The autoimmune response that leads to destruction of pancreatic islet β-cells and insulin-dependent diabetes mellitus (IDDM) has a genetic basis; however, environmental factors may exert profound modulating effects on the genetic predisposition to this autoimmune response. Recent studies in animal models for human IDDM, the genetically diabetes-prone NOD mouse and BB rat, have revealed that microbial agents—including certain viruses and bacteria, fungi, and mycobacteria—often have a protective action against diabetes development. Many of these microbial preparations are immune adjuvants, which are agents that stimulate the immune system. The protective effects of these agents against diabetes appear to involve perturbations in the production of cytokines, which are polypeptides produced by and acting on cells of the immune system. Thus, recent studies in NOD mice suggest that the islet β-cell-directed autoimmune response may be mediated by a T-helper 1 (Th1) subset of T-cells producing the cytokines interleukin-2 (IL-2) and interferon-γ. These studies also suggest that the diabetes-protective effects of administering microbial agents, adjuvants, and a β-cell autoantigen (GAD65 [glutamic acid decarboxylase]) may result from activation of a Th2 subset of T-cells that produce the cytokines IL-4 and IL-10 and consequently downregulate the Th1-cell-mediated autoimmune response. The clinical implication of these findings is that the autoimmune response leading to islet β-cell destruction and IDDM may be amenable to prevention or suppression by therapeutic interventions aimed at stimulating the host's own immunoregulatory mechanisms. *Diabetes* 43:613-621, 1994

The roles of cytokines in the pathogenesis of insulin-dependent diabetes mellitus (IDDM) have been the subject of recent reviews (1-4), which have focused on the cytotoxic effects of cytokines on islet β-cells and mechanisms that may mediate the inhibitory and destructive actions of cytokines on β-cells. However, contrary evidence is accumulating for diabetes-protective effects of cytokines in genetically diabetes-prone NOD mice and BB rats, animal models for human IDDM. Also, despite the well-recognized diabetes-protective effects of immunosuppression, a variety of immunostimulatory agents have been discovered that prevent diabetes development in these animal models for IDDM. In this perspective article, I propose that these apparently conflicting observations regarding the diabetes-promoting versus diabetes-protective actions of cytokines, as well as the diabetes-protective effects of immunostimulation, can be accommodated and reconciled in the paradigm of autoimmune disease as a disorder of immunoregulation.

**Environmental Influences on IDDM Development**
Environmental triggers of IDDM: the traditional view. Both genetic and environmental factors are involved in the pathogenesis of IDDM. The traditional concept is that environmental factors such as microbial agents and chemicals act as triggers of an autoimmune response against pancreatic islet β-cells in a genetically diabetes-prone phenotype (5,6). Indeed, certain viruses have been implicated in the induction of IDDM in humans, notably rubella (7), coxsackie (8,9), and cytomegalovirus (10,11). However, the evidence that human IDDM results from viral infection that affects islet β-cells directly or indirectly through the immune system is incomplete and inconclusive. There is some evidence for a viral etiology of IDDM in animal models with spontaneous autoimmune diabetes resembling human IDDM. Thus, a common retrovirus, Kilham's rat virus, has been identified as a viral trigger of IDDM in the BB rat (12). This viral agent appears to promote diabetes by acting on cells of the immune system rather than on islet β-cells, and the investigators propose that the virus acts on effector or regulatory T-cells to promote somehow an autoimmune response against islet β-cells (12). In the NOD mouse, retrovirus-like particles have been identified in islet β-cells (13,14), and...
differences have been reported between the expression of a retroviral gene in β-cells of diabetes-susceptible NOD mice and those of a control diabetes-resistant mouse strain (15). However, the possible relation of β-cell retroviral gene expression to the pathogenesis of β-cell damage and IDDM remains to be determined. Therefore, present evidence for the traditional view that microbial agents might trigger IDDM, either in human subjects or in animal models, is presently inconclusive.

Nevertheless, recent observations in autoimmune diabetes-prone animal models have reinforced the concept that environmental factors can have a profound influence on the expression of IDDM. Perhaps unexpected, however, is the recent strong evidence that exposure to microbial agents can prevent the development of IDDM.

