Autoimmunity and Genetics Contribute to the Risk of Insulin-dependent Diabetes Mellitus in Families: Islet Cell Antibodies and HLA DQ Heterodimers

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The risk for insulin-dependent diabetes mellitus (IDDM) associated with genetic susceptibility markers at the human leukocyte antigen (HLA) DQA1 and DQB1 loci was evaluated among individuals with and those without islet cell antibodies. A total of 108 antibody-positive parents and siblings of IDDM patients from the Pittsburgh registry were identified among 1,592 who were screened. HLA-DQ molecular typing was performed on 79 of these individuals and on 78 antibody-negative relatives. There were similar proportions of homozygotes for both of the diabetogenic alleles DQA1 arginine-52 (R/R) and DQB1 non-aspartate-57 (nD/nD) among the antibody-positive and antibody-negative relatives (19.0 and 15.4%, respectively). However, subsequent development of IDDM was restricted to individuals who were both antibody positive and carried the potential to make at least one diabetogenic DQ heterodimer. A dose-response effect was observed among the antibody-positive relatives, in which two of 18 capable of generating one diabetogenic heterodimer and six of 29 generating two heterodimers became insulin requiring. Nine of 15 who were homozygous for both R/R and nD/nD, coding exclusively for diabetogenic variants, became diabetic over the course of the follow-up. With a multivariate model, the relative risk for IDDM among those with islet cell antibodies who were also R/R and nD/nD was estimated to be 229.3 compared with those lacking both, after age and sex were controlled for. The data suggest that while autoimmunity, indicated by the presence of cytoplasmic islet cell antibodies, may be relatively common, it progresses only in those with variant HLA-DQ molecules.

Insulin-dependent diabetes mellitus (IDDM) has been recognized as an autoimmune disease, with insulin deficiency resulting from a misdirected immunologic assault...
on the beta cells of the pancreas. Multiple immune system changes are found in newly diagnosed IDDM patients, including auto-antibodies to cytoplasmic antigens of the endocrine cells of the pancreas. These islet cell antibodies were first described in 1974 (1, 2) and have been found to be present in 65–85 percent of all newly diagnosed IDDM patients, in 3–5 percent of family members, and in less than 0.5 percent of the general population. Antibody-positive individuals were found to be 50–200 times more likely to develop diabetes than antibody-negative individuals in several studies (3) so that the presence of islet cell antibodies is considered a potent risk factor. Nonetheless, fewer than 50 percent of nondiabetic subjects have developed diabetes during the course of several follow-up studies among persons who were positive for cytoplasmic islet cell antibodies (4–7). Intensive effort in search of the relevant antigen has led to the identification of two islet-cell moieties, one of with a molecular weight of 64,000 and identified as the enzyme glutamic acid decarboxylase, and the other an apparently unrelated polypeptide with a molecular weight of 37,000 M.

Genetic studies provide additional potent markers of insulin-dependent diabetes mellitus risk. Associations with specific alleles of the genes of the major histocompatibility complex on chromosome 6 were discovered in the mid-1970s, initially among the class I alleles, human leukocyte antigen (HLA)-B8 and HLA-B15 (10) and then with the DR region alleles, DR3 and DR4. Most recently, polymorphisms associated with position 52 of the HLA-DQA1 chain and position 57 of the HLA-DQB1 chain have been found to confer susceptibility to IDDM (11–13). US whites who carry two alleles which code for an amino acid other than aspartate at codon 57 or HLA-DQB1 non-aspartate-57 homozygotes (nD/nD) are approximately 100 times more likely to develop IDDM than are those who are homozygous for aspartate at this position (12). Similarly strong associations have been established for DQA1 alleles coding for arginine at position 52 (R/R) (14–17). Recent work (18) combining genotypes at both loci indicates that susceptibility to IDDM resides in the configuration of the immunologically active DQ gene product: Those heterodimers containing both an Arg-52-positive DQA1 subunit and a non-Asp-57 DQB1 subunit confer susceptibility. Since each person inherits two alleles coding either for susceptible (Arg-52 DQA1 and non-Asp-57 DQB1) or protective (non-Arg-52 DQA1 and Asp-57 DQB1) alleles, every human being has the potential to produce zero, one, two, or four susceptible forms of the active DQ heterodimer. For simplicity, these individuals will be denoted respectively Sus0, Sus1, Sus2, or Sus4.

