Immunologic and Genetic Factors in Type 1 Diabetes*

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Under normal physiologic conditions, the entry of glucose into beta cells triggers the secretion of insulin (1) (Fig. 1). The released insulin is carried in the blood to peripheral tissues where it binds to insulin receptors, which are members of the receptor tyrosine kinase family. This initiates a cascade of transmembrane events resulting in the uptake of glucose by cells and its metabolism into energy or storage as glycogen (2). Defects anywhere along the beta cell-peripheral tissue pathway can result in hyperglycemia, but at the clinical level there are two major forms of diabetes: type 1 diabetes, previously known as juvenile or insulin-dependent diabetes, and type 2 diabetes, previously known as adult or non-insulin-dependent diabetes. Type 1 diabetes is caused by an absolute deficiency in the production of insulin as a result of destruction of pancreatic beta cells. About one million people in the United States suffer from this form of the disease. Type 2 diabetes is the more common form of the disease and afflicts about 16 million people. It is the result of a double defect: inadequate or inappropriate secretion of insulin by beta cells and resistance to the action of insulin in both peripheral tissues (e.g. muscle, adipose tissues) and beta cells. Insulin resistance occurs at the postinsulin receptor level and may be the result of a defect in any one of several genes or pathways as recently demonstrated in transgenic and knock-out mice (1, 2). The precise defect(s) in the human, however, is still not known.

Over the last few years, evidence has accumulated that type 1 diabetes is an autoimmune disease or at least has a major autoimmune component. The evidence comes from three sources: the presence of an inflammatory infiltrate (insulitis) in the islets; a strong linkage between type 1 diabetes and certain alleles of the major histocompatibility complex (MHC)1; and autoantibodies that react with islet cell autoantigens. The purpose of this article on type 1 diabetes is to review some of this evidence with emphasis on the immunological and genetic factors involved in prediction of disease and destruction of beta cells.

**HLA Linkage Found**

In humans, the MHC is known as the HLA complex and contains over 200 genes (3). It is located on chromosome 6 and encodes HLA class I and class II molecules. The main function of these molecules, which are heterodimers made up of α and β chains, is to present antigens that have been processed into peptides to antigen-specific receptors on CD4+ and CD8+ T lymphocytes. Class I molecules, expressed on most nucleated cells, are encoded by genes within the HLA-A, -B, and -C loci, whereas class II molecules, expressed primarily on antigen-presenting cells (e.g. macrophages and dendritic cells), are encoded by genes within the HLA-DR, -DP, -DQ, and -DQ loci.

HLA class I and II genes are highly polymorphic and consist of many different alleles (4). In type 1 diabetes, certain HLA class II alleles or combinations of alleles (haplotypes) show a strong association with the development of diabetes, whereas other haplotypes show a weak or even protective association. For example, individuals with the HLA haplotype DRB1*0302-DQA1*0301, especially when combined with DRB1*0201-DQA1*0501, are highly susceptible (10–20-fold increase) to type 1 diabetes. In contrast, individuals with the haplotype DRB1*0602-DQA1*0102 rarely develop type 1 diabetes. Many other high and low risk haplotypes have been identified, and the frequency of specific haplotypes differs among ethnic groups. Other genes within the HLA complex, particularly class I genes, also have been linked to type 1 diabetes, but the strongest linkage by far is with the DQ and DR class II genes.

Experimental support for the importance of class II genes in the development of diabetes comes from a variety of sources including the deletion of specific MHC loci in mice and their replacement with human HLA homologs. Although the linkage of HLA class II molecules with type 1 diabetes is now well established and the binding of peptides to pockets within the groove of the HLA class II molecule understood, why the binding of peptides to certain HLA class II molecules, and not to others, is associated with autoimmune type 1 diabetes remains unresolved. Regardless of mechanism, HLA typing has proved useful in population screening for identification and follow-up of individuals at high risk for disease.

**Autoantigens Identified**

The initial evidence for autoimmunity in patients with type 1 diabetes came from immunofluorescence studies, which showed that a high percentage of sera from newly diagnosed type 1 patients reacted with pancreatic islets. This led to an intensive search for the actual autoantigens with which the islet cell autoantibodies (ICA) reacted. Three major autoantigens now have been identified. The first is an isofrom of glutamic acid decarboxylase (GAD65) (5). GAD65 is a protein of 585 amino acids with a molecular weight of 65,000 encoded by a gene on chromosome 10p11. It is expressed in neuroendocrine islets, including pancreatic islets, and is located within neuron-like small vesicles. The function of GAD65 in the islets is not known. Between 60 and 80% of newly diagnosed type 1 diabetes patients have autoantibodies to GAD65. These antibodies are directed primarily to the middle and C-terminal portions of the molecule and recognize conformational epitopes.

