A Combination of HLA-DQβ Asp57-Negative and HLA DQα Arg52 Confers Susceptibility to Insulin-dependent Diabetes Mellitus

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Abstract

Family and population studies indicate that predisposition to insulin-dependent (type I) diabetes mellitus (IDDM) is polygenic. It has been shown that the absence of the aspartic acid in position 57 (Asp57) of the DQβ chain is positively correlated to IDDM. However, Asp57-negative haplotypes do not always confer susceptibility and conversely, some Asp57-positive haplotypes seem to be disease associated. It has been suggested that other HLA class II sequences, probably belonging to the HLA DQA1 gene, confer susceptibility to IDDM.

This report, based on extensive oligonucleotide dot blot hybridization of PCR-amplified DQA1 and DQB1 genes, reinforces the importance of the Asp57-negative DQβ chain, but also introduces the possibility that a DQα chain bearing an arginine in position 52 (Arg52) confers susceptibility to IDDM. A molecular model of susceptibility to IDDM is proposed. This model strongly suggests that the disease susceptibility correlates quantitatively with the expression at the cell surface of a heterodimer, composed of a DQα-chain bearing an Arg52 and a DQβ chain lacking an Asp57.

In view of the respective positions of the two residues and their charge, we might anticipate that both residues DQβ Asp57 and DQα Arg52 are critical for modulation of susceptibility, presumably via viral-antigenic peptide and/or autoantigen presentation. (*J. Clin. Invest.* 1990. 85:1315–1319.) HLA • insulin-dependent diabetes mellitus • oligonucleotide typing

Introduction

The HLA class II D region is divided into several distinct subregions, including HLA-DR, −DQ, and −DP. Each subregion contains A and B genes, which encode for the respective α and β chains of the class II α/β heterodimer present on the cell surface (1). HLA class II molecules are highly polymorphic. The greatest polymorphism occurs in the second exon sequences, which code for the amino-terminal extracellular domain (2). Amino acid residues in that domain interact with antigen peptide fragments and with the T cell.receptor (3, 4).

Like many autoimmune diseases, insulin-dependent diabetes mellitus (IDDM)[1] is associated with particular HLA alleles. Studies in the last decade have shown that IDDM is associated with HLA-DR3, DR4, or both (5, 6). It has been shown that HLA-DQ genes, which are in linkage disequilibrium with DR, are also strongly associated with IDDM (7, 8). Recently, Todd et al. (2) have reported that the susceptibility to the disease correlates with the absence of aspartic acid residue at position 57 of the DQβ chain (Asp57). However, Asp57-negative haplotypes do not always confer susceptibility. HLA-DR3 haplotypes, like HLA-DR7 haplotypes, often have the DQB1*0201 alleles (DQw2 serologically detected) that encode for a β chain lacking an Asp57. DR3-DQB1*0201 haplotypes are positively associated with IDDM, while DR7-DQB1*0201 haplotypes are not (9, 10). Moreover, the analysis of the DNA sequences from black IDDM patients (DR7, DR9) indicates that they have the same DQα chain as that of DR4 caucasoid IDDM patients (11). These observations suggest that another gene, probably DQA1, may play a role in IDDM susceptibility. To evaluate this role, we have studied IDDM caucasoid unrelated patients and healthy controls by extensive oligotyping for DQA1 and DQB1 genes.

Methods

Subjects. Unrelated caucasoid patients (n = 50) suffering from IDDM, followed at the Herold Hospital, were genotyped for HLA-A, B, C, and DR, and for Bf, C4A, and CAB complement components (5). Their age at onset was 1–18 yr. Controls (n = 73) were healthy individuals randomly selected and had been previously serologically typed for HLA, A, B, C, and DR antigens using workshop reference reagents.

Cell lines. A total of 30 lymphoblastoid cell lines, homozygous for the HLA-D region fully defined at the Xth International Histocompatibility Workshop (12), were used as reference cells.