**Microbial agents can prevent IDDM: recent observations.** Whereas evidence for induction of IDDM by environmental agents is inconclusive, recent observations have revealed that the environmental influence of microbial agents on IDDM can be protective against disease development (16). Thus, diabetes-prone NOD mice (16) and BB rats (17,18) raised in strictly pathogen-free environments manifest increased diabetes incidence, and viral infections in these animal colonies are associated with decreased diabetes incidence. These findings suggest that deliberate viral infection of diabetes-prone animals might actually protect against IDDM development.

This protection was demonstrated by experimental infection with the lymphocytic choriomeningitis (LCM) virus, which prevented diabetes in NOD mice (19) and BB rats (20). Although LCM virus is lymphotropic, the protection in NOD mice was not a result of production of an immunodefi cient state, because lymphocytes recovered from LCM virus-infected mice failed to release infectious virus. Rather, LCM virus-infected and diabetes-protected NOD mice adoptively transferred protection from diabetes into young NOD mice (19). These findings suggest that viral infection somehow perturbed the immune system so that immunoregulatory or suppressor cells were activated to circumvent or suppress the autoimmune response against islet β-cells (16). In addition to LCM virus infection (19), mouse hepatitis virus (21), encephalomyocarditis virus (22), and lactate dehydrogenase virus (23) infections have been reported to prevent diabetes development in NOD mice.

Not only viruses, but also bacterial and fungal extracts have been discovered to exert protective effects against diabetes development in NOD mice and BB rats. These diabetes-protective microbial agents include a streptococcal extract, OK432 (24,25); staphylococcal enterotoxins (26); a fungal polypeptide, LZ-8 (27); and certain mycobacterial preparations, such as a 65-kDa heat shock protein (hsp 65) of *Mycobacterium tuberculosis* (28), a complete Freund's adjuvant (CFA) comprised of killed *Mycobacterium tuberculosis* in an oil emulsion (29-35), and the bacillus Calmette-Guérin vaccine (BCG) of live *Mycobacterium bovis* (35-39). A common feature of these microbial preparations is their stimulatory action on the immune system. This characteristic has qualified many of these microbial preparations as immune adjuvants, which are used to boost immune responses to antigenic challenges. Furthermore, pan T-cell-stimulatory lectins, such as concanavalin A and phytohemagglutinin have long been known to suppress rejection of tissue grafts and autoimmunity (40-42); and concanavalin A has been reported to prevent diabetes in NOD mice (43), an action we have confirmed in diabetes-prone BB rats (A.R. W.L. Suarez-Pinzon, unpublished observations). It appears paradoxical that stimulation of the immune system should protect against diabetes considering the well-documented protective effects of immunosuppressive therapies in this autoimmune disease (44,45). The answer to this paradox lies in appreciating that the immune system operates in a network that involves finely regulated balances between different types of immune responses. Therefore, activation of one or another of the components of the immune system does not always lead to an increased immune response; rather, the opposite may result.

The strong and reproducible action of CFA to prevent diabetes development in autoimmune diabetes-prone NOD mice and BB rats has provided a very useful paradigm to study the setting of immune responses in genetically diabetes-prone animals and how immunostimulatory procedures may prevent the autoimmune response.

**Immune responses: roles of cytokines**

**The immune response to an antigen.** The initial event in an immune response is the uptake and processing of antigen by macrophages, dendritic cells, or B-cells, which are termed collectively as antigen-presenting cells (APCs) because they present processed antigens to T-cells in association with major histocompatibility complex (MHC) class I or class II molecules at the surface of the APC. T-cells with specific receptors that recognize the antigen, T-cell antigen receptors, bind to the antigen-MHC complex. T-cells that respond to antigens complexed with MHC class I molecules are of the CD8+ phenotype (the CD8 molecule on the T-cell binds to the MHC class I molecule on the APC), and T-cells that respond to antigens complexed with MHC class II molecules are of the CD4+ phenotype (the CD4 molecule on the T-cell binds to the MHC class II molecule on the APC). T-cells also bind, by other ligands, to accessory (or adhesion) molecules on APCs. T-cell binding to the antigen-MHC complex and to accessory molecules on APCs leads to activation of the T-cells. One property of activated T-cells is cytokine production.

