Dose Effect of Cis- and Trans-Encoded HLA-DQαβ Heterodimers in IDDM Susceptibility

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Insulin-dependent diabetes mellitus (IDDM) in whites is strongly associated with particular HLA-DQαβ heterodimers composed of a DQα chain with an arginine at residue 52 ($Arg52^+$) combined to a DQβ chain lacking an aspartic acid at residue 57 ($Asp57^-$). With the aim of confirming this association, clarifying which heterodimers account for the highest risk of IDDM and explaining the excess risk of DR3-DQw2/DR4-DQw8, 115 unrelated white IDDM patients and 108 unrelated healthy nondiabetic control subjects were studied. With polymerase chain reaction and sequence-specific oligonucleotide probes, both patients and control subjects were typed for their HLA-DQA1 and DQB1 alleles and their DQA1-DQB1 haplotype and genotype frequencies were compared. Four major findings emerged from our analysis. *1)* $Arg52^+$ DQα/$Asp57^-$ DQβ heterodimers, formed in cis and/or in trans, are strongly associated with susceptibility to IDDM; 97% of patients and 46% of control subjects had at least one such susceptibility heterodimer (relative risk [RR] 32, confidence interval [CI] 14.25–71.86, $P < 10^{-7}$). *2)* The degree of disease susceptibility depends on the number of such DQ heterodimers that a subject can express according to his or her DQA1-DQB1 genotype. The highest RR was observed in patients with four susceptibility DQ heterodimers (RR 41, CI 17.05–95.9). *3)* Only part of the susceptibility DQ heterodimers were significantly increased in patients, conferring IDDM susceptibility of different strength. The strongest association was with the DQA1*0501-DQB1*0302 combination formed in trans position (RR 35.2, CI 12.88–96.78, $P < 10^{-7}$). *4)* The simultaneous expression of four different susceptibility heterodimers explains the highest risk of DR3-DQw2/DR4-DQw8 heterozygotes. In conclusion, there is a dose effect of cis- and trans-encoded HLA-DQαβ heterodimers in IDDM susceptibility influenced by the diversity of these molecules.
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Insulin-dependent diabetes mellitus (IDDM) results from autoimmune destruction of the insulin-producing β-cells of pancreatic islets. Genetic predisposition to IDDM is partly determined by genes encoded within the MHC, located on chromosome 6 in humans. In whites, IDDM is strongly associated with HLA–DR3-DQw2/DR4-DQw8 heterozygous combination (1–4). Several studies reported that aspartic acid at residue 57 of the DQβ chain ($Asp57^+$) provides resistance to IDDM, whereas other amino acids ($Asp57^-$) are associated with susceptibility (5–9). However, DR1-DQw5, DR2-DQw5, DR13-DQw6, and DR7-DQw2 haplotypes in whites, encoding $Asp57^-$ DQβ chains, are not increased in IDDM patients (5, 6, 10). Therefore, this residue alone is insufficient to determine susceptibility or resistance to IDDM. These observations and comparative studies in different ethnic groups suggested that the DQα chain may play a role in the susceptibility to IDDM (11,12).

By extensive DQA1 and DQB1 oligotyping in white IDDM patients, we reported the probable implication of an arginine at position 52 of the DQα chain ($Arg52^+$) in the susceptibility to IDDM (13). Accordingly, we proposed a molecular model of susceptibility to IDDM based on the expression, at the cell surface in cis and/or trans, of an HLA-DQ heterodimer consisting of an $Arg52^+$ DQα chain associated to an $Asp57^-$ DQβ chain. Biochemical and T-cell studies confirmed that DQαβ heterodimers are encoded by DQA1 and DQB1 genes in both cis and trans positions (14–18). However, our model presents both DR3-DQw2 and DR4-DQw8 homozygous and DR3-DQw2/DR4-DQw8 heterozygous individuals with similar

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TABLE 1
Panel of DQA1 and DQB1 typing sequence-specific oligonucleotide probes