Oligonucleotide typing. From 1-μg DNA samples, the second exons encoding for the first polymorphic domains of the HLA-DQA1 and -DQB1 genes were amplified by polymerase chain reaction (13), using specific DNA flanking primers. The primer sequences are:

PP-DQA1-PL 5'GGTGTAAACTTGTACCAC-3' (position 12–18),
PP-DQA1-PR 5'-CATTGGTAGCAGCGGTAGAG-3' (80–86),
PP-DQB1-PL 5'-CATGTGCTACTTCACCAACG-3' (13–20), and
PP-DQB1-PR 5'-GTAGTTGTGTCTGCACAC-3'(78–83).

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1. *Abbreviations used in this paper:* IDDM, insulin-dependent diabetes mellitus; P, protective; S, susceptible.

After 30 cycles of amplification, an aliquot of each amplified sample was dot blotted on a nylon membrane and alleles were identified by autoradiography after hybridization with (γ-^{32}P) ATP end-labeled oligonucleotide probes. Oligonucleotide sequences probes are displayed in Table I.

Nomenclature. The nomenclature used was according to the official Nomenclature for Factors of the HLA System 1989 (14).

Results

The analysis of amplified DNA from 50 IDDM caucasoid patients and 73 controls, using 9 DQA1 and 10 DQB1 oligonucleotide probes (Table I), defined 8 DQA1 and 12 DQB1 alleles (Table II). As shown in Table II, the DQB1*0201 (DQw2) and DQB1*0302 (DQw8) alleles were significantly increased in IDDM patients. Both DQB1*0201 and DQB1*0302 alleles encode for a DQβ chain lacking Asp57 (2). Moreover, the DQA1*0501 and DQA1*0301 alleles were found highly increased in IDDM patients compared with controls. Conversely, the frequencies of DQA1*0201, 0102, and 0103 alleles were decreased among patients. From the results presented in Table III, we observed that DQA1*0501 and DQA1*0301 alleles are strongly associated with DR3-DQB1*0201 and DR4-DQB1*0302 haplotypes, respectively. We have compared the first domain published sequences (2, 15, 16) of DQA1*0501 and DQA1*0301 alleles (both associated with IDDM) with that of DQA1*0201 allele (negatively correlated with the disease). This revealed that DQA1*0501 and DQA1*0301 encode for an amino acid residue Arg52 of the α chain, while DQA1*0201 encodes for a histidine at this position. Hence, these results suggest that the residue Arg52 may play a crucial role in IDDM susceptibility. The presence of Arg52 was directly confirmed using probe AG3 (Table I).

We defined four DQA1 alleles coding for Arg52-positive as susceptible (S) (DQA1*: 0501, 0301, 0401, and 0601) and four others for Arg52-negative as protective (P) (DQA1*: 0201, 0101, 0102, and 0103). Similarly, five DQB1 genes coding for Asp57-negative were considered susceptible (S) (DQB1*: 0201, 0501, 0502, 0604, 0302) and eight DQB1 genes for Asp57-positive were considered protective (DQB1*: 0503, 0601, 0602, 0603, 0401, 0402, 0301, 0303) (Table III).

To delineate the respective roles of susceptible and protective alleles, the data were analyzed at the molecular level, taking into account all the possible assembled DQ α-β molecules coded by genes in *cis* and *trans* (17, 18). Three kinds of heterodimers can be postulated at the cell membrane, S-S being susceptible, and S-P and P-P being protective. This led to the definition of 10 different cases (Table IV). Our results show that individuals with susceptible heterodimers DQ αS-βS are all affected (case 1). In cases 2 and 3 the percentage of patients is roughly of the same order as the expected frequency of the formation of the DQ αS-βS susceptible heterodimer, assuming that *cis* and *trans* association are equally probable. For cases 4 and 5, the observed percentage of patients is slightly smaller than the calculated frequency of the formation of the DQ αS-βS heterodimer. This could result from a bias due to the small number analyzed. In the absence of a susceptible chain or susceptibility present only on α or β chain, no IDDM patients were observed (cases 6–10). This indicates the absolute requirement of complete susceptible heterodimer expression at the cell surface for the manifestation of IDDM disease.

