

Recent advances in the immunopathogenesis of insulin-dependent diabetes mellitus

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Insulin-dependent diabetes mellitus places a major burden on both affected individuals and society because of its high morbidity and mortality rates and financial costs. That the disease occurs in genetically susceptible individuals as a result of an immunologically mediated process thought to be triggered by environmental factors probably operating in early childhood is well established. Enhanced knowledge of the immunopathogenesis, genetics, and natural history of insulin-dependent diabetes mellitus in nonobese diabetic mice and humans has enabled investigators to better predict disease onset and design therapies aimed at its prevention. Major national and international multicenter trials are currently in progress, engendering cautious optimism that the disease may safely be prevented.

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Whereas just over 10% of patients with diabetes have type 1 or insulin-dependent diabetes (IDD), their contribution to the morbidity and mortality statistics and the health care burden (which approximates \$105 billion or one in seven US health care dollars spent) is disproportionately large, owing to the high rate of microvascular and macrovascular complications [1]. Although the Diabetes Control and Complications Trial in patients with IDD has highlighted the strong association between tight blood glucose control and reduced risk of complications [2], it is clear that intensive insulin therapy is not available, applicable, or affordable for all patients with diabetes. The need is therefore obvious to prevent IDD—the paramount goal for those caring for patients with IDD.

This review focuses on recent advances in the etiology and immunopathogenesis of the disease and the natural history of the prediabetic period. Intervention during the long prediabetic period carries the hope that IDD may be delayed or prevented altogether through therapies that interrupt the causal disease process. Data from both humans and the prototypic animal model, the nonobese diabetic (NOD) mouse, are presented. Despite advances in the study of human IDD, the cellular events that produce β -cell destruction in humans cannot as yet be directly assayed. The NOD mouse is an invaluable model, because access to the pancreas permits a more direct analysis of β -cell destruction. Understanding the cellular events mediating β -cell destruction should ultimately provide the bases for preventive strategies. Additionally, genetic studies in NOD mice have fostered a greater understanding of genetic susceptibility to IDD.

Human insulin-dependent diabetes

The thesis that IDD occurs in genetically susceptible individuals from an indolent autoimmune destruction of the pancreatic β -cells, beginning usually before the age of 10 years, is generally accepted. Triggering of the autoimmune disease process is most likely due to exposure to environmental factors in early childhood. A prodromal period invariably follows, with immunologic and metabolic abnormalities detected long before the onset of clinical disease. Islet cell antibodies (ICAs) have been detected in our own studies up to 12 years before the onset of IDD [3,4]. A decline in insulin secretion 11 to 12 years before the onset of clinical disease has been reported in prediabetic subjects [5,6]. The variable age of onset of disease probably reflects different rates of β -cell destruction. Not all ICA-positive subjects will develop disease, however. Similar to other autoimmune disorders, the disease may wax and wane, resulting in temporary or permanent remissions [3–5,7].

Younger age at onset is associated with greater genetic loading, *ie*, higher frequencies of HLA DR3/4 and DQB1 0201/0302 heterozygotes [8,9]. Concordance rates for identical twins are also much higher when the index case is diagnosed before the age of 10 years [10]. Rapidity of β -cell loss appears inversely related to age, culminating in a peak diabetes incidence at puberty. Histologic examination of the pancreas in toddlers and young patients with IDD [11,12] rarely reveals β -cells, whereas in older, newly diagnosed IDD patients, some functional β -cells are often detected [13]. Approximately 25% of IDD patients develop disease

Abbreviations

GAD—glutamic acid decarboxylase; IAA—insulin autoantibody; ICA—islet cell antibody; IDD—insulin-dependent diabetes; MHC—major histocompatibility complex; NOD—nonobese diabetic.

after the age of 35 years [14]. Many of these patients may not initially require insulin and may be mistakenly labeled as having non-insulin-dependent diabetes for several years prior to progressing to insulin dependency. Recent studies, particularly in such patients with normal or decreased body weight, have documented ICAs, glutamic acid decarboxylase (GAD)₆₅A, low insulin levels, and higher frequencies of DR3 and DR4 [15,16].

