Dendritic Cells and Immunoregulation in the Pathogenesis and Prevention of Type 1 Diabetes

Kelly L. Summers
University of Western Ontario

Annette M. Marleau
University of Western Ontario

Tracey A. Stephens
University of Western Ontario

Jeffrey L. Mahon
University of Western Ontario, jl.mahon@lhsc.on.ca

Bhagirath Singh
University of Western Ontario, bsingh@uwo.ca

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Dendritic Cells and Immunoregulation in the Pathogenesis and Prevention of Type 1 Diabetes

Kelly L. Summers PhD, Annette M. Marleau MSc, Tracey A. Stephens MSc, Jeffrey L. Mahon MD, Bhagirath Singh PhD

Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada
Robarts Research Institute, London, Ontario, Canada

ABSTRACT

Dendritic cells govern the outcome of an immune response toward either tolerance or autoimmunity. Recent evidence has demonstrated that central tolerance is associated with relatively immature dendritic cells or quiescent mature dendritic cells that present self-antigens to autoreactive T cells, thereby silencing their autoreactive potential and/or activating regulatory T cells. Conversely, activated mature dendritic cells that have been instructed to become potent T cell stimulators by adjuvants or pathogens are capable of converting tolerance to immune activation. In combination with genetic and environmental influences, such mature dendritic cells are capable of orchestrating autoimmune responses.

To explore the function of dendritic cells in type 1 diabetes mellitus, the authors evaluated peripheral dendritic cells from both patients with type 1 diabetes and the nonobese diabetic (NOD) mouse model that shares a pathologically analogous disease process.

Dendritic cells in NOD mice are phenotypically comparable to dendritic cells from autoimmune-resistant controls with respect to expression of differentiation molecules. However, in response to maturation stimuli, such dendritic cells in NOD mice are functionally different from those in controls.

Keywords: autoimmune disease, dendritic cells, Ep1.B, immunoregulation, T cells, tolerance
INTRODUCTION

Type 1 diabetes mellitus is an autoimmune disease characterized by the selective destruction of insulin-producing beta cells within the pancreatic islets of Langerhans by autoreactive T cells. There are two main phases of the disease: 1) insulitis refers to the initial infiltration of the pancreatic islets by leukocytes, primarily antigen-presenting cells (APC) and T cells; and 2) clinical disease refers to loss of insulin production and the inability to control and maintain homeostasis of blood glucose levels due to the destruction of islet beta cells (1). The cellular features important at disease onset are difficult to decipher in humans, because humans with prediabetes are asymptomatic irrespective of the presence of insulitis and low beta cell death. Therefore, animal models of type 1 diabetes are used to establish the relative importance of cellular components during the progression from insulitis to diabetes (2).

Nonobese diabetic (NOD) mice share certain characteristics with humans with type 1 diabetes, including a major histocompatibility complex (MHC) class II genetic predisposition to and the spontaneous development of type 1 diabetes. The different stages of disease progression are well defined in NOD mice. Following an unknown environmental trigger, pancreatic islets in NOD mice are infiltrated by leukocytes at 4 to 6 weeks of age. This peri-insulitis progresses to insulitis at 10 weeks of age, which coincides with early beta cell injury and the presence of circulatory autoantibodies to islet cell antigens, such as glutamic acid decarboxylase (GAD), tyrosine phosphatase (IA-2) and islet cell antibodies (ICA). NOD mice progress to diabetes at 15 to 20 weeks of age when they become hyperglycemic because of the elimination of the vast majority of their beta cells (2).

Studies in NOD mice have demonstrated that the transitional period between insulitis and diabetes involves a dynamic process whereby an initial tolerogenic immune response becomes a destructive immune response (2). This appears to result from a progressive imbalance between the migratory regulatory and effector T cell populations. The initial presence of islet cell antigens is thought to induce the infiltration of primarily regulatory T cells into the pancreatic islets. These are heterogeneous populations of T cells that include subsets of CD4+ T cells (T helper [Th] 2-type, CD25+ CD45RB+B) and CD8+ T cells (CD28+) that release regulatory cytokines (interleukin [IL] -4, IL-10, transforming growth factor [TGF] -beta) and induce tolerance. Progressively, islet infiltration by effector T cell populations, primarily Th1-type CD4+ T cells and cytotoxic CD8+ T cells, which release proinflammatory cytokines (IL-2, IL-12, interferon [IFN] -gamma, tumour necrosis factor [TNF] -alpha), predominates, leading to immunity. Genetic defects in the T cells were previously thought to bias this destructive Th1-type immune response in type 1 diabetes. NOD thymocytes have abnormal signalling capabilities, while mature T cells show hyporesponsive proliferation to T cell receptor (TCR) signalling (3,4) and apoptosis resistance (5-9). Parallel T cell defects have been described in humans with type 1 diabetes (10,11). However, important new evidence has emerged to implicate defective dendritic cells as equal contributors driving immunity in type 1 diabetes.

