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Type I (Insulin-Dependent) Diabetes Is a Th1- and Th2-Mediated Autoimmune Disease

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Type I (insulin-dependent) diabetes (IDDM) is an autoimmune disease with an unknown etiology but with a definite outcome, resulting in the progressive misdirected immunologic destruction of insulin-secreting pancreatic β islet cells by autoreactive leukocytes and their mediators (3). Even though the precise cause of the disease remains unclear, a combination of genetic, immunologic, and nongenetic factors contributes to the onset and progression of IDDM (3, 52). Specific HLA antigens, in particular DR3 and DR4, have been associated with increased risk for IDDM development (52, 89), while DR2 alleles generally have been described as "protective" of IDDM (86). In addition to HLA predisposing factors, viral infection (8), psychological factors (73), and dietary factors (8), among others, have been described as predisposing factors. Other investigators failed to demonstrate a strong cause-and-effect link between these factors and IDDM, which highlighted the need for further investigation and identification of causative agents and mechanisms underlying the pathogenesis of IDDM (77).

The frequent coexistence of IDDM with immune disorders is well established and results from an inherent dysregulation in humoral immunity and cell-mediated immunity (3, 8). This is exemplified by the presence of autoreactive antibodies targeting select β-cell constituents and other autoantigens (23, 28), circulating autoreactive T cells (78, 80), heightened expression of adhesion molecules (37, 60), reduced levels of serum cytokine inhibitors (57), and sustained expression of cytokines and their high-affinity receptors (36, 82). The development of hyperglycemia, a hallmark of IDDM, appears at later stages of the disease, months or years after the initiation of targeted autoimmune destruction of β cells (81).

The involvement of T-cell- and macrophage-derived cytokines in IDDM pathogenesis remains the subject of intense investigation; conclusions were largely based on studies with the genetically IDDM-predisposed nonobese diabetic (NOD) mice and BioBreeding (BB) rats, animal models which display many of the characteristics of human type I diabetes (4), and have focused on direct cytotoxic and indirect immunomodulatory effects of cytokines in mediating β-cell destruction (58, 82). Based on such studies, it was concluded that Th1 cytokines exacerbate, while Th2 cytokines protect from, IDDM (70, 72). However, contrary evidence is accumulating which demonstrates that the progression of IDDM from insulitis (pancreatic mononuclear cell infiltration) to frank hyperglycemia is under the control of both Th1 and Th2 cells and their respective cytokines (2, 46, 80, 93). This review focuses on the role of cytokines in IDDM pathogenesis and attempts to reconcile and accommodate the (apparently) conflicting reports pertaining to the protective and damaging roles of Th1 and Th2 cytokines in the context of autoimmune-mediated dysregulation of immunity. For discussion about other facets of altered immunity in IDDM, we refer the reader to excellent reviews published elsewhere (9, 11, 33).

OVERVIEW OF T-CELL ACTIVATION

Antigen-specific activation of naive $CD4^+$ T cells requires two signals. The first signal is imparted by interaction of the multimeric T-cell receptor (TcR)-CD3 complex with processed antigen expressed in conjunction with major histocompatibility complex class II protein by antigen-presenting cells (APC). The second signal is provided by costimulatory molecules which complement TcR-CD3 signals in augmenting T-cell activation (26, 29). At least three major signal transduction pathways operate as a consequence of T-cell activation: (i) phospholipase C-γ1 pathway, resulting in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (18) and the generation of 1,4,5-inositol trisphosphate and diacylglycerol (53, 69), (ii) $p21^{ras}$/RAF kinase pathway, also referred to as the "classical mitogen-activated protein kinase pathway" (38), and (iii) the phosphatidylinositol 3′-OH kinase/GDP-Rac, referred to as the "alternative MAPK-signaling pathway" (12). Depending on the intensity of the signal generated, duration of stimulation, and the contribution of costimulatory molecules, coupling to more than one signal transduction pathway is possible, which determines the outcome of the functional response (17, 42).