Genetic susceptibility

Of patients developing IDD, 85% have no family history of the disease, yet the risk of developing diabetes when one family member is affected increases roughly 15-fold from one in 300 to approximately one in 20. The risk is almost double for those born to affected fathers and reduced by half for children of affected mothers [17]. HLA-identical siblings of a diabetic proband have an absolute risk of developing IDD of approximately one in seven, rising to one in four when HLA-DR3 and DR4 are shared. The highest risk is in identical twins, affecting one in three. The risk of the second twin developing disease is highest in the 3 years following diagnosis in the first and falls to under 2% after 6 years [18].

The HLA class II DR and DQ loci on the short arm of chromosome 6 provide the major genetic influence (Fig. 1). The DR alleles 1, 3, 4, 8, and 16 (DR2) convey susceptibility, whereas DR 5 and 15 (DR2) impart protection [8]. The DQA and DQB gene loci, which are in strong linkage disequilibrium, may have an even more marked influence on diabetogenesis [19]. The DQB1*0302 allele associated with DR4 has the strongest influence on inherited predilection to IDD [20]. Absence of aspartic acid at position 57 (*eg*, a valine, serine, or alanine substitution) or the presence of arginine at position 52 of the DQA1 chain strongly increases diabetes susceptibility [21,22]. DR3-associated DQA*0501/DQB*0201 and DR4-associated DQA*0301/DQB*0302 are the common IDD-prone haplotypes, with one in 20 such heterozygotes affected with IDD [23]. In a recent study in new-onset IDD patients, assessment of the DQB1-DQA1 complete genotype appears to allow for the best prediction of IDD [24]. Forty percent of IDD patients were homozygous for both DQB1 non-Asp and DQA1 Arg alleles, whereas this genotype was not seen in any normal subjects. For the protective DR2 allele, the DR15-associated DQA*0102/0602 haplotype strongly confers protection [25]. Whereas 25% of the white population has this allele, it is found in only 0.5% of IDD patients.

Other loci associated with IDD include the non-HLA insulin gene locus located on chromosome 11 [26,27], the transporter antigen presenting carrier molecules [28], and most recently an additional susceptibility gene on chromosome 2q31 near *HOXD8* [29]. It is probable that multiple genes, both HLA and non-HLA associated, have interactive roles cumulating in aggregate to determine a threshold susceptibility.

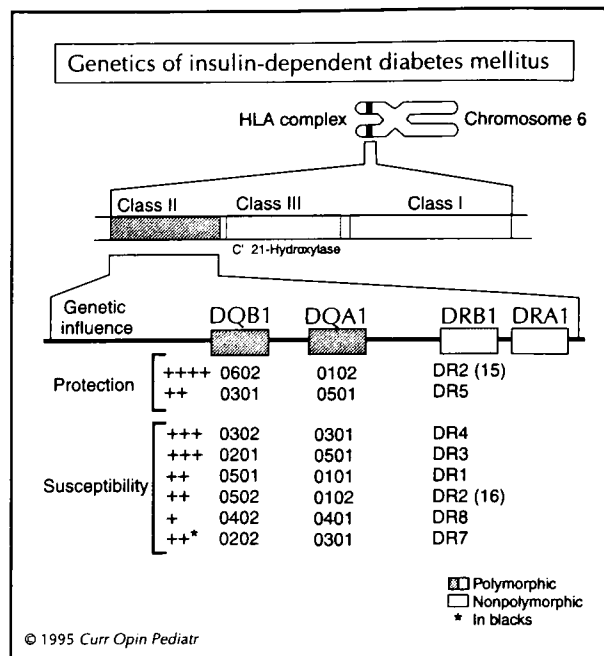


Fig. 1. The major histocompatibility complex in humans, known as the HLA complex, is located on the short arm of chromosome 6. The HLA complex contains three classes of genes. Among the class II genes, DQB1 and DQA1 encode the β and α chains of HLA-DQ molecules, and DRB1 and DRA1 encode the β and α chains of HLA-DR molecules. DRA1 is nonpolymorphic. DQB1, DQA1, and DRB1 alleles are usually in linkage disequilibrium and are therefore inherited together. As shown, various sets of class II major histocompatibility complex alleles influence protection from and susceptibility to insulin-dependent diabetes mellitus to different degrees.

