Specific HLA-DRB and -DQB Alleles and Haplotypes Confer Disease Susceptibility or Resistance in Bahraini Type 1 Diabetes Patients

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Insofar as genetic susceptibility to type 1 diabetes is associated with HLA class II genes, with certain allelic combinations conferring disease susceptibility or resistance, this study assessed the distributions of HLA-DR and -DQ among 107 unrelated patients with type 1 diabetes and 88 healthy controls from Bahrain, all of Arab origin. The HLA-DRB and -DQB genotypes were determined by PCR–sequence-specific priming. The following alleles showed the strongest association with type 1 diabetes among patients versus controls according to their frequencies: DRB1*030101 (0.430 versus 0.097; P < 0.001), DRB1*040101 (0.243 versus 0.034; P < 0.001), DQB1*0201 (0.467 versus 0.193; P < 0.001), and DQB1*0302 (0.229 versus 0.091; P < 0.001). The frequencies of alleles in controls were compared to those in patients, negative associations were seen for DRB1*100101 (0.085 versus 0.014; P < 0.001), DRB1*110101 (0.210 versus 0.060; P < 0.001), DQB1*030101 (0.170 versus 0.075; P = 0.006), and DQB1*050101 (0.335 versus 0.121; P < 0.001). In addition, the DRB1*030101–DQB1*0201 (70.1 versus 22.7%; P < 0.001) and DRB1*030101–DQB1*0302 (21.5 versus 0.0%; P < 0.001) genotypes were more prevalent among patients, thereby conferring disease susceptibility, whereas the DRB1*100101–DQB1*050101 (20.5 versus 2.8%; P < 0.001), DRB1*110101–DQB1*030101 (28.4 versus 8.4%; P < 0.001), and DRB1*110101–DQB1*050101 (30.7 versus 0.9%; P < 0.001) genotypes were more prevalent among controls, thus assigning a protective role. These results confirm the association of specific HLA-DR and -DQ alleles and haplotypes with type 1 diabetes and may underline several characteristics that distinguish Bahraini patients from other Caucasians.

Type 1 diabetes is an autoimmune disease characterized by insulin insufficiency resulting from a progressive immunologic destruction of insulin-secreting pancreatic β islet cells by autoreactive leukocytes and their mediators (2). Although the exact nature of the inducing agent(s) and the sequence of events leading to the destruction of β islet cells and, subsequently, hyperglycemia are not completely understood, it is well established that susceptibility to type 1 diabetes is determined by environmental and genetic factors (7, 25). Many susceptibility loci have been described previously, including the HLA (IDDM1) and insulin (IDDM2) gene regions (2, 5, 18), while other loci will undoubtedly be identified in the future.

Mapping studies with genes from patients with type 1 diabetes confirmed the association of specific major histocompatibility complex class II alleles with the risk of disease development (5, 14, 25). It was proposed that both susceptible and protective alleles at the DRB1, DQA1, and DQB1 loci were associated with the pathogenesis of the disease (14, 20), exemplified by the strong association of the HLA-DR3 and -DR4 and the HLA-DQA1 and -DQB1 alleles with type 1 diabetes (9, 16) and the negative association of the HLA-DR2 and DQB1*0602 alleles with type 1 diabetes (21). It was also apparent that the association (positive, neutral, or negative) of a particular allele at the DRB1, DQA1, and DQB1 loci may vary among various ethnic and racial groups (24). For example, whereas the DRB1*0301–DQB1*0201 and DRB1*0401–DQB1*0302 haplotypes were found to be strongly associated with type 1 diabetes in Caucasians and Japanese individuals, the DRB1*0405–DQB1*0302 and DRB1*0802–DQB1*0302 haplotypes were found to be associated with the disease among Japanese individuals (14).

The prevalence of type 1 diabetes varies significantly, depending on an individual’s geographical location and also ethnic or racial background (2). High disease incidence rates were reported for Caucasians, while Asians generally have low disease incidence rates (6), which may be explained by the selective distribution of susceptible (and protective) HLA-DR and -DQ alleles (6, 14). Although Bahrain has a high incidence rate of diabetes (1), the contribution of HLA class II alleles to insulin-dependent diabetes mellitus and the distribution of the DRB1–DQB1 haplotype among patients with type 1 diabetes and the general population remains unknown. We therefore investigated the associations of the HLA-DRB1 and -DQB1 haplotypes with type 1 diabetes in Bahrain, in particular, with regard to the contribution of the genotypic combination of the DRB1 and DQB1 haplotypes on susceptibility or resistance to type 1 diabetes.

