The Role of Late-Onset Autoimmune Diabetes in White Familial NIDDM Pedigrees

STEVEN C. ELBEIN, MD
KIMBERLEY WEGNER, BA
CINDY MILES, RN

OBJECTIVE — To determine whether autoimmunity is a prominent feature of NIDDM among diabetic members in families with a strong history of NIDDM or in families with a mixture of NIDDM and IDDM.

RESEARCH DESIGN AND METHODS — We determined GAD and islet cell (ICA512) autoantibodies from 215 NIDDM individuals and from 14 individuals with impaired glucose tolerance (IGT) of 68 families, including 1 family with maturity-onset diabetes of the young (MODY) and 3 families ascertained specifically for a mixture of NIDDM and IDDM. We tested 2 control populations: 50 unrelated spouses from Utah families, including 29 spouses with either IGT or NIDDM and 198 random nondiabetic white individuals from Colorado.

RESULTS — We detected either GAD or ICA512 autoantibodies in 11 members of seven families and in one spouse used as a control subject. In two families, two affected individuals showed evidence of autoimmunity, but NIDDM individuals in each of the seven families showed no evidence of autoimmunity. Among the five families with both IDDM and NIDDM individuals (three families ascertained for a mixture and two families ascertained with an incidental IDDM child), antibodies were detected in members of only one family. Antibody-positive individuals were significantly younger at diabetes onset and had low waist-to-hip ratios, but were not more likely to be insulin treated.

CONCLUSIONS — Autoimmunity is an important cause of apparent NIDDM, even among families with a strong history of NIDDM. However, autoimmunity among affected family members appeared to be a chance event and not the manifestation of a different genetic cause of diabetes.

NIDDM has been viewed as a distinct disease from autoimmune IDDM, and most epidemiological and genetic analyses have distinguished IDDM from NIDDM based on the phenotype of onset at ≥40 years, a history of continuous insulin therapy, and ketoacidosis. Nonetheless, many reports suggest that the two diseases occur in the same family more often than expected by chance alone (1,2), and several investigators have suggested an autoimmune destruction of β-cells among unrelated individuals with phenotypic NIDDM (2–4). Recent analyses of IDDM have identified at least 12 loci, most of which do not include obvious candidates for the autoimmune destruction of pancreatic β-cells (5–8). These loci do not appear to include NIDDM susceptibility loci (9), and thus do not explain the apparent increased frequency of IDDM and NIDDM in the same family. With the exception of a Finnish study (10), no data support a role for the HLA region genes in human NIDDM (9,11). Thus shared genetic susceptibility of NIDDM and IDDM seems unlikely.

Two other possibilities might explain the occurrence of IDDM and NIDDM phenotypes in a single family. These families may represent a unique subset of diabetes with a single genetic basis but a pleiotropic phenotype, as has been suggested for mitochondrial DNA mutations (12). Alternatively, they may represent the segregation of autoimmune diabetes and classical NIDDM as different diseases in the same family. Either scenario would complicate attempts to understand the pathogenesis of NIDDM by introducing different causes of diabetes among family members whom we would expect to share a single etiology. Furthermore, the existence of families with autoimmune NIDDM would have implications for clinical management of diabetes if insulin therapy were shown to preserve β-cell function in these individuals.

Because anti-islet cell antibodies often disappear shortly after the onset of IDDM, screening for autoimmune diabetes among apparent NIDDM individuals previously lacked sensitivity (2,4). In contrast, the more recently described autoantibodies to GAD are more often persistent and have a sensitivity in excess of 60% (4), which makes such screening practical. To determine the role of autoimmunity among large families ascertained for apparent NIDDM, we measured both anti-GAD65 autoantibodies and anti-islet cell 512 (ICA512/IA-2) antibodies in 215 diabetic members of 68 families. We suggest that autoimmunity is a significant cause of diabetes among apparently NIDDM individuals from families with a strong history of NIDDM. Surprisingly, the presence of IDDM individuals in the family did not predict autoimmunity in NIDDM members.