Environmental factors

The documented secular trend of increased incidence rates for the disease has intensified the search for environmental triggers. However, when β -cell destruction actually begins is unclear. Based on both autoantibody data and disease peak incidence between the ages of 5 and 15 years, environmental factors must operate in early childhood [14,30•,31]. Molecular mimicry between β -cell autoantigens and environmental proteins has been proposed. Atkinson *et al.* [32•] demonstrated that the major determinant of the β -cell autoantigen GAD included a region (amino acids 27 to 279) with sequence similarity to the P2-C protein of coxsackievirus B. In a provocative paper, Conrad *et al.* [33•] suggest that superantigen expression by pancreatic β -cells may play a vital role in diabetogenesis. In two patients with IDD who died soon after disease onset, the authors found that T cells bearing a particular V β domain (V β 7) selectively infiltrated the islets. In contrast, NOD mice demonstrate a more unbiased V β T-cell repertoire within the islets. Larger confirmatory studies and the identification of the superantigen are necessary to accept the superantigen-diabetes hypothesis. Other examples of potential molecular mimicry include homology between a rubella virus capsid protein and an islet heat shock protein of 52 kD [34] and between ABBOS, a short (17-amino-acid) segment of bovine serum albumin, and a peptide expressed by human islets, p69 [35]. The latter is an attractive hypothesis, because several

epidemiologic studies have shown a greater risk of developing diabetes in children who were not breastfed, whereas breastfeeding for more than 3 months may protect infants from IDD [36,37*]. However, the subject has been hotly debated, with contrasting reports of immunoreactivity to bovine serum albumin observed in IDD [38].

β -Cell autoimmunity

Humoral immunity

Because the risk of developing diabetes is much higher (15- to 20-fold) in families with a diabetic proband than in the general population, most studies have focused on higher-risk relatives. For nonaffected relatives with ICAs alone, diabetes occurs in approximately one in four persons older than 5 years of age [3]. When ICAs are present in younger subjects, being the sibling or child of an affected individual, having a high titer of ICAs, or having DR3/4 heterozygosity dramatically increases the risk of developing IDD [3,4]. Although insulin autoantibodies (IAAs) alone carry a very low risk for progression to disease, their presence in patients with ICAs greatly increases the risk to 50% to 70% over 5 years [4,39]. β -Cell-selective or restricted ICAs reportedly are associated with a lower risk of progression to diabetes than those staining the entire islet [40,41].

Because approximately 85% of patients with IDD have no family history of IDD, it is crucial to determine whether the natural history of the disease in the general population is the same as that in higher-risk relatives. In a recent study in which nearly 10,000 South Florida school children were followed up prospectively over a 10-year period, ICAs (and IAAs) were as predictive of IDD as were their presence in similarly aged nondiabetic relatives of IDD probands [42**]. Just as with relatives, ICA-positive schoolchildren progressing to IDD were likely to be DR3/4 heterozygotes or have the high-IDD-risk DR3 or DR4 alleles. Collectively, these data argue for a similar disease process and natural history in relatives and the general population, and that screening a general population with ICAs and IAAs is feasible for prediction.

Autoantibodies to a 64-kD islet protein, subsequently identified as GAD₆₅, are closely linked to IDD, being previously reported in approximately 80% of relatives before diagnosis [43]. We have recently shown by radioimmunoassay that ICA and GAD₆₅ autoantibodies are found in 79% and 71% of prediabetic relatives, respectively, which was similar to the levels found in newly diagnosed patients [44*]. In nondiabetic relatives, GAD₆₅ autoantibodies greatly increase the predictability of IDD suggested by ICAs or IAAs alone but appear to add little to disease prediction when both ICAs and IAAs are present.

Antibodies to 37- to 40-kD tryptic fragments, distinct from GAD₆₅, are reported in the majority of IDD subjects, yet they are present in only 2% of discordant twins and appear generally absent from ICA-positive polyglandular patients who have not developed diabetes [45]. Antibodies to a 38-kD islet cell secretory granule have also been described in GAD₆₅-neg-

ative, ICA-positive, prediabetic individuals. However, the prognostic utility of these additional autoantibodies remains to be determined.

