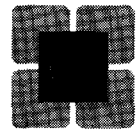

Cytokines and the pathogenesis of insulin-dependent diabetes mellitus

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■ Insulin-dependent diabetes mellitus is an autoimmune phenomenon in humans. At onset, the diabetic pancreas shows a well-characterized insulitis. The inflammatory cells are specifically directed toward beta cells of the pancreatic islets. Several hypotheses link genetic susceptibility for diabetes to immunologic mechanisms. The cytokines interferon gamma and interleukin-6 have essential roles in the progressive destruction of beta cells. Studies with experimental models may improve definition of the pathogenesis of insulin-dependent diabetes mellitus. Combining genetic studies that detect susceptibility to insulin-dependent diabetes mellitus with future therapies aimed at interrupting cytokine production or cytokine receptor expression may lead to prevention of insulin-dependent diabetes mellitus.

□ INDEX TERMS: DIABETES MELLITUS, INSULIN- DEPENDENT; CYTOKINES □ CLEVE CLIN J MED 1992; 59:629–633

INSULIN-DEPENDENT diabetes mellitus (IDDM) is the most common endocrine disorder in childhood, and 30% to 40% of diabetic children will eventually develop nephropathy requiring dialysis and transplantation. Diabetes accounts for a significant proportion of morbidity and mortality among dialysis and transplant patients.[1] Investigation focused on elucidating the pathogenesis of the disease may result in the ability to arrest early pancreatic injury and prevent the entire disease process. Understanding the role of specific cytokines in the pathogenesis of IDDM is one step toward this goal.

IMMUNOLOGIC ACTIVITIY IN DIABETES

Inflammatory pancreatic infiltrate

IDDM in humans is an autoimmune phenomenon. At onset, the diabetic pancreas shows a well-characterized insulitis. The inflammatory cells are predominantly T cells, and a lower percentage are macrophages.[2] These mononuclear cells are closely associated with degenerating beta cells but are absent when there are no beta cells within the islets. The infiltrate is specifically directed toward the beta cells: other endocrine cells within the islets—glucagon-containing alpha cells and somatostatin-producing delta cells—are undamaged.

A large proportion of the infiltrating T lymphocytes are CD8-positive cytotoxic cells (*Table*), thus suggesting cytotoxic activity of the cellular infiltrate. CD4-positive lymphocytes are also present; the majority of these are helper T cells.[3] The infiltrating cells also include lymphocytes or B cells that produce immunoglobulin-G (IgG), indicating that antibody-producing cells also infiltrate the islets.[4]

Antigens

Much investigation has been directed toward detecting the antigens against which these antibodies are formed. It has been known for more than 10 years that islet-cell antibodies are present in sera of patients with recently diagnosed IDDM. Complement-fixing

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TABLE
FACTORS RELATED TO THE PATHOGENESIS OF INSULIN-DEPENDENT DIABETES MELLITUS

Cytokines: Locally active polypeptide mediators secreted by many or all eukaryotic cells. They function in a paracrine or autocrine fashion, modulating behavior of adjacent target cells by binding to specific receptors.
Interferon gamma (IFN-γ): Secreted by antigen-activated T cells; it stimulates cytolytic function in some T cells and macrophages and also stimulates the activation of histocompatibility genes.
Interleukin-1: Protein of 17 kd relative molecular mass produced by activated macrophages, endothelial cells, and mesangial cells. This protein is a co-factor for producing full T cell activation.
Interleukin-2: 15.4 kd lymphokine encoded by a gene on the long arm of chromosome 4 and produced by activated T cells. It serves as a necessary factor for normal T cell proliferation.
Interleukin-4: 15 kd lymphokine produced by T cells; it is a growth factor for B cells and some T cells.
Interleukin-6: Pleiotropic cytokine with multiple biologic activities, including induction of B cell differentiation.
Human leukocyte antigens (HLA): The genes which encode for HLAs are located on the short arm of chromosome 6; genes which encode for class I and class II antigens are separated by class III genes that encode for some of the complement factors.
Class I: Cell-surface antigens that are encoded by three loci: HLA-A, HLA-C, HLA-B.
Class II: Cellular antigens that are encoded by the HLA-D region. They are expressed primarily on B lymphocytes, macrophages, and activated T lymphocytes. Class II antigens are encoded by class II genes: HLA-DP, HLA-DQ, and HLA-DR.
Lymphocytes
B cells: Circulating lymphocytes that manufacture and secrete Immunoglobulin-G antibodies.
Cytotoxic T cells: Circulating and tissue-bound cells that are antigen-triggered. They can lyse cells that bear the sensitizing cell surface antigens; also known as CD8-positive lymphocytes.
Helper T cells: Bear the distinctive CD4 protein; they are activated by HLA class II molecules. Cytotoxic T cell activation is restricted by HLA class I molecules whereas helper T cells are restricted by class II molecules.

