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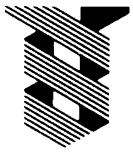

JEFFREY S. FLIER, M.D., *Editor*
LISA H. UNDERHILL, *Assistant Editor*

# TYPE I DIABETES MELLITUS

## A Chronic Autoimmune Disease

GEORGE S. EISENBARTH, M.D., PH.D.

DURING the past decade a wealth of information concerning the pathogenesis of Type I diabetes has become available. Two spontaneous animal models of the disease have been discovered and characterized (the Biobreeding rat and the non-obese diabetic mouse); the importance of a gene or genes in the major histocompatibility complex in Type I diabetes of human beings, of mice, and of rats has been appreciated; and the prognostic importance of selected assays for islet-cell antibodies has been defined. T-cell abnormalities that precede diabetes have been discovered. Evidence has suggested that progressive loss of first-phase insulin secretion precedes diabetes, and immunologic pilot studies have been completed.

Such information is beginning to be used to predict the development of Type I diabetes and has led to randomized immunotherapy trials. In this review I will present the evidence that the Type I diabetes mellitus of humans, Biobreeding rats, and non-obese diabetic mice is a genetically programmed autoimmune disease. Areas in which newer pathogenic information is beginning to affect clinical care will also be discussed.

### THE STAGES OF TYPE I DIABETES

The development of Type I diabetes can conceptually be divided into six stages, beginning with genetic susceptibility and ending with complete beta-cell destruction. The first stage is genetic susceptibility. Some genetically susceptible subjects (less than half of identical twins) have a hypothetical triggering event (Stage II), and active autoimmunity develops (Stage III). Initially, even patients with immunologic abnormalities have normal insulin secretion. During Stage IV, immunologic abnormalities persist but glucose-stimulated insulin secretion is progressively lost, despite normal blood sugar levels. In Stage V, when overt diabetes is first recognized, some residual insulin secretion remains, but is followed by complete beta-cell destruction (Stage VI). Because human pancreatic tissue is rarely available for study, beta-cell mass (Fig. 1[1]) is known only for three stages: Stage I (genetic susceptibility) when islet-cell mass is normal, Stage V (onset of diabetes), and Stage VI (complete beta-cell destruction). At the onset of Type I diabetes, as little as 10 percent of the beta cells remains, and several years after the development of overt diabetes, essentially all beta cells are destroyed.[2]

### STAGE I: GENETIC SUSCEPTIBILITY

Non-obese diabetic mice, Biobreeding rats (named for the Biobreeding Laboratories in Canada, where the strain was discovered), and human beings all have at least one gene in the major histocompatibility region that contributes to susceptibility to diabetes (Table 1). In addition, the inheritance of diabetes in all three of these species is multigenic and involves one or more genes outside the major histocompatibility complex.

Genes within the major histocompatibility region are divided into three classes: Class I genes, which code for the classic transplantation antigens (HLA-A, B, and C in humans); Class II genes (HLA-DP, DQ, and DR in humans; H-2 I-A and H-2 I-E in mice; and RT1.B and RT1.D in rats), which code for dimeric polypeptides that function at least in part as immune-response genes and profoundly affect interactions between monocytes and various lymphocyte subsets; and Class III genes, which code for complement molecules. The major histocompatibility genes are conserved between species (i.e., I-A of mice is similar to DQ of human beings; I-E is similar to DR). These homologies allow DNA probes developed for one species to be used in studying the major histocompatibility complexes of other species. Despite this cross-species conservation, the Class I and Class II genes are extremely polymorphic, and in humans, the various Class II alleles are defined serologically and given numbers (e.g., DR2, DR3, and DR4).

#### Animal Models

The non-obese diabetic mouse strain was developed by Makino and co-workers by selective breeding from an outbred strain of mice in which cataracts develop.[3] Although insulitis develops in essentially all inbred non-obese diabetic mice, overt hyperglycemia develops in only 80 percent of females and 20 percent of males. Castration of female mice decreases their incidence of diabetes, but castration of male mice greatly increases it. Susceptibility to diabetes is determined by T cells, and diabetes can be prevented in non-obese diabetic mice by introducing the nude mouse gene, which produces profound T-cell immunodeficiency. If such "nude" non-obese diabetic mice are reconstituted with immunocompetent T lymphocytes from ge-

From the Section of Immunology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215, where reprint requests should be addressed.