MATERIALS AND METHODS

Subjects. The subjects included in this study comprised 107 type 1 diabetes patients (mean ± standard deviation age, 15.67 ± 9.81 years; age range, 1 to 35 years).
years), of whom 53 were females and 54 were males. Type 1 diabetes was diagnosed according to clinical and laboratory findings. All type 1 diabetes patients were receiving insulin to control hyperglycemia, were not obese (body mass index, 22.08 ± 7.5) (Table 1), were free of any concomitant complication, and were not receiving additional treatment at the time of blood collection. Patients with other forms of diabetes (type 2, latent diabetes, maturity onset diabetes of the young) and who were not related to the diabetic subjects and who were from the same geographical area as the diabetic subjects. All participants were Bahrainis or recently naturalized Bahrainis. None of the participants (patients and controls) was a smoker, consumed alcohol, or was on any drug or medication (including substances of abuse); all participants were asked to sign a consent form according to the study protocol, and all institutional ethics requirements were met.

Autoantibody determination. Serum samples were evaluated for the presence of anti-glutamate decarboxylase (anti-GAD) antibodies, islet cell autoantibodies (ICAs), and insulin autoantibodies (IAAs) by enzyme immunoassay on two different occasions independently by using the BioMerica (Newport Beach, Calif.) and DRG International (Marburg, Germany) assay kits. Each sample was tested twice with each kit system, and the results were scored as positive or negative according to the specifications of the manufacturers. Autoantibodies were measured at the time of initial diagnosis for patients with type 1 diabetes.

HLA genotyping. Total genomic DNA was extracted from the peripheral blood mononuclear leukocytes from patients and controls by the phenol-chloroform method, as is standard, and was used for PCR analysis. The HLA-DRB1 and -DQB1 gene alleles were analyzed by the PCR-sequence-specific priming technique according to the specifications of the manufacturer (One Lambda, Thousand Oaks, Calif.). The PCR products were analyzed on a 2.5% (wt/vol) agarose gel stained with ethidium bromide (0.5 μg/ml).

Data analysis. Allele frequencies were determined by direct counting, and differences in the distributions of individual alleles between patients and controls were determined by Fisher’s exact test or Pearson’s χ² test with Yates’ correction (two tailed). Haplotype frequencies were determined by the maximum-likelihood method with Arlequin (version 2) software (http://anthro.unige.ch/arlequin). P values were calculated for the number of different alleles tested, and significance was determined at a P value of <0.05. Odds ratios (ORs) and 95% confidence intervals (CIs) were also determined. Analysis was performed with the SPSS (version 11.5) for Windows statistical package.

RESULTS

Characteristics of study participants. When the patients were compared to the controls, the patients had elevated fasting glucose levels (12.88 ± 5.52 versus 5.86 ± 3.06 mmol/liter; P < 0.001) and hemoglobin HbA1c levels (9.40 ± 2.94 versus 5.53 ± 1.52 as a percent of total hemoglobin; P < 0.001) (Table 1). The urica, uric acid, and serum lipid profiles were not significantly different between the patients and the controls. Of the 92 type 1 diabetes patients and 54 controls screened, a higher incidence of anti-GAD antibodies (30 of 92 versus 0 of 54; P < 0.001), ICAs (37 of 82 versus 2 of 54; P < 0.001), and IAAs (45 of 82 versus 3 of 54; P < 0.001), as well as the combinations of anti-GAD antibodies and ICAs (16 of 92 versus 0 of 54; P = 0.003), ICAs and IAAs (12 of 92 versus 1 of 54; P = 0.046), anti-GAD antibodies and IAAs (23 of 92 versus 0 of 54; P < 0.001), and anti-GAD antibodies, ICAs, and IAAs (6 of 92 versus 0 of 54; P = 0.14), were seen in the patients than in the controls.

Frequencies of HLA-DRB1 alleles. The distributions of the HLA-DRB1 genotypes in patients and controls are summarized in Table 2. The allelic frequencies of DRB1*030101 (0.430 versus 0.097; P < 0.001) and DRB1*040101 (0.243 versus 0.034; P < 0.001) were significantly higher in type 1 diabetes patients than in controls (Table 2). In addition, the frequencies

| Characteristic                      | Controls | Type 1 diabetes patients | P value* |
|------------------------------------|----------|--------------------------|----------|
| No. (%) of participants            | 88 (45.1)| 107 (54.9)               | 0.469    |
| Gender (no. of M:no. of F [% M: % F]| 41:47 (46.6:53.4) | 54:53 (50.5:49.5) | 0.469    |
| Age (yr [mean ± SD])               | 31.27 ± 14.08 | 15.67 ± 9.81 | <0.01    |
| Duration of diabetes (yr)          | 0.00     | 1.44 ± 0.55              | <0.001   |
| Body mass index                    | 24.82 ± 6.26 | 22.08 ± 7.5  | 0.459    |

Biochemical profile (concn³)

| Parameter           | Controls | Type 1 diabetes patients | P value* |
|---------------------|----------|--------------------------|----------|
| FBS/glucose         | 5.86 ± 3.06 | 12.88 ± 5.52 | <0.001   |
| HbA1c               | 5.33 ± 1.52 | 9.40 ± 2.94  | <0.001   |
| Urea                | 4.03 ± 1.40 | 4.15 ± 1.71  | 0.741    |
| Uric acid           | 248.24 ± 76.30 | 294.83 ± 79.61 | 0.08     |
| HDL cholesterol     | 1.24 ± 0.56 | 1.65 ± 0.98  | 0.042    |
| LDL cholesterol     | 3.16 ± 0.76 | 3.32 ± 1.69  | 0.628    |
| Creatinine          | 57.46 ± 13.92 | 67.64 ± 26.00 | 0.051    |
| Cholesterol         | 4.85 ± 0.91 | 4.24 ± 1.14  | 0.012    |
| Triglycerides       | 1.51 ± 0.74 | 1.86 ± 0.84  | 0.406    |

* Abbreviations: M, male; F, female; FBS, fasting blood sugar; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

b Determined by Pearson’s χ² test.

