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COMMENTARY

Cytokines and Their Roles in Pancreatic Islet β-Cell Destruction and Insulin-Dependent Diabetes Mellitus

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ABSTRACT. Insulin-dependent diabetes mellitus (IDDM) is a disease that results from autoimmune destruction of the insulin-producing β-cells in the pancreatic islets of Langerhans. The autoimmune response against islet β-cells is believed to result from a disorder of immunoregulation. According to this concept, a T helper 1 (Th1) subset of T cells and their cytokine products, i.e. Type 1 cytokines—interleukin 2 (IL-2), interferon gamma (IFNγ), and tumor necrosis factor beta (TNFβ), dominate over an immunoregulatory (suppressor) Th2 subset of T cells and their cytokine products, i.e. Type 2 cytokines—IL-4 and IL-10. This allows Type 1 cytokines to initiate a cascade of immune/inflammatory processes in the islet (insulitis), culminating in β-cell destruction. Type 1 cytokines activate (1) cytotoxic T cells that interact specifically with β-cells and destroy them, and (2) macrophages to produce proinflammatory cytokines (IL-1 and TNFα), and oxygen and nitrogen free radicals that are highly toxic to islet β-cells. Furthermore, the cytokines IL-1, TNFα, and IFNγ are cytotoxic to β-cells, in large part by inducing the formation of oxygen free radicals, nitric oxide, and peroxynitrite in the β-cells themselves. Therefore, it would appear that prevention of islet β-cell destruction and IDDM should be aimed at stimulating the production and/or action of Type 2 cytokines, inhibiting the production and/or action of Type 1 cytokines, and inhibiting the production and/or action of oxygen and nitrogen free radicals in the pancreatic islets. BIOCHEM PHARMACOL **55**;8:1139–1149, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. insulin-dependent diabetes mellitus; autoimmunity; islet β-cell; cytokines; free radicals; nitric oxide

IDDM,† THE RESULT OF DISORDERED REGULATION OF IMMUNE RESPONSES

IDDM is a disease that results from destruction of the insulin-producing β-cells in the pancreatic islets of Langerhans. Current evidence favors the concept that β-cells are destroyed by an autoimmune response directed against certain β-cell constituents (autoantigens) [1]. This autoimmune response is thought to occur in genetically predisposed persons who possess certain "susceptibility" alleles and who lack other "protective" alleles of the *MHC* gene complex, which regulates immune responses. In addition, non-*MHC* genes may contribute to the autoimmune response. The traditional concept is that environmental factors (e.g. microbial, chemical, dietary) may trigger an autoimmune response against β-cells. Recent studies in animal models with spontaneous autoimmune diabetes, however, have revealed that environmental factors (particularly microbial agents) may either *promote or protect against* diabetes development [2]. Therefore, it is believed that both genetic and environmental inputs may be either pathogenic (i.e. IDDM-promoting) or protective against IDDM, and that disease appearance is influenced by the net effects of genetic and environmental factors upon immune responses. According to this concept, IDDM, like other organ-specific autoimmune diseases, results from a dysregulation of immune responses [1]. This posits that T lymphocytes (T cells) specific for islet β-cell molecules (i.e. autoantigens) exist normally, but are restrained by immunoregulatory mechanisms (the self-tolerant state), and that IDDM develops when one or another immunoregulatory mechanism fails, allowing autoreactive T cells directed against islet β-cells to become activated, to expand clonally, and to entrain a cascade of immune/inflammatory processes in the islet (insulitis), culminating in β-cell destruction. A current hypothesis is that the pathogenic immune response is mediated by a Th1 subset of T cells, whereas the protective immune response is mediated by a Th2 subset of T cells [3]. This is illustrated in Fig. 1.

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† *Abbreviations:* APC, antigen-presenting cells; BB, Biobreeding; CAT, catalase; GAD, glutamic acid decarboxylase; GPX, glutathione peroxidase; IDDM, insulin-dependent diabetes mellitus; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; MHC, major histocompatibility complex; NAME, N^G-nitro-L-arginine methyl ester; NMMA, N^G-methyl-L-arginine; $NO^{\cdot}$, nitric oxide; NOD, nonobese diabetic; $O_2^{\cdot -}$, superoxide radical; $OH^{\cdot}$, hydroxyl radical; $ONOO^-$, peroxynitrite; SOD, superoxide dismutase; Th, T helper; and TNF, tumor necrosis factor.