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netically "normal" non-obese diabetic mice, susceptibility to diabetes is restored.[4] When non-obese diabetic mice are bred with normal strains, F1 animals do not become diabetic, whereas 2 percent of F2 and 14 percent of backcrossed animals become hyperglycemic. In all such breeding studies done thus far, all the animals who became diabetic were homozygous for the histocompatibility region of the non-obese diabetic mouse. It is interesting to note that of the two Class II murine "immune-response" genes (I-A and I-E), NOD mice do not express surface I-E and lack E alpha messenger RNA.[5] Thus, the development of diabetes in non-obese diabetic mice cannot be directly affected by I-E molecules, although perhaps their absence is important. Breeding studies indicate that at least one other gene in these mice contributes to diabetes, and that this gene is not linked to the major histocompatibility complex.

Approximately 60 percent of Biobreeding rats manifest Type I diabetes[6,7] between 60 and 120 days of age. Susceptibility to diabetes correlates with the inheritance of an autosomal recessive gene that produces a deficiency of all subsets of circulating T cells[8] and correlates with an independent major histocompatibility complex gene or genes.[9,10] The profound reduction of circulating T lymphocytes in Biobreeding rats is apparent at birth, even though the thymus appears normal. When Biobreeding rats are crossed with other rat strains, the incidence of diabetes in animals that are homozygous for the major histocompatibility complex of the Biobreeding rat is 10 times higher than it is in heterozygous animals. Diabetes develops only in animals that have at least one chromosome similar to that of the biobreeding strain at the Class II gene.[10] In the F2 generation of Biobreeding rats crossed with either Brown Norway or Lewis rats, 1 in 16 animals is homozygous for the histocompatibility complex and the lymphopenia gene of the Biobreeding rat (one fourth times one fourth). Diabetes develops in approximately 30 percent of these homozygous animals, suggesting that other "diabetogenic" genes are also involved. The histocompatibility complex of rats from a control strain (BBN) that lacks the lymphopenia gene and does not manifest diabetes differs from that of the Biobreeding rat in polymorphisms of Class I genes.[10] However, such differences at Class I genes do not influence the diabetogenicity of these chromosomes, suggesting that the actual diabetogenic genes are within the Class II region of the major histocompatibility complex.[10]

Table 1. Genetic Features of Type I Diabetes.

| SPECIES | INCIDENCE OF DIABETES* |
|---|---|
| Non-obese diabetic mouse | Associated with recessive MHC gene<br>Higher in females than in males<br>Associated with at least one other non-MHC gene |
| Biobreeding rat | Associated with functionally recessive MHC gene<br>Associated with autosomal recessive lymphopenia gene<br>Associated with at least one other non-MHC gene |
| Human being | May be associated with recessive MHC gene:<br>Increases with HLA identity and haploidentity of concordant siblings<br>Increased risk in DR3/DR4 heterozygotes<br>Associated with at least one other non-MHC gene |

*MHC denotes major histocompatibility complex.

Multiple forms of immunotherapy can prevent diabetes in Biobreeding rats, probably reflecting the multigenic inheritance of susceptibility to the disease. Suppressing immune function by means of cyclosporine administration, neonatal thymectomy, lymphoid irradiation, or inhibition of macrophage function prevents hyperglycemia.[11,12] Diabetes can also be prevented in Biobreeding rats by restoring T-cell function with bone marrow transplants or transfusions of normal T cells.[13,14] Because Biobreeding rats are so abnormal immunologically, T lymphocytes that are transfused from unrelated strains are accepted and create a chimeric state. The primary importance of T lymphocytes in the development of diabetes in Biobreeding rats is further emphasized by the fact that activated T lymphocytes from acutely diabetic animals can transfer diabetes to other animals.[15]

It is important to note that among Biobreeding rats that are close to being inbred and among non-obese diabetic mice that are inbred, many animals do not become diabetic. In studies of human identical twins, a similar lack of complete concordance for Type I diabetes (concordance is perhaps 50 percent) has been used as evidence that environmental factors influence the development of overt illness. To date, despite multiple studies, few environmental factors have been identified that contribute to the diabetes of Biobreeding rats or non-obese diabetic mice. In particular, rearing Biobreeding rats in a "germ-free" environment does not influence the age of onset of diabetes or the percentage of animals that become diabetic.[16]

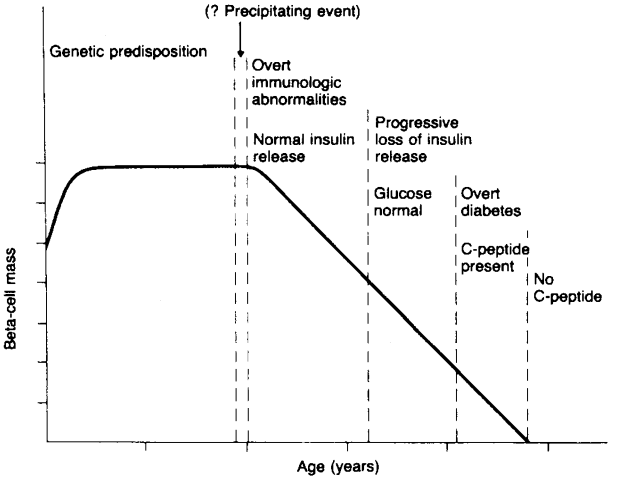


Figure 1. Stages in the Development of Diabetes Mellitus.
The stages of diabetes are listed from left to right, and hypothetical beta-cell mass is plotted against age. (Reproduced from Eisenbarth[1] with the permission of the publisher.)