Cellular immunity

Enhanced islet cell-reactive T-lymphocyte responses [46], peripheral blood mononuclear cell reactivity to GAD₆₅ [47] and insulin [48], increased β -cell cytoadherent T lymphocytes [49], and defective CD4+ CD45RO immunoregulatory abnormalities [50] have all been described in prediabetic subjects. Abnormal lymphocyte subsets, including increased CD4+ CD45RA suppressor-inducer and decreased CD4+ CD45RO helper-inducer subsets (naive and memory cells, respectively), have also been noted [50–52], and elevated levels of the circulating adhesion molecules intercellular adhesion molecule-1 and L-selectin [53] and decreased peripheral blood monocyte major histocompatibility complex (MHC) class I expression have been suggested in at-risk relatives [54]. Although these findings may give insight into disease pathogenesis, they have not been helpful in prediction of IDD.

Metabolic derangements

When serologic markers are considered together with metabolic changes, the predictability of IDD increases even further [55]. In our studies, which form the basis for the recent National Institutes of Health-funded Diabetes Prevention Trial, persistent loss of first phase insulin secretion measured during intravenous glucose tolerance testing in ICA-positive relatives carries at least a 50% risk of progression to disease over 5 years. Higher rates of progression are observed in those with the greatest ICA titers and those in whom ICAs are first detected at a very young age. Changes in glucose tolerance measured during oral glucose tolerance testing occur late in the disease process, reflecting significant β -cell destruction and impending clinical disease.

Nonobese diabetic mice

Immunopathogenesis

Autoantigens

Defining the target antigens in IDD appears critical for developing strategies to induce immune tolerance (downregulation of the self-immune process that otherwise causes β -cell destruction). Generation of suppressive cytokines such as interleukin-4, interleukin-10, and transforming growth factor- β could play a role in tolerance. If human IDD and NOD mouse IDD share similar pathogenesises, they should share target autoantigens. Just as with humans, NOD mice produce GAD autoantibodies. However, whereas human IDD sera predominantly react with the 65-kD molecular weight form of GAD (GAD₆₅), mouse islets predominantly express GAD₆₇ and appear to express lower concentrations of GAD than do rat and human pancreata [56,57]. These differences raise the question of commonality of pathogenesis in NOD mouse IDD and human IDD. Tisch *et al.* [58] demonstrated that the first autoantibodies in NOD mice are directed against GAD₆₅ and GAD₆₇ at 4 weeks of age, coincident with

the onset on insulinitis. These responses occur earlier than antibody responses to carboxypeptidase H, peripherin, or heat shock protein 60. In 16-week-old mice, compared with other strains, only NOD mice displayed cellular responses to GAD₆₅ and GAD₆₇, suggesting that GAD is a critical autoantigen. Intrathymic injection of GAD₆₅ delays the onset and decreases the frequency of IDD by about half, suggesting that tolerization of GAD is beneficial. Gelber *et al.* [59] has also shown that unprimed peripheral blood T cells from NOD mice respond to islet proteins other than GAD. These T cells also responded to proteins from human islets, suggesting other shared autoantigens.

Cellular events in insulinitis and diabetogenesis

Insulinitis and its consequent β -cell destruction clearly involves macrophages and T cells. Macrophages not only serve as antigen-presenting cells but may also mediate β -cell damage through liberation of toxic products (eg, cytokines and reactive oxygen species). Increased numbers of macrophages are found in the peri-islet areas at 18 to 22 days, which precedes the histologic observation of insulinitis. By the time early insulinitis is observed at 30 to 40 days, increased numbers of macrophages are also found within the islet and exocrine pancreas [60].

Langmuir *et al.* [61] have suggested that the observed impairment in macrophage development results from deficient Ly-6C expression in the NOD mouse bone marrow. Deficient bone marrow responses to interleukin-3 were also demonstrated, suggesting intrinsic defects in the development of the NOD mouse immune system. This finding adds to the earlier report that NOD bone marrow is intrinsically intolerant to β -cell antigens. In LaFace and Peck's [62] studies, bone marrow stem cells transplanted into lethally irradiated C57BL/6 mice permitted the development of insulinitis in a strain otherwise displaying no tendency to β -cell autoimmunity.