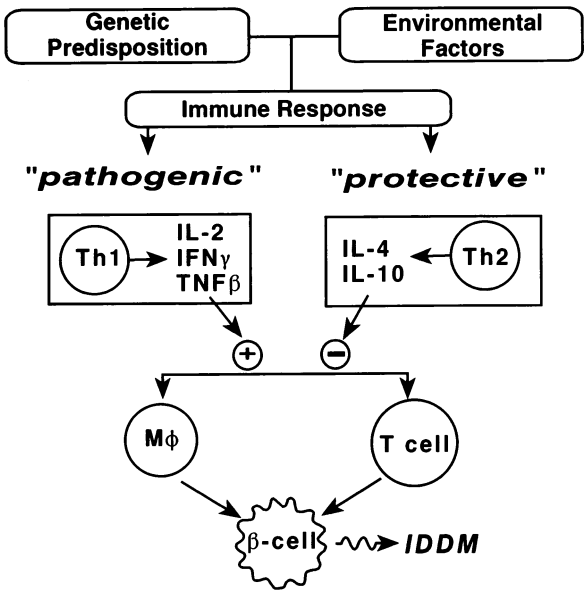

FIG. 1. Current formulation of the pathogenesis of IDDM. Both genetic and environmental factors may influence the direction taken by the immune response (pathogenic or protective). The pathogenic immune response that leads to pancreatic islet β-cell destruction and IDDM is mediated by T cells autoreactive to islet β-cell antigen(s). A current hypothesis is that the autoreactive T cells are a Th1 subset of T cells identified by their distinct cytokine products, IL-2, IFNγ, and TNFβ, whereas the protective limb of the immune response may be mediated by a Th2 subset of T cells that produce IL-4 and IL-10. According to this concept, Th1 cells and their cytokine products (IL-2, IFNγ, and TNFβ) activate macrophages (Mϕ) and cytotoxic T cells to destroy β-cells, causing IDDM, whereas Th2 cells and their cytokine products (IL-4 and IL-10) down-regulate (suppress) Th1 cells and cytokines and thereby prevent IDDM.

CYTOKINE REGULATION OF IMMUNE RESPONSES

Th1 and Th2 cells are characterized by their distinct cytokine secretory products [4]. Th1 cells secrete IL-2, IFNγ, and TNFβ, whereas Th2 cells secrete IL-4 and IL-10. Also, other cytokines are produced by both Th1 and Th2 cells, and Th cell phenotypes other than Th1 and Th2 exist and have other patterns of cytokine secretion. The functional significance of Th1 and Th2 cell subsets is that their distinct patterns of cytokine secretion lead to strikingly different T cell actions [4–6]. Th1 cells and their cytokine products (IL-2, IFNγ, TNFα, and TNFβ) are the mediators in cell-mediated immunity (formerly termed delayed-type hypersensitivity). Th1 cell-derived IFNγ, TNFα, and TNFβ activate vascular endothelial cells to recruit circulating leukocytes into the tissues at the local site of antigen challenge, and they activate macrophages to eliminate the antigen-bearing cell. In addition, Th1 cell-derived IL-2 and IFNγ activate cytotoxic T cells to destroy target cells expressing the appropriate MHC-associated antigen, and they activate natural killer cells to destroy target cells in an MHC-independent fashion. Thus, Th1 cytokines activate cellular immune responses. In contrast, Th2 cytokines are much more effective stimulators of humoral immune responses: that is, immunoglobulin (antibody) production, especially immunoglobulin E, by B lymphocytes. Furthermore, responses of Th1 and Th2 cells are mutually inhibitory. Thus, the Th1 cytokine IFNγ inhibits the production of the Th2 cytokines IL-4 and IL-10; these, in turn, inhibit Th1 cytokine production [4–6].

CYTOKINES: FRIENDS OR FOES IN IDDM PATHOGENESIS?

Cytokine Studies in Isolated Islets

It is now well documented that cytokines can be cytotoxic to pancreatic islets *in vitro* [7, 8]. IL-1, TNFα, TNFβ, and IFNγ (in pico- to nanomolar concentrations) are *cytostatic* to β-cells: that is, the individual cytokines inhibit insulin synthesis and secretion, but these largely recover after the cytokine is removed. In addition, the cytokines may be *cytocidal*: i.e. IL-1, TNFα, TNFβ, and IFNγ, usually when added in combination, destroy the β-cells in both rodent and human islets. Because the cytodestructive effects of cytokines on islet β-cells *in vitro* are not specific to β-cells (e.g. α-cells in the islets are also damaged), cytokines may not qualify as mediators of β-cell destruction in IDDM, which is β-cell specific. Even agents with known β-cell specificity *in vivo* (e.g. alloxan, streptozocin), however, can damage other islet endocrine cells *in vitro*, possibly because of nonspecific damage to the non-β-cells adjacent to damaged β-cells *in vitro*. For example, β-cells separated from non-β-cells in islets are destroyed by streptozocin and alloxan, but non-β-cells are not [9]. Similarly, IL-1 is cytotoxic to both β- and α-cells in isolated rat islets, but it selectively inhibits β-cell secretion of insulin and not α-cell secretion of glucagon in separated purified preparations of these islet endocrine cells [10]. Moreover, cytokine applications to islets *in vitro* may not mimic the molecular pathology of the pancreatic insulitis lesion *in vivo*. Thus, cytokine products of islet-infiltrating macrophages and T cells could be delivered in a targeted fashion into the microenvironment of the β-cell or even directly into the β-cell by contiguous cytotoxic T cells. Highly localized and directed delivery of cytokines from T cells and macrophages to β-cells might explain why rejection of islet allografts in rats did not destroy syngeneic islets mixed in with the allogeneic islets (whole islets, not single cell preparations, were admixed) [11]. Also, syngeneic islets were not destroyed after cotransplantation with allogeneic or xenogeneic islets in mice; however, insulin secretory responses from the syngeneic islets cotransplanted with xenogeneic islets were severely impaired, suggesting inhibitory effects of xenogeneic macrophage-derived products (e.g. IL-1, TNFα, $NO^{\cdot}$) on islet β-cell function [12].

