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Immunogenetics of HLA class II in Israeli patients with adult-onset Type 1 diabetes mellitus

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Summary The distribution of *HLA* class II alleles and genotypes in Israelis of different ethnic origin with adult-onset type 1 diabetes (T1D) was examined. The results were compared with published findings in healthy Israelis and childhood-onset T1D Israelis. An additional comparison was made between subgroups of patients with rapidly and slowly progressive adult-onset T1D (LADA). A DNA-based low-resolution analysis was performed for *DRB1** and *DQB1** alleles and a high-resolution analysis for *DRB1*04* and *DQB1*1* alleles.
In all, 87% of the study group was positive for *DRB1*03* or *DRB1*04* compared with 36% of the healthy controls. The main alleles accounting for susceptibility to T1D were *DRB1*0402*, found in 77.9% of carriers of *DRB1*04* and *DQB1*0302*, found in 74.6% of carriers of *DQB1*03*. The *DQB1*0602* was not detected in any patient. The distribution was similar to that reported in Israeli children with T1D and significantly different from healthy Israelis. There was no significant difference in the distribution of *HLA* class II alleles between patients with rapidly progressive T1D or LADA.
It may be concluded that the different ages of onset of T1D and its different forms of development in Israeli patients are apparently not caused by a different prevalence of *HLA* class II alleles.

Introduction

Type 1 diabetes mellitus (T1D) develops as a result of the cellularly mediated autoimmune destruction of insulin-secreting pancreatic beta cells, a process called insulitis [1]. Evidence of organ-specific autoimmunity is provided by the presence of antibodies against islet cells (ICA), insulin (IAA), glutamic acid decarboxylase (GADA), and tyrosine-phosphatase (IA2-Ab), and infiltration of pancreatic islets by B and T lymphocytes. Immune-mediated diabetes may occur at any age [2,3]. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus had divided the autoimmune type 1 diabetes into a rapidly (classic

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ABBREVIATIONS

BMI	body mass index
GADA	glutamic acid decarboxylase antibodies
HLA	human leukocyte antigen
ICA	antibodies against islet cells
LADA	latent autoimmune diabetes in adults
T1D	type 1 diabetes mellitus

type 1 diabetes) and slowly progressive form [4]. The diabetes in the latter group is categorized as latent autoimmune diabetes in adults (LADA).

Both adult-onset and childhood-onset types are known to be associated with HLA class II genes. The susceptible and the protective *HLA* alleles vary by ethnic/racial group. In Israel, the distribution of *HLA* class II alleles has been examined in Ashkenazi Jews, non-Ashkenazi Jews, and Arabs with childhood-onset T1D [5]. The aim of the present study was to investigate the association of *HLA-DRB1* and *DQB1* haplotypes with adult-onset T1D in the main Israeli Jewish ethnic groups and to compare the findings among patients with adult-onset classical T1D and LADA and reported patients with juvenile-onset T1D.

Subjects and methods

Subjects

The study population consisted of 82 patients with T1D who were >20 years of age at onset of the disease. All were attending the Diabetes Clinic of the Endocrine Institute of Rabin Medical Center, a major tertiary facility. The following data were recorded for each patient: age, sex, ethnic origin, age at diagnosis, family history of diabetes, symptoms at diagnosis, presence of other autoimmune diseases, body mass index (BMI), fasting C-peptide, GADA and ICA levels, and diabetes treatment. Patients were diagnosed with T1D if they fulfilled at least three of the following criteria: family history of T1D in a first-degree relative, BMI <25, presence of other autoimmune diseases, event of diabetic ketoacidosis, C-peptide level <1 ng/ dL, and detection of GADA and/or ICA (the present study did not include IAA or IA2-ab). In addition, at least one of the criteria should have been an event of diabetic keroacidosis, low C-peptide, or positive anti-pancreas antibodies. Patients were diagnosed with LADA according to the criteria recently proposed by the Immunology of Diabetes Society: namely, age ≥30 years at diagnosis; positivity for at least one of the four antibodies commonly found in patients with type 1 diabetes; and no insulin treatment within the first 6 months of diagnosis [6].