In light of these recent immunologic and genetic findings, we hypothesized that combining islet cell antibody testing with DQ-typing would provide an extremely powerful means of predicting subsequent disease. This possibility was evaluated among the high-risk first-degree relatives of insulin-dependent diabetes mellitus patients from the Allegheny County, Pennsylvania, registry.

MATERIALS AND METHODS

A matching strategy was employed to determine the relation of DQ alleles to the presence of islet cell antibodies and the re-
lation of these markers together to the development of IDDM.

**Study population**

The Pittsburgh registry includes all IDDM patients diagnosed in Allegheny County, Pennsylvania, since 1965 and comprises a well-defined population which has served as the basis for many investigations of the etiology of IDDM (19). Current ascertainment (1985-1990) is estimated to be 94.8 percent county-wide (T. Dokheel, University of Pittsburgh, personal communication, 1992). Nondiabetic first-degree relatives were followed with annual blood draws for glucose and/or glycosylated hemoglobin, and sera were stored for future viral testing. Some people also participated in more specialized protocols. The underlying population thus consisted of 3,270 nondiabetic parents or siblings of patients who had one or more yearly blood samples drawn (7). Families who were not seen regularly were contacted approximately every 2 years to determine diabetes and mortality status. Follow-up was calculated from the date of the initial blood draw for islet cell antibodies until conversion to IDDM or the last date of contact. The average length of follow-up was 4.9 years (range, 0.2–8.5 years) for the islet cell antibody-positive subjects and 5.7 years (range, 0.2–8.6 years) for the antibody-negative relatives.

Individuals were considered to have converted to IDDM if, in the absence of obesity, they started and have continued to use insulin after having at least one previous blood sample with a glucose value which was not in the diabetic range. Diabetes was diagnosed if a fasting plasma glucose reading exceeded 140 mg/dl on two occasions or if a postprandial reading exceeded 200 mg/dl, with or without an elevated stable glycosylated hemoglobin value (greater than 7.3 percent). Alternatively, the results of an oral glucose tolerance test were used; the standard National Diabetes Data Group criteria were applied (20). Eight relatives (five antibody positive and three antibody negative) reported a diagnosis of non-insulin-dependent diabetes mellitus (NIDDM), and one antibody-positive sister had gestational diabetes. Because of the inherent difficulties of distinguishing NIDDM from slowly progressive IDDM in these subjects, they were excluded from the analyses of subsequent conversion to insulin reliance. However, six of these individuals were DQ typed.

**Laboratory methods**

Islet cell antibody testing was completed for 1,592 of the overall group of first-degree relatives. The University of Florida Department of Pathology and Laboratory Medicine, Gainesville, Florida, evaluated the majority of the relatives (n = 1,412), who were selected randomly. Forty-nine individuals (3.5 percent) were positive for islet cell antibodies, resulting in a prevalence rate similar to those reported elsewhere (3). Islet cell antibody testing of the remainder of the samples was performed by the Department of Endocrinology, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, and focused on multiple case family members, families with HLA-A- and HLA-B-identical siblings, and the stored sera of those who subsequently became diabetic; a further 59 positive subjects were found. From the 1,484 antibody-negative relatives, we matched 108 subjects on the basis of age (± 2 years), sex, and parent/sibling status with the 108 parents and siblings who were antibody positive.

Assay for islet cell antibodies was performed in Florida, using an immunofluorescent procedure on human pancreas substrate (21) and in Pittsburgh using an immunoperoxidase technique on a rat pancreas substrate (22). In the two most recent Juvenile Diabetes Foundation islet cell antibody proficiency tests, the Pittsburgh data showed 100 percent sensitivity and 100 percent specificity compared with that in Gainesville. Agreement between the two assays on a further 20 samples was 90 percent (data not shown).