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A disease is usually thought to be genetic if concordance is 100 percent in identical twins. A lack of complete concordance is thought to implicate a critical environmental factor or factors. However, recent studies in molecular biology have revealed that during lymphocyte ontogeny, a "semi-random" process of gene rearrangements occurs in lymphoid cells, resulting in the huge genetic diversity of both immunoglobulin molecules and T-cell receptors. Thus, key immunologic genes are not identical in identical twins and in inbred strains of animals, and although all the predisposing genetic factors may be present, genetic disease does not always develop. This perhaps explains why, although lymphocytes infiltrate the islets in all non-obese diabetic mice, diabetes develops in only 80 percent. In some animals, despite autoimmunity, beta-cell destruction is apparently not severe enough to result in hyperglycemia.

### Humans

The genetics of Type I diabetes in humans is not as well understood as it is in the two animal models just discussed. However, it is clear that genes within the major histocompatibility complex of humans contribute to the development of diabetes.[17-19] Ninety-five percent of whites with Type I diabetes express the histocompatibility alleles — DR3 or DR4, or both. These alleles are only expressed in 40 percent of the general white population. Expression of DR2 is decreased in persons with Type I diabetes. A worldwide effort to define the D-locus genes (Class II) in relation to diabetes is now under way, with use of such methods as lymphocyte typing,[20] analysis of polymorphisms of DNA restriction-fragment length,[21,22] and study of extended histocompatibility haplotypes.[23] Such typing will refine the definition of genetic risk for Type I diabetes.

In addition to population studies, inheritance studies of Type I diabetes within families also demonstrate the importance of the major histocompatibility region.[17-19] For example, if one child in a family has Type I diabetes, the risk of diabetes in the siblings is related to the number of HLA haplotypes the sibling shares with the diabetic proband. If two haplotypes are shared with the diabetic sibling, the risk of diabetes is approximately 10 to 20 percent; if one haplotype is shared it is 5 percent, and if neither haplotype is shared it is 1 percent (Fig. 2). In addition, subjects who express both the high-risk HLA-alleles DR3 and DR4 have the highest risk of Type I diabetes, both in the general population and within families. Expression of both DR3 and DR4 alleles even increases the concordance rate for Type I diabetes in identical twins.[24]

Attempts to characterize the diabetogenic genes of humans have been complicated by the fact that until recently, overt diabetes was used as the end point. Thus, it has not been possible to identify non-diabetic parents who are genetically susceptible. Some of these

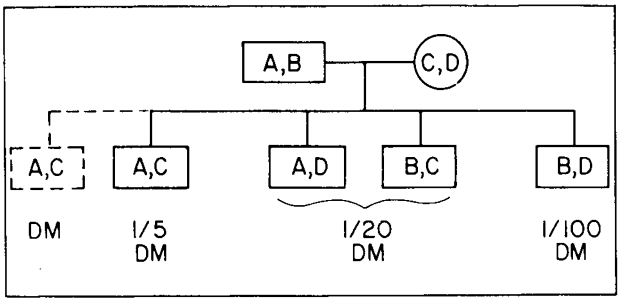


Figure 2. Risk of Type I Diabetes Mellitus among Siblings of a Proband, Relative to HLA Type (A,C).

The broken line denotes the proband with diabetes mellitus (DM), and the fractions indicate the risk of diabetes mellitus in siblings. The letters denote HLA identity. (Reproduced from Eisenbarth[1] with the permission of the publisher.)

parents can now be identified by the immunologic and endocrinologic assays that will be discussed later.[1]

Currently, one can only speculate about the number of genes involved and their mode of inheritance in human Type I diabetes. The speculation that I favor is that at least two genes (one of which is recessive) within the HLA region (DR3 associated and DR4 associated) are necessary but not sufficient for diabetes to develop. In addition, an independent gene probably exists outside the major histocompatibility complex. The location of such a non-HLA-linked gene is unknown, although recent studies of the association of Type I diabetes with DNA polymorphisms of the insulin gene suggest that it may be on chromosome 11.[25] In part, my hypothesis concerning the genetics of human Type I diabetes is influenced by the genetic analysis of Biobreeding rats and non-obese diabetic mice. However, although animal models provide important pathogenic insights, they may not accurately mimic the human disease.