**Characteristics of cytokines.** Cytokines are peptide molecules synthesized and secreted by activated lymphocytes (lymphokines), macrophages/monocytes (monokines), and cells outside the immune system (e.g., endothelial cells, bone marrow stromal cells, and fibroblasts). Cytokines are used mainly by immune system cells to communicate with each other and to control local and systemic events of immune and inflammatory responses. More than 30 immunologically active cytokines exist and are generally grouped as interleukins (ILs), interferons (IFNs), tumor necrosis factors (TNFs), and colony-stimulation factors (CSFs) (46). Both the production of cytokines by cells and the actions of cytokines on cells are complex. Thus, one cell may produce several different cytokines; a given cytokine may be produced by one or several different cell types; and a cytokine may act on one or more cell types. Also, cytokine actions are usually local, occurring between two cells that are conjugated to one another, on neighboring cells (paracrine), and on the cell that secretes the cytokine (autocrine). In some cases (notably, the macrophage-derived inflammatory cytokines IL-1, IL-6, and TNF-α), cytokines exert actions on distant organs.
TABLE 1
Cytokines produced by Th1- and Th2-cell subsets of T-cells

<table>
<thead>
<tr>
<th>Cytokine produced</th>
<th>Th1-cell subset</th>
<th>Th2-cell subset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse</td>
<td>Human</td>
</tr>
<tr>
<td>IL-2</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>TNF-β</td>
<td>++</td>
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<tr>
<td>TNF-α</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>IL-3</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>IL-4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>IL-5</td>
<td>--</td>
<td>+</td>
</tr>
<tr>
<td>IL-10</td>
<td>--</td>
<td>+</td>
</tr>
</tbody>
</table>

Values for mouse Th1- and Th2-cell subsets are proportions of mouse CD4+ T-cell clones producing a given cytokine (50). Values for human Th1- and Th2-cell subsets are proportions of human T-cell clones producing a given cytokine (47). ++, Large proportion; +, small proportion; -, none.

(endocrine). Cytokine synthesis is regulated by the differentiation of cells into the various cytokine-secreting phenotypes and by the selective activation of different cell types to produce some or all of their characteristic set of cytokines.

T-cell subsets, cytokine profiles, and immune response regulation. Antigen-activated T-cells are termed T-helper (Th) cells because they help to mediate both cellular and humoral (antibody) immune responses. Th-cells are generally identified as CD4+; however, CD8+ Th-cells also exist (47). At least two distinct Th-cell types, Th1 and Th2, have been described, both in mice (48–50) and in humans (47). Th1- and Th2-cells are distinguished by their distinct cytokine secretion patterns (Table 1). Th1-cells produce IL-2, IFN-γ, and TNF-β (lymphotoxin), whereas Th2-cells produce IL-4, IL-5, and IL-10. Other cytokines are produced by both Th1- and Th2-cell populations. Also, 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 (47–50). Th1-cells and their cytokine products (IL-2, IFN-γ, and TNF-β) are the mediators in cell-mediated immunity, formerly termed delayed-type hypersensitivity. Th1-cell-derived IFN-γ and TNF-β 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 IFN-γ activate cytotoxic T-cells to kill target cells expressing the appropriate MHC-associated antigen and activate natural killer cells to kill 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, i.e., immunoglobulin (antibody) production, especially immunoglobulin E, by B-cells. Furthermore, responses of Th1- and Th2-cells are mutually inhibitory. Thus, the Th1 cytokine IFN-γ inhibits the production of Th2 cytokines; these (IL-4 and IL-10), in turn, inhibit Th1 cytokine production.

Among signals that may orient the immune response in the direction of either a Th1- or a Th2-cell response, the macrophage-derived cytokines, IL-10 and IL-12, have been discovered to play important roles (51–53). IL-12 is a potent stimulant of Th1-cells and cytokines, notably IFN-γ. Thus, IL-12 can initiate cell-mediated immunity (54). In contrast, IL-10 (derived from macrophages or Th2-cells) exerts anti-inflammatory effects by inhibiting production of IL-12 and other pro-inflammatory macrophage cytokines (IL-1, IL-6, IL-8, TNF-α) by increasing macrophage production of IL-1 receptor antagonist and inhibiting the generation of oxygen and nitrogen free radicals by macrophages. In addition, IL-10 may favor Th2- over Th1-cell differentiation and function by inhibiting expression of MHC class II molecules and the B7 accessory molecule on macrophages, a major co-stimulator of T-cells (55). The combination of IL-4 and IL-10 is particularly effective in inhibiting Th1 effector function (cell-mediated immunity) in vivo (56).

