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T Helper Type 1 and 2 Cytokines Mediate the Onset and Progression of Type I (Insulin-Dependent) Diabetes

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ABSTRACT

Type I (insulin-dependent) diabetes mellitus (IDDM) is an autoimmune disease that results from the destruction of insulin-secreting pancreatic islet β -cells by autoreactive cells and their mediators. Although its exact cause is not completely understood, it is well established that IDDM is associated with dysregulated humoral and cellular immunity, exemplified by altered production of and response to macrophage- and T cell-derived cytokines and a shift in T helper (Th) cell differentiation in favor of a pathogenic Th1 pathway. Th1 cytokines, including interleukin-2 and interferon- γ , induced islet β -cell destruction directly by accelerating activation-induced cell death (apoptosis) and by up-regulating the expression of select adhesion molecules. Th1 cytokines facilitated the pancreatic homing of autoreactive leukocytes, hence enhancing β -cell destruction. More

recently, a role for Th2 cytokines in IDDM pathogenesis was described. Accordingly, local production of Th2 cytokines, in particular interleukin-10, accelerated β -cell destruction by enhancing autoreactive cell infiltration of the pancreas (insulinitis) through modulation of the release of other cytokines and by modulating the microvasculature. Whereas both Th1 and Th2 cytokines are present in peripheral T cells and in the pancreas in IDDM, the mechanism of action and the kinetics of a cell damage induced by Th1 and Th2 cytokines appeared to be distinct. Collectively, this supports the idea that IDDM is not an exclusive Th1-mediated disorder as was suggested, and that both Th1 and Th2 cells and their respective mediators participate and cooperate in inducing and sustaining pancreatic islet β -cell destruction in IDDM. (*J Clin Endocrinol Metab* 84: 1497–1502, 1999)

TYPE I (insulin-dependent) diabetes (IDDM) is an autoimmune disease characterized by insulin insufficiency that results from a progressive immunological destruction of insulin-secreting pancreatic islet β -cells by autoreactive leukocytes and their mediators (1). Although the exact nature of the inducing agent(s) and the sequence of events leading to the autoimmune destruction of islet β -cells and subsequently hyperglycemia are currently not completely understood, it is well established that genetic, nongenetic, and immunological factors contribute to the pathogenesis of IDDM (1–3). Specific human leukocyte antigen alleles, in particular DR3 and DR4, were associated with an increased risk of IDDM development (2, 4). Other human leukocyte antigens, such as DR2, were described as protective of IDDM development (5). In addition to genetic factors, nongenetic factors were shown to predispose for and accelerate the development of overt diabetes in IDDM-prone individuals. These included viral infection (6), psychological factors (7), and dietary factors (such as cow milk) (6) among others. Other reports could not confirm a strong cause and effect link between these factors and IDDM, thereby highlighting the need for further investigation into identifying the causative agent(s) and the mechanisms underlying the onset and progression of IDDM (8).

IDDM is associated with altered humoral and cellular immunity (1, 6). This was evidenced by the presence of autoreactive antibodies targeting select β -cell constituents and other autoantigens (9, 10), circulating autoreactive T cells (11, 12), and heightened expression of adhesion molecules (13, 14). In particular, IDDM was associated with altered regulation of cytokine expression manifested in part by reduced levels of serum cytokine inhibitors (15) coupled with sustained expression of proinflammatory and immunoregulatory cytokines and their high affinity receptors (16–18). The development of hyperglycemia, a hallmark of IDDM, appears later in the course of the disease, frequently following months or years of the initiation of the T cell-targeted autoimmune destruction of islet β -cells (19, 20).

The exact roles T cell- and macrophage-derived cytokines play in the pathogenesis of IDDM remain the subject of intense investigation (20, 21). Conclusions reached were largely based on studies on the genetically IDDM-predisposed animals, the nonobese diabetic (NOD) mice and Bio-Breeding (BB) rats, both of which display many of the characteristics of human IDDM (22). Based on these and other studies, cytokines were shown to induce and/or exacerbate IDDM through direct and indirect mechanisms. The former was through direct cytotoxicity, whereas the latter included modulation of the activation, homing, and effector (cytotoxic) functions of proinflammatory cells (17, 18, 23).

