FREQUENCY OF HLA CLASS II DR AND DQ ANTIGENS IN BRAZILIAN PATIENTS WITH TYPE I DIABETES

FREQÜÊNCIA DOS ANTÍGENOS HLA-DR E DQ EM PACIENTES BRASILEIROS COM DIABETES MELLITUS TIPO I

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ABSTRACT: The association of type I diabetes mellitus with genes of the HLA class II system has been well established. These genes may be different among populations due to the genetic background predominating in each ethnic group. The objective of the present study was to characterize HLA class II antigens in a Brazilian population of type I diabetics. The study was conducted on 58 diabetic patients and 102 healthy controls of the same geographic area and of similar ethnic background. B lymphocytes were separated from total lymphocytes by nylon wool. HLA antigens were typed by a complement-dependent microlymphocytotoxicity method. Data were analyzed statistically by the two-tailed exact Fisher test. The frequencies of HLA-DR3 and -DR4 antigens, and of the combinations of HLA-DR3/DR4 antigens were significantly higher in diabetics than in controls ($P < 0.05$). The frequencies of the HLA-DQ2 and DQ3 antigens did not show significant differences when analyzed separately; however, the combinations of HLA-DR3 and HLA-DQ3, HLA-DR4 and HLA-DQ2 or HLA-DQ3, and HLA-DR3 HLA-DR4 and HLA-DQ3 antigens were significantly higher in the diabetic group than in the control ($P < 0.05$). Even though HLA-DR2 and -DR7 antigens (associated with protection against the disease) were less frequent than in the control group, no significant difference was detected after P correction.

UNITERMS: Histocompatibility Antigens. HLA Antigens. Diabetes Mellitus, Insulin-Dependent. Pathogenesis.

1. INTRODUCTION

Studies on different ethnic groups have strongly suggested that susceptibility to type I diabetes (insulin-dependent diabetes mellitus, IDDM) is associated with certain histocompatibility antigens (HLA). HLA class II molecules are implicated in the predisposition to autoimmune disease such as IDDM since they participate in the determination of the repertory of antigenic peptides that T cells (T helper CD4+ lymphocytes) will be able to recognize.

Previous studies[(1)] have shown that the HLA-DR3 and HLA-DR4 specificities of the class II HLA complex increase the risk for IDDM, whereas

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HLA-DR2 is less frequent in these patients. These associations of the HLA-DR locus with IDDM have been detected in diabetic European and North American Caucasians and also in diabetic Black Americans when compared to non-diabetic controls. However, these associations were not detected in a diabetic Chinese population. Brazilian studies[2] have also confirmed the higher frequency of DR3 and DR4 among type I diabetics when compared to healthy controls.

More recent studies[3/19] have shown that susceptibility to IDDM is associated more strongly with the alleles of the HLA-DQ locus. Certain molecules expressed by the genes of the HLA-DQ locus can present peptides derived from beta cells of pancreatic islets which are recognized by T CD4 lymphocytes[17], causing cell destruction in the organism itself. Specifically, these alleles of the HLA-DQ locus are DQB1*0302 (former HLA-DQ3.2 allele or HLA-DQ8 antigen) and DQA1*0301 alleles, usually linked to the HLA-DR4 locus, forming the HLA-DRB1*04/DQB1*0302 haplotype, and are frequent among European and Northern Caucasian diabetics and among Black diabetics. Among Japanese diabetics, the most frequently observed association with DR4 is with DQB1*0402 (HLA-DQ4 antigen). With respect to the HLA-DR3 locus, a frequently detected association is with DQB1*0201 and DQA1*0501 alleles among North American and European Caucasians. In contrast, among Black diabetics, HLA-DR3 antigens seems to be frequently associated with HLA-DQB1*0201 (HLA-DQ2 antigen) and HLA-DQB1*0402 (HLA-DQ4 antigen). Among Japanese individuals, the association of IDDM with HLA-DR3 is infrequent, perhaps owing to the low frequency of HLA-DR3 in this population. On the other hand, HLA-DR9 is quite frequent among Japanese diabetics and is usually associated with HLA-DQ1*0301 (HLA-DQ7 antigen)[8,13,15].

The objective of the present study is to characterize HLA class II antigens of the DQ and DR loci in a Brazilian population of type I diabetics from the Southeastern region of the country.