It is evident, then, that activation of either Th1- or Th2-cells will result in either cellular or humoral immune responses, respectively. Protective responses to pathogens are dependent on activation of the appropriate Th subset accompanied by its characteristic set of immune effector functions. For example, human Th1-cells develop in response to intracellular bacteria and viruses, whereas Th2-cells develop in response to allergens and helminth components (47–50). Th1- and Th2-cells play different roles not only in protection against exogenous offending agents but also in immunopathology. Th1-cells are involved in contact dermatitis, organ-specific autoimmunity, and allograft rejection, whereas Th2-cells are responsible for initiation of the allergic cascade (47–50).

Evidence is accumulating that the islet β-cell-directed autoimmune response in IDDM may be a Th1 response, and that prevention of IDDM by immunostimulatory procedures may result from activation of an opposing Th2 response.

Cytokines implicated in IDDM pathogenesis

Studies over the last decade have examined through several different approaches the possible involvement of cytokines...
in the autoimmune pathogenesis of IDDM and in islet β-cell damage (Table 2). Studies in vitro have demonstrated that certain cytokines (IL-1, TNF-α, TNF-β, IFN-γ) can be directly cytotoxic to islet β-cells, inhibiting insulin secretion, and that, usually in combination, these cytokines can injure and destroy β-cells (1–4). Because these (and other) cytokines have been found to be expressed in the pancreatic islets of human subjects with recent-onset IDDM, cytokines may qualify as mediators of β-cell damage in IDDM. Also, IFN-γ has been detected in lymphocytes infiltrating islets of human subjects with recent-onset IDDM. Also, IFN-γ has been detected in lymphocytes infiltrating islets of human subjects with recent-onset IDDM (69). Further evidence for IFN-γ being a β-cell cytotoxic cytokine in IDDM comes from the findings that transgenic expression of IFN-γ by β-cells in normal mice leads to an autoimmune, lymphocyte-dependent infiltration of the islets by mononuclear cells (insulitis), β-cell destruction, and IDDM (64,65). In addition, monoclonal antibodies to IFN-γ protect against diabetes development in NOD mice (57,66) and BB rats (67). Interestingly, IFN-α has been detected in β-cells of human subjects with recent-onset IDDM (68). Also, β-cell transgenic expression of IFN-α elicits an immune-mediated destruction of islet β-cells, and anti-IFN-α antibody prevents this β-cell damage and IDDM (69). However, IFN-α is a product of many cells that are virally infected or otherwise stressed, and this cytokine may recruit immune system cells (and their cytokines, e.g., IFN-γ) to damage the IFN-α-producing islet β-cells (69).

Studies involving the administration of cytokines to diabetes-prone NOD mice and BB rats in vivo have revealed that several cytokines can prevent diabetes development, including IL-2 (70,71), IL-4 (72), and IL-10 (73). Even some of the cytokines that are cytotoxic to β-cells in vitro (IL-1, TNF-α, and TNF-β) can prevent diabetes development in NOD mice and BB rats (74–79). Because deficiencies in the endogenous production of IL-1 (80), IL-2 (72,80), IL-4 (72), TNF-α (75,76,81), and TNF-β (79) have been reported in diabetes-prone NOD mice and/or BB rats, the diabetes-protective effects of chronic administration of these cytokines may represent corrections of immunoregulatory deficits in the diabetes-prone animals. However, systemic cytokine administration also may act indirectly on 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 (82).

Taken together, these studies (Table 2) indicate that the roles of certain cytokines in IDDM pathogenesis are uncertain; e.g., IL-1 and TNF may be cytotoxic to islet β-cells in the islet microenvironment (studies in vitro), but may prevent an islet β-cell-directed autoimmune response by acting on immunological or possibly neuro-endocrine cells (studies in vivo). On the other hand, the actions of other cytokines are more consistent; e.g., IFN-α and IFN-γ appear to have only diabetes-promoting roles, and IL-2, IL-4, and IL-10 appear to be diabetes-protective.