Cytokine Studies In Vivo

A variety of cytokines implicated in the pathogenesis of IDDM have been found to be expressed at the gene or

protein level, or both, in the insulitis lesion of animals with autoimmune diabetes, the NOD mouse and the BB rat, and in the pancreata of IDDM patients [3]. The simple presence of a cytokine in the insulitis lesion, however, does not identify the role of that cytokine in IDDM pathogenesis. Thus, a cytokine may be either proinflammatory or, alternatively, it may be responding to regulate (i.e. suppress) the inflammatory process. In recent studies of cytokine gene expression in pancreatic islet-infiltrating mononuclear cells of NOD mice, IFNγ mRNA expression was found to correlate with β-cell destructive insulitis [13, 14]. Further evidence for a β-cell cytotoxic role for IFNγ comes from the finding that transgenic expression of IFNγ by β-cells in normal mice leads to an immune-mediated insulitis, β-cell destruction, and IDDM [15]. Also, monoclonal antibodies to IFNγ protect against diabetes development in NOD mice [16, 17] and BB rats [18]. In addition, IFNγ has been detected in lymphocytes infiltrating islets of human subjects with recent-onset IDDM [19]. Interestingly, another proinflammatory cytokine, IFNα, has been detected in β-cells of human subjects with recent-onset IDDM [20], and IFNα mRNA expression is increased significantly in the pancreata of IDDM patients as compared with control human pancreata [21]. Also, islet expression of IFNα precedes insulitis and diabetes in BB rats [22]. Furthermore, islet β-cell transgenic expression of IFNα in normal mice elicits an immune-mediated destruction of the β-cells, and anti-IFNα antibody prevents this β-cell damage and IDDM [23]. Because IFNα is a product of many cells that are virally infected or otherwise stressed, these findings suggest that an initial β-cell stress (possibly viral) may induce IFNα production, which would recruit immune system cells, and these, in turn, could damage the IFNα-producing islet β-cells. Other studies in transgenic mice that ectopically express immunoregulatory cytokines in islet β-cells suggest proinflammatory roles for IL-2 [24], IL-10 [25], TNFα [26, 27], and TNFβ [28] produced locally in the islet, in addition to IFNα [23] and IFNγ [14, 15].

In contrast to the actions of cytokines as mediators of β-cell injury, suggested from the *in vitro* and transgenic studies described above, studies involving systemic administration of cytokines to diabetes-prone NOD mice and BB rats *in vivo* have revealed that several cytokines can *prevent* diabetes development: these include IL-1 [29, 30], IL-2 [31, 32], IL-4 [33], IL-10 [34], TNFα [30, 35, 36], and TNFβ [37, 38]. Because deficiencies in the endogenous production of IL-1 [39], IL-2 [33, 39], IL-4 [33], TNFα [30, 35, 40], and TNFβ [38] have been reported in diabetes-prone NOD mice and/or BB rats, the diabetes-preventive effects of chronic administrations of these cytokines may result from corrections of specific deficits in cytokine production in the diabetes-prone animals. Systemically administered cytokines, however, may act on targets outside the immune system. For example, IL-1 and TNF can stimulate the hypothalamic-pituitary axis, leading to secretion of adrenocorticotropic hormone and consequently adrenal corticosteroids, which suppress inflammatory cells and cytokines [41]. Also, although IL-1 may decrease IDDM incidence in BB rats [29], this effect might not be observed if studies were controlled for the effects of IL-1 on decreased food intake [42]. These findings highlight the pleiotropic effects of cytokines that complicate interpretations of their actions. Cytokines are highly interdependent; therefore, a given cytokine may affect the production and action of other cytokines, resulting in physiologic or pathologic changes different from those induced by the original cytokine. For example, IL-1, TNFα, TNFβ, and IL-2 appear to be cytotoxic to islet β-cells in the islet microenvironment (in studies *in vitro* and in transgenic studies), whereas systemic administration of these cytokines may prevent an islet β-cell-directed autoimmune response by acting on immunologic circuits outside the islet or possibly on neuroendocrine cells (in studies *in vivo*). Finally, studies using NOD mice and BB rats to examine the effects on diabetes incidence of administering antibodies to cytokines or cytokine receptors to block cytokine actions *in vivo* have supported diabetes-promoting roles for IL-2 [43, 44], IL-6 [16], and IFNγ [16, 17].

It is important to recognize that most of our current information on cytokines implicated in IDDM pathogenesis comes from studies using NOD mouse and BB rat models. There is evidence, however, for cytotoxic actions of IL-1, TNFα, and IFNγ on human islets *in vitro*, and IFNα and IFNγ have been detected in islets of patients with recent-onset IDDM [19–21]. Reports on plasma levels of IL-1, TNFα, and IFNγ, as well as secretion of these cytokines by peripheral blood mononuclear cells from patients with IDDM, have not provided consistent results, and it is not clear whether changes in cytokine levels preceded or resulted from IDDM [45, 46]. Also, peripheral blood cells probably do not adequately represent the cell infiltrate within the islet. Because the pancreas may not be accessible for immunologic investigation in humans, and considering that immunoregulatory actions of cytokines are exerted at short distances (paracrine and autocrine), it may be difficult to study the intraislet roles of cytokines in the evolution of β-cell destructive insulitis in human IDDM.

