Update on the Genetics and Pathophysiology of Type I Diabetes Mellitus

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DIAGNOSIS
Diabetes mellitus includes several disorders, all of which result in hyperglycemia. The diagnostic criteria require hyperglycemia on two separate occasions and include either fasting hyperglycemia (>127 mg/dL), random hyperglycemia (>200 mg/dL), or abnormal glucose tolerance (>200 mg/dL). Fasting hyperglycemia is the most reproducible criterion, and, for that reason, glucose tolerance testing is rarely needed to confirm the diagnosis.

INCIDENCE
Type I diabetes mellitus (previously referred to as insulin-dependent diabetes) is the second most common chronic disease in childhood, and the most common form of diabetes in children (1.7 of 1,000 children). The prevalence exhibits a strong age dependence, ranging from 1 in 2,500 children at 5 years of age to 1 of 300 children by 18 years of age. Seventy-five percent of these patients are diagnosed before their 15th birthday. For these reasons, type I diabetes mellitus is a condition with which all pediatricians should be completely familiar.

EDUCATIONAL OBJECTIVES
1. Review the diagnostic criteria for diabetes mellitus.
2. Discuss the age-dependent incidence of diabetes mellitus in children.
3. Describe the genetic factors that have an impact on the risk for having type I diabetes mellitus.

GENETICS
It has long been recognized that type I diabetes mellitus has a strong genetic component (i.e., it "runs in families"). Studies of monozygotic and dizygotic twins have shown that concordance approaches 50% for monozygotic twins, but only 30% for dizygotic twins. If a father has diabetes, the risk of his children having the disease is 4% to 6%. The risk is lower (2% to 3%) for the children of mothers with diabetes. Overall, children with one affected parent have a 5% risk.

These incidence statistics, the frequent familial clustering among first-degree relatives, the large differences in incidence among different populations, and the recognition that environmental factors trigger or cause this disease have established its multifactorial etiology. Regardless of the etiology, diabetes is characterized by progressive pancreatic β-cell destruction mediated by T-cells and resulting in insulin deficiency. This process is of clinical importance to the pediatrician because the rate of islet cell destruction is greatest in young children and because secondary complications occur within 10 to 20 years after onset of the disease.
Two lines of evidence suggested the autoimmune etiology of type I diabetes mellitus. First, only type I diabetes mellitus was associated with other autoimmune endocrinopathies such as Addison’s disease, thyroid disorders, and pernicious anemia. Second, organ-specific, cell-mediated immunity was observed only in type I diabetes mellitus. These early observations were precursors for the genetic exploration of this disease. Many groups subsequently established a relationship among autoimmune, type I diabetes mellitus, and the human leukocyte antigen (HLA) haplotypes on human chromosome 6, leading to delineation of the genetics or inheritance of type I diabetes mellitus.

The current taxonomy of type I diabetes mellitus assumes the presence of an immune mechanism involving the HLA genes. Advances in molecular genetics permit identification of “genetic predisposition,” whereas previous assessments were based on “aggregate” or “population” data. Epidemiologic data have established a 30-fold difference in the incidence of type I diabetes mellitus across racial groups and countries.

The HLA region contributes 30% to 60% of the genetic susceptibility for type I diabetes mellitus. Examination of specific HLA haplotypes has led to the recognition that 95% of patients with type I diabetes mellitus have either HLA DR3 or HLA DR4 compared with a 50% prevalence in the general population. Clinical investigations have suggested that certain HLA haplotypes increase susceptibility, whereas others make an individual less susceptible. Several investigators have established a strong linkage to the HLA-DQ (beta) locus. Within this locus, a relationship has been defined between the amino acid residue at position 57 and the risk of type I diabetes mellitus. Any amino acid other than aspartic acid confers susceptibility to the disease and the autoimmune response against the insulin-producing islet cells. A recent study documented that 96% of diabetic probands were homozygous at this locus compared with 19.5% of unaffected control subjects. The difference in incidence in racial and geographic groups correlates with the HLA-DQ polymorphism.

Application of molecular oligonucleotide markers to examine DNA haplotypes has revealed multiple genetic markers for type I diabetes mellitus. Markers linked to susceptibility have been localized to chromosomes 11q15.5, 11q13, 15q26, 6q25, 14q24, and 2q33. The chromosomal locus 11q15.5 is of special interest because this region contains the insulin gene. The linkage polymorphism is adjacent to the insulin gene (5’ prime flanking end) and contains a variable number of tandem repeat (VNTR) 14-bp oligonucleotides.