Probe	Allele	Sequence	Amino acid position
DQA1			
AG1-52	101, 102, 103	5′-CCTGAGTTCAGCAAATTTGG-3′	49-55
AG3-52	501, 601, 401	5′-GTGTTTGCCTGTTCTCAGAC-3′	46-53
AG5-52	301	5′-CAGTTGCCTCTGTTCCGC-3′	47-52
AG6-52	201	5′-CCTCTGTTCCACAGACTTAGA-3′	50-55
AG8m	103, 201, 601	5′-TGGCCAGTTCACCCATGAA-3′	22-28
AG9m	102, 103, 501	5′-GGAGATGAGCAGTTCTACG-3′	31-37
AG10	101	5′-GGAGATGAGGAGTTCTACG-3′	31-37
AG11	101, 102, 401, 501	5′-TGGCCAGTACACCCATGAA-3′	22-28
AG12	201, 401, 601	5′-ACTTGAACATCCTGATTAAACG-3′	73-79
DQB1			
BG1m	602, 603	5′-GCGGCCTGATGCCGAGTAC-3′	54-60
BG2m	601, 503	5′-GGCGGCCTGACGCCGAGT-3′	54-60
BG3m*	301, 303	5′-GCCGCCTGACGCCGAGTA-3′	54-60
BG4m	401, 402	5′-GGCGGCTTGACGCCGAGTA-3′	54-60
BG5M	302	5′-GGCCGCCTGCCGCCGAGT-3′	54-60
BG6	501, 604, 605	5′-GGCGGCCTGTTGCCGAGTA-3′	54-60
BG7	501, 503, 502, 402	5′-GCGTGCGGGGTGTGACCAGA-3′	23-29
BG8*	301, 601	5′-GCGTGCGTTATGTGACCAGA-3′	23-29
BG9*	201	5′-GAGAAGAGATCGTGCGCTTC-3′	34-40
BG11	502	5′-GCGGCCTAGCGCCGAGTAC-3′	54-60
BG12*	603, 604	5′-TGCGTCTTGTAACCAGACAC-3′	24-31
BG13	602, 302, 303	5′-GTGCGTCTTGTGACCAGACAC-3′	24-30
BG14	402	5′-GACCGAGCGCGTGCGGGG-3′	20-26

All DQA1 probes were washed for 15 min at 58°C, and all DQB1 probes were washed for 15 min at 64°C, except at 62°C.
m, Probe sequences as reported in ref. 13, with modifications.

risk to develop the disease without considering the excess of heterozygous individuals among IDDM patients. Therefore, this study is to confirm our proposed model and attempts to clarify the excess risk of DR3-DQw2/DR4-DQw8 among IDDM patients. With the polymerase chain reaction (PCR) and sequence-specific oligonucleotide (SSO) probes, we studied 115 unrelated white IDDM patients and 108 healthy nondiabetic control subjects for their DQA1 and DQB1 alleles, haplotypes, and genotypes.

RESEARCH DESIGN AND METHODS

One hundred fifteen unrelated white IDDM patients, followed at the pediatric diabetic unit of the Sick Children's Hospital (Hôpital Enfants-Malades, Paris) were consecutively recruited for the study with no selection criteria other than their informed consent. The results of 50 IDDM patients reported previously are included in this study (13). All patients had insulin-dependent and ketosis-prone diabetes with age at onset <30 yr (mean ± SD age of onset 9.9 ± 5.3 yr). Twenty-seven patients had a first-degree relative with IDDM (14 had an affected sibling, 11 had an affected parent, and 2 had an affected child).

The control population consisted of 108 randomly selected healthy nondiabetic blood donors with similar origins. None had a family history of IDDM, and blood glucose was measured in these subjects. All subjects were serologically typed for HLA-A, -B, -C, and -DR, -DQ antigens with Histocompatibility Workshop reference reagents (19). The families of the patients also had been serologically typed, enabling us to deduce patients' haplotypes.

Cell lines. DNA from 30 HLA-D homozygous lymphoblastoid cell lines, fully defined during the 10th International Histocompatibility Workshop (19), were used as reference controls for each hybridization.

Amplification of HLA class II. DNA was isolated from fresh or frozen peripheral blood lymphocytes by a nonionic detergent method (20). One microgram DNA was amplified by PCR with thermus aquaticus *Taq* DNA polymerase as described elsewhere (21,22). The second exon of the DQA1 and DQB1 genes was amplified with the following primers:

PP-DQA1-PL: 5′-GGTGTAAACTTGTACCAG-3′ (12-18)
PP-DQA1-PR: 5′-CATTGGTAGCAGCGGTAGAG-3′ (80-86)
PP-DQB1-PL: 5′-CATGTGCTACTTCACCAACG-3′ (13-20)
PP-DQB1-PR: 5′-GTAGTTGTGTCTGCACAC-3′(78-83)

Amplifications were accomplished by 30 cycles of incubation at 92°C for 1 min, 50°C for 1 min, and 72°C for 2 min.