TH1 CELLS AND CYTOKINES AS MEDIATORS OF β-CELL DESTRUCTION IN IDDM

Abundant evidence now suggests that autoreactive T cells are present in the normal immune system but are prevented from expressing their autoreactive potential by other regulatory (suppressor) T cells [47]. The opposing actions of autoreactive and regulatory T cells are regulated by their respective cytokine products [4–6]; one study has provided direct evidence for the operation of such a cytokine immunoregulatory balance in the avoidance of autoimmune diabetes [48]. Diabetes was induced in a nonautoimmune rat strain by rendering the animals relatively T cell deficient, using a protocol of adult thymectomy and sublethal γ-irradiation. Importantly, insulitis and diabetes could be prevented in these rats by injecting them with a

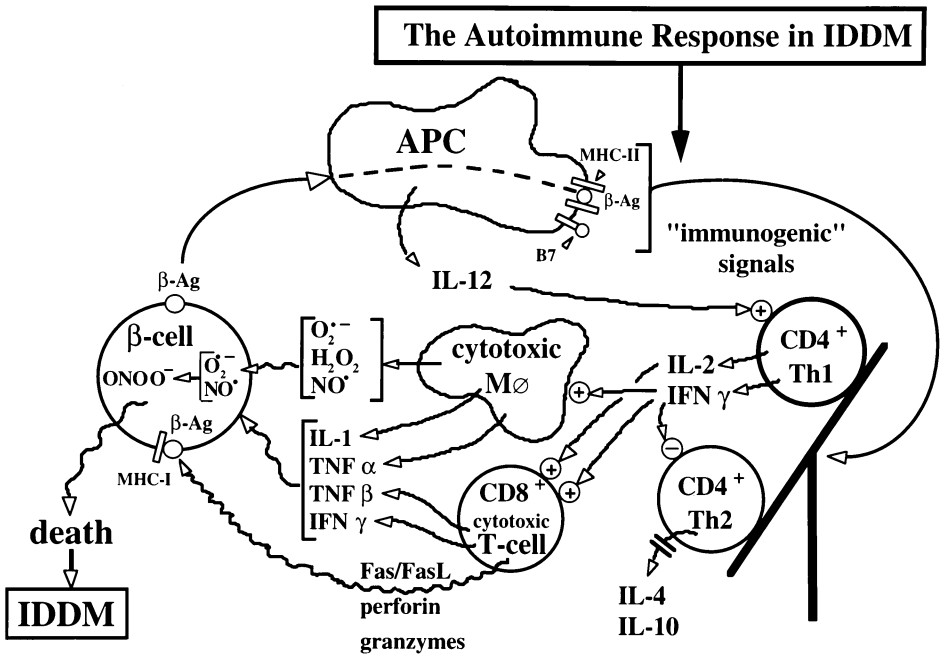

FIG. 2. Scheme of the proposed circuitry of immune system cells and cytokines that may be involved in the autoimmune response leading to destruction of pancreatic islet β-cells and IDDM. The concept illustrated posits that certain β-cell proteins act as autoantigens (β-Ag) after being processed by APC, such as macrophages and dendritic cells, and presented in a complex with MHC class II molecules on the surface of the APC. Collectively, the β-Ag–MHC II complex, accessory molecules on the APC (e.g. the B7 molecule), APC-derived IL-12, and perhaps other signals would constitute immunogenic signals that activate $CD4^+$ T cells, predominantly of the Th1 subset. Antigen-activated Th1 cells produce IL-2 and IFNγ, which inhibit Th2 cell production of IL-4 and IL-10. Also, IL-2 and IFNγ activate macrophages (Mϕ) and cytotoxic $CD8^+$ T cells, and these effector cells may kill islet β-cells by one or both of two types of mechanisms: (1) direct interactions of antigen-specific cytotoxic T cells with a β-cell autoantigen-MHC class I complex on the β-cell (involves Fas/Fas L, or perforin and granzyme-mediated killing mechanisms), and (2) non-specific inflammatory mediators, such as free radicals/oxidants ($O_2^{\cdot-}$ and H_2O_2), $NO^{\cdot}$, and cytokines (IL-1, TNFα, TNFβ, IFNγ).

particular $CD4^+$ T cell subset that is isolated from healthy syngeneic donors and produces IL-4 and IL-2 but not IFNγ [48]. In another study, $CD4^+$ T cell lines that react to rat insulinoma cells and secrete either IFNγ or IL-4 have been developed from spleens of diabetic NOD mice. The IFNγ-secreting $CD4^+$ T cells (Th1-like phenotype) adoptively transferred β-cell destructive insulitis and diabetes in neonatal NOD mice, whereas the IL-4 secreting $CD4^+$ T cells (Th2-like phenotype) induced a non-destructive peri-islet insulitis [49]. Similarly, Th2 cells expressing a diabetogenic T cell receptor adoptively transferred β-cell destructive insulitis and diabetes in neonatal NOD mice, whereas Th2 cells expressing the same T cell receptor did not; however, the Th2 cells could not prevent the Th1 cells from transferring diabetes [50]. Nevertheless, the protective effects against insulitis and diabetes of IL-4 [33], IL-4-producing $CD4^+$ T cells [48], and IL-10 [34] suggest that Th2 cells producing IL-4 and IL-10 may be responsible for preventing β-cell destructive insulitis, possibly by suppressing IFNγ-producing Th1 cells. Thus, suppression of IFNγ production is a recognized action of both IL-4 and IL-10 [51–53], and IFNγ is a mediator of islet β-cell destruction *in vitro* as well as β-cell-destructive insulitis and IDDM [3].