DENDRITIC CELLS AND IMMUNOREGULATION

Dendritic cells are a heterogeneous group of bone marrow-derived cells that specialize in the uptake, processing and presentation of antigenic peptides to T cells. Dendritic cells have complex roles in immunoregulation since they can regulate both immune responses against foreign antigens and central and peripheral tolerance to self-antigens (12-15). How dendritic cells direct these opposing T cell responses remains unclear. Several mechanisms, including dendritic cell subset, dendritic cell maturation and activation states, dendritic cell-derived cytokines and costimulator signals, have been proposed as influences on the differential generation or activation of regulatory and effector T cells (Table 1, Figure 1). Notably, although human and mouse dendritic cell subsets confer heightened activation of T cells and excessive production of pro-inflammatory cytokines. These findings highlight a putative contribution of unabated dendritic cell activation to the loss of self-tolerance and to chronic, self-directed responses that define type 1 diabetes. Moreover, effective manipulation of dendritic cell activation state provides a promising avenue for regulating autoimmunity. Using a novel self-derived peptide that programs dendritic cell maturation and activation from monocytic precursors, the authors demonstrated suppression of autoreactivity in the NOD mouse model of type 1 diabetes. Collectively, these data are consistent with a model in which dendritic cells at different maturation and activation states regulate peripheral tolerance vs. autoimmunity.
have differences in their surface marker expression and cytokine profiles, they have similar functional characteristics, including plasticity in directing both immunity and tolerance, and the ability to respond to microbial products and other threats (12).

Immature dendritic cells are most efficient at internalizing antigen and processing it into peptides. This event triggers the migration of antigen-carrying dendritic cells to regional lymph nodes where peptides bound to MHC molecules are presented to peptide-specific TCRs on T cells. During migration, the dendritic cell matures, which coincides with increased APC capacity (13-15). Properties of the antigen dictate the strength of the MHC-peptide signal and resultant activation state of the dendritic cell. A high dose, affinity or density of antigen is thought to activate the dendritic cell and stimulate its development into an immunogenic dendritic cell. Otherwise, the dendritic cell remains in an immature or quiescent state and induces tolerance (12,16,17). The influence of the dendritic cell subset remains unclear. Originally, dendritic cell subsets were thought to have unique functions: myeloid dendritic cells induced immunity and lymphoid dendritic cells mediated tolerance. However, there is increasing evidence that it is not the dendritic cell subset but, more likely, the dendritic cell maturation state, activation state and microenvironment that dictate the outcome of the immune response (Table 1, Figure 1) (12,13).

In normal situations, immunity occurs when activated mature dendritic cells stimulate cytotoxic T lymphocytes or induce the differentiation of naïve CD4+ T cells into Th1-type effector T cells. Th1 cell differentiation is also influenced by

| Table 1. Summary of factors considered important in dendritic cell immunoregulation of tolerance and immunity |
|---------------------------------------------------------------|-----------------|-----------------|
| **Immunoregulatory factors**                                  | **Tolerance**   | **Immunity**    |
| **Features of dendritic cells**                               |                 |                 |
| Subset (controversial)                                        | Lymphoid        | Myeloid         |
| Expression of MHC molecule                                   | High (mature)   | High            |
| Costimulator molecules                                        | CD80, CD86, B7h/B7RP-1, OX40L | CD80, CD86, 4-1BB |
| Antigen affinity and dose                                     | Unknown         | High            |
| Molecular interactions between dendritic cells and T cells    |                 |                 |
| TCR signal strength                                           | Weak            | Strong          |
| Costimulator signal strength                                  | Weak            | Strong          |
| Microenvironment of dendritic cells and T cells               |                 |                 |
| Cytokines released by dendritic cells                         | IL-10, IL-4, IL-6 | IL-12, IL-15, IL-18, IFN-gamma, TNF-alpha |
| Inflammatory mediators                                        | PGE₂, corticosteroids | Unknown         |
| Chemokines                                                    | MCP-1           | Unknown         |
| Dendritic cell-mediated T cell effects                        | T cells activated | Th1, CD8^+ CTL |
| Mechanisms                                                    | Anergy or lysis of pathogenic T cells | Activate effector T cells |