## Stage II: Triggering

Environmental factors that may trigger the development of beta-cell immunity are poorly defined. However, there are drugs and infectious agents that trigger the development of autoimmunity: procainamide and hydralazine hydrochloride induce lupus erythematosus, methyldopa triggers hemolytic anemia, amiodarone triggers thyroiditis and Graves' disease,[26] wheat protein gliadin triggers celiac disease, streptococci trigger rheumatic valve disease, and mononucleosis triggers hemolytic anemia. In many instances, removal of these environmental factors abrogates the autoimmune process. My colleagues and I are beginning a trial to determine whether removing dietary proteins (and substituting amino acids) will alter the autoimmunity of Type I diabetes, as it did in studies of Biobreeding rats done by Elliott and Martin.[27]

A number of viruses are known to be diabetogenic in animals, and interest has focused on a possible viral origin of diabetes. Several studies and observations

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suggest that a virus may trigger a latent or potential diabetic state. Coxsackievirus was obtained from a child who died from viral encephalitis and diabetes,[28] and when this virus was isolated from the child and injected into mice, the animals became hyperglycemic. However, recent reports of the pathological findings in the child's pancreas indicate that extensive beta-cell damage appears to have preceded the infection.[2] This suggests two possible scenarios, in addition to the original suggestion that the virus itself directly infected beta cells and directly caused diabetes. The virus could have been diabetogenic, but only in the context of a previously reduced beta-cell mass, or alternatively, the virus could have been a nonspecific stress that hastened an event that would have occurred anyway.

If environmental factors that contribute to diabetes act years before hyperglycemia develops, identifying such triggering events at the time of clinical diabetes will certainly be difficult. New strategies are needed to identify such remote triggering events.

Congenital rubella infection is a well-defined environmental trigger for Type I diabetes. Approximately 20 percent of persons born with congenital rubella have diabetes later in life. Such persons also have a high incidence of thyroiditis and other immunologic disorders. Type I diabetes occurs in children who express the HLA allele DR3 or DR4, or both.[29] How congenital rubella increases the incidence of diabetes in persons who express HLA susceptibility alleles is not known. One hypothesis is that rubella virus leads to tissue destruction by infecting multiple target tissues (e.g., islets and the thyroid). We have found persistent abnormalities of T-cell subsets in a group of young adults with a history of congenital rubella infection.[30] Such a T-cell abnormality by itself may predispose to multiple autoimmune illnesses by disrupting normal immunoregulation.

### Stage III: Active Immunity

Table 2 shows seven immunologic abnormalities that can precede the development of overt Type I diabetes mellitus.[31-39] Patients with diabetes and their relatives can also have additional abnormalities, such as antibodies to thyroglobulin, to thyroid microsomal antigens, to single-stranded DNA, and to unidentified components of Bouin's-fixed pancreas (pancreatic tissue that is treated with picric acid, embedded in paraffin, and extracted with organic solvents). Such additional abnormalities in families of patients with Type I diabetes appear to be associated with an increase in the prevalence of autoimmune phenomena. They are inherited independently of the susceptibility to diabetes that is determined by the major histocompatibility complex and are found in persons who have no clinical evidence of diabetes.

Antibodies that react with the islets of unfixed sections of normal human pancreas are strongly associated with the development of Type I diabetes.[40-42] Using a modification of the classic technique for measuring such antibodies, we found that approximately 70 percent of patients with recent-onset diabetes and a similar proportion of those with pre-diabetes had anti-islet-cell antibodies.[43] The presence of antibodies may precede the development of overt diabetes by more than nine years.[44]

The antigen or antigens to which these anti-cytoplasmic antibodies are directed have not been purified, but biochemical evidence suggests that they are similar to the glycolipids[45] that react with the experimentally raised anti-islet-cell monoclonal antibodies 3G5 and A2B5, as well as with tetanus toxin.[46,47] In particular, studies of frozen sections and human serum have shown that the antigenicity is sensitive to gentle periodate oxidation, is restored by borohydride, is acetone stable, and is sensitive to chloroform and methanol.[45] Carbohydrate determinants and gangliosides in particular appear to be major targets of autoantibodies in three other organ-specific autoimmune diseases: peripheral neuropathy associated with paraproteinemia,[48,49] Graves' disease,[50] and cold agglutinin disease.[51] Because Bouin's-fixed human pancreas (which was proposed as a reagent for measuring "cytoplasmic" islet-cell antibodies) is extracted with organic solvents, it does not contain glycolipid antigens and, in our studies, does not react with serums that react strongly to non-fixed pancreas.[45] Differences in assay technique contribute to some of the confusion in the literature about assays for islet-cell antibody, but all assays suffer from a lack of quantitation and from the use of human pancreata, which can vary tremendously. Although some commercial tests for islet-cell antibodies are now advertised in the United States, reliable assays will probably not be widely available until the target antigens are purified.