**AUTOIMMUNE DIABETES: A TH1-CELL-MEDIATED IMMUNE PROCESS**

Abundant evidence now suggests that autoreactive T-cells are present in the normal immune system but prevented from expressing their autoreactive potential by other regulatory (suppressor) T-cells (83). There is also good evidence for endogenous regulatory T-cells that oppose the emergence of autoimmune diabetes in both NOD mice and BB rats (44). The opposing actions of autoreactive and regulatory T-cells are mediated by their respective cytokine products (47–50); and direct evidence for the operation of such a cytokine immunoregulatory balance in the avoidance of autoimmune diabetes has been provided recently (84). Diabetes was induced in a nonautoimmune rat strain by rendering the animals relatively T-cell-deficient using a protocol of adult thymectomy and sublethai γ-irradiation. Then, insulitis and diabetes were prevented in these rats by injection of a particular CD4+ T-cell subset that is isolated from healthy syngeneic donors and produces IL-4 and IL-2 but not IFN-γ (84). These findings are in accord with reports that IL-2 (72,80) and IL-4 (72) production are decreased in NOD mice and that administration of IL-2 (70,71) and IL-4 (72) can prevent insulitis and diabetes development. In addition, IL-10 administration recently has been found to decrease significantly insulitis severity and diabetes incidence in NOD mice (73). IL-4 and IL-10 are cytokine products of Th2-cells and inhibit cell-mediated immune responses, partly by downregulation of IFN-γ production by Th1-cells (51–52). Given the importance of IFN-γ as a mediator of β-cell destruction in vitro and of insulitis and diabetes in vivo (Table 2), it follows that IFN-γ-producing Th1-cells may contribute to the immune insulitis process that mediates islet β-cell destruction. Furthermore, the protective effects against insulitis and diabetes of IL-4 (72), IL-4-producing CD4+ T-cells (84), and IL-10 (73) suggest that Th2-cells producing IL-4 and IL-10 may be the T-cell subset responsible for preventing the autoimmune response, possibly by suppressing the IFN-γ-producing Th1 subset. Therefore, the concept arises that the autoimmune response in IDDM involves some disturbance(s) in immunoregulatory circuits that leads to a dominance of Th1 over Th2 T-cell subset function and cytokine production.

This concept that an immunoregulatory defect is associated with the autoimmune response to islet β-cells in IDDM posits that certain β-cell antigen(s) are processed by macrophages or other APCs and presented together with MHC class II molecules on the surface of the APC, resulting in the delivery of an immunogenic signal(s) that involves activation of CD4+ Th1-cells and suppression of CD4+ Th2-cells and their respective cytokines (Fig. 1). Although the molecular nature of the putative immunogenic signals involved in the autoimmune response of IDDM is unknown, these signals likely relate to mechanisms involved in recognition of MHC-antigen complex by T-cells. Thus, the immunogenicity of a β-cell protein may depend on the peptide fragment derived from processing by the APC (85), the amino acid sequences of the MHC class II molecules that bind and present the β-cell peptide (antigen), and the precursor frequency of autoreactive T-cells with T-cell receptors to match the β-cell antigen-MHC complex (86). In addition to the MHC-antigen complex interaction with T-cell receptors, co-stimulation of T-cells by interaction with APC accessory (or adhesion) molecules is necessary for full T-cell activation (87). For example, transfer of diabetes in NOD mice is prevented by blockade of an adhesion-promoting receptor on macrophages (88). Also, pancreatic islet grafts survived xenogeneic transplantation (from rats to streptozocin-induced diabetic mice) when the mice were treated with an immunoligand that binds the B7 adhesion molecule on APCs (89). Finally,
The autoimmune response in IDDM

FIG. 1. A scheme illustrating the immune system cells that may be involved in the autoimmune response leading to destruction of pancreatic islet β-cells. The concept illustrated posits that certain β-cell (β-Ag) act as autoantigens (β-Ag) after being processed by macrophages (MΦ) or other antigen-presenting cells and presented in a complex with MHC class II molecules on the surface of the MΦ. The β-Ag-MHC II complex, accessory molecules on the MΦ (e.g., the B7 molecule), and perhaps other signals together may comprise immunogenic signals that activate T-cells, predominantly of the Th1 subset. Also, MΦ-derived IL-12 activates Th1-cells. The antigen-activated Th1-cells produce IL-2 and IFN-γ, which inhibit Th2-cell production of IL-4 and IL-10. Also, IL-2 and IFN-γ activate MΦ and cytotoxic T-cells to kill islet β-cells by a variety of mechanisms, including oxygen free radicals (O₂⁻, and H₂O₂), NO, cytokines (IL-1, TNF-α, TNF-β, IFN-γ), and cytotoxic T-cells that interact with β-cell autoantigen-MHC class I complex on the β-cell.