Engagement of the TcR in the absence of appropriate costimulation results in a transient activation with very little interleukin-2 (IL-2) production, followed by a sharp decline in activation (19). The provision of secreted (49, 62) and cell-bound (7, 51) costimulatory molecules, in synergy with TcR-CD3 signals, significantly augments cytokine expression at the transcriptional and posttranscriptional levels. This results in stabilization of IL-2 and other cytokine mRNA transcripts (50, 51), abrogation of anergy (31), and enhancement of cell viability as a result of inhibition of activation-induced cell death, or apoptosis (71).

Insofar as costimulatory signals determine whether TcR recognition of antigen will lead to activation or anergy, a role for altered costimulation in the pathogenesis of autoimmune disorders, including IDDM (see below) (41, 88), was proposed. This was supported by the findings that (i) blockade of cell-bound costimulatory molecules by chimeric toxin-immunoglobulin (Ig) fusion proteins induced hyporesponsiveness (13,

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48) and (ii) aberrant expression of costimulatory signals may activate autoreactive T cells, thereby inducing and/or exacerbating autoimmunity (48). This has revived interest in manipulating costimulatory pathways as new strategies for controlling autoimmune diseases, including IDDM (9, 88).

IMMUNOLOGY OF Th CELLS

In 1986, Mosmann et al. reported that upon activation $CD4^+$ T cells will differentiate into two distinct T helper (Th) cell clones expressing distinct cytokine profiles and effector functions (64), thus giving rise to a unifying Th1/Th2 paradigm. Central to this are the specific requirements for induction of Th1 and Th2 activities, including the nature of APC (macrophages, dentritic cells, or B cells) (21, 54), strength of TcR binding to processed antigen, and Th1 and Th2 cytokines (65, 74). Th1 cells produce IL-2 and gamma interferon (IFN-γ), while Th2 cells produce IL-4, IL-5, IL-10, and IL-13 (65, 73). Th0 cells, which produce both Th1 and Th2 cytokines, are generally regarded as precursors for Th1 and Th2 cells, being swayed into differentiating into either pathway in response to external stimuli (76) and also in response to Th1 and Th2 cytokines (55, 84). It should be noted that these two polarized patterns of cytokine expression represent extremes of many possible outcomes (40, 61).

Th1 and Th2 cells negatively cross-regulate the function of one another through their respective cytokines (55, 74). Th1 cytokines induce Th1 activity and block Th2 activity (34, 59), whereas Th2 cytokines promote Th2 activity while inhibiting Th1 activity (83). This indicates that induction of one Th program is accompanied by a corresponding decline in the activation of the other Th program (40). It remains to be seen whether this results from a shifting from one Th subset to another or, alternatively, from suppression of the growth of cells with committed phenotypes (1, 40). In any event, difference in cytokine secretion between Th1 and Th2 cells translates into functional differences, as Th1 cells, by producing IFN-γ, activate $CD8^+$ T cells and macrophages and promote cell-mediated immunity (1). Th2 cells stimulate IgM, IgG1, and IgE synthesis by B cells and activate eosinophils, thus promoting hypersensitivity reactions due to their capacity to produce IL-4 and IL-5 (15, 74).

PATHOPHYSIOLOGY OF CYTOKINES IN IDDM

In view of their role in macrophage activation and induction of delayed-type hypersensitivity reactions, Th1 cells were regarded as proinflammatory, while Th2 cells, which inhibit Th1 activity (see above), were considered anti-inflammatory (65, 84). Consistent with this characterization were the findings that IL-4 and IL-10—exclusive products of Th2 cells—inhibited IL-2-mediated responses and suppressed the production of the (proinflammatory) Th1 cytokines (20, 85). Accordingly, it was speculated that Th1 cytokines play a direct role in the pathogenesis and progression of IDDM, while Th2 cytokines should afford protection against Th1-mediated destruction of β islet cells. However, recent reports argued against this oversimplification (61), as Th2 cells and their mediators were shown to be involved in IDDM pathogenesis through facilitation of pancreatic mononuclear-cell infiltration (32, 87) and acceleration of β islet cell destruction (44, 68). This prompted the conclusion that IDDM is a Th1- and Th2-mediated disease (see below).

