The Low Prevalence of Immunogenetic Markers in Korean Adult-Onset IDDM Patients

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OBJECTIVE — IDDM is an autoimmune disease that occurs among genetically susceptible individuals. In Asian populations, it is not uncommon for adult patients with NIDDM to eventually lose β-cell function and develop IDDM. These individuals may be characterized by autoantibodies to GAD and high-risk HLA-DQ alleles, which are unlikely to be prevalent among patients with true NIDDM or in the general population. The objective of the present study was to evaluate and compare the prevalence of these immunogenetic markers in NIDDM patients and healthy nondiabetic individuals from Korea.

RESEARCH DESIGN AND METHODS — The prevalences of anti-GAD antibodies and HLA-DQA1 and DQB1 alleles among 121 patients with newly diagnosed NIDDM identified from a population-based study in Yonchon, Korea, and 100 matched healthy control subjects were evaluated and compared.

RESULTS — The overall prevalence of anti-GAD antibodies was 1.7% (2 of 121) in patients with previously undiagnosed NIDDM, whereas 1 of 100 control subjects had a positive test for antibodies. Among those who tested positive, titers of antibodies to GAD were not high. No statistically significant differences in the distributions of either mean levels of anti-GAD antibodies or DQA1 and DQB1 alleles were found comparing NIDDM patients with control subjects. Interestingly, the frequency of DQB1*non-Asp-57 and DQA1*Arg-52 alleles in the Korean adult control population was similar to that in the U.S. white population (DQB1*non-Asp-57: 0.431 vs. 0.475; DQA1*Arg-52: 0.492 vs. 0.463).

CONCLUSIONS — The low prevalence of anti-GAD antibodies and HLA-DQA1 and DQB1 susceptibility alleles among recent-onset NIDDM patients, which was similar to observations in control subjects, suggests that diabetes in Korean adults is unlikely to have an autoimmune component to its pathogenesis.

IDDM is an autoimmune disease, generally occurring in children and characterized by an absolute deficiency of insulin caused by destruction of the β-cells of the pancreas (1). NIDDM is common in older obese individuals and is associated with less severe insulin deficiency of unknown etiology, although insulin resistance is suspected as the prime abnormality (2,3). However, IDDM is also known to occur among adults (4). Moreover, a group of patients who present with what appears to be NIDDM are unable to achieve euglycemia with diet and oral hypoglycemic agents and require insulin shortly after diagnosis (5,6). Among whites, this type of diabetes is characterized as a late-onset and slowly evolving form of IDDM, since several studies have shown associations with markers of autoimmunity, including antibodies to GAD (7–10) and the presence of high-risk HLA-DQ alleles (i.e., DQA1*Arg-52 and DQB1*non-Asp-57) (11–13).

Contrary to observations in Western countries, ~2/3 of Korean NIDDM patients are not obese (14). In addition, a significant number of Korean diabetic adults who were not initially treated with insulin eventually require daily injections to control their high blood glucose levels. However, they are not prone to ketoacidosis (15). Therefore, the clinical distinction between IDDM and NIDDM among Korean adults is sometimes difficult. If we assume that the etiology of adult-onset IDDM is associated with the autoimmune and genetic markers that characterize childhood IDDM in whites and probably in Koreans, we may be able to predict future insulin dependency in adult NIDDM patients using immunogenetic determinants. Therefore, the objective of the current study was to compare the prevalence of anti-GAD antibodies and high-risk HLA-DQ genotypes among adult-onset NIDDM patients and healthy nondiabetic control subjects. This may lead to the identification of a group of individuals likely to develop IDDM in the future.

RESEARCH DESIGN AND METHODS

Study population

A population-based survey of diabetes, using the standard oral glucose tolerance test protocol, in Yonchon County, Korea, was recently completed. Yonchon County is located in the northern part of Kyunggi Province, 60 km away from Seoul. It oc-

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PCR, polymerase chain reaction; SSPE, saline-sodium phosphate-EDTA buffer; WHR, waist-to-hip ratio.

cupies 733 km^2 and includes ~60,000 people. Details about the survey procedures are described elsewhere (16). Briefly, a population-based cross-sectional study was performed to estimate the prevalence of diabetes among residents above 30 years of age. Of the 3,804 eligible residents, 2,520 participated in the survey. The prevalence of diabetes in Korean adults above age 30 who live in Yonchon County, age-standardized by the world population, was 8.0%, and the prevalence of impaired glucose tolerance was 10.6%. Among the diabetic patients, diabetes was newly diagnosed during the survey in 121 individuals. Of these, 62 were men and 59 were women; their mean current age was 57.4 ± 12.5 years (BMI: 25.2 ± 3.8 kg/m^2; waist-to-hip ratio [WHR]: 0.89 ± 0.1). We plan to prospectively follow these individuals to determine the percentage that will eventually develop insulin dependence.