Although it is accepted that IDDM resulted from altered balance between T helper type 1 and 2 (Th1 and Th2) cells, the exact roles Th1 and Th2 cells play in IDDM pathogenesis

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remain to be established. It was suggested that Th1 cytokines promote whereas Th2 cytokines protect from the onset and progression of IDDM (24–26). However, in some cases Th2 cells and their cytokines accelerated β -cell destruction, hence arguing against this exclusive and oversimplistic conclusion. It appears that the onset and progression of IDDM from insulinitis (pancreatic mononuclear cell infiltration) to overt diabetes are controlled by Th1 and Th2 cells together with their cytokines and other mediators (12, 27–29). This review will focus on the roles Th1 and Th2 cytokines play in the pathogenesis of IDDM; for discussion about other aspects of altered immunity in IDDM, the reader is referred to reviews published previously (30–32).

Biology of Th1 and Th2 cells

Antigen-specific T cell activation requires two signals. One is imparted by interaction of the T cell receptor (TcR)/CD3 complex with the antigen: major histocompatibility complex class II protein complex expressed by antigen-presenting cells (APC). The second signal is provided by cell-bound and secreted costimulatory molecules which, while not imparting any antigenic specificity, synergize with TcR/CD3 signals in augmenting T cell activation (33, 34). Several signal transduction pathways operate as a result of T cell activation. These include phospholipase C- γ 1 pathway, p21^{ras}/RAF kinase (the classical mitogen-activated protein kinase) pathway (35), and the phosphatidylinositol 3'-hydroxykinase/GDP-Rac (the alternative mitogen-activated protein kinase) signaling pathway (36). Coupling to more than one signaling pathway is possible depending on the intensity of the signal generated, the duration of stimulation, and the contribution of costimulatory molecules, which, in turn, affects the duration and outcome of the functional response (37, 38).

Secreted (39, 40) and cell-bound (41, 42) costimulatory molecules synergized with TcR/CD3 signals in augmenting cytokine expression at the transcriptional and posttranscriptional levels. This resulted in stabilization of interleukin-2 (IL-2) and other cytokine messenger ribonucleic acid transcripts (42, 43), abrogation of anergy (44), and enhancement of cell viability largely as a result of antagonism of activation-induced cell death/apoptotic signals (45). Insofar as costimulatory signals determine whether TcR recognition of antigen will lead to activation or to clonal anergy, a role for altered costimulation in the pathogenesis of autoimmune diseases such as IDDM (see below) (46, 47) was proposed. For example, blockade of cell-bound costimulatory molecules by chimeric toxin/Ig fusion proteins induced hyporesponsiveness (48, 49). In addition, it was suggested that aberrant expression of costimulatory signals (in particular CD28/CTLA-4) by activated autoreactive T cells may induce and/or exacerbate autoimmunity (49). Accordingly, manipulating costimulatory pathways was proposed as a potential strategy for managing autoimmune diseases, including IDDM (32, 47).

Antigen-specific T cell activation results in the differentiation of naive CD4⁺ Th cells into Th1 and Th2 clones based on their pattern of cytokine production and effector functions. Th1 produce IL-2, interferon- γ (IFN- γ), and tumor necrosis factor- α and promote cell-mediated responses and

delayed-type hypersensitivity reactions (50, 51). Th2 cells produce IL-4, IL-5, IL-10, and IL-13 and stimulate humoral immunity (50, 51). Th0 cells, which produce both Th1 and Th2 cytokines, represent either a distinct Th subset or a common precursor for Th1 and Th2 cells that differentiates into either Th subset in response to external stimuli (52) and to Th1 and Th2 cytokines (53, 54).