IDDM: A Th1-MEDIATED EVENT

Evidence from human studies and animal models supports a direct role for Th1 cells and their respective cytokines in the pathogenesis and progression of IDDM. This conclusion is based on the findings that recent-onset IDDM was associated with an increase in the expression of Th1 cytokines and a corresponding decline in the production of Th2 (IL-4) cytokines (6, 35, 79). Destruction of β cells was suggested to be due to a frank Th1-driven insulitis (22), and it was suggested that IDDM could be abrogated by induction of Th2 cytokine expression (30) or by treatment with the Th2 cytokines IL-4 and IL-10 (22, 72). The latter were described to act through inhibition of the production of Th1 cytokines. Furthermore, the predominance of Th1 cytokines in β-islet 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 mice littermates (25).

Mechanistically, Th1 cytokines induced and accelerated β-cell destruction through direct and indirect mechanisms. Th1 cytokines, including IFN-γ, exerted their effects primarily at the level of macrophage and $CD8^+$ T-cell activation, enhancing infiltration of these cells into the islets, thus accelerating β-cell destruction through the release of preformed and de novo-synthesized cytotoxic mediators (nitric oxide, oxygen radicals, serine esterases, etc.) (24). In addition to these direct effects, and owing to their capacity to suppress the production of Th2 cytokines, Th1 cytokines facilitated β-cell destruction indirectly by several mechanisms. These included induction of the activation and expansion of bystander autoreactive T cells, which increased their overall proportion (47), and suppression of the production of soluble cytokine antagonists, including the IL-1 receptor antagonist (22). The latter resulted in stimulation of IL-1 production by macrophages (22) and, in conjunction with continued autoantigenic stimulation, significant augmentation in the expression of IL-2 and other Th1 cytokines. Insofar as IDDM is associated with reduction in the production and activation of serum cytokine inhibitors (57), and as Th1 cytokines potentiated the production and effector functions of monokines (IL-1 and tumor necrosis factor alpha) (56), this eventually amplified the cascade of β-cell destruction.

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 hypothesis were the findings that insulitis associated with new-onset IDDM involved pancreatic homing of Th2 cells (39, 60) and the predominance of Th2 cytokines (45, 60, 90). Pancreatic expression of Th2 cytokines did not overcome autoimmune destruction of the pancreas (46, 66) but rather accelerated it (5, 68, 93). In addition, induction of Th2-mediated antibody responses to a β-cell constituent led to a rapid spread of Th2 immunity to unrelated β-cell antigens and, in conjunction with Th1 cytokines, to exacerbation of IDDM (87). In addition, peri-insulitis and insulitis were prevented by treatment of NOD mice with anti-IL-10 antibodies (44). Furthermore, IDDM was not prevented by adoptive transfer of Th2 cells (even at a 10-fold excess relative to Th1 cells [39]) or by induction of Th2 activity by a neutralizing anti-IL-12 monoclonal antibody (MAb) (91).

It was of interest that this "Th2-induced" component of anti-β-cell immunity appeared to be mediated principally by IL-10 but not by IL-4, thus making it unclear whether this effect was

a generalized feature of Th2 cytokines or, alternatively, unique to IL-10. In this regard, it was demonstrated that local production of IL-10, but not IL-4, accelerated autoimmune destruction of β islet cells (46, 63, 68). In addition, NOD mice were protected from development of overt diabetes by a neutralizing anti-IL-10 MAb but not anti-IL-4 MAbs, which were described to be ineffective in altering the course of Th2 autoimmune destruction of pancreatic β islet cells (68). Furthermore, in contrast to IL-10 (68), tissue expression of IL-4 (67) led to a nondestructive insulitis. These and other results underscore the fact that the role of Th2 cytokines in IDDM pathogenesis is a complex one and depends on the relative contribution of individual cytokines in the process. This warrants further scrutiny in assigning a generalized pathogenic role for Th2 cytokines (versus a specific effect of IL-10) in the pathogenesis and progression of IDDM.