HLA-DQ typing was performed by subjecting genomic DNA extracted from lymphocytes to the polymerase chain reaction.
The second exons of the DQA1 and DQB1 genes, each of which encoded the first external domain of their respective chains, were amplified. Dot-blot analyses were performed with allele-specific oligonucleotide probes (12, 17, 23, 24).

Among the 108 antibody-positive subjects, 91 (84 percent) had blood drawn for DNA analysis, and 79 were DQA1 and DQB1 typed. There were 12 subjects who refused further blood sampling, three who were lost to follow-up, and two who were deceased. The antibody-negative relatives were matched irrespective of whether a sample for DNA was obtained on the corresponding antibody-positive subject. There were 104 (96 percent) of 108 antibody-negative relatives who had blood drawn for DNA analysis, 78 of whom were DQ typed.

**Statistical methods**

Chi-square tests were used to evaluate the distribution of susceptibility markers in islet cell antibody-positive and -negative groups using the SPSSX statistical package (25). The BMDP 1L and 2L programs (26) were used for survival analyses of conversion to IDDM. For the Cox proportional hazards analysis, the number of potential susceptible DQ heterodimers was coded ordinally (SusO = zero, Sus1 = one, Sus2 = two, Sus4 = three).

**RESULTS**

Males and females were equally represented in the islet cell antibody-positive group (53 percent were female). Positive subjects had a mean age of 29.9 years at the time the blood sample was taken, while the mean age in the 1,484 antibody-negative individuals was 32.3 years (p = 0.17). The antibody-positive group was made up of 59 parents and 49 siblings of registered patients, which was nearly identical to the parent/sibling distribution in the group of islet cell antibody-tested subjects as a whole. Thus, the antibody-positive subjects generally resembled the entire group of subjects with respect to the conventional demographic variables.

Relatives positive for islet cell antibodies were identified and matched with antibody-negative relatives as described. The results reported here focus on the 157 individuals with molecular typing at DQA1 and DQB1. The relatives with DQ typing were similar to the 216 original study subjects with respect to age, sex (not shown), and time of follow-up. Of particular importance, the proportion of subjects who subsequently converted to IDDM was similar (table 1). However, a higher percentage of siblings (80.6 percent) than of parents (66.7 percent) were DQ typed (p = 0.02).

The analysis of HLA-DQ alleles by islet cell antibody status demonstrated that the potential for producing diabetogenic DQ heterodimers was similar among antibody-positive and antibody-negative relatives (table 2). Approximately 15 percent of each group lacked the ability to produce a susceptible heterodimer altogether, despite in many cases being homozygous R/R at DQA1 or nD/nD at DQB1. Similar frequencies of Sus1, Sus2, or Sus4 were noted in the antibody-negative and -positive groups (p = 0.816) (table 2). It should be noted that this analysis included those individuals who reported NIDDM or gestational diabetes.

### TABLE 1. Characteristics of DQ-tested subjects and the entire study group, first-degree relatives of IDDM* probands from the Pittsburgh registry