Despite the limitations of the current assays for

Table 2. Immunologic Abnormalities That Can Precede Diabetes.

| Abnormality | Prediabetic Prevalence | Specific? | Reference No. |
|---|---|---|---|
| Anti-cytoplasmic islet-cell antibodies ("anti-glyco-conjugate") | 70% | Yes | 31<br>32 |
| Anti-insulin antibodies | 30% | ?Yes | 33 |
| Ia-positive T cells | 70% | No (Graves', Addison's disease) | 34 |
| Anti-cell-surface antibodies | ? | No (thyroiditis) | 35 |
| Cytotoxic islet-cell antibodies | ? | No | 36<br>37 |
| Anti-64K antibodies | 60% | ?(thyroiditis, ?identical molecule) | 38 |
| Lymphocyte inhibition of insulin secretion | ? | ? | 39 |

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cytoplasmic islet-cell antibody, a number of research groups have prospectively evaluated relatives of patients with Type I diabetes, persons with Type II diabetes, persons with gestational diabetes, and normal subjects. There is some controversy about the percentage of positive subjects in each group, but approximately 2 percent of first-degree relatives,[42,52] 10 percent of patients with Type II diabetes,[53] 10 percent of patients with gestational diabetes,[54] and less than 0.5 percent of normal subjects appear to have islet-cell antibodies.[55] Data from several groups indicate that islet-cell antibodies rarely disappear before overt diabetes occurs.[42]

It is not certain whether diabetes will develop in all persons with islet-cell antibodies. Nevertheless, since the prodromal stage can last more than a decade and the majority of persons with islet-cell antibodies have profoundly abnormal insulin secretion, it is likely that overt diabetes will develop in the majority of such subjects.

Maclaren and co-workers have recently reported identifying 12 subjects with islet-cell antibodies among 1445 normal schoolchildren.[55] Among the 12, the parents of 2 and an aunt of another had Type I diabetes, and the parents of 4 others had a goiter or pernicious anemia. Like patients with Type I diabetes, the 12 children expressed a high-risk HLA allele, DR3 or DR4. Thus, even in unselected non-diabetic persons, islet-cell antibodies appear to distinguish a group of subjects at increased risk for Type I diabetes. Among subjects who are clinically categorized as having Type II diabetes, the presence of islet-cell antibodies predicts future insulin dependence.[53]

Antibodies that immunoprecipitate insulin also appear before the onset of Type I diabetes.[33] In a collaborative study with Palmer and co-workers, we found such antibodies in approximately 30 percent of subjects who later had Type I diabetes.[56] Eighty percent of the persons with anti-insulin antibodies also had islet-cell antibodies. Like the amount of cytoplasmic islet-cell antibodies, the amount of anti-insulin antibodies varied little before diabetes occurred.[56] Antibodies to insulin appear to have prognostic importance.

There are additional assays for anti-islet-cell antibodies that do not use sectioned pancreatic tissue (Table 2). The clinical usefulness of most of these assays is limited by false positive reactions in related autoimmune diseases, and their difficulty has precluded their use in large-scale studies of susceptible and control populations.

Despite the intense interest in humoral immunity and the evidence that islet-cell antibodies that react with the islet-cell surface can inhibit insulin secretion,[57] it is unlikely that they are the major determinant of beta-cell destruction. Most islet-cell antibodies are not specific for beta cells. In addition, no one has transferred diabetes with antibodies, and there is no evidence that islet-cell antibodies have transplacental effects (in contrast to the autoantibodies associated with myasthenia gravis and Graves' disease).

There are reasons to believe that T lymphocytes are the major determinants of beta-cell destruction, although subsets of standard resting T cells show no consistent abnormality in Type I diabetes.[58-60] In the Biobreeding rat, it is concanavalin-A–activated lymphocytes that transfer diabetes.[15] Boitard and co-workers have presented data showing that T lymphocytes from patients with Type I diabetes can specifically inhibit the insulin secretion of rat islet cells.[39] In the pancreatic transplants done between identical twins by Sutherland and co-workers, in which pancreata from identical twins without diabetes were transplanted to the related twins with diabetes, islet-cell destruction in association with massive T-cell infiltration occurred within weeks.[61] Before transplantation, the pancreata of the non-diabetic twins were qualitatively normal; however, the T cells within the islets of these transplanted pancreata (as well as in one patient with new-onset diabetes) expressed the DR or Ia antigen, suggesting that the infiltrating T lymphocytes were activated.