Several factors influence the development of Th1 and Th2 cells, including the APC type (macrophages, dendritic cells, or B cells) (55–57), the avidity of TcR interaction with antigen, and Th1/Th2 cytokines (50, 51, 58). Th1 and Th2 cells reciprocally regulate the function of one another through their respective cytokines (51, 54). Th1 cytokines, in particular IFN- γ , induce the development of Th1 cells and block the differentiation of Th2 cells and Th2-driven responses (59, 60). In contrast, Th2 cytokines promote the differentiation of Th2 cells while inhibiting Th1 activation and Th1-induced responses (61), indicating that induction of one Th program is accompanied by a corresponding decline in the activation of the other Th program (62, 63). It remains to be seen whether this is the result of a frank shifting to a specific Th subset or is due to suppression of the growth of cells with committed phenotypes (62, 64). It should be noted that these two polarized patterns of cytokine expression represent extremes of many possible outcomes (62, 65).

Pathophysiology of Th1 and Th2 cells in IDDM

Understanding of the roles that Th1 and Th2 cells play in the pathogenesis of IDDM was based on adoptive transfer experiments demonstrating the capacity of Th1 cells to transfer diabetes to NOD mouse recipients (66–68). Th2 cells generally protected from the development of IDDM in NOD recipients, the latter acting presumably by inhibiting local Th1 cell activity. Oral administration of insulin, in particular the immunodominant B chain (68–70), was associated with progressive reduction in β -cell destruction in NOD mice and BB rats concomitant with decreased expression of Th1 cytokines (71–73) and a corresponding increase in Th2 cytokine expression (67, 73, 74). This was due to the induction of an insulin-reactive Th clone that protected recipient NOD mice from the development of IDDM in a cotransfer experiment (67). Interestingly, both Th1 (70) and Th2 (67, 73) insulin-reactive IDDM-protective Th cells were described. The latter inducing a Th2 cytokine-secreting profile in recipient animals, and the former allegedly acted by stimulation of transforming growth factor- β activity (70), which, in turn, altered a pathogenic Th1 to a protective Th2 phenotype (55).

However, the generality of this conclusion remains to be seen in light of reports documenting the failure of Th2 cells to alter the progression of IDDM (66), but in some cases to precipitate overt diabetes (29). Evidence implicating both Th1 and Th2 cells, in particular their respective cytokines, in mediating β -cell destruction is a reflection of the dual role of Th1 and Th2 cytokines in IDDM pathogenesis, depending on the cytokine and time after disease onset (21). For example, IDDM was shown to be prevented by induction of Th2 cells (75) or by treatment with the Th2 cytokines, IL-4 and IL-10, which, in turn, blocked the production of Th1 cytokines (24, 76). In addition, IL-10 (a Th2 cytokine) reportedly exacer-

bated IDDM by facilitating pancreatic mononuclear cell infiltration (69, 77) and also by accelerating islet β -cell destruction by necrosis (78, 79). This prompted the conclusion that IDDM is a Th1- and Th2-mediated autoimmune disease (see below).

IDDM: a Th1-mediated event

Evidence from human and experimental animal studies supported a direct role for Th1 cells and their cytokines in the onset and progression of IDDM. These included the findings that recent-onset IDDM was associated with the predominance of Th1 cytokines concomitant with a corresponding decline in the production of Th2 (IL-4) cytokines (80–83). Destruction of β -cells resulted from a frank Th1-driven insulinitis (76, 83), as revealed by the capacity of adoptively transferred diabetogenic Th1 cells to provoke insulinitis in NOD/SCID mice (68). In addition, IDDM was abrogated by the induction of Th2 cytokine expression (75) or by treatment with the Th2 cytokines, IL-4 and IL-10 (24, 76), the latter acting through inhibition of production of Th1 cytokines. Furthermore, the predominance of Th1 cytokines in islet β -cell infiltrates in female, but not male, NOD mice was described as a major predisposing factor for developing anti- β -cell immunity, and subsequently overt diabetes, in female, but not male, NOD littermates (84).