CTL = cytotoxic T lymphocyte
IFN = interferon
IL = interleukin
MCP = monocyte chemotactic protein
MHC = major histocompatibility complex
PGE₂ = prostaglandin E₂
TCR = T cell receptor
Th = T helper
TNF = tumour necrosis factor
Tr1 = T regulatory type 1
other factors, including IL-12 secretion by dendritic cells and strong costimulatory signals via specific molecules. In contrast, tolerance results in the induction of anergy in or the killing of autoreactive T cells, selective activation of regulatory T cells or differentiation of naïve T cells into regulatory T cells or Th2-type cells (Table 1, Figure 1). In autoimmunity—the breakdown of tolerance—dendritic cells acquire autoantigens, mature and defectively stimulate normally quiescent autoreactive T cells that express a TCR either specific for or able to cross-react with the presented self-peptide.

Tolerance normally involves dendritic cells in an immature or quiescent mature state that are predominantly IL-10 producers and express low levels of inhibitory-type costimulatory molecules. Variations in costimulatory molecule expression on dendritic cells are key determinants in whether tolerance or immunity ensues. Notably, CD80 and CD86 on dendritic cells are both increased in response to microbial and inflammatory stimuli (18). Interactions between CD28 on T cells with CD80 and CD86 provide cues for T cell proliferation and survival (19). However, there are conflicting reports concerning the functional differences between CD80 and CD86. Although there are data suggesting that CD80 and CD86 are equally effective costimulatory molecules (20,21), other lines of evidence concede that their effects are divergent (22,23). CD86 is expressed earlier on maturing dendritic cells, at higher levels than CD80, and has been associated with positive regulation of T cell activation (24). Conversely, at later stages of immune response, upregulated CD80 represents the preferential ligand for cytotoxic T lymphocyte antigen (CTLA) -4 to limit T cell activation and generate regulatory T cells (25). Moreover, in the steady state, constitutively low levels of CD80 and CD86 on dendritic

Dendritic cell factors that can regulate the type of T cell that is activated and subsequent immune response that is induced include the maturation and activation states of the dendritic cells, specificity of the molecular interactions or strength of the costimulatory signals to the T cell, type of cytokines secreted by the interacting dendritic cells and presence of inflammatory mediators in the dendritic cell-T cell microenvironment. The relationship between dendritic cell subset and the type of immune response induced remains unclear. Potential areas of intervention are proposed that could manipulate dendritic cells to direct the induction of tolerance and protection from type 1 diabetes. This primarily involves therapies that suppress the activation of dendritic cells or stimulate dendritic cells to express or secrete immunosuppressive molecules or cytokines, respectively. Ideally, this would encourage dendritic cells to activate regulatory T cells and/or induce anergy or apoptosis in autoreactive T cells.

IFN = interferon
IL = interleukin
NF = nuclear factor
PGE₂ = prostaglandin E₂
TNF = tumour necrosis factor
cells maintain self-tolerance by stably sustaining regulatory T cell populations (26). Cumulatively, this evidence suggests both immunogenic and tolerogenic roles for CD80 and CD86 on dendritic cells.

**IMMUNOREGULATION IN TYPE 1 DIABETES**

T cell autoreactivity in type 1 diabetes appears to result from an inability of dendritic cells to activate immunoregulatory T cells (2). Dendritic cells are likely involved in the initiation of type 1 diabetes, since they are one of the first cell populations to infiltrate the pancreas during insulitis in NOD mice (27,28) and BioBreeding (BB) rats (29). Dendritic cells have also been found in the pancreas of an infant with diabetes (30) and in the pancreatic islets of people with a long duration of type 1 diabetes (31). Dendritic cells accumulate in T cell areas near islets in the exocrine pancreas. It has been proposed that these infiltrating dendritic cells orchestrate the transition of tolerance to immunity in type 1 diabetes by inefficiently stimulating tolerance in autoreactive T cells. Alternatively, dendritic cells may present beta cell peptides to autoreactive T cells that selectively destroy insulin-secreting pancreatic beta cells. Various factors have been suggested to be involved in defective dendritic cell regulation of tolerance in type 1 diabetes, including reduced numbers of dendritic cells, increased myeloid:lymphoid dendritic cell subset ratios and dendritic cell maturation and activation states (32-36).