Both CD4⁺ T cells and CD8⁺ T cells are necessary for both the development and adoptive transfer of IDD [63]. Regulatory CD4⁺ T cells can be subdivided according to their expression of different CD45 isoforms. In mice, the CD45R⁺ population appears dominantly as the Th1 subset that secretes interleukin-2 and interferon gamma, spawning cell-mediated responses that may lead to insulinitis and β -cell destruction. CD45R⁻ T cells function more like Th2 cells, liberating interleukin-4 and interleukin-10, which induces humoral (protective) responses. CD3^{bright} CD4⁺ CD8⁺ and CD4⁺ CD8⁻ thymic CD45RA⁺ cell numbers are increased in NOD mice, with CD45RA⁺ expression being increased in all peripheral blood T cells [63]. Furthermore, most islet-infiltrating T cells are positive for CD45RA. Anti-CD45RA antibodies protect against the development of IDD.

In contrast to humans, in which IDD affects both genders fairly equally, 70% to 90% of female NOD mice and only 40% to 50% of males are affected. McDuffie and Ostrowska [64] proposed a "suppressive" role for

CD8 V β 8.3 T cells in the development of IDD. They enumerated T-cell receptor V β CD4 and CD8 T-cell subsets in male and female NOD mice between 2 and 17 weeks of age. In half the males between 16 and 20 days of age (just prior to the initiation of insulinitis), the percentage of CD8 T cells bearing V β 8.3 declined. At 28 to 30 days of age (earliest onset of insulinitis), male mice had increased percentages of V β 8.3 CD8 T cells. Female mice had no such increases. This hypothesis is bolstered by the ability of anti-V β 8 monoclonal antibody-activated acutely diabetic T cells to prevent diabetes on transfer to susceptible 2- to 3-week-old nondiabetic NOD mice.

Potential mediators of β -cell destruction

The precise mechanisms involved in immunologically mediated selective β -cell destruction remain elusive. T cells are clearly involved in the immunoregulatory process and possibly the destructive process, and macrophage liberation of reactive oxygen intermediates appears to further contribute to β -cell necrosis. Horio *et al.* [65], mixing isolated islets and either T cells or macrophages *in vitro*, demonstrated that whereas T cells alone did not induce reactive oxygen production, macrophages had a marked effect. A role for reactive oxygen in diabetogenesis was strongly suggested in studies showing that free radical scavenger (superoxide dismutase-polyethylene glycol or catalase-polyethylene glycol)-treated NOD mice displayed a 50% to 60% reduction in insulinitis.

Toxic mediators of β -cell destruction may also be induced in β -cells via local cytokine release. Corbett *et al.* [66] observed that on transfer of spleen cells from diabetic female NOD mice into recipient irradiated male mice, β -cells expressed increasing concentrations of nitric oxide. Coincident with rising β -cell nitric oxide content, glucose-induced insulin secretion declined, suggesting a causal relationship.

Although autoantibodies directed against islet cell antigens have long been recognized, the potential role of antibody-directed, complement-mediated lysis in the destruction of β -cells has not been fully resolved. Baxter and Cooke [67] recently demonstrated that NOD mice are C5 deficient. Deficiency of C5 would block complement-mediated lysis of targets via the membrane attack complex, making such a mechanism therefore extremely unlikely.

Genetics

Genetic characteristics of IDD in humans and NOD mice include 1) polygenic inheritance, 2) major contribution of class II MHC genes, and 3) contribution by several other non-MHC genes to IDD susceptibility. In NOD mice, it is now accepted that 10 or more loci are involved [68], including the critical MHC class II gene *H-287* or *Idd-1*; genes on chromosomes 3 (centromeric, *Idd-3*; telomeric, *Idd-10*), 11 (*Idd-4*), 7 (*Idd-7*), and 14 (*Idd-8*); and less important genes on chromosome 1 (*Idd-5*), 6 (*Idd-6*), and 4 (*Idd-9*) (Table 1). Studies of outcross-backcross breedings of NOD and NON mice have also identified *Idd-2* near *Thy-1* on chromosome 9. For all of these loci, potential human homologies require extensive investigation [69].