Th1 cytokines induced and aggravated β -cell destruction through direct and indirect mechanisms. Th1 cytokines, including IL-2 and IFN- γ , acted primarily at the level of macrophage and CD8⁺ T cell activation, enhancing infiltration of these cells into the islets. Infiltrating cells induced and/or accelerated β -cell destruction largely by releasing preformed and newly synthesized cytotoxic mediators (nitric oxide, oxygen radicals, serine esterases, *etc.*) (85). Th1 cytokines also facilitated β -cell destruction indirectly by several mechanisms as a result of their capacity to inhibit the production of Th2 cytokines and Th2 cell activity. Th1 cytokines induced the activation and expansion of bystander autoactive T cells, resulting in an increase in their overall proportions (86). Th1 cytokines also inhibited the production of soluble cytokine antagonists, including the IL-1 receptor antagonist (76), which resulted in stimulation of IL-1 production by macrophages (76) and, coupled with sustained autoantigenic stimulation, resulted in a significant augmentation in the expression of IL-2 and other Th1 cytokines. Insofar as IDDM is associated with reduction of the production and activation of serum cytokine inhibitors (15), and as Th1 cytokines potentiated the production and effector functions of monokines (IL-1 and tumor necrosis factor- α) (87), this eventually amplified the cascade of anti- β -cell immunity.

IDDM: a Th2-mediated event

Whereas the role of Th1 cytokines in IDDM pathogenesis is well established, a role for Th2 cytokines in precipitating certain aspects of IDDM in the NOD mouse was recently proposed. Central to this role were the findings that 1) insulinitis associated with new-onset IDDM involved pancreatic homing of Th2 cells (14, 66) and the predominance of Th2 cytokines (14, 88, 89). Pancreatic expression of Th2 cytokines did not overcome autoimmune destruction of the pancreas

(28, 90), but, rather, accelerated it (27, 79, 91). In addition, induction of Th2-mediated antibody responses to a β -cell constituent led to a rapid spreading of Th2 immunity to unrelated β -cell antigens and, in association with Th1 cytokines, to exacerbation of IDDM (69). Furthermore, whereas IDDM was not prevented by adoptive transfer of Th2 cells (even if present in a 10-fold excess of Th1 cells) (66) or by induction of Th2 activity by neutralizing anti-IL-12 monoclonal antibodies (92), periinsulinitis and insulinitis were prevented by treatment of NOD mice with anti-IL-10 antibodies (78).

It was of interest to note that this Th2-induced component of anti- β -cell immunity was mediated principally by IL-10, but not by IL-4, thus questioning whether this effect was a generalized feature of Th2 cytokines or, alternatively, unique to IL-10. In this regard, it was shown that local production of IL-10, but not IL-4, accelerated autoimmune destruction of β -islets (28, 79, 93). NOD mice were protected from the development of overt diabetes by neutralizing anti-IL-10 mAb, but not anti-IL-4, monoclonal antibodies, which were ineffective in altering the course of Th2 autoimmune destruction of pancreatic islet β -cells (79). Furthermore, in contrast to IL-10 (79), tissue expression of IL-4 (94) led to nondestructive insulinitis. This underscores the fact that the role Th2 cytokines play in the pathogenesis of IDDM is complex and depends on the relative contributions of individual cytokines in the process. This warrants further scrutiny in assigning a generalized pathogenic role for Th2 cytokines (*vs.* a specific effect of IL-10) in the pathogenesis and progression of IDDM.