When a T lymphocyte is stimulated to divide, either in vivo or in vitro, it acquires a series of cell-surface molecules, including the 120,000-dalton 4F2 antigen (the p120 calcium moderator),[62] the insulin receptor,[63] the transferrin receptor, the receptor for the T-cell growth factor interleukin-2,[64] and the Ia antigen (HLA Class II molecule). Endocrine cells within the thyroid and a subset of beta cells[65] can also express surface Ia molecules after in vitro activation, or in Graves' disease.[66] The functional importance of "aberrant" T-cell expression of the Ia antigen has not been defined for T lymphocytes and beta cells, but thyroid cells that express Ia antigen can stimulate T lymphocytes. In both humans and Biobreeding rats, an elevation of circulating Ia-positive cells precedes the development of Type I diabetes[34] and is a marker of disordered immunity. A similar increase in Ia-positive T cells is seen in DR3-associated autoimmune diseases such as Graves' disease[67] and Addison's disease.[68] Mandrup-Poulsen and colleagues have recently reported that interleukin-1 can directly kill islet cells, and these investigators suggest that beta-cell destruction may be mediated by lymphokines.[69]

## Stage IV: Progressive Loss of Glucose-Stimulated Insulin Secretion

My colleagues and I studied islet-cell antibody status, fasting glucose, and first-phase insulin secretion (the peak insulin response one to three minutes after an intravenous glucose injection) after intravenous glucose in two triplets who were genetically "identical" to the third triplet, who became diabetic in 1965.[44] One triplet has remained without islet-cell antibodies and is not diabetic. Over the 17 years of follow-up, his first-phase insulin secretion varied within the normal range. In contrast, the other triplet had islet-cell antibodies from the first measurement in

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1970 and continued to have them until overt diabetes developed in 1974. His first-phase insulin release was initially stable and was within the low normal range, but before the onset of hyperglycemia it declined until it was well below any normal response.

Such a dramatic loss of first-phase insulin secretion has preceded the development of hyperglycemia in all subgroups of diabetic patients whom we have studied (subjects with or without cytoplasmic islet-cell antibodies, Ia-positive T cells, anti-insulin antibody, DR3, or DR4).[70,71] We measure cytoplasmic islet-cell antibodies to identify first-degree relatives who are immunologically abnormal. When intravenous glucose tolerance is tested for the first time in relatives with islet-cell antibodies, insulin secretion is profoundly abnormal in half the subjects.[52] Despite their inability to secrete insulin in response to intravenous glucose, these subjects are not yet overtly diabetic, probably because their insulin response to secretagogues such as oral glucose, tolbutamide, arginine, and glucagon is relatively preserved. This selective loss of response to intravenous glucose may simply reflect a reduction in beta-cell mass. Weir and co-workers have produced similar abnormalities in insulin secretion in rats by partial pancreatectomy.[72] In contrast to the findings in immunologically abnormal relatives of patients with Type I diabetes, first-phase insulin secretion is normal in subjects who are genetically susceptible (identical twins and HLA-identical siblings) but do not have islet-cell antibodies.[52]

We have studied 18 young subjects with a history of transient hyperglycemia or glucosuria. At the time of evaluation, glucose tolerance in several of the subjects was either normal or only partially impaired (not to the extent seen in overt diabetes). Six of the subjects had islet-cell antibodies, and in 12 subjects, first-phase insulin secretion was below the first percentile. After less than two years of follow-up, overt diabetes developed in 6 of these 12 patients, whereas none of the other patients whose insulin secretion was above the first percentile progressed to overt disease.

A combination of specific immunologic screening and assessment of insulin secretion using glucose-tolerance testing may enable persons who are at high risk for Type I diabetes to be identified, and may also enable us to predict when overt diabetes will occur. We are currently testing this hypothesis in a randomized study, and we are also determining whether alternate-day prednisone, which suppresses Ia-positive T cells, will retard or prevent progression to overt diabetes.[73]

## Stage V: Overt Diabetes

By the time that overt hyperglycemia develops, extensive beta-cell destruction has already occurred. At this stage, most islets have cells that contain glucagon, somatostatin, and pancreatic polypeptide but have no beta cells.[2] Inflammation within islets seems to correlate with the presence of residual beta cells. A number of pilot studies of immunotherapy at the onset (Stage V) of Type I diabetes have been completed. Prednisone given after onset increases C-peptide secretion[74] and suppresses Ia-positive T cells[34] but has little metabolic benefit (perhaps because of the insulin resistance associated with its use).[74] Horse antithymocyte globulin and a monoclonal antibody (T12) that reacts with human T cells (given for approximately one week) have only short-term effects on metabolic control. Administration of antithymocyte globulin has been associated with severe transient thrombocytopenia.[75] Plasmapheresis,[76] levamisole,[77] and inosiplex[78] have little clinical effect.