In all, 46 patients were Ashkenazi Jews, 33 were non-Ashkenazi Jews, and three were Israeli Arabs.

The results of the genetic analysis of the patients were compared with those of Israeli patients with juvenile-onset type 1 diabetes mellitus and to a control group. The juvenile-onset T1D group included 165 unrelated Israeli patients: 95 non-Ashkenazi Jews and 70 Ashkenazi Jews. The control group comprised 425 healthy, unrelated Israeli volunteers, of whom 132 were Ashkenazi Jews and 293 were non-Ashkenazi Jews. The genetic data of the juvenile-onset T1D patients as well as the control group were previously examined and published by other investigators [5]. The *HLA* allele frequencies of the three Israeli Arabs with adult-onset T1D were not compared with a control group because of their small number. However, we included their genetic results in the analysis of the whole study sample.

HLA class II typing

After the patients and control subjects signed an informed consent form, blood samples were collected. Tissue typing for *HLA* was performed on peripheral blood mononuclear cells. The distribution of *HLA* class II alleles was examined by DNA-based low-resolution analysis for *DRB1** and *DQB1** alleles using polymerase chain reaction (PCR) amplification with sequence-specific primers (SSP) and with Tepnel-Luminex sequence specific oligonucleotide probes. High-resolution analysis for *DRB1*04* and *DQB1*1* alleles was performed by PCR-SSP (One Lambda, Canoga Park, CA).

Statistical analysis

All data are expressed in frequencies. *DRB1* and *DQB1* typing were compared between adult-onset and childhood-onset T1D patients and between adult-onset TIDM patients and controls, separately for Israeli Jews of Ashkenazi and non-Ashkenazi origin. The same methods were used for the analysis of patients with adult-onset T1DM presented in the current study and for the controls and patients with childhood-onset T1DM that were analyzed and published previously [5]. In addition, *DRB1* and *DQB1* typing were compared between the LADA and the classical adult-onset T1D subgroups. Comparisons between patients and control groups are expressed as odds ratios and were statistically tested using the Fisher exact probability test, which is the most exact test for testing odds ratios in 2×2 tables. Comparisons between patients groups (*i.e.,* adult-onset and childhood-onset) were tested using the χ^2 test. For all analyses, Bonferroni-adjusted p values are provided. The Bonferroni adjustment was based on the number of comparisons of frequencies in each table. Thus, ethnically based tables are corrected only within each ethnic group.

Results

Patient characteristics

Mean age at diagnosis was 35.4 years; mean BMI was 24.8 kg/m^2; and mean C-peptide level was 0.81 ng/ml. A total of 61 patients (74.4%) were positive for GADA and/or ICA; 14 patients (17%) were negative for these antibodies; and in 7 patients (8.5%) data on the antibody profile were not available.

Of the 82 patients, 32 met the above-mentioned criteria of the Immunology of Diabetes Society for LADA. Their mean age at diagnosis was 43.3 years; mean BMI was 23.7 kg/m^2; and mean C-peptide level was 0.97 ng/ml. The remaining 50 patients were categorized as having rapidly progressive T1D.

HLA class II alleles positively associated with adult-onset T1D

The *DRB1* and *DQB1* typing of the patients with adult- or childhood-onset T1D and the controls are presented in Tables 1 and 2. *DRB1* and *DQB1* typing of the whole sample and the patients with LADA and classical adult-onset T1D separately are presented in Table 3.

In the *DRB1* group of alleles, *DRB1*03* and *DRB1*0402* were found to be positively associated with adult-onset T1D. Rates for *DRB1*03* in the Ashkenazi subjects (present and reported) were 34.7% in those with adult-onset T1D vs. 40% in those with childhood-onset T1D (p = NS), and 12.8% in