Th2 cytokines can no longer be viewed as protective of IDDM, and their claimed use as immunotherapy needs reassessing in view of their direct role in promoting insulinitis and β -cell destruction. Functionally, Th2 cytokines exerted their affects through direct and indirect mechanisms. First, Th2 cytokines, in particular IL-10, may promote necrosis through occlusion of the microvasculature, thereby reducing the viability of the larger islets. Second, IL-10 may exert immunostimulatory effects on activated T and B cells due to its role as a stimulatory and differentiation factor for B cells (95, 96) and cytotoxic T cells (97), respectively, coupled with the differential responsiveness of different APC types (macrophages, B cells, and dendritic cells) to antigenic stimulation (57) and to IL-10 action (56). Third, Th2 cytokines promote periinsulinitis and frank insulinitis by enhancing major histocompatibility complex class II expression (93, 98) or by altering the expression of endothelium-bound addressin, thereby stimulating the accumulation of macrophages, B cells, and eosinophils (27). Fourth, by augmenting cytokine production by endothelial cells and other cell types (99, 100), local production of Th2 cytokines amplified the cascade of anti- β -cell immunity through activation of resident immune cells and by facilitating the pancreatic infiltration of other cell types.

Functional considerations

It is evident that Th1 cells are not the sole mediators of islet β -cell destruction, and that Th2 cells are not inhibitory or benign as was previously suggested, as they are capable of inducing islet β -cell destruction. Th1 and Th2 cytokines ap-

pear to cooperate in driving islet β -cell destruction, eventually leading to hyperglycemia. Functionally, the lesion morphology differs between Th1- and Th2-driven insulinitis (66, 79). Th1 lesions comprised focally confined insulitis consisting primarily of CD8⁺ and CD4⁺ T cells, and islet β -cells die by apoptosis, thereby sparing surrounding exocrine tissue (101). In contrast, Th2 lesions are more dispersed and consisted primarily of eosinophils, macrophages, and fibroblasts, with a notable scarcity of T cells (90), and islet β -cells die by necrosis. In addition, there is the accumulation of fibroblasts and the generation of extensive extracellular matrix and adipose tissue in Th2 lesions that subsequently promoted tissue necrosis.

In addition to morphological differences in lesions, the kinetics of β -cell destruction differ between Th1- and Th2-driven autoimmune attacks (89). Compared to Th2-mounted attacks, Th1-driven attacks are more rapid and aggressive and are sustained for a longer time period. This suggested that Th2-mediated attacks are responsible for the early phase of IDDM (29), whereas Th1-driven responses are responsible for the persistent and sustained attacks (78). It remains to be seen whether the predominance of Th1 attacks seen in advanced IDDM is a reflection of the expansion of Th1 clones and/or is due to the incapacity of Th2 clones to sustain an immunological attack, as previously suggested (69).

Concluding remarks

The assignment of a pathogenic role for Th1 cells and a protective role for Th2 cells and their respective cytokines in the onset and progression of IDDM was largely based on artificial conditions that did not reflect the delicate balance and relative contribution of each Th subset throughout the disease. Accordingly, Th1 cells can no longer be the sole instigators of IDDM, and Th2 cells appear to be more harmful than previously believed. A number of points are worth considering in this context. First, many studies were largely based on *in vitro* observations using well defined experimental conditions that were not representative of the cytokine milieu or the cellular network that operates in the pancreas during the autoimmune attack. Second, the designation of Th1 and Th2 cytokine-secreting profiles represents the extreme of many possible outcomes. Accordingly, pushing the differentiation of one Th subset to the extreme using monoclonal antibodies or recombinant cytokines *in vitro* cannot be duplicated *in vivo* (102, 103). Third, assignment of a protective role for Th2 cytokines, including IL-10, was based on a well documented effect of IL-10. However, cytokines such as IL-10 are pleiotropic; a given cytokine may be produced by more than one cell type and may exert its effect on several target cells (96, 97, 99, 104). Thus, the assignment of a specific role for Th1 and Th2 cytokines cannot be addressed fully using these isolated conditions.

In conclusion, the onset and progression of IDDM are under the control of both Th1 and Th2 cells and their respective cytokines. Although it is desirable and tempting to manipulate Th1-Th2 balance in favor of a benign or a protective immune response, future immunotherapies must take into consideration the delicate balance between Th1 and Th2 cells during distinct phases of IDDM.

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