The results of CD4⁺ Th1-cell activation are induction of IL-2 and IFN-γ production; inhibition of Th2 cytokine production; and activation of macrophages, cytotoxic T-cells, and natural killer cells. These activated effector cells may be cytotoxic to islet β-cells through a variety of mechanisms (Fig. 1), including oxygen free radicals (O₂⁻ and H₂O₂), nitric oxide (NO), cytokines (IL-1, TNF-α, TNF-β, IFN-γ), and cytotoxic T-cells that interact with β-cell autoantigen-MHC class I complex on the β-cells, and natural killer cells that may damage β-cells without MHC restriction. The predominance of one or another of these potential immune/inflammatory mediators of islet β-cell destruction is the subject of continuing studies in animal models (1-4, 92-101) and in human subjects with IDDM (102-105).

In addition to macrophages and T-cells, other cellular elements in and around the islet (not shown in Fig. 1) are likely participants in the insulitis lesion. For example, vascular endothelial cells may contribute cytokines (IL-1 and IL-6) and may respond to inflammatory cytokines (IL-1, TNF-α, and IFN-γ) by expressing adhesion molecules to circulating leukocytes (106). This response would permit migration of macrophages and lymphocytes from the circulation into the islet. Also, endothelial cells may respond to inflammatory cytokines by expressing MHC class II molecules (107), which could allow endothelial cells to act as APCs and possibly present β-cell autoantigen(s) to T-cells. Thus, intra- and peri-islet vascular endothelial cells could participate actively in amplifying the β-cell-directed autoimmune process (108).

IMMUNOSTIMULATORY PROCEDURES PREVENT IDDM: CORRECTION OF A CYTOKINE BALANCE?

The concept has been presented above that the autoimmune response in IDDM involves some disturbance(s) in immunoregulatory circuits that is manifested as dominance of Th1 over Th2 T-cell subset function and cytokine production (Fig. 1). A corollary of this proposition is that measures leading to reversal of this Th subset balance, with Th2-cells cytokines dominating over Th1-cells cytokines, should block the autoimmune response and prevent IDDM. Evidence is building to support this possibility. Thus, a variety of immunostimulatory procedures that include certain microbial agents and extracts, immune adjuvants, and T-cell mitogens (19-39, 43) recently have been discovered to prevent the development of insulitis, β-cell destruction, and IDDM in genetically diabetes-prone animals (see Microbial agents.) These procedures may provide tolerogenic signals that substitute for the immunogenic signals operant in autoimmune IDDM and thereby reset the Th subset balance so that Th2-cells cytokines now dominate over Th1-cells cytokines (Fig. 2).

Autoimmunity is generally viewed as a failure of the immune system to develop tolerance or nonreactivity to self molecules (potential antigens). Tolerance of the immune...
system to self may be established by different mechanisms, including clonal deletion (elimination) of autoreactive T-cells, clonal anergy (paralysis) of autoreactive T-cells, and suppression of autoreactive T-cells by other cells or products of the immune system, e.g., by nonspecific suppressor cells, antigen-specific T regulatory (suppressor) cells, and immunoregulatory cytokines. One or more of these mechanisms of T-cell tolerance (except clonal deletion) may be involved in the actions of microbial agents and adjuvants that lead to the prevention of autoimmune diabetes. Certainly, these immunostimulatory procedures prevent diabetes development in genetically diabetes-prone NOD mice and BB rats (19-39,43) without structural changes or complete remodelling of the immune system—unlike procedures that involve bone marrow, thymic, or lymphoid cell replacement or deletion, e.g., anti-lymphocyte serum, cyclosporine, monoclonal antibodies to T-cells, silica, and anti-macrophage antibodies (44).