Table I. Synthesized Oligonucleotide Probes

Probe	Allele	Sequence	Amino acid position
DQB1			
BG1	0602, 0603	5′-GGCGGCCTGATGCCGAGTAC-3′	54–60
BG2	0601, 0503	5′-GGCGGCCTGACGCCGAGTAC-3′	54–60
BG3	0301, 0303	5′-GGCCGCCTGACGCCGAGTAC-3′	54–60
BG4	0401, 0402	5′-GGCGGCTTGACGCCGAGTAC-3′	54–60
BG5	0302	5′-GGCCGCCTGCCGCCGAGTAC-3′	54–60
BG6	0501, 0604	5′-GGCGGCCTGTTGCCGAGTAC-3′	54–60
BG7	0501, 0503, 0502, 0401, 0402	5′-GCGTGCGGGGTGTGACCAGA-3′	23–29
BG8	0301, 0602	5′-GCGTGCGTTATGTGACCAGA-3′	23–29
BG9	0201	5′-GAGAAGAGATCGTGCGCTTC-3′	34–40
BG10	0604, 0602, 0301, 0302, 0303, 0603	5′-GGGCGGAGTTGGACACGGTG-3′	72–78
DQA1			
AG1	0101, 0102, 0103	5′-GCCTGGCGGTGGCCTGAG-3′	45–50
AG2	0103	5′-GGACCTGGAGAAGAAGGAGAC-3′	37–44
AG3	0501, 0601, 0401	5′-GTGTTTGCCTGTTCTCAGAC-3′	46–53
AG4	0501	5′-CTTGAACAGTCTGATTAAACGC-3′	72–79
AG5	0301	5′-GTCTGGCAGTTGCCTCTG-3′	45–50
AG6	0201	5′-CTGTTCCACAGACTTAG-3′	50–55
AG7	0601, 0401	5′-CATCGCTGTGACAAAACATAAC-3′	65–72
AG8	0103, 0201, 0601	5′-CTGGCCAGTTCACCCATGA-3′	22–28
AG9	0102, 0103, 0501, 0601	5′-GGAGATGAGCAGTTCTACGTG-3′	31–37

Table II. Sequence-specific Oligonucleotide Dot Blot Analysis of 50 IDDM Patients and 73 Controls

	IDDM		Controls		P
	n	%	n	%	
DQA1*					
0101	10	20	21	29	NS
0102	3	6	24	33	10^{-4}
0103	0	0	15	21	10^{-3}
0201	2	4	19	26	10^{-3}
0301	39	78	18	25	10^{-7}
0401	0	0	6	8	NS
0501	40	80	28	38	10^{-6}
0601	0	0	1	1	NS
DQB1*					
0501	10	20	16	22	NS
0502	3	6	1	1	NS
0503	0	0	5	7	NS
0601	0	0	1	1	NS
0602	0	0	19	26	10^{-4}
0603	0	0	13	18	10^{-3}
0604	0	0	8	11	NS
0201	37	74	31	42	10^{-5}
0301	5	10	21	29	10^{-1}
0302	37	74	12	16	10^{-7}
0303	0	0	0	0	NS
4**	1	2	6	8	NS

n = the number of individuals carrying the indicated alleles; % = the phenotypic frequency of the corresponding chain.
$P > 0.05$.
** = 0401 + 0402 alleles.

Discussion

Susceptibility to IDDM has been previously shown to be highly correlated with the absence of an Asp57 on the DQβ chain (2). Nevertheless, the presence or the absence of this residue in the DQβ sequences of IDDM patients cannot entirely determine the susceptibility or the resistance to the disease (19, 20). In this report we confirmed the importance of DQβ Asp57 residue in the disease susceptibility. Moreover, we tested the probable implication of the DQα chain in IDDM disease. Our results, based on oligonucleotide typing of the DQα chain of 123 individuals including 50 IDDM patients, demonstrate the implication of a DQα Arg52 residue in IDDM susceptibility.