<table>
<thead>
<tr>
<th></th>
<th>DQ-tested</th>
<th>All subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of persons</td>
<td>157</td>
<td>216</td>
<td></td>
</tr>
<tr>
<td>Age (years) ± standard deviation</td>
<td>31.6 ± 14.2</td>
<td>28.9 ± 15.3</td>
<td>0.10</td>
</tr>
<tr>
<td>% ICA* positive</td>
<td>50.3</td>
<td>49.8</td>
<td>0.79</td>
</tr>
<tr>
<td>% developing IDDM</td>
<td>12.1</td>
<td>10.2</td>
<td></td>
</tr>
<tr>
<td>% developing NIDDM*†</td>
<td>3.8</td>
<td>4.2</td>
<td>0.31</td>
</tr>
<tr>
<td>% of parents tested</td>
<td>66.7</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>% of siblings tested</td>
<td>80.6</td>
<td>100.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean follow-up interval (years) ± standard deviation</td>
<td>5.6 ± 2.2</td>
<td>5.3 ± 2.1</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* IDDM, insulin-dependent diabetes mellitus; ICA, islet cell antibodies; NIDDM, non-insulin-dependent diabetes mellitus. † Includes one sister with gestational diabetes mellitus.
five of the parents who subsequently reported NIDDM were Sus2, four of whom were antibody positive and one of whom was antibody negative. The sister who was islet cell antibody positive and was diagnosed with gestational diabetes mellitus was found to be Sus1. Therefore, in none of the groups was an individual found to have converted to diabetes without the ability to produce at least one diabetogenic heterodimer of the four potential DQ configurations.

As discussed above, the six relatives with DQ typing who reported diabetes in the absence of insulin treatment were excluded from the analyses of subsequent conversion. Of the remaining 151 individuals, 74 were antibody positive and 77 were antibody negative. During the interval since islet cell antibody testing, 17 of the remaining 74 antibody-positive subjects with molecular typing (23 percent) have converted to insulin-receiving diabetes (figure 1). None of the Sus0 relatives developed diabetes, while two of 18 Sus1 (11.1 percent), six of 29 Sus2 (20.7 percent), and nine of 15 Sus4 relatives (60.0 percent) have converted to diabetes over an average of 4.9 years follow-up ($p < 0.001$). The data suggest a dose-response relation, with those producing greater proportions of diabetogenic DQ heterodimers experiencing greater risk (Mantel-Haentzel test for linear trend, $p < 0.0001$).

In the 77 nondiabetic antibody-negative relatives with DQ typing, two parents (2.6 percent) became insulin-receiving diabetic patients without having a previous blood sample which was positive for islet cell antibodies. Their samples were tested 0.7 and 2.7 years prior to the onset of diabetes. Further samples from these subjects were tested for islet cell antibodies: one of the parents had negative readings at 1 month and at 6 years postdiagnosis, while the other had an equivocal result which was read as negative 4 months after diagnosis, followed 1 year later by another negative reading. Both of these individuals were classified into the higher risk groups on the basis of DQ typing, one as Sus2 and the other as Sus4 (figure 1).
FIGURE 1. Number of nondiabetic relatives converting to diabetes on the basis of islet cell antibodies and the number of susceptible DQ heterodimers. ICA, islet cell antibodies; SUS, number of potential diabetogenic heterodimers; IDDM, insulin-dependent diabetes mellitus; RR, relative risk adjusted for age and sex.

who were Sus2 and antibody positive ($n = 29$); and those who were Sus4 and antibody positive ($n = 15$). Those who were both islet cell antibody positive and Sus4 suffered a cumulative risk over 6 years of 65 percent, compared with the risk of 23 percent for antibody-positive Sus2 and 7 percent for antibody-positive Sus 0 or Sus1. Based on just two antibody-negative cases, the cumulative risk was 8 percent for Sus4 antibody-
negative subjects and 3 percent for Sus2 relatives who were antibody negative ($p < 0.0001$, generalized Wilcoxon chi-square). The mean follow-up interval for the antibody-negative group was 6.0 years; no relatives who were antibody negative and either Sus0 or Sus1 have become diabetic thus far.

A Cox proportional hazards model was constructed including age at the beginning of follow-up, sex, DQ status, and islet cell antibodies (table 3). The multivariate model, controlling for the duration of follow-up, confirmed the importance of both genetic and immunologic processes in the etiology of IDDM. Controlling for other variables, the relative risk of being Sus4 compared with being Sus0 was 25.4 (figure 1), while being antibody positive alone carried a relative risk of 9.0. Using coefficients from this model, relatives who were both antibody positive and most genetically susceptible (Sus4) were estimated to be 229.3 times more likely to develop IDDM than those who were Sus0 and antibody negative.