Dendritic cells in humans with type 1 diabetes Few studies have examined directly isolated dendritic cells in diabetes. The majority of reports in both animal models of diabetes (32-34) and in humans (35,36) describe dendritic cells generated in vitro by incubating monocytes or bone marrow cells in cytokines (granulocyte-macrophage colony-stimulating factor [GM-CSF] and IL-4) for extended periods of time. This procedure activates cells and promotes the selective differentiation of myeloid dendritic cells. Clearly, dendritic cells generated in culture are highly manipulated cells that are not representative of naturally occurring dendritic cells.

The authors avoided the use of in vitro-generated dendritic cells and instead compared the frequency and activation state of naturally occurring dendritic cell subsets in subjects with type 1 diabetes (all positive for antibodies to GAD65 and/or IA-2) to those of controls without diabetes. Whole blood was directly labelled with a panel of monoclonal antibodies to identify CD11c^+ myeloid dendritic cells and CD11c^- lymphoid dendritic cells. Surface expression of the CD83 activation antigen and several costimulator molecules was determined in each dendritic cell subset.

The authors found that the total number of dendritic cells per litre of blood was similar between subjects with type 1 diabetes and controls. The proportions of myeloid dendritic cells and lymphoid dendritic cells in the circulating dendritic cell population were normal in type 1 diabetes. The level of activation and density of costimulator molecules on each dendritic cell subset varied significantly between subjects with type 1 diabetes and controls.

### Table 2. Properties of myeloid dendritic cells in diabetic mice and humans with type 1 diabetes compared to controls.* † Arrows (↑↓) indicate changes relative to controls

<table>
<thead>
<tr>
<th>Effect</th>
<th>NOD mice*</th>
<th>Humans with type 1 diabetes†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of CD80 and CD86</td>
<td>Normal</td>
<td>↑ Normal</td>
</tr>
<tr>
<td>T cell proliferation</td>
<td>↑</td>
<td>↑↑ Normal</td>
</tr>
<tr>
<td>IL-12/IFN-gamma secretion</td>
<td>↑</td>
<td>↑↑ NE</td>
</tr>
</tbody>
</table>

*Immature dendritic cells were generated from bone marrow cells cultured in GM-CSF and IL-4 for 5 days. Activated dendritic cells were generated from immature dendritic cells activated by coculture with autologous T cells. Controls were dendritic cells from autoimmune-resistant mouse strains (BALB/c, C57BL/6, NOR).

†Monocyte-dendritic cells were immature dendritic cells generated from monocytes cultured in GM-CSF and IL-4 for 7 days. Fresh dendritic cells were lineage^- HLA-DR^ CD11c^- cells in blood. Controls were dendritic cells from healthy individuals without diabetes.

GM-CSF = granulocyte-macrophage colony-stimulating factor
HLA = human leukocyte antigen
IFN = interferon
IL = interleukin
NE = not evaluated
NOD = nonobese diabetic
NOR = nonobese diabetes-resistant
dendritic cell subset were also similar between groups (Table 2). Therefore, circulatory dendritic cell subset numbers and phenotype are not impaired in type 1 diabetes (31). These data do not support the results of a previous report that showed impaired dendritic cell numbers and phenotype in human type 1 diabetes (35). This difference is likely explained by the use of in vitro-generated dendritic cells in the study conducted by Takahashi and colleagues (35). The authors’ study does not exclude the possibility that circulatory dendritic cells may have abnormalities in the mature or activated state that result in altered T cell stimulation and cytokine production, leading to type 1 diabetes.

In support of published data in animal models of type 1 diabetes (27-29) and an infant with type 1 diabetes (30), the authors observed that dendritic cells were present in the exocrine pancreatic tissue of subjects with type 1 diabetes but were absent in controls without diabetes (31). Pancreatic dendritic cells likely represent dendritic cells that bear beta cell-specific peptides and activate pathogenic T cells autoreactive to islet beta cells. These pancreatic dendritic cells may also directly facilitate beta cell destruction.