The above findings have formed the concept that the autoimmune response in IDDM involves some disturbance (or disturbances) in immunoregulatory circuits that leads to a dominance of Th1 cells and their cytokine products over Th2 cells and their cytokines (Fig. 2). The scheme depicted in Figure 2 emphasizes that the direction taken by the T cell response, in terms of Th phenotype, is largely regulated by cytokines. Thus, naive T cells are not precommitted to any particular Th phenotype; the Th phenotype varies with the cytokines in the microenvironment. The presence of IL-12, a macrophage and B cell product, favors Th1 cell differentiation, and anti-IL-12 antiserum blocks expression of the Th1 phenotype [54, 55]. On the other hand, IL-4, a Th2 and mast cell product [55], favors Th2 cell differentiation, and anti-IL-4 monoclonal antibody promotes expression of a Th1 phenotype [56, 57]. The results of Th1 cell activation are induction of IL-2 and IFNγ production, inhibition of Th2 cytokine production, and activation of macrophages, cytotoxic T cells, and natural killer cells. There is evidence that these cytokine-dependent pathways may operate in the expression of IDDM. Thus, administration of IL-12 to prediabetic NOD female mice was found to accelerate the onset of diabetes, and this was associated with enhanced IFNγ and decreased IL-4 production by islet-infiltrating lymphocytes, and selective β-cell destruction [58]. Furthermore, IL-12 appears to be required for diabetes development, because (1) administration of anti-IL-12 monoclonal antibody significantly decreased IDDM incidence in NOD mice, and (2) NOD mice made IL-12

deficient by backcrossing with IL-12 gene knockout mice did *not* develop insulitis and diabetes [59]. Not shown in Fig. 2 is the possible direct activation of cytotoxic T cells by IL-12 [60]; this must be considered as a potential effector mechanism in β-cell destruction because IL-12 appears to be necessary for the development of diabetes in NOD mice [59], whereas IFNγ may not be absolutely necessary since diabetes did develop (albeit delayed) in NOD mice backcrossed to mice with targeted disruption of the IFNγ gene [61].

The scheme depicted in Fig. 2 illustrates that both β-cell antigen-specific and nonspecific immune/inflammatory responses appear to participate in mediating islet β-cell destruction in IDDM. The β-cell antigen-specific immune response involves direct binding of $CD8^+$ cytotoxic T cells to β-cells. The T cells specifically recognize β-cell antigen(s) presented by MHC class I molecules on the β-cells, and this is followed by activation of the cytotoxic T cells, which may then kill the β-cells by receptor (Fas/FasL)-mediated mechanisms or by secretion of cytotoxic molecules (granzymes and perforin). These mechanisms of β-cell destruction in IDDM remain to be demonstrated. The nonspecific immune/inflammatory response involves β-cell destruction by T cell and macrophage-derived molecules—proinflammatory cytokines (IL-1, TNFα, TNFβ, IFNγ) and free radicals/oxidants, e.g. $O_2^{\cdot -}$, H_2O_2, and $NO^{\cdot}$. These inflammatory mediators are produced by T cells (both $CD4^+$ and $CD8^+$) and macrophages activated by cytokines from T cells, particularly IFNγ, and also IL-2, TNFα, and TNFβ (Fig. 2). In a recent study, mRNA levels of iNOS were found to correlate with mRNA levels of IL-1α and IFNγ in islets of prediabetic NOD mice, and iNOS protein was expressed in islet-infiltrating macrophages and β-cells [62]. These findings suggest that IL-1 (from macrophages) and IFNγ (from T cells) induce iNOS and consequent $NO^{\cdot}$ production by macrophages and by the β-cells themselves. In this way, macrophage-derived $NO^{\cdot}$ would be destructive to adjacent β-cells, and β-cell-derived $NO^{\cdot}$ would be self-destructive. Indeed, $NO^{\cdot}$ has been identified as an important mediator of β-cell damage by activated macrophages [63]. Also, intraislet release of IL-1 by passenger macrophage activation *in vitro* leads to iNOS expression within β-cells and consequent impaired insulin secretion [64].

MOLECULAR MECHANISMS OF CYTOKINE ACTIONS IN ISLET β-CELLS

The actions of IL-1β (as a single agent) on β-cells have been studied the most, but the actions of TNFα and IFNγ (usually in combination with IL-1β) have also been examined, and a variety of mechanisms have been proposed to mediate the cytostatic and/or the cytotoxic effects of these cytokines (IL-1β, TNFα, and IFNγ) on islet β-cells. Most current evidence points to oxygen and/or nitrogen free radicals as mediators of cytokine-induced islet β-cell destruction [7, 8, 65] (Fig. 3).