The strongest association for disease susceptibility, other than for the HLA loci, is to the polymorphism on the long arm of chromosome 14. The 14q24 locus is known as the IDDM-11 marker. In large correlative studies of disease susceptibility in individual families, the presence of either the HLA markers or the IDDM-11 marker is predictive of diabetes. Such clinical investigations have resulted in identification of 18 different chromosomal regions that show linkage to type I diabetes mellitus, thus providing evidence that it is inherited in a polygenic (multifactorial) fashion.

The documentation of islet cell cytoplasmic antibodies provides additional support for the polygenic inheritance pattern of type I diabetes mellitus. Approximately 80% to 90% of Caucasians with new-onset type I diabetes mellitus have islet cell antibodies. These may also be detected in the relatives of affected patients as early as 12 years before clinical manifestations appear. Not all relatives with positive islet cell antibodies will have the disease. Diet, viral infections, and active immunization have been incriminated as environmental factors. Molecular mimicry has been proposed as a causative factor because it could allow cross-reacting immune responses. Shared antigens could occur as a consequence of structural similarities such as:

- cross-reactivity among the β-cell auto-antigen, glutamic acid decarboxylase (GAD), and a protein in coxsackievirus
- cross-reactivity between islet cell and rubella viral proteins
- cross-reactivity between islet cell peptides and bovine serum albumin
- duration of breastfeeding less than 3 months (possibly associated with the early introduction of cows’ milk formula).
The development of new biotechnologies such as oligonucleotide microarrays ("chip technology" for DNA genotype analysis) and their application to the study of complex diseases such as diabetes will enhance our capacity to offer more precise counseling to families at risk. Currently, genetic counseling consists of defining empiric risk estimates and detecting the presence or absence of islet cell autoantibodies.12

IMMUNOLOGY OF TYPE I DIABETES MELLITUS

Despite the importance of these genetic factors, compelling evidence has shown that additional environmental factors must act on the genetically predisposed individual for type I diabetes mellitus to develop.14,13,20 These factors result in autoimmune destruction of the pancreatic β-cells, as evidenced by lymphocytic infiltrate (insulitis) in the islets of newly diagnosed patients; the specificity of β-cell destruction; the observation that transplanted β-cells from identical donors are rapidly and selectively destroyed; and the presence of antibodies specific for islet antigens.4,13

One of the most powerful factors in predicting diabetes is the presence of specific HLAs, DR3 or DR4, because they confer a tenfold increased risk for diabetes.1 The importance of these HLA haplotypes derives from the central role of the major histocompatibility complex (MHC) in the immune response. Adaptive immunity requires the selection and expansion of individual lymphocyte clones that recognize a specific antigen. During ontogeny, each lymphocyte expresses a single set of receptor genes and thus a unique cell-surface receptor. Early in development, if the receptor recognizes a self-antigen, binding leads to programmed cell death and depletion of all lymphocytes that respond to self-antigens. The remaining lymphocytes express unique receptors that collectively bind a wide array of foreign antigens. Binding of the specific antigen induces lymphocyte proliferation, forming a clone with identical specificity. Further differentiation generates effector cells that destroy the antigen and long-lived memory cells that provide the amnestic response.

Lymphocytes are divided into two functional groups, including B-cells, which are precursors to antibody-producing cells, and T-cells, which recognize antigens produced inside infected cells. This is possible because an infected cell will digest foreign proteins, releasing small (8–15 amino acids) peptides. These peptides are covalently bound to the MHC on the cell surface and presented by this complex to surrounding T-cells. Class I MHC molecules present antigen exclusively to lymphocytes of the CD8 subclass. The CD8 molecule allows the lymphocyte to bind to the MHC and kill the infected cell. Class II MHC molecules bind and present peptides to T-lymphocytes of the CD4 subtype. CD4 lymphocytes destroy the infected cell and activate antibody-producing cells.

The human MHC proteins are referred to as HLAs, and class II MHC molecules (HLA-D proteins) are subdivided into HLA-DR, HLA-DQ, and HLA-DP. The expression of specific HLA molecules is critical for the development of diabetes, because a given T-cell can recognize its peptide only if the peptide is bound by the correct HLA molecule. This restriction would allow only specific HLA types to present β-cell autoantigens to CD4 cells. For diabetes, this concept is strongly supported by the observation that homozygous absence of aspartic acid at position 57 in the HLA-DQ (beta) chain confers a striking 100-fold increased risk of diabetes.4,13 In contrast, homozygous aspartic acid at position 57 offers virtually complete protection against diabetes.1,13

Presumably, this reflects the ability of the former to present β-cell antigen and the absence of this with the latter.

