
30. Karges W, Pechhold K, Al Dahouk S, et al.: **Induction of autoimmune diabetes through insulin (but not GAD65) DNA vaccination in nonobese diabetic and in RIP-B7.1 mice.** *Diabetes* 2002, **51:**3237–3244.
31. Pechhold K, Karges W, Blum C, et al.: **Beta cell-specific CD80 (B7-1) expression disrupts tissue protection from autoantigen-specific CTL-mediated diabetes.** *J Autoimmun* 2003, **20:**1–13.
32. Tisch R, Wang B, Weaver DJ, et al.: **Antigen-specific mediated suppression of beta cell autoimmunity by plasmid DNA vaccination.** *J Immunol* 2001, **166:**2122–2132.
33. Weaver DJ, Jr, Liu B, Tisch R: **Plasmid DNAs encoding insulin and glutamic acid decarboxylase 65 have distinct effects on the progression of autoimmune diabetes in nonobese diabetic mice.** *J Immunol* 2001, **167:**586–592.
34. Di Lorenzo TP, Peakman M, Roep BO: **Translational mini-review series on type 1 diabetes: systematic analysis of T cell epitopes in autoimmune diabetes.** *Clin Exp Immunol* 2007, **148:**1–16.
35. Harlan DM, Hengartner H, Huang ML, et al.: **Mice expressing both B7-1 and viral glycoprotein on pancreatic beta cells along with glycoprotein-specific transgenic T cells develop diabetes due to a breakdown of T-lymphocyte unresponsiveness.** *Proc Natl Acad Sci U S A* 1994, **91:**3137–3141.
36. Pechhold K, Chakrabarty S, Harlan DM: **Cytotoxic T cell-mediated diabetes in RIP-CD80 transgenic mice: autoantigen peptide sensitivity and fine specificity.** *Ann N Y Acad Sci* 2007, **1103:**132–142.
37. Vitiello A, Marchesini D, Furze J, et al.: **Analysis of the HLA-restricted influenza-specific cytotoxic T lymphocyte response in transgenic mice carrying a chimeric human-mouse class I major histocompatibility complex.** *J Exp Med* 1991, **173:**1007–1015.
38. Pascolo S, Bervas N, Ure JM, et al.: **HLA-A2.1-restricted education and cytolytic activity of CD8(+) T lymphocytes from beta2 microglobulin (beta2m) HLA-A2.1 monochain transgenic H-2Db beta2m double knockout mice.** *J Exp Med* 1997, **185:**2043–2051.
39. Ureta-Vidal A, Firat H, Perarnau B, Lemonnier FA: **Phenotypical and functional characterization of the CD8+ T cell repertoire of HLA-A2.1 transgenic, H-2KbnullDbnull double knockout mice.** *J Immunol* 1999, **163:**2555–2560.
40. Marron MP, Graser RT, Chapman HD, Serreze DV: **Functional evidence for the mediation of diabetogenic T cell responses by HLA-A2.1 MHC class I molecules through transgenic expression in NOD mice.** *Proc Natl Acad Sci U S A* 2002, **99:**13753–13758.
41. Serreze DV, Marron MP, Dilorenzo TP: **"Humanized" HLA transgenic NOD mice to identify pancreatic beta cell autoantigens of potential clinical relevance to type 1 diabetes.** *Ann N Y Acad Sci* 2007, **1103:**103–111.
42. Figdor CG, de Vries IJ, Lesterhuis WJ, Melief CJ: **Dendritic cell immunotherapy: mapping the way.** *Nat Med* 2004, **10:**475–480.
43. Clare-Salzler MJ, Brooks J, Chai A, et al.: **Prevention of diabetes in nonobese diabetic mice by dendritic cell transfer.** *J Clin Invest* 1992, **90:**741–748.
44. Krueger T, Wohlrab U, Klucken M, et al.: **Autoantigen-specific protection of non-obese diabetic mice from cyclophosphamide-accelerated diabetes by vaccination with dendritic cells.** *Diabetologia* 2003, **46:**1357–1365.
45. Shinomiya M, Fazle Akbar SM, Shinomiya H, Onji M: **Transfer of dendritic cells (DC) ex vivo stimulated with interferon-gamma (IFN-gamma) down-modulates autoimmune diabetes in non-obese diabetic (NOD) mice.** *Clin Exp Immunol* 1999, **117:**38–43.
46. Papaccio G, Nicoletti F, Pisanti FA, et al.: **Prevention of spontaneous autoimmune diabetes in NOD mice by transferring in vitro antigen-pulsed syngeneic dendritic cells.** *Endocrinology* 2000, **141:**1500–1505.
47. Tacken PJ, de Vries IJ, Torensma R, Figdor CG: **Dendritic-cell immunotherapy: from ex vivo loading to in vivo targeting.** *Nat Rev Immunol* 2007, **7:**790–802.
48. Machen J, Harnaha J, Lakomy R, et al.: **Antisense oligonucleotides down-regulating costimulation confer diabetes-preventive properties to nonobese diabetic mouse dendritic cells.** *J Immunol* 2004, **173:**4331–4341.
49.• Han B, Serra P, Yamanouchi J, et al.: **Developmental control of CD8 T cell-avidity maturation in autoimmune diabetes.** *J Clin Invest* 2005, **115:**1879–1887.
This study examined the fate of β-cell (islet-specific glucose-6-phosphatase catalytic subunit-related protein)–specific TCR transgenic CD8+ T cells during diabetes development. Some of these T cells escaped thymic negative selection and contributed to islet inflammation and diabetes development. The authors observed a (presumable protective) mechanism that operated during the autoimmune response in the periphery, which resulted in a deselection of CD8+ T cells with higher avidity, explaining the (paradoxical) accumulation of T cells with lower avidity during T-cell–mediated autoimmunity.
50.•• Olcott AP, Tian J, Walker V, et al.: **Antigen-based therapies using ignored determinants of beta cell antigens can more effectively inhibit late-stage autoimmune disease in diabetes-prone mice.** *J Immunol* 2005, **175:**1991–1999.
Careful examination of I-Ag7–restricted autoantigenic epitopes in the NOD mouse allowed the authors to distinguish target determinants (TDs) found in spontaneous T-cell responses during NOD diabetes from other immunogenic epitopes that were not involved (termed ignored determinants, IDs). Immunizations using IDs, but not TDs, ameliorated diabetes progression when administered late in the prediabetic phase.