According to these results, we proposed a molecular model based on the expression, at the cell surface, of a DQ αS-βS heterodimer consisting of DQβ chain Asp57 negative, associated to a DQα chain Arg52 positive. In IDDM, patients who carry the PS/SP where the susceptible positions are on separate haplotypes may indicate an important role for the class II dimer, rather than one chain alone in susceptibility to IDDM. Further analysis concerning these rare IDDM genotypes should be done to confirm this role.

It is likely that the three-dimensional conformation of the DQ molecule will dictate T cell recognition and its function in the immune response. By analogy with the HLA-A2 class I crystallographic structure recently proposed (21), a model for class II molecule structure was hypothesized (22). In this model, residues on the α-helices and β-sheets encoded by the second exon sequences of the A and B genes form the walls and floor of a cavity. This cavity binds an antigen for its presentation to the T cell receptor. These two residues (DQβ Asp57 and DQα Arg 52) are located at the opposite extremities of the α-helical sides of the antigenic groove. Moreover, the replacement of the aspartic acid by a neutral amino acid (serine, valine, and alanine) and the presence of arginine residue might therefore modify the antigenic peptide presentation. This led us to suggest that both residues may simultaneously interact in IDDM susceptibility.

We showed that in the absence of the DQ αS-βS heterodimer, no IDDM patients are observed. Nevertheless, the presence of this DQ association in some healthy individuals dem-

Table III. Association of DR with DQA1 and DQB1 Alleles Detected by Oligotyping

DR**	DQB1*	DQβ-Asp57	DQα-Arg52	DQA1*
1	0501	S	P	
10	0501	S	P	0101
14	0503	P	P	
15	0602	P	P	
16	0502	S	P	0102
13	0604	S	P	
8	0601	P	P	
15	0601	P	P	0103
5	0601	P	P	
13	0603	P	P	
7	0201	S	P	0201
7	0201	S	P	
4	0301	P	S	
4	0302	S	S	
4	0401	P	S	0301
9	0303	P	S	
8	0402	P	S	
3	0402	P	S	0401
11	0301	P	S	
12	0301	P	S	
14	0301	P	S	0501
16	0301	P	S	
3	0201	S	S	
8	0301	P	S	0601

S, Susceptible chain (DQβ Asp57 negative; DQα Arg52 positive).
P, Protective chain (DQβ Asp57 positive; DQα Arg52 negative).
** *DR*, serologically determined as assigned during the Xth International Histocompatibility Workshop for the cell lines (12).

Table IV. Distribution of Susceptible and Protective HLA-DQ Molecules among IDDM Patients and Controls

Cases	HLA-DQ α	HLA-DQ β	Possible expressed heterodimer	Patients (n = 50)	Controls (n = 73)
1	S	S	SS, SS, SS, SS	32	0
	S	S			
2	S	S	SS, SS, SP, PS	4	6
	S	P			
3	S	S		12	7
	P	S			
4	P	S	SS, PP, SP, PS	2	6
	S	P			
5	S	S		0	17
	P	P			
6	P	S	SP, SP, PS, PS	0	10
	P	S			
7	S	P		0	5
	S	P			
8	P	S	SP, PS, PP, PP	0	11
	P	P			
9	P	P		0	6
	S	P			
10	P	P	PP, PP, PP, PP	0	5
	P	P			

SS, HLA-DQ molecules susceptible; *SP, PS, PP*, HLA-DQ molecules protective.

onstrates that this factor is necessary but not sufficient for the disease induction and may have a quantitative effect. However, it might be important to measure the amount of molecules expressed on the cell surface to verify this point. These findings and the observations previously supported by HLA-DR typing of twins (23, 24) suggest that an HLA-linked gene(s) is not the only diabetogenic gene, and that environmental factors might play a role.

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