PRECLINICAL β-CELL AUTOIMMUNITY

The appearance of clinical diabetes is preceded by a stage of β-cell autoimmunity accompanied by progressive loss of insulin secretion, and marked by the presence of islet cell antibodies (ICAs). This preclinical stage may last up to 13 years and recent observations suggest that β-cell autoimmunity begins early in life.1,2,4,13,15,21,22 ICAs are frequently identified in the sera of school-age children (0.2% in Holland, 3% in the United States, and 4.4% in Sardinia) and the incidence of ICAs is roughly proportional to the incidence of diabetes in each population.4 In the BABY-DIAB study, children born to parents with
diabetes were observed over time.\textsuperscript{13,20,21} Although cord sera were positive for autoantibodies, these resolved by 9 months of age. However, it is noteworthy that antibodies reappeared before 2 years of age in all children (n = 37) born to parents with diabetes. The Diabetes Autoimmunity Study in the Young also prospectively observed children to determine the timing of β-cell autoimmunity.\textsuperscript{1,2,22} β-cell antibodies appeared between 0.7 and 7.1 years of age. In a recent study of first-degree relatives, the incidence of ICA seroconversion was strongly correlated with age: it was most frequent (3.7% per year) in children younger than 5 years of age, intermediate (0.5% per year) in children 5 to 9 years of age, and never observed in children older than 10 years of age.\textsuperscript{21}

The tempo of islet cell destruction may also be related to the age of onset. In children younger than 7 years of age, 80% of pancreatic islets are damaged by the time of diagnosis, compared with 60% in children 7 to 14 years of age and 40% in children older than 14 years of age.\textsuperscript{1,20} β-cell destruction is complete within 3 years of diagnosis in most young children, but as many as 15% of older patients retain β-cell function for up to 10 years. These data suggest that β-cell autoimmunity is well developed by 5 years of age, and progresses more rapidly in younger patients.

**ENVIRONMENTAL TRIGGERS**

For environmental factors to induce β-cell autoimmunity, they must have an effect early in life. Viruses, particularly the enteroviruses, have been strongly implicated.\textsuperscript{1,13,16,18} This was originally based on (1) the seasonal variability of diabetes, (2) outbreaks occurring 4 to 5 years after viral epidemics, and (3) the direct pancreatic effects of mumps and coxsackie B4. Great interest has been recently generated by the discovery of marked epitope (or active antigenic site) similarity between the coxsackie viral protein, P2-C, and the β-cell autoantigen, glutamic acid decarboxylase (GAD 65).\textsuperscript{1,13,14} Antibody cross-reactivity between these two molecules (molecular mimicry) could lead to β-cell destruction following the immune response to a coxsackie viral infection. The initiating viral infection may even occur in utero. In congenital rubella syndrome, prenatal exposure to rubella virus leads to an increased incidence of diabetes 5 to 20 years after birth. A 52-kd β-cell autoantigen has been shown to cross-react with rubella viral antigens, and may be another example of molecular mimicry.

Infant nutritional factors may also serve as environmental triggers. Early weaning from breastfeeding, early institution of cows' milk formula, and early institution of solid food have all been implicated.\textsuperscript{1,3,13,15,20,22} Breastfeeding of less than 3 months' duration has been associated with a 50% increase in the risk of diabetes. Perez-Bravo et al. compared children who had diabetes with HLA-matched, nondiabetic control subjects and found that cow's milk feeding increased the relative risk of diabetes (odds ratio, 13.1).\textsuperscript{1,23} Antibodies directed against bovine serum albumin have been identified in the sera of patients with diabetes, and recently a distinct 17 amino acid epitope in the bovine serum albumin molecule, which shares significant homology with the β-cell surface protein, p69, has been identified. Although it is possible that antibodies directed against bovine serum albumin could initiate β-cell destruction, other studies have failed to support this concept. Currently, controversy continues to surround this possibility.