2. MATERIAL AND METHODS

The study was conducted on 58 type I diabetics (34 women and 24 men), all of them insulin-dependent, predisposed to diabetic ketoacidosis, non-obese, ranging in age from 8 to 60 years (mean, 24.5 ± 1.6 years). The patients were from similar geographic regions, especially the Northeastern region of the state of São Paulo and the Southern region of Minas Gerais, with an ethnic background consisting of a miscegenation of European Caucasians, Brazilian Indians and African Negroes. The control group consisted of 102 healthy individuals (22 women and 80 men) from the same geographic area and with a similar genetic background.

Total peripheral blood lymphocytes from these patients were extracted by centrifugation at 1800 rpm for 20 min and B lymphocytes were separated from total lymphocytes by the nylon wool method (cotton tip technique). HLA class II antigens were serologically typed by the microlymphotoxicity method[20]. The following HLA class II antigens were typed: DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR11, DR13, DR14, DR52, DR53, DQ1, DQ2, DQ3 and DQ7.

Data were analyzed statistically by the two-tailed exact Fisher test [21] and the P value obtained was later corrected by multiplying by the number of specificities tested (corrected P).

3. RESULTS

The frequency of HLA-DR and HLA-DQ antigens in diabetic patients and controls is presented in Table I, with significantly higher values detected in diabetic patients for HLA-DR3 antigen (60.3% for patients versus 18.6% for controls, $P < 0.05$), HLA- DR4 antigen (53.4% for patients versus 27.4% for controls, $P < 0.05$), and for the combination of HLA-DR3/DR4 antigens (37.6% for patients versus 1.97% for controls, $P < 0.05$). The HLA-DQ2 and HLA-DQ3 antigens showed no significant differences when analyzed separately. However, a significantly higher frequency among diabetic patients was obtained for the combinations of HLA-DR3 and HLA-DQ3 antigens (39.6% for patients versus 9.8% for controls, $P < 0.05$), and for the combination of HLA-DR3/DR4/DQ3 antigens (24.1% for patients versus 0.98% for controls, $P < 0.05$). The haplotype HLA-DR4/DQ3 was found in 39.6% of patients and in 18.6% of controls, $P < 0.05$. In contrast, the HLA-DR2 and HLA-DR7 antigens were less frequently observed in type I diabetics compared to controls (13.8% versus 31.4%, and 5.2% versus 20.6%, respectively), although no significant differences were detected after the calculation of the corrected P.

Table I - Frequency of HLA DR and DQ antigens among 58 Brazilian type I diabetics and 102 healthy control individuals

Antígenos HLA	Pacientes (N = 58) (%)	Controles (N = 102) (%)
DR1	13 (22.4)	29 (28.4)
DR2	8 (13.8)**	32 (31.4)
DR3	35 (60.3)*	19 (18.6)
DR4	31 (53.4)*	28 (27.4)
DR5	5 (8.6)	18 (17.2)
DR6	1 (1.7)	8 (7.8)
DR7	3 (5.2)**	21 (20.6)
DR8	0 (0)	2 (1.9)
DR9	5 (8.6)	14 (13.7)
DR11	1 (1.7)	7 (6.8)
DR13	0 (0)	2 (1.9)
DR14	0 (0)	3 (2.9)
DR52	48 (82.7)	61 (59.8)
DR53	34 (58.6)	49 (48.0)
DQ1	28 (48.3)	65 (63.7)
DQ2	19 (32.7)	28 (27.4)
DQ3	36 (62.0)	49 (49.0)
DQ7	12 (20.7)	20 (19.6)
DR3/DR4	16 (27.6)*	2 (1.97)
DR3/DQW3	23 (39.6)*	10 (9.8)
DR4/DQW3	23 (39.6)*	19 (18.6)
DR4/DR3/DQW3	14 (24.1)*	1 (0.98)

* P < 0.05 compared to control (Fisher test);

** P < 0.05 compared to control only before correction (Fisher test).

4. DISCUSSION

Recent studies[11/19] have shown that susceptibility to IDDM is strongly associated with combinations of alleles at the HLA-DQ locus in Caucasian, Black and Japanese subjects (HLA-DQA1 and HLA-DQB1 genes). HLA-DQA1*0301 associated with the DQB1*0302 allele, and HLA-DQA1*0501 associated with DQB1*0101 are more frequently detected among diabetics of the three ethnic groups (in the *cis* position of the combination) compared to controls. In Blacks, a significant association also occurs with DQA1*0301 and DQB1*0201 at the *trans* position. In Caucasians, the genotypes most frequently involved in the susceptibility to the disease are HLA-DR3/DQ2 antigens (DQB1*0201/DQA1*0501 haplotype) and HLA-DR4/DQ8 antigens (DQB1*0302/DQA1*0301 haplotype) [8,13,15]. In the Japanese population, patients with type I diabetes are also associated with DQA1*0301/DQB1*0303 and DQA1*0301/DQB1*0401 haplotypes, both at the *cis* position. Blacks and Caucasians present a weak association with DQA1*0301 and DQB1*0402 alleles. Among Asiatic Indian, Caribbean Black, Scandinavian [7,18,19] and New Zealand diabetics [9,12] there is also a high frequency of the combination of DQB1*0302 alleles with the HLA-DR4 locus.