The second major autoantigen, IA-2 (also known as ICA512), is a member of the transmembrane protein-tyrosine phosphatase (PTP) family (6). It is 979 amino acids in length, has a molecular weight of 106,000, and is encoded by a gene on chromosome 2q35. Because of a critical amino acid replacement at position 911 (Asp for Ala), which is required for enzymatic activity, IA-2 is catalytically inactive. IA-2 is a transmembrane...
Subjects with type 1 diabetes have autoantibodies to one or more of their autoantigens. It is now estimated that up to 90% of newly diagnosed Caucasian subjects with type 1 diabetes have autoantibodies to one or more of their autoantigens. Between 30 and 50% of subjects with type 1 diabetes have autoantibodies against insulin (11). The frequency of autoantibodies against insulin is substantially lower in individuals who develop type 1 diabetes at an older age.

Autoantibodies to IA-2, GAD65, and insulin were among the first autoantibodies to appear in the prediabetic state and are usually found in very young children. Between 30 and 50% of young children with type 1 diabetes have autoantibodies against insulin (11). The frequency of autoantibodies against insulin is substantially lower in individuals who develop type 1 diabetes at an older age. The identification of these autoantigens and the ability to prepare them in recombinant form has made it possible to develop rapid, sensitive, and reproducible radioimmunoassays, especially for IA-2 and GAD65, which have been standardized by a series of international workshops. Tests using these recombinant molecules have replaced the ICA immunofluorescence assay for most routine studies. By use of these tests, it is now estimated that up to 90% of newly diagnosed Caucasian subjects with type 1 diabetes have autoantibodies to one or more of the three major autoantigens.

Autoantibodies Predict Disease

At first, the autoantibodies to IA-2, GAD65, and insulin were used for diagnosis and classification of patients with type 1 diabetes. It soon became apparent, however, that these autoantibodies appeared many months or years before the onset of clinical disease. This made it clear that type 1 diabetes is not an acute but a long term chronic disease. Both prospective and retrospective studies demonstrated that the presence of these autoantibodies could be used to predict individuals at risk of developing clinical disease. In fact, the number of autoantibodies, rather than the titer of the autoantibodies proved to be the key in predicting type 1 diabetes. Estimates based on first degree relatives of diabetic patients showed that the likelihood of developing type 1 diabetes within 5 years was ~10% in the presence of one autoantibody, ~50% in the presence of two autoantibodies, and ~60–80% in the presence of three autoantibodies (9). By screening a population for individuals who are positive for two or more of these autoantibodies, it is now possible to readily select subjects at high risk of developing type 1 diabetes for entry into therapeutic intervention trials long before their beta cell reserve is depleted.

Autoantibodies also have provided new insight into adults classified as having type 2 diabetes. Five to ten percent of these individuals have autoantibodies to GAD65, 2–4% have autoantibodies to IA-2, and 1% or less have autoantibodies to insulin (12). Based on these findings, GAD65 appears to be the predominant autoantigen in the older age group. Thus, either a number of patients classified as having type 2 diabetes have been misclassified and really have type 1 diabetes or some of these individuals have a combination of type 1 and type 2 diabetes. Although these numbers seem small, the issue is not trivial. Because about 16 million people in the United States have type 2 diabetes, if only 5% of these have been misclassified, the number of patients with type 1 diabetes would be almost doubled.

Mechanisms of Beta Cell Destruction

Although autoantibodies have turned out to be excellent diagnostic and predictive markers for type 1 diabetes, it is generally thought that they play only a minor role, if any, in the actual pathogenesis of the disease. Instead, the cell-mediated immune response is believed to be responsible for beta cell killing. Inflammatory cells are found in and around the pancreatic islets. However, in some individuals these inflammatory cells are present for years without clinical symptoms. In
fact, some individuals with autoantibodies and insulitis do not go on to develop clinical disease. The outcome appears to be related to the amount of beta cell destruction. It is estimated from animal studies that between 80 and 90% of the beta cells must be destroyed before the diabetes becomes clinically apparent. In humans, however, the temporal and quantitative relationships between inflammatory cells, beta cell damage, and clinical diabetes have been difficult to determine because in the United States pancreatic biopsies are not performed. As a consequence, much of our information about cell-mediated immune pathogenesis and beta cell killing comes from animal models, particularly NOD mice and BB rats. These animals spontaneously develop an autoimmune disease similar, although not identical, to human autoimmune type 1 diabetes (13).