islet-cell antibodies are also considered to be highly predictive of active pancreatic beta cell destruction.

Islet-cell surface antibodies appear to have greater pathologic significance than complement-fixing antibodies, since they may represent an initial attack on the plasma membrane. The nature of the antigen toward which these antibodies are directed is not known; however, Baekkeskov et al[5] have identified a human islet-cell protein of relative molecular mass 64 kd which is precipitated by antibodies found in sera from patients with newly diagnosed IDDM. Assays for these antibodies vary a great deal from laboratory to laboratory, and their results are operator-dependent.

Antibodies directed against the islet-cell membrane and intracellular antigens would presumably be important in the pathogenesis of IDDM; however, some investigators believe that the presence of such antibodies in patients with IDDM is an epiphenomenon not directly related to the pathogenesis of the disease.[1]

Patient susceptibility

Investigators at the Children's Hospital of Pittsburgh[1] have proposed that some patients are susceptible to the clinical syndrome of diabetes and that the process leading to islet-cell destruction is initiated due to some environmental phenomenon. Epidemiologic studies have shown that patients with class I human leukocyte antigens (HLA) B-8 and B-15 are more susceptible to diabetes than patients who do not have these antigens.[6] Further studies indicate that HLA class II antigens encoded by DR genes (antigens DR3 and DR4) are also predictive for patients who may develop diabetes.[7] Patients with one such DR antigen have a risk of developing IDDM that is four times that of the general population; patients with both DR antigens have 12 times the risk.

HYPOTHESES OF PATHOGENESIS

Several hypotheses link genetic susceptibility for diabetes to immunologic mechanisms (including the involvement of cytokines) that are responsible for the destruction of the beta cells.

Environmental factor hypothesis

The first hypothesis, as reviewed by Trucco and Dorman,[8] proposes that islet cells are initially damaged by an environmental factor (chemicals such as nitrosamines, or viruses such as coxsackievirus, mumps, or rubella) associated with the expression of IDDM (*Figure 1*). The damaged beta cell releases an antigen usually not encountered by immunocompetent cells. The antigen then becomes an auto-antigen: a tissue macrophage processes the antigen and presents it to helper T cells in conjunction with a HLA class II molecule. The helper T cells induce B lymphocytes to secrete antibodies directed against specific epitopes of the beta-cell antigen. The antibodies bind to the beta cell, activating a complement cascade and producing the cytotoxic effect. The cytotoxic activity is supported by cytotoxic T cells which infiltrate the damaged islets and continue to attack beta cells either directly or via an antibody-dependent mechanism.

Bottazzo's hypothesis

Bottazzo's hypothesis[9] is similar to that of Trucco and Dorman, except that it eliminates the macrophage