SSO analysis of amplified DNA. The combination of the DQA1 and DQB1 SSO probes used allowed the determination of the known DQA1 and DQB1 alleles in both homozygous and heterozygous individuals (Table 1). Amplified DNA samples were dot blotted onto nylon filter (Biotrace, Gelman Science, Ann Arbor, MI) and hybridized for 1 h at 50°C in a solution containing 5× SSPE (0.9 M NaCl/50 mM $Na_2H_2PO_4$/5 mM EDTA), 5× Denhardt's, 0.5% sodium dodecyl sulfate (SDS), and the [γ-^{32}P]ATP end-labeled SSO probes. Filters were washed for 20 min

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TABLE 2
Distribution of DQA1 and DQB1 alleles among insulin-dependent diabetes mellitus (IDDM) patients and healthy nondiabetic control subjects

		IDDM (n = 115; %)	Control (n = 108; %)	Relative risk	Confidence interval	P
DQA1*	Arg52					
0101	P	19	27			
0102	P	10	36	0.23	0.12–0.41	$2 \cdot 10^{-5}$
0103	P	2	20	0.07	0.02–0.22	$2 \cdot 10^{-5}$
0201	P	10	17			
0301	S	71	29	6.8	3.88–12.47	10^{-7}
0401	S	5	6			
0501	S	67	44	2.9	1.56–5.33	$9 \cdot 10^{-5}$
0601	S	1	1			
DQB1*	Asp57					
0501	S	19	22			
0502	S	3	3			
0503	P	0	7	0.09	0.01–0.54	0.007
0601	P	1	1			
0602	P	1	23	0.03	0.008–0.11	10^{-7}
0603	P	1	18	0.04	0.001–0.17	$3 \cdot 10^{-5}$
0604+	S	6	9			
0201	S	71	32	5.8	3.32–10.48	10^{-7}
0301	P	9	39	0.17	0.08–0.33	10^{-6}
0302	S	63	16	10.9	5.84–20.35	10^{-7}
0303	P	0	3			
0401	P	1	0			
0402	P	5	6			

S, susceptible chains (DQα $Arg52^+$, DQβ $Asp57^-$); P, protective chains (DQα $Arg52^-$, DQβ $Asp57^+$).
+, 0604 and 0605.

in 5× SSPE/0.1% SDS at 58°C for all DQA1 SSO probes and at 64 or 62°C for DQB1 SSO probes (Table 1).

HLA class II alleles nomenclature. The latest nomenclature of the World Health Organization committee for factors of the HLA system was used (23).

Statistical analysis. The DQA1-DQB1 haplotypes were calculated from phenotype frequencies according to Mattiuz et al. (24). The number of patients and control subjects positive for an allele and the haplotypes and genotypes were compared by χ^2 analysis with Yates' continuity correction. Fisher's exact test was used when theoretical numbers were ≤3. P ≤ 0.05 was significant. Relative risk (RR) was estimated according to Wolf's formula, and Haldane's modification of the formula was used in sets containing 0 (25).

RESULTS

HLA-DQA1 and DQB1 alleles. The distribution of the 8 HLA-DQA1 and 13 DQB1 alleles among the 115 IDDM patients and 108 healthy nondiabetic control subjects is shown in Table 2. As previously described (5,13), alleles encoding $Arg52^+$ DQα and $Asp57^-$ DQβ chains are susceptibility alleles (S), whereas those encoding $Arg52^-$ DQα and $Asp57^+$ DQβ chains are protective (P). The gene frequencies of DQA1 and DQB1 susceptibility alleles were 80 and 91%, respectively, in patients compared with 44 and 45%, respectively, in control subjects. However, the various susceptibility alleles were not equally present among patients. Only the frequencies of DQA1*0301, 0501 and DQB1*0302, 0201 alleles were significantly increased in patients compared with control subjects. Conversely, the frequencies of DQA1*0102, 0103, and DQB1*0503, 0602, 0603, and 0301 P alleles were significantly decreased among patients.

DQA1-DQB1 haplotypes. The various haplotypes in patients and control subjects show that the susceptibility to IDDM is more strongly related to a DQA1-DQB1 haplotype rather than to one allele alone (Table 3). The most frequent haplotypes among patients were those consisting of DQA1 and DQB1 susceptibility alleles (S-S; 74 vs. 16% in control subjects, RR 14, CI 9.31–21.32, $P < 10^{-7}$), mostly including the DQA1*0501-DQB1*0201 and DQA1*0301-DQB1*0302 haplotypes associated with DR3-DQw2 and DR4-DQw8, respectively. The DQA1*0301-DQB1*0201 (S-S) haplotype, in linkage disequilibrium with DR9, is rare in whites but is increased in black IDDM patients (11). On the other hand, haplotypes consisting of DQA1 and DQB1 protective alleles (P-P) were highly significantly decreased among patients (1 vs. 27% in control subjects, RR 0.04, CI 0.01–0.09, $P < 10^{-7}$). Haplotypes including a protective DQA1 and a susceptibility DQB1 allele (P-S) induced a neutral effect with respect to IDDM (20 vs. 28% in control subjects, NS), whereas those composed of a susceptibility DQA1 and a protective DQB1 allele (S-P) were significantly decreased among patients (8 vs. 27% in control subjects, RR 0.23, CI 0.13–0.4, $P < 10^{-7}$). This suggests that the effect of DQB1 alleles predominates over that of DQA1 in susceptibility or resistance.