For the present study, 100 nondiabetic control subjects matched by age (56.2 ± 12.5 years), sex (men: 60; women: 40), locality, BMI (24.9 ± 3.4 kg/m^2), and WHR (0.88 ± 0.05) were randomly selected from the prevalence survey for comparison. Those with a family history of diabetes were excluded.

Laboratory methods

GAD antibody immunoprecipitation assay. Soluble porcine GAD was purified from fresh pig brain and prepared for the GAD enzyme assay as previously described (17). The purified and enzymatically active GAD showed both isoforms (67,000 and 65,000 M_r) by SDS-PAGE. For immunoprecipitation, after preadsorption with pooled normal sera, 40 μl of ^{125}I-GAD (50,000 cpm) was added to 25 μl of test plasma diluted 1:2 in cold wash buffer (20 mmol/l Tris, pH 7.4; 150 mmol/l NaCl; and Triton X-100, 0.5% wt/vol). Samples were incubated overnight at 4°C, further incubated with 50 μl of 50% protein A-Sepharose (1 h, 4°C), and then centrifuged. The precipitate was washed three times in 750 μl wash buffer, and radioactivity was counted. A positive standard serum, defined to contain 100 U of antibody, was included in every assay. The activity of test sera was expressed as a percentage of the counts precipitated by the reference serum. The upper limit for a normal result, as determined using sera from Australian blood donors, is 18 U, which exceeds the mean + 3 SD (18).

DQA1 and DQB1 gene amplification. DNA was extracted from peripheral blood leukocytes (10 ml blood in 1% EDTA) using IsoQuick commercial kits from Microprobe (Garden Grove, CA) and used as a template for DQA1 and DQB1 gene polymerase chain reaction (PCR) amplification, as described by Saiki et al. (19). The second exons encoding the first polymorphic domains of the HLA-DQA1 and DQB1 genes were selectively amplified, as described previously (20,21). The procedure was carried out using the following primers for amplification: DQA-ampA 5′-ATG GTG TAA ACT TGT ACC AGT-3′, DQA-ampB 5′-TTG GTA GCA GCG GTA GAG TTG-3′, DQB-ampA 5′-CAT GTG CTA CTT CAC CAA CGG-3′, DQB-ampB 5′-CTG GTA GTT GTG TCT GCA CAC-3′. Successful PCR amplifications were obtained from each individual's genomic DNA using the thermostable *Taq* polymerase and a thermocycler from Perkin-Elmer/Cetus (Norwalk, CT). Thirty cycles of amplification were performed: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and a 30-s extension at 72°C. Then 1 μl of amplified DNA was dot-spotted on nylon membrane filters, denatured with 0.4 N NaOH for 20 min, and then neutralized with 2× saline-sodium phosphate-EDTA buffer (SSPE) (1.5 mmol/l NaCl, 0.1 mmol/l NaH_2PO_4, and 10 mmol/l EDTA, pH 7.4). After the filters were dried at room temperature, the DNA was fixed to the filters by exposure to ultraviolet light for 5 min. The filters were prehybridized at 54°C for 2 h in a solution of 3 mmol/l tetramethylammonium chloride, 50 mmol/l Tris (pH 8.0), 2 mmol/l EDTA (pH 8.0), 0.1% SDS, 5× Denhardt's solution (5 g Ficoll, 5 g polyvinylpyrrolidone, 5 g bovine serum albumin, 500 ml H_2O diluted 10-fold into prehybridization buffer), and Herring's sperm DNA (100 mg/l). The filters were hybridized at 54°C overnight in a prehybridization solution with ^{32}P-labeled oligonucleotide probes. After hybridization, filters were washed twice nonstringently for 10 min in 2× SSPE-0.1% SDS at room temperature. One stringent wash was subsequently performed for 10 min at room temperature and one wash at 58°C in a solution of 3 mmol/l tetramethylammonium chloride, 50 mmol/l Tris (pH 8.0), 2 mmol/l EDTA (pH 8.0), and 0.1% SDS. The filters were exposed to X-ray film (Kodak XAR-5, Eastman Kodak, Rochester, NY) with an intensifying screen at −80°C for 1–4 h. Eight DQA1 alleles and 14 DQB1 alleles were evaluated using the sequence-specific oligonucleotide probes described previously (20,21). The nomenclature used to define the HLA-DQ alleles was according to the official Nomenclature for Factors of the HLA System, 1991 (22).