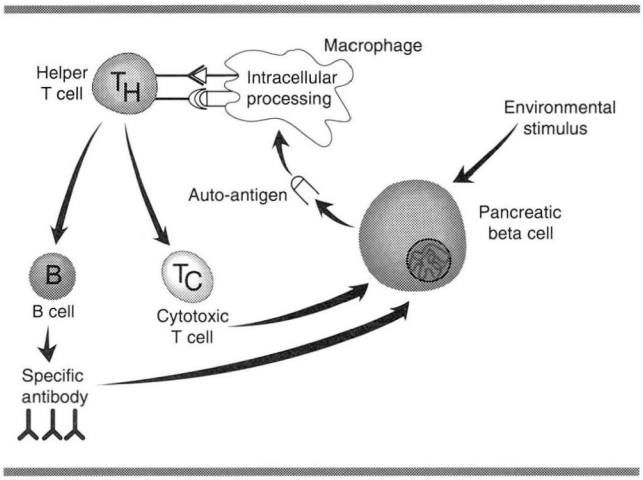

FIGURE 1. The hypothesis of Trucco and Dorman. Under the influence of an environmental stimulus, a pancreatic beta cell releases an auto-antigen. This antigen is taken up and processed by a macrophage which presents it, together with a class II DR antigen, to a helper T cell. The helper T cell becomes activated, inducing B cells to produce antibodies specific to the antigen, and also inducing cytotoxic T cells to directly attack the pancreatic beta cell.

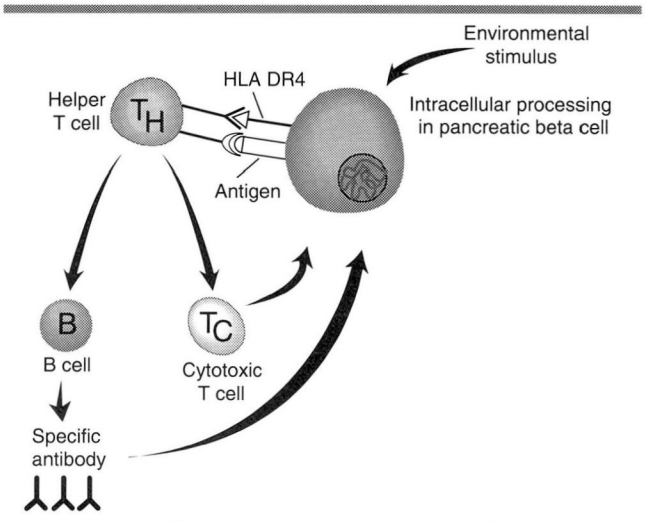

FIGURE 2. Bottazzo's hypothesis. An environmental stimulus to a pancreatic beta cell induces cell surface expression of an HLA-DR antigen and an auto-antigen. These are recognized by a helper T cell, which activates cytotoxic T cells and B cells to attack the beta cell. This hypothesis differs from that of Trucco and Dorman in that the intermediary macrophage is not required.

as an intermediary in the process. Based on laboratory data, Bottazzo has proposed that after the environmental stimulus (which he calls "factor X") the beta cell itself expresses class II molecules normally hidden to helper T cells (*Figure 2*). The process then continues much as in the Trucco and Dorman hypothesis. However, specific class II DR antigens must be present: whereas DR2 does not allow activation of the helper T cells, DR4 will. (Thus, Bottazzo's hypothesis takes into account the susceptibility of the patient to certain DR antigens.) Cytotoxic T cells, activated through signals from the helper T cell, directly attack class I molecules expressed on the surface of the beta cell.

Comprehensive hypothesis

Trucco and Dorman[8] have proposed a third hypothesis in which the HLA molecule involved in IDDM susceptibility is located on the HLA DQ molecule in close association with the DR locus on the 6th chromosome (*Figure 3*). This hypothesis once again suggests that there is a foreign protein which is processed and presented by the macrophage to the CD4-positive helper T cell. This helper T cell recognizes the antigen bound to the expressed class II molecule. The activated helper T cell can then secrete interleukin-2 (IL-2), thus stimulating proliferation of cytotoxic T cells. Activated cytotoxic T cells then express the IL-2 receptor and class II molecules at their surface. Interaction between the CD4 molecule of the helper T cell and the class II molecules expressed on the cytotoxic T cell brings the helper and cytotoxic T cells in closer contact, causing IL-2 to be effective. B lymphocytes also divide and produce specific antibodies with the help of activated helper T cells. Other cytokines produced by activated helper T cells stimulate the expression of class I and class II molecules at the beta-cell surface, thus providing targets for CD8-positive cytotoxic T cells and for CD4-positive helper T cells, respectively. The major cytokines released by this autoimmune cascade include IL-1, tissue necrosis factor, and interferon gamma (IFN-γ).