Evidence has been presented for CFA-induced protection against diabetes development in NOD mice and BB rats in association with increases or induction of antigen-nonspecific or natural suppressor (NS) cells (29,31,34). NS cells are generally considered to be large granular lymphocytes belonging to the T-cell lineage but lacking mature T-cell markers; i.e., NS cells are CD4-CD8- (109). How NS cells exert their suppression of T- and B-cell functions is not known. However, the suppression may be effected by one or more factors and cytokines produced by NS cells (110,111). Macrophages with nonspecific suppressor activity have also been reported to be induced by the immune adjuvant BCG in association with protection from diabetes in NOD mice (37,38).

Most studies, however, have identified T regulatory cells and cytokines as mediators of the diabetes-protective effects of immune adjuvants (33,35). T-cells induced after CFA treatment of NOD mice can prevent both the inductive and effector phases of the autoimmune response that leads to islet β-cell destruction and IDDM (33,35). Thus, lymph node or splenic cells from CFA-treated NOD mice transferred protection from diabetes in young NOD mice; also, adoptive transfer of spleen cells from CFA-treated NOD mice, together with spleen cells from acutely diabetic NOD mice, delayed disease induction in irradiated recipient NOD mice (35). Depletion of the Thy1.2+ (total T) cells or the CD4+ (Th) cells from the CFA-treated NOD donor splenic cells abrogated the protective effects of these cells, indicating that the CFA-induced protective cells were CD4+ Th cells (35). In addition, CFA-treated old NOD mice were resistant to passive transfer of disease by spleen cells from acutely diabetic NOD mice; however, diabetes could be induced in the CFA-protected mice by cyclophosphamide treatment, which suggests that T regulatory cells (presumably depleted by cyclophosphamide) accounted for the protective effects of CFA against the autoimmune response. Similarly, protection against diabetes in NOD mice infected with staphylococcal enterotoxins, called superantigens because they stimulate a large fraction of T-cells, was attributed to activation of CD4+ T suppressor cells (26).

The ability of CFA to inhibit the effector phase of diabetes in the studies above (35) confirmed an earlier report that treatment of already diabetic NOD mice with CFA at the time of syngeneic islet transplantation prevented islet β-cell destruction and disease recurrence (32). In these experiments, mononuclear and lymphocytic cells still accumulated around the transplanted islets (peri-insulitis) in the CFA-treated NOD mice, but these cells did not invade the islet, and insulin-containing β-cells remained intact even 200 days after islet transplantation (32). These findings suggest that autoreactive T-cells exist in the CFA-treated NOD mice but cannot function as effectors of β-cell destruction. Similar results were reported in another study where autoreactive T-cells were considered to be dormant in the CFA-treated NOD mice (33).

Taken together, these studies suggest that the mycobacterial immune adjuvants CFA and BCG (and possibly other microbial agents and T-cell mitogens) may deliver tolerogenic signals, i.e., activate regulatory (suppressor) T-cells that would render islet β-cell autoreactive T-cells nonresponsible. Furthermore, the regulatory T-cells are of the CD4+ Th1 type and may belong to the Th2 subset that produces IL-4 and IL-10, as suggested by the following studies.

Protection against β-cell destructive insulitis and diabetes in NOD mice, provided by injecting the mice with CFA, was reported to be associated with a relative increase in IL-4-producing T-cells and a decrease in IFN-γ-producing T-cells recovered from sentinel syngeneic islet grafts placed under the renal capsule in NOD mice (112). In addition, by using a polymerase chain reaction assay to measure cytokine mRNA expression in tissues, we have found that IL-10 mRNA expression is significantly increased and expression of IL-2 and IFN-γ mRNAs is significantly decreased in syngeneic islet grafts of CFA-injected NOD mice compared with saline-injected NOD mice (112a). Therefore, we concluded that the β-cell destructive infiltrate in syngeneic islet grafts transplanted into diabetic NOD mice contained IL-2 and IFN-γ-producing Th1-cells and that CFA treatment of the diabetic NOD mice at the time of islet transplantation induced IL-10-producing cells that downregulated the Th1-cells, converting a β-cell destructive infiltrate into a nondestructive one and thereby preventing islet graft rejection and diabetes recurrence. This interpretation is supported by the finding that administration of IL-10 significantly prolonged survival of syngeneic islet grafts in diabetic NOD mice (112a). Also, another study reports that IL-10 administration can significantly decrease insulitis severity and the incidence of spontaneous diabetes in NOD mice (73). These effects of IL-10 are in accord with the known actions of this cytokine to downregulate inflammatory responses mediated by monocytes/macrophages and their cytokine products, as well as to downregulate cell-mediated immune responses triggered by Th1-cells and their cytokine products (51–55). Also, our finding of increased IL-10 mRNA expression in nondestructive islet infiltrates (CFA-protected syngeneic islet grafts in NOD mice, 112a) is in accord with a report that IL-10 mRNA expression in the central nervous system of mice with experimental autoimmune encephalitis (a model for multiple sclerosis) correlates with recovery from disease (113). Interestingly, transgenic expression of IL-10 by islet β-cells in mice leads to pronounced vascular endothelial cell changes and leukocyte extravasation into the pancreas without infiltration of cells into the islets, β-cell destruction, or diabetes (114).