RESEARCH DESIGN AND METHODS

Study population
The study population consisted of 244 non-founding members of 68 families: 64 families (227 people) ascertained for at least 2 NIDDM siblings and for no more than 1
parent known to be diabetic at the time of sampling, 1 family (7 members) with probable maturity-onset diabetes of the young (MODY), and 3 families (10 members) ascertained for a mixture of IDDM and NIDDM. Nonfounding family members were defined as second- and third-genera-

tions of the proband sibling pair. The study population consisted of 215 individuals with NIDDM, 14 individuals with impaired glucose tolerance (IGT), and 15 euglycemic nonfounding family members all ascertained for Northern European ancestry. The control population consisted of 50 spouses of family members; these spouses comprised 24 with NIDDM, 5 with IGT, and 21 with normal glucose tolerance tests. The spouses were not known to be related to any family member. The study population is comparable to other populations of Northern European descent. All nondiabetic family members and spouses underwent a standard 75-g oral glucose tolerance test. Both ICA512 and GAD65 antibodies levels were determined on all available individuals with NIDDM from plasma stored at -80°C. Individuals with clinical IDDM were not tested. No measures of insulin secretion were available for NIDDM subjects, who did not undergo glucose tolerance testing. An independent control population of unrelated nondiabetic subjects from the University of Colorado was tested for GAD65 and ICA-512 autoantibodies.

All subjects were studied at the General Clinical Research Center of the University of Utah under a protocol approved by the Institutional Review Board after providing informed consent.

**GAD and ICA assay**

GAD65 and ICA512 autoantibodies were assayed by combined radioassay using recombinant GAD65 and ICA512 (amino acids 256–979), as described in detail elsewhere (13). Both positive and negative control sera for both GAD65 and ICA512 autoantibodies were included in each 96-well plate, and antibody levels were expressed as an index calculated as described (13). An SD score was calculated based on the mean ± SD of 198 healthy control subjects as follows: (sample index − control mean)/control SD. Upper limits of normal for the SD score were 0.032 for anti-GAD65 and 0.071 for anti-ICA512, corresponding to the 99th percentile and 100th percentile for anti-GAD65 and anti-ICA512, respectively, in 198 normal individuals. We considered values exceeding these levels to be positive. Among individuals with diabetes onset at <30 years, sensitivity and specificity for anti-GAD65 are 82 and 99%, respectively. For anti-ICA512, sensitivity and specificity for diabetes at <30 years are 73 and 100%, respectively. Comparable figures for late-onset autoimmune diabetes are uncertain.

**Statistical analysis**

For comparison, IGT and NIDDM individuals were both considered to be affected. Either positive anti-ICA512 or anti-GAD65 antibodies were considered to represent autoimmune diabetes. Continuous variables for patients with nonautoimmune diabetes were compared with patients with autoimmune diabetes using the Student’s t test for normally distributed samples or the Mann-Whitney U test for skewed variables. Frequencies of autoimmune diabetes and diabetes with onset ≤40 years were compared using the χ² test. All analyses were performed with SPSS/Win software (SPSS, Chicago, IL) on a personal computer.

**RESULTS** — Anti-GAD65 antibodies were present in a single IGT control subject among 50 control spouses (29 IGT or NIDDM). In contrast, positive anti-GAD65 antibodies were present in 9 NIDDM and 1 IGT individuals of 237 family members tested (211 affected), and anti-ICA512 autoantibodies were present in 3 out of 237 family members, including 2 individuals with positive anti-GAD65 autoantibodies. Of 68 families tested, 9 families had at least 1 member with evidence of either anti-GAD65 or anti-ICA512 antibodies. Two families each had two diabetic members with either positive anti-GAD65 or positive anti-ICA512 antibodies. None of seven members of the probable MODY family showed positive antibodies. Of the five families with both IDDM and NIDDM members, only one family had a single individual with anti-GAD65 autoantibodies who had IDT. The prevalence among families with a known IDDM individual was not significantly different (P = 0.63) from the 9 out of 62 families with only typical late-onset NIDDM, in which one phenotypic NIDDM member had positive autoantibodies. Furthermore, autoantibodies were not significantly more common among family members than among our spouse control individuals, of whom over half had NIDDM or IGT. In contrast, we found a significant increase in autoantibody positivity among NIDDM family members when compared with a nondiabetic control population (2 out of 198 vs. 11 out of 215; P = 0.02).