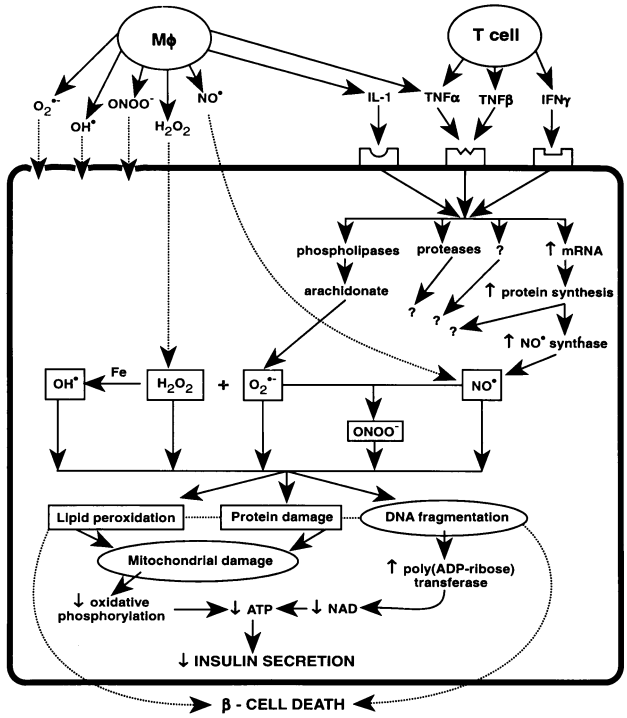

FIG. 3. Possible biochemical mechanisms of islet β-cell damage by cytokines and free radicals produced by pancreatic islet-infiltrating cells in IDDM. Activated macrophages (Mϕ) produce oxygen free radicals ($O_2^{\cdot -}$, $OH^{\cdot}$, and H_2O_2), the nitrogen free radical, $NO^{\cdot}$, and $ONOO^-$. These free radicals may damage the β-cell plasma membrane, and H_2O_2 and $NO^{\cdot}$ may also diffuse intracellularly to cause further damage. Activated Mϕ also produce IL-1 and TNFα, and activated T cells produce TNFα, TNFβ, and IFNγ. These cytokines bind to specific receptors on β-cells and elicit signals (not fully characterized) that activate different pathways. Phospholipases (e.g. PLA_2) are activated and arachidonic acid and its metabolites produced, leading to excessive formation of oxygen free radicals ($O_2^{\cdot -}$, H_2O_2, and $OH^{\cdot}$). Proteases and other (?) mediators are also activated. Also, IL-1, TNF, and IFNγ activate synthesis of nitric oxide synthase, with consequent formation of $NO^{\cdot}$. Oxygen free radicals ($O_2^{\cdot -}$, H_2O_2, and $OH^{\cdot}$) and nitrogen free radicals ($NO^{\cdot}$) may act separately and/or interact (e.g. $O_2^{\cdot -} + NO^{\cdot} \rightarrow ONOO^-$, a strong oxidant). These radicals can inactivate mitochondrial and cytosolic proteins (enzymes), leading to decreased oxidative phosphorylation, decreased glycolysis, and consequently decreased ATP levels and impaired insulin secretion. Higher levels and/or longer lasting production of free radicals may increase damage to cellular constituents (e.g. membrane phospholipids, enzymes, and DNA) essential for cell survival, resulting in β-cell death.

Oxygen Free Radicals as Mediators of β-Cell Destruction

There are several lines of evidence to support the hypothesis that oxygen free radicals participate in mediating islet β-cell dysfunction and destruction in IDDM. First, pancreatic islet β-cells are highly vulnerable to injury by free radicals, such as those produced by alloxan [66], and this has been attributed, at least in part, to the low activities of oxygen free radical scavenging enzymes in islet cells, notably mitochondrial Mn SOD [67] and GPX [66]. Also,

gene (mRNA) expressions of several antioxidant enzymes—Mn SOD, cytoplasmic Cu-Zn SOD, GPX, and CAT have been reported to be lower in islets than in various other mouse tissues [68]. Furthermore, lower than normal levels of total SOD activity have been reported in islets of autoimmune diabetes-prone BB rats [69] and NOD mice [70]; however, the claim for NOD islets has not been substantiated [71]. Second, a variety of antioxidants (deferoxamine, nicotinamide, SOD, α-tocopherol, probucol, and lazaroid) have been reported to provide some protection against IDDM development in autoimmune diabetes-prone BB rats and/or NOD mice [72–79]. Third, oxygen free radical scavengers have been used *in vitro* to protect islet β-cells from the cytotoxic effects of cytokines. Thus, an oxygen free radical scavenger combination of dimethylthiourea and citiolone significantly inhibited the islet cytotoxic effects of combinations of IL-1, TNFα, and IFNγ [80]. In subsequent studies using rat islets [81] and human islets [82], formation of a lipid peroxidation product (malondialdehyde) was reported in islet cells incubated with a cytotoxic combination of cytokines (IL-1, TNFα, and IFNγ), and a novel lazaroid antioxidant reduced both lipid peroxidation and β-cell destruction induced by the cytokines. On the other hand, IL-1, TNFα, and IFNγ, as individual cytokines, all inhibited insulin release without any increase in lipid peroxidation or cytodestructive effects in rat islets [81]. Similarly, others have demonstrated that free radical scavengers do not prevent IL-1-induced inhibition of insulin release in rat islets [83] or a hamster insulinoma cell line [84]. Taken together, these findings suggest that cytokine-induced inhibition of insulin release (a functional, not a destructive effect) may not be oxygen free radical-mediated, whereas the *cytodestructive* effects of cytokines on islet β-cells appear to involve oxygen free radical-mediated events, as described for the cytotoxic effects of IL-1, TNFα, and IFNγ in a variety of other cell types [85, 86]. In a recent study using rat islets, the cytokine combination of IL-1β, TNFα, and IFNγ was reported to induce the formation of a broad spectrum of aldehydes in the islet cells, and certain aldehydes—4-hydroxynonenal (4-HNE), hexanal (C6), and malondialdehyde (MDA)—were found to be highly toxic to the β-cells [87]. These findings suggest that cytokine-induced islet β-cell destruction involves oxygen free radical production, lipid peroxidation, and the generation of aldehydes that are toxic to the β-cells.