Although any of these hypotheses may be correct, questions remain: What is the antigen? Is it the same antigen in each individual? Is it a foreign protein or a hidden antigen that is uncovered?

EXPERIMENTAL CONFIRMATION

Recently, Campbell and associates[10] confirmed that the cytokines IFN-γ and IL-6 are essential for the development of IDDM in the non-obese diabetic Wehi mouse model. After administration of cyclophos-

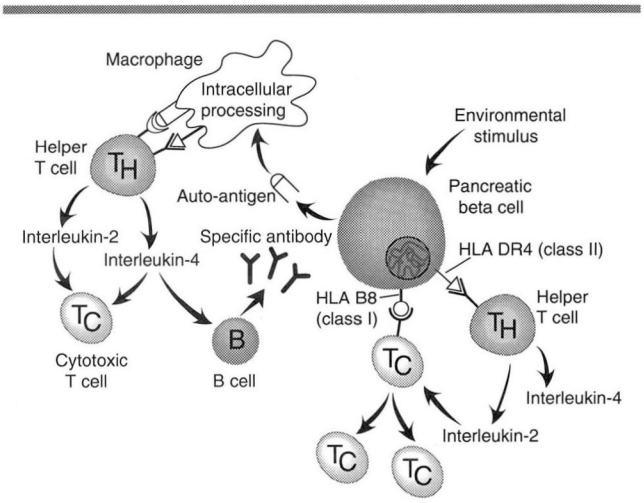

FIGURE 3. Comprehensive hypothesis. An environmental stimulus is processed by a macrophage and presented to a CD4-positive helper T cell, together with class II molecules. In addition, the beta cell expresses class I and class II HLA molecules which are recognized by cytotoxic T and helper T cells, respectively. Helper T cells are activated to secrete lymphokines interleukin-2 and interleukin-4, which induce activated CD8-positive cytotoxic T cells to proliferate and activated B-cells to produce specific antibodies.

phamide, this model develops a mononuclear infiltrate in the pancreas, followed by beta-cell destruction. The cytokine IFN-γ was initially detected in minimal amounts in supernatant of the islet-cell culture at day 7. Alternatively, IL-6 activity in such supernatant cultures progressed to extremely high levels by day 10. Mice infused with either anti-IFN-γ or anti-IL-6 antibody had significantly reduced frequency of diabetes (1 of 20 and 3 of 19, respectively, vs 9 of 19 in the control population) and a decreased severity of insulitis. Campbell et al concluded that these two cytokines have essential roles in the progressive destruction of beta cells that ultimately leads to overt IDDM. These studies provide the first in vivo demonstration of the importance of these cytokines in the pathogenesis of beta-cell destruction.

PREVENTING DIABETES

By combining genetic studies that allow detection of susceptibility to IDDM with future therapies aimed at interrupting cytokine production or cytokine receptor expression, prevention of diabetes may become a reality. Drugs that interfere with cytokine function are already available. Cyclosporine acts primarily by blocking helper T-cell production of IL-2 through inhibition of mRNA.[11] Thus, cyclosporine blocks both the activation of stimulated cytotoxic T lymphocytes and the proliferation of helper T lymphocytes, processes that depend upon the availability of IL-2. Newer immunosuppressants such as FK506 depress lymphocyte cytokine production at least 10 times more than cyclosporine. Rapamycin,[12] a macrocyclic triene antibiotic, suppresses IL-2- or IL-4-driven T-cell proliferation.

Previous studies in non-obese diabetic mice similar to those used by Campbell et al[10] have shown that cyclosporine at a dose of 25 mg/kg can prevent diabetes, whereas rapamycin prevented the disease at significantly lower doses of 6 and 12 mg/kg. Rapamycin has also been used in other autoimmune disease models. These studies emphasize the importance of cytokines in the pathogenesis of development of autoimmune diseases in general and diabetes in particular.

Clinical trials with cyclosporine have been disappointing; but with studies (such as those by Campbell et al[10]) specifically identifying cytokines of importance, future drug trials may have more positive results.

From these studies with experimental models the pathogenesis of IDDM may become better defined. Earlier detection and intervention may become possible and may prevent the disastrous morbidity and mortality associated with this lifelong, life-threatening disease.

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