Taken together, these studies suggest immunostimulatory procedures, such as certain microbial agents and immune adjuvants, may stimulate the production of regulatory cytokines, such as IL-4 (by Th2 and/or mast cells) and IL-10 (by Th2 and/or macrophages). These cytokines could
contribute to tolerogenic signals, i.e., signals for the immune system to recognize islet β-cell potential autoantigen(s) as self. Thus, tolerogenic signals would substitute for immunogenic signals that direct an autoimmune response against islet β-cells. These tolerogenic signals would favor T-cell differentiation along a Th2 pathway, downregulate Th1-cells and cytokines (IFN-γ, IL-2, TNF-β), inhibit cytotoxic macrophage and T-cell functions, and consequently preserve islet β-cells and avoid IDDM (Fig. 2).

FUTURE PROSPECTS: CLINICAL CONSIDERATIONS

The clinical hope from the observations that certain immunostimulatory procedures prevent autoimmune diabetes development in genetically diabetes-prone animals is that clinically safe means of immune stimulation may be similarly effective in preventing IDDM in human subjects at risk for this disease. Immunostimulatory agents that have a broad spectrum of immune stimulation, affecting macrophages and T-cells (such as the immune adjuvant BCG) and polyclonal T-cell activators (such as microbial superantigens and lectins) may not be optimal for clinical trials because of possible undesirable side effects from generalized immunostimulation.

However, recent findings demonstrate that more selective immunostimulation may be at hand. Thus, administration of the peptide GAD65 (glutamic acid decarboxylase), 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 (115–117). Moreover, GAD-responsive T-cells from diabetes-prone NOD mice were characterized as Th1, IFN-γ-producing (116), whereas IFN-γ 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 (117). 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 (117). 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 in patients at risk for IDDM (118); also, a strong humoral response to GAD correlates with a slow progression to IDDM (118,119).

Therefore, the paradigm of autoimmune diabetes as a Th1-cell-mediated immune response involving IL-2 and IFN-γ-induced activation of cytotoxic macrophage and T-cell killing of islet β-cells, based on recent evidence in animal models, may also apply to human IDDM. Conclusive evidence for similar involvement of Th1-cells and cytokines in the pathogenesis of human IDDM, however, remains to be obtained. Some evidence exists for other organ-specific autoimmune disorders, including Hashimoto's thyroiditis (120) and progressive multiple sclerosis (121).

In summary, nonspecific stimulation of the immune system, by administration of microbial extracts and adjuvants, as well as specific immune stimulation by administration of an islet β-cell autoantigen, GAD65, can prevent autoimmune diabetes development in genetically diabetes-prone NOD mice. Both nonspecific and specific immune stimulations appear to prevent diabetes by downregulating a Th1 subset of T-cells and their cytokine products (IFN-γ and IL-2) and by upregulating a Th2 subset of T-cells and their cytokines (IL-4 and IL-10). Although it remains to be demonstrated that human IDDM involves similar Th1-cell and cytokine-mediated autoimmune processes, these findings in NOD mice provide a rationale to consider immune modulation therapies involving immunostimulation in attempts to prevent IDDM in human subjects at risk for this disease. In addition, identification of the cytokine mediators and suppressors of the islet β-cell–directed autoimmune response in animal models with IDDM suggests the use of these peptides (or their antagonists) for therapeutic intervention in human IDDM prevention.

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