We examined at least one other NIDDM individual in each of the nine families with an apparent autoimmune diabetic individual with the NIDDM phenotype (range 1–6 NIDDM individuals tested; average number tested, 4.8). In each family, including the two families with two siblings with autoantibodies, at least one individual (range 1–5) had no evidence of autoantibodies and thus appeared to have typical NIDDM.

To determine whether family members with autoimmune diabetes had any distinctive phenotypic features, we compared family members with NIDDM who had evidence of autoimmunity with those who had no evidence of autoimmune antibodies (Table 1). No significant differences were present in sex, treatment with insulin, lipid
\textbf{CONCLUSIONS} — Although several investigators have demonstrated autoimmunity among individuals with typical NIDDM (2-4), the role of autoimmune diabetes among families with multiple NIDDM individuals has not been investigated. We would expect a single major gene to segregate with diabetes within such families. Indeed, defects in mitochondrial DNA can cause both NIDDM and IDDM from the same mutation (12). HLA-mediated autoimmune β-cell destruction could potentially act similarly as a major gene causing both IDDM and NIDDM in the same family. Were this the case, the presence of an IDDM member would suggest that NIDDM family members might have autoimmune diabetes requiring insulin therapy. Although we found evidence for late-onset autoimmune diabetes in one out of seven families ascertained for two or more NIDDM siblings, the presence of an IDDM family member did not predict the presence of anti-GAD or anti-ICA512 autoantibodies among relatives. Furthermore, among each family with one antibody-positive, member, other diabetic members were tested who had no evidence of autoimmunity. Together, these data suggest that the appearance of IDDM and NIDDM, or classic NIDDM and late-onset autoimmune diabetes, represents the chance occurrence of two common diseases rather than genetic overlap. Thus, the presence of either known IDDM or autoimmune NIDDM in a family does not predict that other family members have the same pathogenesis of diabetes. In support of this conclusion, our genetic analysis of proposed IDDM loci in NIDDM families also failed to support evidence for common genetic determinants of these diseases (9). However, since even anti-GAD autoantibodies may not be present in up to 40% of IDDM patients, a second possibility is that patients with late-onset autoimmune diabetes were present but not detected in these studies. A third possibility is that the genetic determinants of IDDM and NIDDM are complex and that we were unable to detect overlapping genetic loci within families. From the clinical standpoint, this last possibility still implies that autoimmunity in one family member is not helpful in determining autoimmunity among other apparent NIDDM family members.

Not surprisingly, individuals with positive antibodies had a lower waist-to-hip ratio and earlier onset than typical NIDDM patients. However, they were not significantly more likely to be treated with insulin (64% with autoantibodies, 42% without autoantibodies), and we found considerable overlap with NIDDM, with respect to age of onset. Thus, these patients are not easily distinguished from typical NIDDM patients except by measurement of antibodies. Our data would suggest that these patients could represent a significant source of misclassification and thus misdiagnosis, even among families with a strong history of NIDDM. Autoimmune mechanisms would be expected to lead to impaired insulin secretion (4,14,15). We have chosen not to measure insulin secretion in NIDDM patients because of concerns in distinguishing primary defects from glucotoxic effects, and thus cannot evaluate this hypothesis directly among those with positive antibodies. In population-based studies, low insulin secretion has correlated with positive antibodies (4,14).

We were only able to test for anti-GAD65 and anti-ICA512 autoantibodies at a single point in time. Whether these antibodies will be persistent and lead to eventual insulin deficiency (4) is thus unknown. Nonetheless, our data suggest that autoimmune diabetes is common in this non-Finnish Northern European population with a prevalence of nearly 5% (10% of families), when either anti-GAD65 or anti-ICA512 autoantibodies are accepted as evidence for autoimmune β-cell destruction. A strong family history of NIDDM did not reduce this prevalence. These data are important to the ongoing genetic analyses of diabetes and may have implications for treatment of NIDDM if early insulin therapy can be shown to preserve the remaining β-cell reserve.

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