Statistical analysis

The genotype distributions for the control populations were tested for Hardy-Weinberg equilibrium using the χ^2 goodness-of-fit test. Differences between the HLA allele distributions for the patients and control subjects were determined using the χ^2 test with Yates' correction (two-tailed). When the expected frequency for one of the alleles was <5, the Fisher's exact test was used. Corrected *P* values were obtained by multiplying the *P* value by the number of alleles tested at each locus.

RESULTS

Anti-GAD antibodies

Three subjects had borderline elevated anti-GAD levels: two were diabetic patients and one was a normal control subject. Neither of the diabetic patients who tested positive for anti-GAD antibodies had a history of diabetic ketoacidosis or used insulin. One anti-GAD$^+$ patient was a 50-year-old man (BMI = 25.8 kg/m^2, WHR = 0.98, fasting serum insulin concentration = 12.2 μIU/ml) who had an antibody level of 24 U. The other patient was a 63-year-old man (BMI = 27.6 kg/m^2, WHR = 0.95, fasting serum insulin concentration = 16.2 μIU/ml) who had an antibody level of 23 U. One of 100 control subjects also had an anti-GAD antibody level of 24 U. The HLA-DQA1 genotypes for the diabetic patients who tested positive for GAD antibodies were DQA1*0401/0301 and DQA1*0301/0501. The HLA-DQB1 alleles carried by these individuals were DQB1*0401/0302 and DQB1*0301/0302, respectively. The HLA-DQ genotypes of the anti-GAD$^+$ control subject were DQA1*0401/0501 and DQB1*0303/0201.

DQA1 and DQB1 allele and genotype distribution

The observed genotype distributions for the control populations were not significantly different from the expected proportions under the assumption of Hardy-Weinberg equilibrium. This indicated

Table 1—*Distribution of HLA-DQA1 and DQB1 alleles among patients with newly diagnosed NIDDM and related nondiabetic control subjects*

Alleles	Yonchon County		White population
	Diabetic patients	Control subjects	
HLA DQA1			
0101 (nR)	28 (11.7)	29 (14.7)	14.4
0102 (nR)	47 (19.6)	34 (17.2)	11.9
0103 (nR)	37 (15.4)	23 (11.7)	2.3
0201 (nR)	13 (5.4)	14 (7.1)	15.4
0301 (R)	63 (26.3)	57 (28.9)	19.9
0401 (R)	15 (6.3)	8 (4.1)	3.0
0501 (R)	28 (11.7)	27 (13.7)	20.7
0601 (R)	9 (3.7)	5 (2.5)	2.7
HLA DQB1			
0201 (nD)	22 (9.3)	29 (14.9)	24.3
0301 (D)	40 (17.0)	24 (12.3)	16.0
0302 (nD)	33 (14.0)	17 (8.7)	8.1
0303 (D)	12 (5.1)	15 (7.7)	0.0
0401 (D)	22 (9.3)	24 (12.3)	0.8
0402 (D)	6 (3.5)	5 (2.6)	2.4
0501 (nD)	28 (11.9)	25 (12.8)	12.5
0502 (nD)	12 (5.1)	6 (3.1)	1.0
0503 (D)	14 (5.9)	9 (4.6)	1.6
0601 (D)	13 (5.5)	14 (7.2)	2.4
0602 (D)	20 (8.5)	15 (7.7)	6.5
0603 (D)	4 (1.7)	5 (2.6)	7.6
0604 (nD)	10 (4.2)	7 (.3.6)	1.6

Data are *n* (%) or %. Percentages for the white population are from 11th International Histocompatibility Workshop, 1991 (21). For HLA DQA1, only one allele of two NIDDM patients and three control subjects was readable. For HLA DQB1, only one allele of six NIDDM patients and five control subjects was readable. R, Arg-52; nR, non-Arg-52; D, Asp-57; nD, non-Asp-57.

that the control samples were likely representative of the Korean general population. The DQA1 and DQB1 allele distributions for the NIDDM patients and nondiabetic control subjects are shown in Table 1. No statistically significant differences were found comparing the NIDDM patients with the control subjects.