Maternal nutrition has also been implicated in type I diabetes mellitus. Children of Icelandic women, who consume large quantities of smoked mutton during pregnancy, have an increased risk of diabetes. Smoked foods rich in nitrates, nitrites, and nitrosoamines could provide free radicals that could damage fetal β-cells and initiate the autoimmune response.\textsuperscript{1,13,20}

**PROGRESSION TO DIABETES**

Although genetic factors and environmental triggers are important in the initiation of β-cell autoimmunity, clinical diabetes does not universally develop in those who have these. Up to 10% of the general population have β-cell autoimmunity; however, less than 1% will have diabetes. In first-degree relatives and school-age children, remission rates for the presence of ICA range from 10% to 78%, and the risk of development of diabetes is greatest in children younger than 10 years of age.\textsuperscript{1,13,17}

The non-obese diabetic strain of mice is an excellent model for type I diabetes mellitus and
has been used to observe progression of the β-cell immune response. β-cell autoimmunity sequentially develops against an orderly set of islet antigens in these animals, beginning with GAD and then spreading to involve other antigens, including heat shock protein-60, carboxypeptidase H, and insulin.13,13,24,25

In humans, β-cell autoimmunity does not appear to follow a set sequence. However, higher antibody titers and a greater number of antibodies are associated with the greatest risk of progression. First-degree relatives with high titers of ICA (> 80 Juvenile Diabetes Foundation units) have a 53% probability of having diabetes within 5 years. Individuals with only 1 type of antibody rarely have diabetes. In contrast, 50% or more of those with two antibodies (ICA, GAD, IA-1, or IA-2) will have diabetes, and the risk is even greater in those with three or more antibodies.13,15

Factors that might suppress β-cell autoimmunity are not yet known. However, clues may reside in the subsets of CD4 cells and the immunomodulators each produces.13,14 The T-helper 1 subset of CD4 lymphocytes support cell-mediated immunity, secrete interferon-γ and interleukin (IL)-2, and are believed to be the cells involved in type 1 diabetes mellitus. In contrast, T-helper 2 cells support humoral immunity, secrete IL-4, IL-5, IL-10, and IL-13, and down regulate the diabetic immune process. Thus, diabetes may result from a functional imbalance between these two subsets of CD4 cells.13,14

**SUMMARY**

Type 1 diabetes mellitus results from the genetically predetermined autoimmune destruction of pancreatic β-cells, resulting in gradual, but complete, loss of insulin secretion. There are strong associations with specific HLA haplotypes, but environmental triggers are also required to initiate β-cell autoimmunity. These could possibly include enteroviral infection, early weaning from breast-feeding, early exposure to cow's milk antigens, and free radical damage. Once initiated, β-cell autoimmunity does not always lead to clinical diabetes, suggesting that immunomodulators may be important in the control of β-cell destruction. Current interventions designed to prevent type 1 diabetes mellitus are based on attempts to alter this immune response and to preserve β-cell function. It is important for the pediatrician to understand the background of these trials and to be able to answer parents' questions regarding study participation.

**REFERENCES**


**CALENDAR**

**September 23-24, 1999:** Pediat-ics for the Practitioner—Update ‘99. To be held at the Thomas B. Turner Building, Johns Hopkins University School of Medicine, Baltimore, Maryland. For more information, contact Office of Continuing Medical Education, Johns Hopkins University School of Medicine, Turner 20, 270 Rutland Avenue, Baltimore, MD 21205-2195; telephone: (410) 955-2959; fax: (410) 955-0807; e-mail: cmnet@jhu.edu; Website: www.med.jhu.edu/cme.

**October 20-23, 1999:** Pediatric Infectious Disease Seminar. To be held at the Wyndham Palace & Resort Spa, Walt Disney World, Florida. For more information, contact George M. Converse, III, MD, The Lloyd Noland Foundation, 701 Lloyd Noland Parkway, PO Box 925, Fairfield, AL 35044-0925; telephone: (205) 783-5276.

**November 20-21, 1999:** Practical Management of Common Problems in Ambulatory Pediatric Patients. To be held at the Searle Center for Continuing Medical Education, Duke University Medical Center, Durham, North Carolina. For more information, contact Joseph Marc Majure, MD, Assistant Professor, Pediatrics Course Director, 26th Annual Spock Symposium, Duke University Medical Center, Room 302 Bell Building, Box 2994 DUMC, Durham, NC 27710; telephone: (919) 684-2289; fax: (919) 684-2292.

**January 29-February 5, 2000:** Update: Controversies in Emergency & Primary Care. To be held at the Sheraton Poipu Beach Resort, Kauai, Hawaii. For more information, contact Edith S. Bookstein, PO Box 2586, La Jolla, CA 92038; telephone: (619) 454-3212; fax: (619) 454-3556.

**February 26-March 4, 2000:** Pediatric Emergencies. To be held at the Renaissance Wailea Beach Resort, Maui, Hawaii. For more information, contact Edith S. Bookstein, PO Box 2586, La Jolla, CA 92038; telephone: (619) 454-3212; fax: (619) 454-3556.