In mice and humans, there are two major classes of T lymphocytes: CD8+ cytotoxic lymphocytes, which recognize processed antigens (i.e. peptides) bound to MHC class I molecules on the surface of cells (e.g. beta cells), and CD4+ helper lymphocytes, which recognize processed antigens bound to MHC class II molecules on the surface of antigen-presenting macrophages and dendritic cells (APCs). In type 1 diabetes, the direct (cell-to-cell) interaction between antigen-specific CD8+ cytotoxic T lymphocytes and autoantigens on beta cells results in beta cell killing (Fig. 2A). In contrast, antigen-specific CD4+ helper T lymphocytes do not recognize autoantigens on beta cells because beta cells do not express MHC class II molecules. Instead, they act by recognizing autoantigens that have been picked up and processed by APCs expressing class II molecules. This indirect mechanism results in the release of a variety of effector molecules and is known as bystander killing (Fig. 2B).

Both direct and indirect killing (14–16) are thought to occur by apoptosis following activation of caspases, but necrosis also might play some role. Based on animal models, it is now generally believed that multiple effector molecules and pathways are involved in beta cell killing.

**Triggers of Autoimmunity**

A critical question, independent of the mechanism by which the immune response kills beta cells, is what actually triggers the autoimmune cascade. Immunologic, genetic, and environmental factors have been implicated. Normally an individual’s T lymphocytes are immunologically anergic or tolerant to self-antigens (3). T lymphocyte education and selection takes place in the thymus. T cells that do not receive a signal from an HLA-autoantigen complex die by neglect. T cells that receive a signal from an HLA-autoantigen complex that is too strong die by apoptosis. However, T cells that receive a weak, low affinity signal from an HLA-autoantigen complex are positively selected. These positively selected autoantigen-specific T cells, generally present in very low numbers, escape from the thymus and migrate to peripheral organs throughout the body including the pancreas. Under ordinary circumstances they remain dormant and are kept under strict regulatory control by still poorly defined regulatory mechanisms (e.g. CD4+CD25+ and/or NKT lymphocytes) (17, 18). If, however, these antigen-specific T cells come in contact with cognate autoantigens presented by beta cells or APCs in the pancreas and if the regulatory controls fail, these dormant, antigen-specific T cells will be activated and the autoimmune cascade of beta cell killing will be initiated. Thus, immune dysregulation may serve as one of the triggers for autoimmunity.

Genetic and environmental factors also have been implicated as possible initiating triggers (19, 20). The fact that in identical twins the concordance rate for type 1 diabetes is less than 50% argues for a genetic predisposition upon which an environmen-
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**Concluding Comments**

Type 1 diabetes is caused by autoimmune destruction of pancreatic beta cells. Although still not understood in detail in humans, extensive studies in animals indicate that the destruction of beta cells is the result of a T lymphocyte-mediated immune response and that more than one effector pathway is involved. At least 20 different genes have been linked to type 1 diabetes, but with the exception of HLA genes, most of these linkages have been weak. Immunologic studies, however, have identified three major autoantigens: GAD65, IA-2, and insulin. Autoantibodies against these autoantigens appear years before clinical symptoms and are valuable markers for identifying individuals at high risk of ultimately developing clinical disease. To a very large degree, this has made type 1 diabetes a predictable disease, and autoantibodies now are being widely used to identify high risk subjects for recruitment into therapeutic intervention trials.

Of particular importance, the knowledge gained from type 1 diabetes in regard to autoantibodies as predictors of disease may be applicable to many of the 30 or more other chronic autoimmune diseases. Taken as a group, autoimmune diseases are the third leading cause of morbidity and mortality after heart disease and cancer. For some of these diseases, it is already known that specific autoantibodies appear long before clinical symptoms. If in the future prospective studies validate the predictive value of these autoantibodies, high throughput procedures for measuring these autoantibodies will almost certainly become an integral part of the routine medical examination (28).

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**REFERENCES**

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