**Dendritic cell function in NOD mice**

To assess the contribution of putative dendritic cell anomalies to the development of type 1 diabetes, Marleau and Singh evaluated dendritic cell maturation and function using in vitro-generated, bone marrow-derived dendritic cells from NOD mice (33). This allowed for the determination of inherent genetic predispositions in a pure myeloid dendritic cell population. The phenotype of NOD dendritic cells was compared to that of dendritic cells derived from diabetes-resistant control mice, C57BL/6 (a Th1-biased strain) and BALB/C (a Th2-biased strain). Both immature and mature (stimulated with lipopolysaccharide [LPS] and IFN-gamma) NOD dendritic cells expressed MHC and costimulator molecules within the range of that of controls. Upregulation of costimulator molecules was also examined in dendritic cells cocultured with syngeneic T cells plus anti-CD3 to act as an antigen surrogate for stimulation of the TCR. This system allowed T cell-derived signals to activate the dendritic cell (37,38). In comparison to dendritic cell-T cell combinations from BALB/C and C57BL/6, NOD dendritic cells cocultured with T cells plus anti-CD3 exhibited substantially elevated expression of costimulator molecules, suggestive of a more mature state (Table 2).

Dendritic cell maturation, specifically, elevated costimulator molecule expression, has been associated with both heightened and prolonged T cell responses as well as induction of more strongly Th1-biased immunity (15). In support of these findings, agents that inhibit maturation of myeloid dendritic cells, such as IL-10, corticosteroids and prostaglandin E2, convert dendritic cells from Th1- to Th2-skewing cells. To demonstrate the functional outcome of enhanced maturation of dendritic cells from NOD mice, stimulation of T cell proliferation by NOD dendritic cells was compared to stimulation by C57BL/6 dendritic cells. Consistent with their elevated expression of costimulator molecules, NOD dendritic cells were capable of eliciting elevated T cell proliferation, with fewer NOD dendritic cells required to induce maximal responses. Moreover, both IL-12 p70 and IFN-gamma were significantly elevated in cocultures containing NOD dendritic cells and T cells (Table 2). Importantly, in the absence of T cells, NOD dendritic cells activated by LPS and IFN-gamma also exhibited higher IL-12 p70 production than C57BL/6 dendritic cells. The higher responses induced by NOD dendritic cells could be interpreted as excessive, since C57BL/6 is the prototypic strain for strong Th1 immunity. The results of studies of dendritic cells in other autoimmune disorders also suggest that alterations in dendritic cell signalling and maturation underlie an observed loss of self-tolerance (39,40).

The relevance of NOD dendritic cell hyperactivity was exemplified through comparison with dendritic cells derived from nonobese diabetes-resistant (NOR) mice. The NOR mouse strain is NOD-related and MHC-syngeneic, but is resistant to the development of spontaneous and cyclophosphamide-induced type 1 diabetes (41,42). Although NOR mice develop a mild inflammatory infiltrate at the islet periphery, minimal T cell infiltration follows (42). Since potentially deleterious responses in NOR islets are halted at the level of antigen presentation prior to T cell involvement, genetic loci that differ between NOR and NOD genomes are probably related to dendritic cell function. Marleau and Singh demonstrated that elevated costimulator molecule expression and Th1-polarizing abilities are unique characteristics of NOD dendritic cells that are not shared by NOR dendritic cells (33). Therefore, the aberrance of NOD dendritic cell function appears to be intrinsic, genetically controlled and related to the disease process.

The authors’ finding of an elevated functional capacity for NOD dendritic cells supports the results of recent reports showing that NOD dendritic cells have persistent hyperactivation of the transcription factor nuclear factor (NF)-kappa B, which controls IL-12 synthesis (43,44). A diabetes susceptibility locus, IDDM18, has been identified in proximity to the gene encoding the p40 subunit of IL-12 (45). Functionally, this allelic polymorphism in patients with type 1 diabetes leads to increased levels of IL-12 p40 protein. Moreover, NOD macrophages, derived from the common myeloid precursor of both dendritic cells and macrophages, also produce aberrantly elevated IL-12 (46). These findings, in combination with those of the authors, are strongly suggestive of generalized myeloid lineage defects in type 1 diabetes.

**DENDRITIC CELL DIFFERENTIATION FROM MONOCYTES BY A PEPTIDE OF APOLIPOPROTEIN E**

The authors recently discovered a novel way to induce monocyte to dendritic cell differentiation using a peptide fragment...
Distinct treatments are used progressively during different stages of the pathogenesis of type 1 diabetes, including immunoregulation treatments during peri-insulitis, immunosuppression treatments during early beta cell destruction and, ultimately, exogenous insulin supplementation, islet cell transplantation or pancreas transplantation following beta cell elimination. However, treatments that either suppress the initial disease trigger in genetically susceptible individuals or inhibit early events in autoimmune diseases would be preferred. The importance of dendritic cells in these early stages of disease and their ability to both immunize and tolerate T cells make them unique candidates for immunotherapy. Thus, therapies that promote the differentiation or activation of dendritic cells that induce tolerance or a protective Th2-type immune response would be ideal immunoregulatory regimens. The therapeutic potential of dendritic cells for use in cancer vaccines, transplantation and autoimmune diseases is therefore currently under intensive investigation.