Nitric Oxide as a Mediator of β-Cell Destruction

$NO^{\cdot}$ is a free radical demonstrated in recent years to be a potent and pleiotropic mediator with physiological as well as toxic activities [88]. Inhibition of insulin secretion by IL-1 appears to be mediated by $NO^{\cdot}$ [65]. Thus, inhibition of nitric oxide synthase (both the constitutive and the inducible isoform) by analogues of L-arginine, NMMA and NAME, blocked IL-1-induced $NO^{\cdot}$ formation and prevented IL-1-induced inhibition of insulin release in rat islets [89, 90]. A mechanism(s) by which IL-1-induced $NO^{\cdot}$ formation inhibits insulin release appears to be the inactivation of iron–sulfur centers of iron-containing enzymes such as mitochondrial aconitase, required for glucose oxidation and insulin release [91]. Thus, the nitric oxide synthase inhibitor NMMA prevented IL- 1β-induced inhibition of glucose oxidation and insulin release by purified rat islet β-cells, and also prevented IL-1β-induced inhibition of mitochondrial aconitase activity of dispersed rat islet cells and a rat β-cell tumor line (RINm5F) [92]. Collectively, these findings demonstrate that $NO^{\cdot}$ qualifies as a mediator of the inhibitory effects of IL-1β on glucose-stimulated insulin secretion in rat islets. On the other hand, $NO^{\cdot}$ appears to have only a partial role or no role in mediating the cytodestructive effects of a combination of cytokines (IL-1β + TNFα + IFNγ) in islet β-cells. Thus, by using a selective inhibitor of nitric oxide synthase, NMMA, it was reported that stimulation of $NO^{\cdot}$ production was not a sufficient condition for cytokine-induced destruction of β-cells in a rat insulinoma cell line, RINm5F [93]. Also, cytokine-induced $NO^{\cdot}$ production in human islets was found to be neither necessary nor sufficient to destroy β-cells; rather $NO^{\cdot}$-independent cytotoxic mechanisms were involved, and these could be inhibited by nicotinamide, an oxygen free radical scavenger and inhibitor of ADP ribosylation [94]. Similarly, another group reported that cytokines (IL-1β + TNFα + IFNγ) suppress glucose-stimulated insulin release and destruction of human islet cells irrespective of their effects on $NO^{\cdot}$ generation [95].

Interactions of Oxygen Free Radicals and $NO^{\cdot}$ in the Mediation of β-Cell Destruction

$ONOO^{-}$ is a highly reactive oxidant species produced by the combination of the oxygen free radical $O_2^{\cdot -}$ and the nitrogen free radical $NO^{\cdot}$ [96, 97]. The production of $ONOO^{-}$ has been demonstrated in various forms of shock and inflammation, and current evidence suggests that $ONOO^{-}$ is a more potent oxidant and cytotoxic mediator than $NO^{\cdot}$ or $O_2^{\cdot -}$ alone [98–100]. $ONOO^{-}$ has been reported to be highly cytotoxic to rat and human islet cells, when added *in vitro* [101]. Recently, $ONOO^{-}$ formation was detected in pancreatic islets *in vivo*, and this occurred in conjunction with β-cell destruction and IDDM development in NOD mice [102]. In that immunohistochemical study, one "footprint" of $ONOO^{-}$ formation was detected as nitrotyrosine (formed by $ONOO^{-}$-induced nitration of tyrosine residues on proteins) in islet-infiltrating macrophages as well as in β-cells of prediabetic NOD mice; moreover, $ONOO^{-}$ was detected in the majority of β-cells of acutely diabetic NOD mice [102]. Because $ONOO^{-}$ is a reaction product of $O_2^{\cdot -}$ and $NO^{\cdot}$, these findings implicate both oxygen and nitrogen free radicals as mediators of islet β-cell destruction in autoimmune diabetes (Fig. 3).

THERAPEUTIC PERSPECTIVES

Present and future approaches to prevention of human IDDM are based on recent findings regarding the roles of cytokines in the autoimmune pathogenesis of β-cell destruction. Thus, current evidence suggests that IDDM results from an immunoregulatory imbalance in which Th1 cells and their cytokine products, IFNγ, IL-2, and TNFβ (Type 1 cytokines), dominate over immunoregulatory (suppressor) Th2 cells and their cytokine products, IL-4 and IL-10 (Type 2 cytokines). Therefore, the current notion is that therapies aimed at IDDM prevention should be directed at increasing Th2 cell function and Type 2 cytokine production, and/or decreasing Th1 cell function and Type 1 cytokine production.

A variety of microbial products and immune adjuvants have been discovered to prevent IDDM in genetically diabetes-prone animals (NOD mice and BB rats) [2], and some of these agents have been reported to preferentially stimulate Type 2 over Type 1 cytokine responses [3]. Immunostimulatory agents that prevent IDDM in animal models, however, have a broad spectrum of immune stimulation affecting macrophages and T cells [e.g. the immune adjuvant bacillus Calmette-Guerin (BCG)], or these agents are polyclonal T cell activators (e.g. microbial superantigens and lectins) and, therefore, may not be optimal for clinical trials of IDDM prevention because of possible undesirable side-effects from generalized immunostimulation.

Recent findings, however, demonstrate that more selective immunostimulation may be at hand. Thus, administration of the peptide glutamic acid decarboxylase (GAD65), an islet β-cell autoantigen, can prevent autoimmune diabetes development in NOD mice, and this prevention is associated with the induction of specific tolerance to this peptide [103–105]. Moreover, GAD-responsive T cells from diabetes-prone NOD mice were characterized as Th1, IFNγ-producing [104], whereas IFNγ production in antigen-stimulated spleen cell cultures from GAD65-tolerant (and diabetes-protected) NOD mice was reduced significantly, indicating that tolerance may result from suppression of GAD65-responsive Th1 cells [105]. Because this effect was not accompanied by a corresponding reduction of the humoral (antibody) response to GAD and other IDDM autoantigens, a GAD65 induction of Th2 cells with suppression of Th1 cells was suggested [105]. These findings are directly relevant to the observation that in humans there is an inverse relation between humoral (Th2 cell-mediated) and cellular (Th1 cell-mediated) autoimmunity to GAD in subjects at risk for IDDM [106]; also, a strong humoral response to GAD correlates with a slow progression to IDDM [106, 107].