Table 1. Diabetogenic loci in nonobese diabetic mice

Susceptibility locus	Chromosome	Marker or gene	Relative influence
<i>Idd-1</i>	17	<i>H-2</i>	+++++
<i>Idd-2</i>	9	<i>Thy-1</i>	++
<i>Idd-3</i>	3	<i>Glut2/IL-2</i>	+++
<i>Idd-4</i>	11	<i>D11Nds1</i>	++
<i>Idd-5</i>	1	<i>IL-1R, Lsh/Ity/Bcg, Vil, Cd28</i>	+
<i>Idd-6</i>	6	<i>D6Mit14</i>	+
<i>Idd-7</i>	7	<i>D7Nds6 (Ckmm)</i>	++
<i>Idd-8</i>	14	<i>D14Nds1 (Plau)</i>	++
<i>Idd-9</i>	4	<i>D9Nds16</i>	+
<i>Idd-10</i>	3	<i>Tshβ, IgG-FcR</i>	+++

Other than *H-2^{s7}*, there are no genes that function as absolute recessives for the development of IDD in NOD mice [70]. Combinations of multiple genes of lesser importance "conspire" to provide susceptibility to IDD. Therefore, IDD appears to function as a threshold trait; when *H-2^{s7}* is in the homozygous state, any number of other genes of NOD or non-NOD origin in combination can provide sufficient susceptibility to induce IDD.

Idd-3 is near the genes for Glut-2 and interleukin-2. Although interleukin-2 in NOD mice contains an amino acid sequence variation, Chesnut *et al.* [71] could not demonstrate any aberration in NOD interleukin-2 function. On this basis, it is unlikely that interleukin-2 is the susceptibility gene in this region. Furthermore, there may be two susceptibility genes in this centromeric portion of chromosome 3. In studies of [(NOD \times NZW) F_1 \times NOD] backcross mice, Obata *et al.* [72] found that Glut-2 was associated with insulinitis, whereas interleukin-2 could not serve as a susceptibility gene, because NOD and NZW share identical interleukin-2 structural genes [71].

Idd-4, which influences early-onset IDD, is located near *D11Nds1*. A candidate gene for this region has not been identified. *Idd-7*, located on chromosome 7 near *D7Nds6 (Ckmm)*, is absent in NOD mice but is contributed by the C57BL/10 background. *Idd-8*, located on chromosome 14 near *D14Nds1 (Plau)*, likewise is not carried by NOD mice but is supplied by the C57BL/10 background.

Idd-10 is located on the telomeric portion of chromosome 3 near *Tsh β* . However, *D3Nds11 (Fcgr1)*, near the IgG-Fc receptor gene, is even more interesting. Prins *et al.* [73] showed that NOD mice carry a defective IgG-Fc receptor. In turn, there is a marked reduction in the turnover of cell surface receptor-antibody complexes.

Idd-5 is interesting in that this region contains the interleukin-1 receptor (*IL-1r α*) as well as the immune response locus *Lsh/Ity/Bcg*, the calcium-regulated actin-binding protein villin (*Vil*), and CD28 (*Cd28*), which is the T-cell ligand that is bound by B7 present on antigen-presenting cells.

Conclusions

Enhanced knowledge of the immunopathogenesis, genetics, and natural history of IDD in NOD mice and humans has enabled investigators to better predict disease onset. Disease prediction has engendered cautious optimism that IDD may be prevented. Several national and international multicenter trials are currently in progress that will attempt to prevent IDD through antioxidant therapy (with nicotinamide), β -cell rest and tolerization (with insulin), or environmental modulation (by avoidance of cow's milk).

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The major determinant of GAD recognized by newly diagnosed subjects and persons at increased risk for IDDM are amino acids 27 to 279, a region that has significant sequence similarity to the P2-C protein of coxsackievirus B. These findings support the epidemiologic evidence suggesting an inductive role of coxsackievirus B in the autoimmune process underlying IDDM. The concept of mimicry between islet antigens and potential environmental triggers is further strengthened.

Provocative study causally relating superantigens to the immunopathogenesis of IDDM. Larger confirmatory studies and identification of the superantigen are awaited.

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