**DISCUSSION**

This study reaffirms the central role played by both genetic and immunologic processes in the etiology of IDDM. It was undertaken with the idea that genetic susceptibility, as encoded in HLA DQA1 and DQB1 alleles, permits the development of an autoimmune reaction to the pancreatic beta cells and eventually a deficiency of insulin. We expected that by following the antibody-positive and -negative relatives for subsequent diabetes, we would be able to determine the interplay of islet cell antibodies with DQ. It is of critical importance to discern whether the genetic lesion permits the initial establishment of pancreatic autoimmunity or whether it is the progression or a more rapid progression of autoimmunity which occurs only in the presence of variant DQ types. The most striking finding in this analysis was the apparent quantitative relation between the proportion of DQ gene products with "susceptible" configurations and the risk of subsequent diabetes.

A potential confounding factor in this study is that a sample of first-degree relatives is likely to include a larger proportion of high-risk subjects than a sample drawn from the population at large. Using previous results from this population (12), we compared the prevalence of DQB1 nD/nD homozygosity among the 175 relatives in this cohort who had DQB1 typing with other local groups (12, 27). The prevalence of DQB1 nD/nD among first-degree relatives was three times higher than that among Allegheny County blood donors (table 4) and resembled that among a group of insulin-dependent probands. However, the prevalence was lower than among IDDM pa-

**TABLE 3. Relative risk for insulin-dependent diabetes mellitus in first-degree relatives of probands from the Pittsburgh registry: results of Cox regression models**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative risk</th>
<th>95% confidence interval</th>
</tr>
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<tbody>
<tr>
<td><strong>Relatives with antibody assay and DQ molecular typing (n = 151)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (+ 10 years)</td>
<td>0.84</td>
<td>0.60–1.18</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>1.88</td>
<td>0.73–4.83</td>
</tr>
<tr>
<td>No. of susceptible DQ gene products (Sus4* vs. Sus0)</td>
<td>25.39</td>
<td>3.61–178.73</td>
</tr>
<tr>
<td>Islet cell antibodies (positive vs. negative)</td>
<td>9.03</td>
<td>2.06–39.53</td>
</tr>
<tr>
<td><strong>Antibody-positive parents and siblings (n = 74)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (+ 10 years)</td>
<td>0.82</td>
<td>0.57–1.19</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>1.98</td>
<td>0.72–5.41</td>
</tr>
<tr>
<td>No. of susceptible DQ gene products (Sus4 vs. Sus0)</td>
<td>21.37</td>
<td>2.79–163.65</td>
</tr>
</tbody>
</table>

* Sus, susceptible forms.
TABLE 4. Distribution of DQB1 nD* alleles among 175 relatives compared with other DQB1-typed groups, Allegheny County, Pennsylvania

<table>
<thead>
<tr>
<th></th>
<th>nD/nD</th>
<th>nD/D†</th>
<th>D/D</th>
<th>(x^2) (2 df)</th>
<th>(p) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relatives ((n = 175))</td>
<td>97</td>
<td>55</td>
<td>69</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>Multiple case IDDM‡,§ ((n = 68))</td>
<td>60</td>
<td>88</td>
<td>8</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Single case IDDM‡ ((n = 49))</td>
<td>30</td>
<td>61</td>
<td>19</td>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>Blood donors‡ ((n = 123))</td>
<td>24</td>
<td>20</td>
<td>57</td>
<td>46</td>
<td>42</td>
</tr>
</tbody>
</table>

* nD, nonaspartate: susceptible alleles at DQB1 code for a residue other than aspartic acid at position 57. They are DQB1 0501, 0502, 0604, 0605, 0201, 0302.
† D, aspartate: protective alleles at DQB1 code for an aspartic acid residue at position 57. They are DQB1 0503, 0601, 0602, 0603, 0301, 0303, 0401, 0402.
‡ See reference 12.
§ IDDM, insulin-dependent diabetes mellitus; NS, nonsignificant.