Concentrations are in millimole per liter unless indicated otherwise.

a Measured as percentage of total hemoglobin (high-pressure liquid chromatography method).
The distributions of the HLA-DRB1 and -DQB1 haplotypes in patients and controls were assessed next. The frequencies of DRB1*0301-DQB1*0201 (70.1 versus 22.7%; P < 0.001; relative risk [RR] = 7.97) and DRB1*0301-DQB1*0302 (21.5 versus 0.0%; P < 0.001) were higher in the patients than in the controls (Table 4). In contrast, the frequencies of DRB1*1001-DQB1*0501 (20.5 versus 2.8%; P < 0.001; RR = 0.11), DRB1*1101-DQB1*0301 (28.4 versus 8.4%; P < 0.001; RR = 0.23), and DRB1*1101-DQB1*0501 (30.7 versus 9.0%; P < 0.001; RR = 0.02) were higher in the controls than in the patients (Table 4).

**DISCUSSION**

This study is the first report on the association of HLA class II alleles with type 1 diabetes in Bahrain, an island in the Arabian (Persian) Gulf (estimated population, 700,000). The results showed that haplotypes that conferred susceptibility to type 1 diabetes appeared in a large number of patients with diabetes, exemplified by the strong association of DRB1*0301-DQB1*0201 with type 1 diabetes, with a high RR (7.97); the frequency of this haplotype was 70.1% among the patients with diabetes, whereas it was 22.7% among the controls. By contrast, DRB1*1101-DQB1*0301 was negatively associated with type 1 diabetes, thereby assigning protective and susceptible HLA class II alleles. These findings were reminiscent of earlier findings of an association of DRB1*0301-DQB1*0201 with type 1 diabetes among Caucasian patients (11, 19) but not non-Caucasian patients (14).

In contrast to the strong susceptibility conferred by the DRB1*0301-DQB1*0201 haplotype, the DRB1*1101-DQB1*0301 haplotype was negatively associated with type 1 diabetes, hence suggesting a protective role for this haplotype. Whereas “protective” haplotypes were previously described for Caucasians (11, 26) and non-Caucasians (14), this is the first indication of a protective aspect of this haplotype. The DRB1*1001 allele also afforded some protection not only when it was combined with protective DQB1 alleles, including DQB1*0501 (P < 0.001; RR = 0.11), but also when it was combined with the susceptible allele DQB1*0302. Collectively, this suggests an intricate interplay between individual DRB1 and DQB1 alleles in determining susceptibility to type 1 diabetes.

The strong association between select DRB1 and DQB1 alleles and diabetes among Bahraini type 1 diabetes patients is similar to that found among Caucasians of Arab descent, including individuals in Kuwait (12) and Egypt (10), and also non-Arab descent, such as individuals in Turkey (22), Yemen (13), Spain (8), Greece (17), the United Kingdom (11, 15), and Italy (19). The notable difference between Bahraini and other Caucasian populations was the weak association of DRB1*0401-DQB1*0302 with type 1 diabetes (11, 19, 22) and the absence of a negative association with DRB1*1501-DQB1*06 with type 1 diabetes (4, 22), which distinguished Bahraini diabetics from other Caucasians and which points to differences in the frequencies of the other DQB1 alleles were not statistically different between the patients and the controls.
the differential distributions of certain HLA class II alleles among diabetes by race, as was suggested previously (23).

Insofar as HLA class II molecules direct the presentation of antigens, including autoantigens, to autoreactive T cells, thereby launching the T-cell activation cascade and the subsequent destruction of pancreatic β islet cells, it is tempting to speculate that the strong association of DRB1*0301-DQB1*0201 and the negative association of DRB1*1101-DQB1*0301 as well as DRB1*1001-DQB1*050101/0302 with type 1 diabetes is likely due to differences in affinity to autoantigenic fragments presented by each haplotype, as was suggested previously (3). Accordingly, the DRB1*0301-DQB1*0201 haplotype may bind to and present β cell islet peptides to autoreactive T cells, thereby precipitating β-cell-directed immunity. On the other hand, DRB1*1101-DQB1*0301 and DRB1*1001-DQB1*050101/0302 may have a reduced or no affinity for autoantigenic fragments, thereby explaining their dominant protective nature. A larger study which addresses the class II genotype distribution among type 1 diabetics will be needed to confirm or rule out this interesting speculation.

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