The data were pooled and expressed as DQA1*Arg-52 (or DQA1* non-Arg-52)– and DQB1*non-Asp-57 (or DQB1*Asp-57)–containing alleles. Although individual allele frequencies differed from those obtained for white subjects (22), the prevalence of all DQB1*non-Asp-57 (0.431 vs. 0.475) and DQA1*Arg-52 (0.492 vs. 0.463) alleles was similar for these groups. No statistically significant differences in the combined frequency of DQA1*Arg-52 alleles or DQB1*non-Asp-57 alleles were found when comparing the NIDDM patients with the control subjects. Furthermore, we could not find any significant differences in the distributions of DQA1* Arg-52 homozygotes and heterozygotes or in those for DQB1*non-Asp-57 homozygotes and heterozygotes when comparing the NIDDM patients with the control subjects (Table 2). When the different genotypes were grouped according to the number of diabetogenic heterodimers potentially formed, no statistically significant differences were found when comparing the NIDDM patients with the control subjects. When we compared the frequency distribution of the heterodimers in the nondiabetic control subjects with that obtained from white control subjects, we were not able to detect any statistically significant differences (data not shown).

CONCLUSIONS — While it is convenient to categorize the usual types of diabetes as IDDM and NIDDM, it is becoming more difficult to determine the limits of NIDDM (4–6). IDDM occurs more frequently in families with a strong history of NIDDM than in the general population (23). Moreover, among nonobese patients with NIDDM, there is likely a subgroup of individuals who will pass through a phase of NIDDM en route to true insulin dependence (5,6). Among whites, this type of diabetes has been shown to be a late-onset and slowly evolving form of IDDM and is frequently associated with antibodies to GAD (7) and the presence of HLA-DR3/DR4 antigens (5,8). However, little is known about this type of diabetes and the associations with HLA-DQ alleles and anti-GAD antibodies in an Asian population.

In addition to the strikingly low prevalence of childhood IDDM in Korea (24), more than half of the Korean adults with NIDDM are considered to have partial insulin deficiency (25). While they exhibit wasting and polydipsia at onset, they are not sick until they develop mild ketosis, which occurs occasionally under stressful conditions. Otherwise, they do not need insulin for preventing ketosis but often require it for blood glucose control. Their basal plasma insulin levels are generally close to the lower limits of normal, but their response to glucose loading is minimal. Therefore, the clinical distinction between IDDM and NIDDM among Korean adults is particularly difficult to make.

We previously demonstrated that a low C-peptide response along with low BMI may be good predictors of a patient's subsequent clinical course (26). However, if we assume that β-cell–directed autoimmunity may contribute to the in-

Table 2—*Distributions of HLA-DQA1 and DQB1 genotypes among patients with newly diagnosed NIDDM and unrelated nondiabetic control subjects*

Genotype	Diabetic patients	Control subjects
DQA1		
R/R	38 (32)	31 (32)
R/nR	59 (50)	52 (54)
nR/nR	22 (18)	14 (14)
DQB1		
nD/nD	22 (19)	20 (21)
nD/D	58 (50)	38 (40)
D/D	35 (31)	37 (39)

Data are *n* (%). R, Arg-52; nR, non-Arg-52; D, Asp-57; nD, non-Asp-57.

sulin insufficiency present in patients with what appears to be adult-onset IDDM, then the presence of anti-GAD antibodies and high-risk HLA alleles, which are among the best markers of IDDM (9–13), may also have good predictive value for future insulin dependence among individuals with NIDDM. Thus, earlier treatment with insulin in these subjects may improve their immediate well-being and lessen the risk of long-term complications of diabetes.