patients from multiple case families. Similarly, the prevalence of being islet cell antibody positive in these relatives was approximately seven times higher than is found in the population at large (3). It is reasonable to expect that a relation between islet cell autoimmunity and genetic susceptibility markers would be obscured in a study population enriched with subjects positive for both. The adjusted relative risk of IDDM for being both antibody positive and Sus4 was comparable with that recorded elsewhere for islet cell antibodies alone and for DQB1 nD/nD (3, 4, 12, 13). Our subjects, however, were drawn from a uniquely high-risk population, and they refer to the risk differential among particular groups within this high-risk category. For this reason, validation of the interrelation of DQ alleles and islet cell antibodies must await study in unrelated non diabetic subjects with and those without islet cell antibodies.

The results of the survival analyses suggest that, at least within these high-risk families, being genetically at risk was not directly associated with islet cell antibodies, but that the presence of both susceptible DQA1 and DQB1 alleles and islet cell antibodies was required for diabetes to occur. Using the beta coefficients from the first Cox regression model in table 3, including all 151 relatives, we find that those relatives with both islet cell antibodies and Sus4 were over 200 times more likely than the least susceptible relatives to become diabetic (figure 1). In this analysis, DQ typing was available on just 77 of the 1,484 antibody-negative relatives. We may speculate that if more of these subjects had been typed the risk differential associated with the number of inherited susceptible DQ alleles may have been even greater. Yet, only those who were both positive for islet cell antibodies and who carried the potential to make at least two of four susceptible DQ heterodimers were at increased risk. While several investigators have used multiple immunologic markers (insulin autoantibodies, islet-cell surface antibodies, etc. (6, 8, 28)) to distinguish antibody-positive subjects who are destined to progress to overt IDDM from those who are not, genetic markers have not been widely used (29), perhaps because the serologically defined HLA markers within a family do not contribute anything beyond the islet cell antibody assay in predicting disease. However, the newly identified HLA-DQ molecular markers clearly increase the predictive ability for IDDM beyond that of islet cell antibodies alone.

The data within the high-risk families of this study are subject to varying interpretations. They imply that the production of islet cell antibodies in an individual is not directly related to DQA1 and DQB1 alleles. Antibody titers were not available on these subjects. Since we have demonstrated an effect of titer on development of IDDM (5), the addition of titer to susceptibility alleles may have improved our predictive ability.
Alternatively, these factors may be interactive. We might infer that variant DQ molecules represent one of several factors permitting the autoimmune beta cell damage for which islet cell antibody positivity is a nonspecific marker. The concept that genetic differences play a role primarily in fostering, rather than initiating, an aberrant immune response is not new. Nerup et al. (30) proposed that beta cells elaborate an autoantigen in response to some sort of damage and that this autoantigen is presented to the T lymphocytes by macrophages, thus initiating a full-blown inflammatory response. It is fair to conclude that DQ molecules which are structurally different might function with greater efficiency in presenting an autoantigen to T lymphocytes, thus enhancing the autoimmune response (18).

These data, then, support the premise that both genetic susceptibility and immunologic activation must exist for IDDM to develop. They show that conversion to diabetes is restricted to those islet cell antibody-positive individuals who carry the potential to produce a large proportion of variant DQ heterodimers. The data support theories of aberrant immune response, but they contribute nothing to hypotheses which propose that a variant DQ molecule initiates the autoimmune process. Among these high-risk subjects, the Arg-52 non-Asp-57 DQ heterodimer appears to permit the exacerbation of autoimmunity, or it may inhibit the suppression of the autoimmune response.

Intensive follow-up of subjects who are positive for islet cell antibodies and Sus4 is necessary to determine which events distinguish the 60 percent who develop IDDM from those who do not. It is possible that eventually all of these individuals will convert to IDDM. In any case, the study of highly susceptible individuals promises to help identify the environmental/immunologic events which lead to diabetes.

REFERENCES

18. Trucco M. To be or not to be Asp 57, that is the question. (Commentary). Diabetes Care 1992;15:705–15.