Several studies have demonstrated that the transfer of exogenous dendritic cells, e.g. pancreatic lymph node dendritic cells, IFN-gamma-stimulated dendritic cells and in vitro-generated dendritic cells, into prediabetic NOD mice was protective against development of type 1 diabetes. These dendritic cells appeared to protect either through the induction of tolerance, by activating regulatory T cells and driving a Th2-type response, or by downregulating the activity of autoreactive T cells. The protective effect of transferred dendritic cells might appear unexpected in light of their established roles as the earliest islet infiltrators and initiators of the disease process. Nevertheless, it appears that dendritic cell therapy affords protection from the inherent regulatory defect in NOD mice, the hallmark feature of which is a prominent Th1 bias. It is noteworthy to consider that a more general feature of dendritic cell transfer might be responsible for the observed protective effects, since diabetes prevention appears to be relatively independent of dendritic cell maturation state upon transfer or prior pulsing with autoantigen.

Although the use of dendritic cells in immunotherapy shows promise, the trace levels of dendritic cells in vivo present serious limitations. Several approaches have been used to expand dendritic cell numbers, including FLT3 ligand and cytokines such as GM-CSF. However, these techniques are very costly, have potential side effects when administered in high quantities and, importantly, do not specifically induce the differentiation of protective dendritic cells. Such limitations could be overcome with Ep1.B immunization. Ep1.B-immunized NOD mice showed significantly reduced insulitis and incidence of disease, indicating that Ep1.B can protect NOD mice from the onset of type 1 diabetes. Ep1.B immunization resembled other protective agents, such as bacille Calmette-Guérin, M. tuberculosis andcomplete Freund’s adjuvant, that primarily act by stimulating dendritic cell and macrophage function. Ep1.B induced similar monocyte to dendritic cell differentiation in NOD mice and control strains, suggesting that Ep1.B immunization can correct the central defect in NOD dendritic cells by expanding the number of dendritic cells and upregulating dendritic cell function necessary to prevent the development of type 1 diabetes, such as activation of regulatory T cells.

Dendritic cells also have the ability to regulate T cell responses based on cytokines present in the priming environment. The authors propose that Ep1.B-stimulated dendritic cells induce a Th2-type immune response in vivo by either secreting IL-10 to induce Th2 cell differentiation or by directly activating regulatory T cells, which release IL-4 and IL-10 into the priming environment. Moreover, the beneficial effects of increased IL-10 production and inhibition of
IFN-gamma secretion have been demonstrated in the NOD mouse, whereby exogenous administration of either recombinant IL-10 or IFN-gamma antagonists could prevent the onset of type 1 diabetes (69-71). The authors anticipate that a naturally processed, nonimmunogenic, self-peptide termed Ep1.B has this therapeutic potential, since it stimulates the generation of protective dendritic cells in vivo and prevents type 1 diabetes.

CONCLUSION
The authors propose the following role for dendritic cells in type 1 diabetes, based on the results of their studies and the published literature. In the quiescent or immature state, dendritic cells exist in a phenotypically normal resting state. Following an undefined triggering event, dendritic cells infiltrate the islets during insulitis where they recruit and present beta cell peptides to T cells. Signalling events occur between the interacting dendritic cells and T cells, causing abnormally high activation and maturation states of the dendritic cells. This results in the increased production of IL-12 and predisposes the dendritic cells to lead to an elevated Th1-type immune response. The extreme Th1 propensity of activated dendritic cells does not allow for the generation of regulatory T cell responses that would prevent type 1 diabetes. A potential therapeutic approach would therefore be to influence dendritic cells toward a more tolerogenic phenotype, possibly with the use of Ep1.B immunization. This would skew the immune response toward a regulatory or Th2-type response and prevent type 1 diabetes (Figure 1).

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do not deliver identical costimulatory signals, since B7-2 but not B7-1 preferentially costimulates the initial production of IL-4. *Immunity*. 1995;2:523-532.