One of us has previously shown that GAD autoantibody status at the onset of NIDDM is a good predictor of late autoimmune diabetes in white adults (7). There was also a report suggesting that GAD autoantibodies can differentiate, in patients in whom NIDDM is clinically diagnosed, a subset of patients who have ongoing autoimmune destruction and often develop a requirement for insulin (9). According to that study, ~9% of the population-based sample of patients aged 6–79 years with new-onset diabetes had markers of autoimmunity. From our investigation, however, seropositivity of anti-GAD antibodies was present in only 1.7% of the patients with newly diagnosed diabetes. Nearly all the samples were negative, suggesting that diabetes in Korean adults is unlikely to have an autoimmune component to its pathogenesis. This appears to have put to rest an erroneous belief that there are a large number of "type 1 1/2" diabetic patients in this group. It may also reflect the fact that not all adult-onset IDDM is caused by autoimmune mechanisms, especially among Koreans. Alternatively, in some adult-onset IDDM patients, autoimmunity may stabilize after partial destruction of β-cell mass (27), which may contribute the partial insulin deficiency without autoimmune markers.

Although antibodies to GAD appear to be more predictive of adult IDDM than other islet-cell antibodies among whites, seropositivity to GAD is not universal at the onset of IDDM, and the prevalence of anti-GAD antibodies in Asians and particularly Koreans is lower than among white patients (28). This suggests the involvement of multiple pancreatic autoantigens in the IDDM autoimmune process and/or genetic differences within and between ethnic groups that contribute to the heterogeneous autoimmune response to GAD. Alternatively, some cases of IDDM could have an etiology unrelated to autoimmunity: for example, the occurrence of mutations of mitochondrial DNA (29). The small number of seropositive patients with adult-onset IDDM limited our ability to evaluate potential differences between the seropositive and seronegative patients.

IDDM is much less frequent in Korea than in countries with predominantly white populations. According to the Seoul Registry, the incidence of diabetes among children younger than age 15 in Korea was 0.6/100,000 per year (~1/30 the rate observed in Scandinavian countries) (24). A study of five populations suggested that the variation in the frequency of IDDM susceptibility genes across populations is a major determinant of the worldwide patterns of IDDM incidence (30). Recent studies of the HLA-DQB1 gene have revealed that the presence of DNA sequences coding for an amino acid other than aspartate in the 57th position of the molecule (non-Asp-57) was highly associated with IDDM susceptibility. In contrast, an aspartic acid sequence in position 57 (Asp-57) appeared to be protective against the development of IDDM. The presence of sequences coding for arginine in position 52 of the DQA1 gene (Arg-52) is also significantly related to increased IDDM risk, particularly in the presence of DQB1*non-Asp-57. The heterodimers formed by the combination of DQB1*non-Asp-57 and DQA1*Arg-52 are most highly associated with the disease (13,31). Although the high-risk HLA-DQA1 and DQB1 alleles are unlikely to be the only genes associated with IDDM susceptibility, they are currently the genetic markers that are most strongly related to the disease among whites. This association appears to be consistent across populations, confirming the importance of this polymorphism in determining IDDM susceptibility. A study of the association between HLA-DQB1 alleles and IDDM in China, another country with a low incidence rate, found a low frequency of DQB1*non-Asp-57 alleles among healthy control subjects (32). However, susceptibility to IDDM does not appear to be an all-or-none phenomenon but is related to the number and type of high-risk DQB1/DQA1 alleles that an individual carries. There is evidence for a hierarchical susceptibility scale among the DQB1*non-Asp-57 alleles, ranging from the most protective Asp-57 alleles (DQB1*0602) to the more permissive non-Asp-57 alleles (DQB1*0201 and DQB1*0302). Although the prevalence of all DQB1*non-Asp-57 and DQA1*Arg-52 alleles in Korean normal populations was similar to that of whites, the differences in the frequency distribution of individual DQB1 alleles might explain the actual incidence differences for IDDM. Interestingly, we found a similar frequency of the high-risk DQB1 alleles among both NIDDM patients and nondiabetic Korean control subjects.

The ultimate goal for those who care for people with IDDM is prevention. Efficient tests screening for autoantibodies to multiple islet antigens along with PCR-based HLA typing may increase our ability to identify high-risk individuals from the general population for β-cell function testing and immunointervention. However, in Korea, the low prevalence of islet-cell autoimmunity in patients with newly diagnosed NIDDM and the lack of significant differences in HLA-DQA1 and DQB1 allele frequencies between patients and control subjects indicate that such individuals are unlikely to be good candidates for immune intervention. When we consider the low prevalence of anti-GAD antibodies in recent-onset NIDDM patients, as well as the low frequency of DQB1*non-Asp-57 alleles, we can estimate that diabetes in Korean adults is unlikely to have an autoimmune component to its pathogenesis.

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