This association of the HLA-DQ locus with IDDM seems to be due to the substitution of one amino acid (aspartic acid) at position 57 in the beta chain of the HLA- DQ molecule (HLA-DQ beta chain) by alanine, valine or serine but not aspartic acid [6]. However, more recent studies have demonstrated that the situation is much more complex, since Japanese diabetics (type I) frequently present DQ beta chains with an aspartic acid residue [8,13].

À lower susceptibility to the development of IDDM has been detected in individuals with various combinations of DQA1 and DQB1 alleles at the *cis* position, with a strong association of DQA1*0102 allele with the HLA-DQB1*0602 allele (HLA-DQ6 antigen) [13, 18,19] . These alleles related to a lower susceptibility to IDDM are frequently associated with the HLA-DR2 locus.

In the present study there was a higher frequency of HLA-DR3 and HLA-DR4 specificities among diabetics, as also reported in a previous study conducted on Brazilian patients[2] . The association of the HLA-DR3 and HLA-DR4 antigens with the HLA-DQ3 antigen was significantly more frequent among diabetics, as also was the association of HLA-DR3 or HLA-DR4 with the HLA-DQ2 antigen. With respect to the alleles that "protect" against the disease, there was a higher frequency of loci DR2 and DR7 in the controls, although the difference was not significant after P correction. HLA class II typings, as reported here, were performed solely by serological methods, using antibodies which reacted with broad HLA-DQ

specificities such as HLA-DQ1 and HLA-DQ3 antigens. Certainly, the evaluation of HLA class II specificities at the molecular levels will discriminate particular associations with HLA-DQB1 alleles in the Brazilian population. Although the Brazilian population is highly miscegenated, preliminary molecular evaluations of the HLA-DRB1 and DQB1 loci conducted by us [22] or molecular studies of the HLA-DRB1 locus conducted by the University of Campinas, São Paulo, Brazil[23], indicate that immunogenetic susceptibility to type I diabetes in Brazilians has a similar immunogenetic profile, in terms of HLA class II alleles, as has been reported for other Caucasian and Caucasoid populations throughout the world[8,13,16/19].

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RESUMO: A associação entre diabetes mellitus do tipo I com os genes HLA de classe II está bem estabelecida. Os genes podem ser diferentes de acordo com a etnia do grupo estudado. Neste estudo, foram tipificados os antígenos HLA de classe II em uma população brasileira de pacientes com diabetes do tipo I. Para tal, foram estudados 58 pacientes diabéticos e 102 indivíduos sadios, procedentes da mesma área geográfica e com etnia semelhante à dos pacientes. Os linfócitos B foram separados, utilizando-se lã de *nylon*. Os antígenos HLA foram tipificados por intermédio de um método de microlinfocitotoxicidade dependente de complemento. A análise estatística foi realizada pelo teste exato de Fisher bicaudal. As freqüências dos antígenos HLA-DR3 e HLA-DR4 e as combinações dos antígenos HLA-DR3 e DR4 estavam significantemente elevadas nos pacientes ($P < 0,05$). As freqüências dos antígenos HLA-DQ2 e HLA-DQ3 não foram significantemente diferentes daquelas observadas em controles, quando analisadas separadamente, no entanto, as freqüências das combinações dos antígenos HLA-DR3 com HLA-DQ3, HLA-DR4 com HLA-DQ2 ou HLA-DQ3, e, ainda, HLA-DR3, HLA-DR4 com HLA-DQ3 estavam significantemente elevadas nos pacientes diabéticos ($P < 0,05$). Embora as freqüências dos antígenos HLA-DR2 e HLA-DR7 (associados com proteção contra o desenvolvimento da doença) estivessem menores nos pacientes em relação aos controles, a significância não foi atingida após a correção do valor do P.

UNITERMOS: Antígenos de Histocompatibilidade. Antígenos HLA. Diabetes Mellitus Insulino-Dependente. Patogenia.

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