Administration of β-cell candidate autoantigens other than GAD may also induce self-tolerance and prevent IDDM. For example, insulin (and insulin B-chain) can prevent diabetes development in NOD mice and BB rats, and possibly in human subjects at high risk for IDDM [108]. The protective effects of insulin in NOD mice have been attributed to regulatory cells that inhibited intraislet expression of IFNγ-producing T cells [14]. In another study, diabetes-inducing, IFNγ-secreting $CD4^+$ T cell lines and clones from NOD mice were reported to recognize a 38-kDa protein in a rat insulinoma membrane fraction [49], possibly the 38-kDa protein islet antigen against which T cell reactivity has been described in human subjects with IDDM [109, 110]. Collectively, these studies suggest that the cytokine production profile of peripheral blood T cells specifically reactive to islet autoantigens (GAD, insulin, 38-kDa protein) may serve as predictors of IDDM development. Also, these studies suggest that administration of islet autoantigen(s) in subjects at risk of IDDM development may prevent disease onset by inducing Th2-type responses and/or inhibiting Th1-type responses. A large randomized prospective clinical trial is presently underway in the United States to test the effectiveness of insulin (parenteral and oral, in separate groups) in preventing IDDM in subjects at risk [111].

Another approach to shifting the balance of cytokines in favor of Th2 over Th1 cells and cytokines may be by manipulating expressions of costimulatory molecules (e.g. B7) on APC, thereby influencing the subsets of T cells that are elicited in response to islet autoantigens. For example, treatment of autoimmune diabetes-prone NOD mice at an early age with CTLA4 immunoglobulin (binds to the B7 molecule on APCs and prevents the binding of B7 to its natural ligand, CD28, on T cells) has been reported to protect the NOD mice from developing IDDM [112]. Thus, B7/CD28 interactions between APCs and T cells influence Th1/Th2 cell differentiation and the development of autoimmune disease.

Cytokine-based therapies for IDDM prevention may take one or more of several forms. Type 1 cytokines (in particular IFNγ), and the macrophage-derived proinflammatory cytokines, IL-1 and TNFα, may be blocked by administering (1) antibodies to these cytokines or to the corresponding cytokine receptors, (2) soluble cytokine receptors, and (3) receptor antagonists. Alternatively, Type 2 cytokines (e.g. IL-4 and IL-10) may be administered. A limitation of these approaches for IDDM prevention, however, is the need for parenteral therapy (e.g. subcutaneous injections) because presently available cytokine and anti-cytokine preparations are not active after enteric administration. Another problem is the short duration of action of the cytokine/anti-cytokine preparations, necessitating frequently repeated parenteral administrations. Therefore, cytokine/anti-cytokine preparations with longer durations of action would be highly desirable. Recently, a long-lasting biologically active form of IL-10 (an IL-10/Fc fusion protein) has been produced, and this form of IL-10 prevented diabetes development in NOD mice, suppressed Th1-type cytokines, and up-regulated Th2-type cytokines; furthermore, diabetes prevention outlasted the administration of IL-10/Fc therapy [113].

Another approach to protecting islet β-cells from dam-

age by proinflammatory cytokines (IFNγ, IL-1, TNFα/β) would be to block the production and/or action of oxygen and nitrogen-based free radicals induced in macrophages, and in β-cells themselves, by these proinflammatory cytokines. Indeed, a variety of antioxidants (nicotinamide, deferoxamine, SOD, α-tocopherol, probucol, and lazaroid) have been reported to provide some protection against IDDM development in autoimmune diabetes-prone NOD mice and BB rats [72–79]. Based on these findings, a large multinational randomized placebo-controlled prospective trial has been launched to test the effectiveness of nicotinamide to prevent IDDM in human subjects at risk [114]. Also, based on the evidence that $NO^{\cdot}$ is involved in autoimmune β-cell destruction in animal models [89–92], IDDM prevention should be directed at selective inhibition of the cytokine-inducible isoform of nitric oxide synthase, iNOS. Development of pharmacological inhibitors of NOS with isoform selectivity appears to be a realistic goal. For example, certain non-amino acid-based small molecules, such as aminoguanidine, mercaptoalkylguanidines, and *S*-alkylisothioureas are highly selective iNOS inhibitors and have potent anti-inflammatory and anti-shock properties [115]. In preliminary studies, we have found that oral administration of guanidinoethyldisulphide, a mercaptoalkylguanidine compound [116], significantly delayed diabetes onset in NOD mice, and this was associated with decreased formation of $ONOO^{-}$, a reaction product of $O_2^{\cdot -}$ and $NO^{\cdot}$, in islet β-cells (unpublished data).

In summary, recent evidence suggests that destruction of pancreatic islet β-cells and consequent IDDM is the result of disordered immune regulation. The current concept is that islet β-cell autoreactive T cells that mediate β-cell destruction are composed of a Th1 subset of T cells that produce IFNγ, IL-2, and TNFβ (Type 1 cytokines), and these cytokines activate macrophages and cytotoxic T cells to destroy β-cells by mechanisms that include production of free radicals. Furthermore, it is believed that autoreactive Th1 cells and their cytokine products (IFNγ, IL-2, TNFβ) are pathogenic to islet β-cells because they are not sufficiently regulated (suppressed) by a Th2 subset of T cells that produce IL-4 and IL-10 (Type 2 cytokines). Based on this concept, approaches to preventing pancreatic islet β-cell destruction and IDDM should be aimed at stimulating the production and/or action of Type 2 cytokines, inhibiting the production and/or action of Type 1 cytokines, and inhibiting the production and/or action of oxygen and nitrogen free radicals.

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