The most promising pilot study of immunotherapy in Type I diabetes has involved the drug cyclosporine,[79] which selectively blocks the activation of T lymphocytes. Stiller et al.[79] in Canada have treated more than 50 patients with recent-onset diabetes. The group treated within six weeks of diagnosis appears to have had the best response to therapy, but several subjects who were treated later also responded. After one year of treatment, approximately half the patients treated within six weeks of the onset of diabetes were no longer taking insulin and had an improvement in glucose homeostasis (levels of glycosylated hemoglobin were near normal). In several patients in whom cyclosporine was discontinued, diabetes recurred, often preceded by a reappearance of islet-cell antibodies. No immunologic measure (in particular, the presence or absence of islet-cell antibodies) predicted who would respond to therapy.

The common side effects of cyclosporine included gingival hyperplasia, hirsutism, and a small but notable decrease in hematocrit and creatinine clearance. All side effects disappeared when the drug was discontinued. When used at higher doses or in combination with other immunosuppressive drugs (or both), cyclosporine has been associated with side effects that were more severe than those seen in the Canadian trial or in other trials of cyclosporine in autoimmune disease. These effects included the development of lymphoma and irreversible renal damage.[80,81]

Despite the generally positive findings of the Canadian trial, it must be appreciated that the trial was not randomized, and that cyclosporine has been used in humans for only approximately six years. The true risks and benefits associated with this therapy must be defined by controlled randomized trials, which are under way at a number of research centers in the United States and Europe.

Azathioprine, a powerful antiproliferative and immunosuppressive drug, appears to have blocked the recurrence of insulitis and diabetes in a fourth identical-twin transplant studied by Sutherland and coworkers.[61] Trials of this immunosuppressant in patients with new-onset Type I diabetes are under way.

## Conclusions

During the past decade tremendous advances have been made in basic immunology, including the discovery of gene rearrangements that create antibody di-

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versity; the isolation of the T-cell receptor; the biochemical characterization of Class I, II, and III genes within the major histocompatibility complex; production of monoclonal antibodies to define resting and activated T cells; and the discovery of interleukin-2 (T-cell growth factor) and its use in the creation of continuous T-cell lines. The study of Type I diabetes has obviously benefited from these immunologic advances, and yet remarkably little is understood about the immunology of this disease. Nevertheless, because of these advances in immunology and the data that link autoimmune phenomena to Type I diabetes in human beings, Biobreeding rats, and non-obese diabetic mice, some directions for future research on Type I diabetes are obvious. Putative target antigens must be biochemically characterized; T-cell lines that are capable of transferring diabetes in rodent models must be developed, and the actual gene within the major histocompatibility region that contributes to susceptibility to diabetes must be isolated. If a limited clonal response creates the autoimmunity of Type I diabetes, immunization to prevent the disease may some day be a reality. Barring the discovery of environmental factors that specifically trigger the disease, the immunization I envision would use selected T-cell–receptor peptides that might lead to the generation of antibodies against beta-cell autoreactive T-cell clones.

## Discussion

Dr. Jeffrey S. Flier: The good news, as you pointed out, is that over the past few years it has become increasingly clear that autoimmunity has a key role in Type I diabetes. The bad news is that the knowledge that other diseases are autoimmune has not led to a cure of the disease. Do you think that Type I diabetes, because the antigen has been so well defined, will be one of the first of such diseases to succumb to immune therapy, or do you think that in general the kinds of tools that are now available will apply to the cure of all autoimmune diseases?

Dr. Eisenbarth: My answer is a little of both. There are very few islet cells, and yet in diabetes it takes the body nine years to destroy them. However, when beta cells are transplanted, the immune response destroys these cells within months. So I believe there is something unique about Type I diabetes. Maybe it is more akin to polymyalgia rheumatica, which responds to some safe therapies that we have now. Also, the Biobreeding rat is more or less a unique model, and diabetes in this animal is preventable with many types of immune therapy. In addition, cyclosporine is starting to be used at a low dose for many diseases. Many problems are associated with it, but in patients with uveitis, patients with Type I diabetes, and 800 patients treated for autoimmunity, low doses have not led to lymphomas or progressive renal toxicity. It may well be that the first generation of many drugs such as cyclosporine used at low doses would be helpful. Diabetes may be a very slow autoimmune disease that responds like thyroiditis.