In any event, Th2 cytokines can no longer be viewed as "protective" of IDDM, and their use as immunotherapy needs reassessment in view of their direct role in promoting insulitis and β-cell destruction. Functionally, Th2 cytokines exert their effects 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, due to its role as a B-cell (27, 75)- and cytotoxic-T-cell (14)-stimulatory or differentiating factor, IL-10 may stimulate activated T cells and B cells. Differential responsiveness of different APC types (macrophages, B cells, dendritic cells) to antigenic stimulation (21) and to IL-10 action (54) has been reported. Third, Th2 cytokines promote peri-insulitis and frank insulitis by enhancing major histocompatibility complex class II expression (63, 92) or by altering the expression of endothelium-bound addressin, thereby stimulating accumulation of macrophages, B cells, and eosinophils (93). Fourth, by augmenting cytokine production by endothelial cells and other cell types (10, 16), local production of Th2 cytokines amplifies the cascade of anti-β-cell immunity through activation of resident immune cells and by facilitating the pancreatic infiltration by other cell types.

Th1- VERSUS Th2-DRIVEN INSULITIS

It is evident that Th1 cells are not the sole mediators of β-islet cell destruction, that Th2 cells are not inhibitory or benign, as they are capable of inducing β-islet destruction, and that both Th1 and Th2 cytokines appear to cooperate in driving β-islet cell destruction, eventually leading to hyperglycemia. However, the types of lesions differ between Th1- and Th2-driven insulitis (39, 68). Th1 lesions comprised focally confined insulitis consisting primarily of $CD8^+$ and $CD4^+$ T cells, and β islet cells die by apoptosis, thereby sparing surrounding exocrine tissue (43). On the other hand, Th2 lesions are more dispersed and consist primarily of eosinophils, macrophages, and fibroblasts, with a notable sparsity of T cells (67), and β islets die by necrosis. Also, there is the accumulation of fibroblasts and the generation of the extensive extracellular matrix and adipose tissue in Th2 lesions which subsequently leads to tissue necrosis.

In addition to differences in lesion morphology, the kinetics of β-cell destruction differ between Th1- and Th2-driven autoimmune attacks (45). Compared to a Th2-mounted attack, Th1-driven injuries are more rapid and aggressive and are sustained for a longer period, which suggests that a Th2-mediated attack is responsible for the early phase of IDDM (2) while Th1-driven responses are responsible for the persistent and sustained attacks (44). It remains to be determined whether the predominance of Th1 attacks in advanced IDDM is a reflection of the expansion of Th1 clones and/or due to the incapacity of Th2 clones to sustain an immunologic attack, as has been suggested (87).

CONCLUDING REMARKS

The previous assignment of a pathogenic role to Th1 cells and a protective role to Th2 cells and their respective cytokines in the pathogenesis and progression of IDDM was largely based on artificial conditions. This did not reflect the delicate balance and relative contribution of each Th subset at distinct stages of the disease. Accordingly, Th1 cells are not the sole instigators of IDDM, and Th2 cells are more harmful than previously believed.

A number of points are worth considering in this context. First, many studies were based largely on in vitro observations using well-defined experimental conditions which were not representative of the cytokine milieu and/or the cellular network that are operative in the pancreas during the autoimmune attack. Second, the Th1/Th2 cytokine-secreting profile represents the extreme of many possible outcomes. Accordingly, pushing the differentiation of one Th subset to the extreme by using MAbs or recombinant cytokines is an exaggeration since this cannot be duplicated in vivo. Third, assignment of a protective role to Th2 cytokines, including IL-10, was based on a well-documented effect of IL-10. However, cytokines are pleiotropic; a cytokine may be produced by more than one cell type and may exert its effect on several target cells. Thus, assignment of a specific role to Th1 and Th2 cytokines cannot be fully addressed by 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. While it is desirable and tempting to manipulate the Th1-Th2 balance in favor of a benign or a protective immune response, future immunotherapy must take into consideration the delicate balance between Th1 and Th2 cells during distinct phases of IDDM.

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