Dr. Flier: I wanted to go back to some earlier data of Irvine. He looked at islet-cell antibodies by similar techniques and reported that among the adults with diabetes who were apparently not insulin-dependent but who were given sulfonylureas without benefit, a good number had islet-cell antibodies. Can you comment on whether this finding has been supported in subsequent studies?

Dr. Eisenbarth: Such results have been confirmed by a number of studies. About 15 percent of patients who are classified clinically as having Type II diabetes have islet-cell antibodies, and on the average, within four years they become insulin dependent. I think that if you have islet-cell antibodies, you have a disease akin to Type I diabetes, and you have the HLA types DR3 and DR4. The same is true of studies of gestational diabetes. I think the assay for islet-cell antibodies will be used prognostically in at least four settings: Type II diabetes, gestational diabetes, so-called pre-diabetes, and potential renal-transplant donors.

Dr. Flier: That would mean that Type II diabetes with islet-cell antibodies is more prevalent than the usual Type I diabetes, given the 10 to 1 predominance of Type II versus Type I.

Dr. Eisenbarth: Right. There are many patients with adult-onset Type I diabetes. When we screen, we often find that a parent has islet-cell antibodies.

A Physician: Do you think it is reasonable to screen patients with adult-onset diabetes and then look at their children as a predictor if the patients are positive?

Dr. Eisenbarth: I think the real issue is whether you change their therapy. Someone with islet-cell antibodies can still have the disease managed with oral agents for a while. My own bias is that I would like to know whether islet-cell antibodies are present because that tells me something about the disease course. Screening the relatives, I think, is also a good idea. However, the islet-cell antibodies disappear in 90 percent of subjects after the onset of diabetes, so if someone has had diabetes for 15 years, one cannot determine whether antibodies were once present. The odds are 10 to 1 against you. When the antigen is gone, it looks as though the antibody disappears in most cases.

Dr. Flier: In vitro studies of cell-mediated immunity were reported in the late 1970s but have been limited in number in recent years. Why are these studies lagging? Is it because there is no appropriate antigen to study with activated lymphocytes in vitro?

Dr. Eisenbarth: I think it is because the studies are so difficult. Also, many of us here think that T-cell–mediated mechanisms are going to prove most imporant, as they are in Biobreeding rats. In this model it is very clear that if T cells are transferred from diabetic animals, the disease is also transferred. We would like to have the antigen so that we could drive T-cell clones. There is a lot of ongoing work in this area.

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DR. GEORGE CAHILL: You made a conflicting statement. You stated that HLA-identical siblings have about the same concordance rate as identical twins. That leaves little room for polygenic inheritance, except for HLA.

DR. EISENBARTH: The concordance rate for identical twins is apparently higher than it is for HLA-identical siblings. I think that the polygenic part is also going to be in the HLA region. The reason I say this is that if you take identical twins who have Type I diabetes and you look at concordance rates, even though they are identical, if they are DR3/DR4 positive the concordance rate is 70 percent, whereas it is 40 percent if they are not DR3/DR4 positive.[24] So there is a synergism of presumably at least two different genes in that region. In addition, if you look at family typing, it is not DR3 and DR4 that are so important; if you really want to predict, you just find out what haplotype they have inherited. There are families in which only the diabetic has inherited DR2/DR1. One question is: Is there another gene, perhaps recessive, in that histocompatibility complex in addition to the HLA-D specificities that determines susceptibility to diabetes? My working hypothesis is that there is more than one gene in the histocompatibility complex.

A PHYSICIAN: Along those lines then, what is known about localization of the lymphopenia gene in the Biobreeding rat? Is it linked to the major histocompatibility complex?

DR. EISENBARTH: The inheritance of the lymphopenia gene is totally independent of the histocompatibility region. The other interesting thing from the viewpoint of multigenetic inheritance is that different persons have different syndromes. For instance, there is a syndrome — polyendocrine autoimmune syndrome Type I with Addison's disease, mucocutaneous candidiasis, and hypoparathyroidism in young babies — that is purely an autosomal recessive illness. Type I diabetes develops in about 5 percent of the patients with this syndrome. Its inheritance is totally different from that of diabetes and is not related to HLA or DR3. But I think there is room for different genes in different people, and my best guess is that the histocompatibility region is the area of choice, but the whole genome needs to be looked at.

A PHYSICIAN: What about the combination of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness?

DR. EISENBARTH: It is not HLA-related. However, I haven't seen good immunologic studies.

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