DQA1-DQB1 genotypes. Table 4 presents the frequences of DQA1-DQB1 genotypes among patients and control subjects. The genotypes are listed from highest to lowest frequencies in patients. Only 26 different DQA1-DQB1 genotypes were observed in IDDM patients com-

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TABLE 3
DQA1-DQB1 haplotype frequencies in 115 insulin-dependent diabetes mellitus (IDDM) patients and 108 control subjects

DR+	DQA1*-DQB1*		IDDM (n = 230; %)	Control (n = 216; %)	Relative risk	Confidence interval	P
3	0501–0201	S-S	34	9	5.1	3.09–8.42	10^{-7}
4	0301–0302	S-S	36	7	6.9	4.07–11.65	10^{-7}
9	0301–0201	S-S	2	0.4			
1	0101–0501	P-S	10	12			
7	0201–0201	P-S	5	9			
13	0102–0604	P-S	3	5			
2	0102–0502	P-S	2	2			
13	0103–0604	P-S	0	0.4			
8	0401–0402	S-P	3	3			
5	0501–0301	S-P	3	16	0.14	0.06–0.30	10^{-6}
4	0301–0301	S-P	1	7	0.18	0.06–0.55	$2 \cdot 10^{-3}$
8	0601–0301	S-P	0.4	0.4			
4	0301–0401	S-P	0.4	0			
9	0301–0303	S-P	0	0.4			
2	0102–0602	P-P	0.4	13	0.03	0.008–0.11	10^{-7}
13	0103–0603	P-P	0.4	9	0.04	0.009–0.17	10^{-5}
2	0103–0601	P-P	0.4	0.4			
14	0101–0503	P-P	0	3	0.12	0.02–0.69	0.01
14	0103–0503	P-P	0	0.4			

S, susceptible chains (DQα $Arg52^+$; DQβ $Asp57^-$); P, protective chains (DQα $Arg52^-$; DQβ $Asp57^+$).
+, Serologically determined.

pared with 57 in healthy nondiabetic control subjects. The DR3-DQA1*0501-DQB1*0201/DR4-DQA1*0301-DQB1*0302 heterozygous genotype was the only one significantly increased among patients (37 vs. 2% in control subjects, RR 32, CI 11.45–89.38, $P < 10^{-7}$). Both DR3-DQA1*0501-DQB1*0201 and DR4-DQA1*0301-DQB1*0302 homozygous genotypes were increased in patients without reaching statistical significant (RR 8 and 15, respectively). Of the 115 IDDM patients investigated, 58 (50%) had one of three DQ genotypes compared with 3% of control subjects (RR 36, CI 14.9–86.4, $P < 10^{-7}$). Of the remaining 57 IDDM patients, 46 (81%) carried

TABLE 4
Distribution of DQA1-DQB1 genotypes among 115 insulin-dependent diabetes mellitus (IDDM) patients compared with 108 control subjects

DQA1*-DQB1*/DQA1*-DQB1*	IDDM (%)	Control (%)	Relative risk	Confidence interval	P
0301–0302/0501–0201	36	2	32	11.45–89.38	10^{-7}
0301–0302/0101–0501	7	2			
0501–0201/0501–0201	7	1			
0301–0302/0301–0302	6	0			
0301–0302/0201–0201	5	0			
0501–0201/0101–0501	5	1			
0501–0201/0201–0201	3	2			
0301–0302/0102–0604	3	2			
0501–0201/0102–0604	3	0			
0301–0302/0501–0301	3	0			
0501–0201/0401–0402	3	2			
0101–0501/0301–0301	3	1			
0301–0302/0301–0201	2	0			
0501–0201/0301–0201	2	0			
0501–0201/0102–0502	2	0			
0101–0501/0501–0301	2	3			
0301–0302/0102–0502	1	0			
0102–0604/0601–0301	1	0			
0301–0302/0401–0402	1	1			
0501–0201/0301–0401	1	0			
0301–0201/0401–0402	1	1			
0201–0201/0401–0402	1	1			
0101–0501/0201–0201	1	3			
0101–0501/0102–0602	1	3			
0102–0502/0103–0603	1	2			
0501–0301/0103–0601	1	0			
Others	0	72			

*, Forty-two different genotypes found in healthy nondiabetic subjects and absent in IDDM patients.

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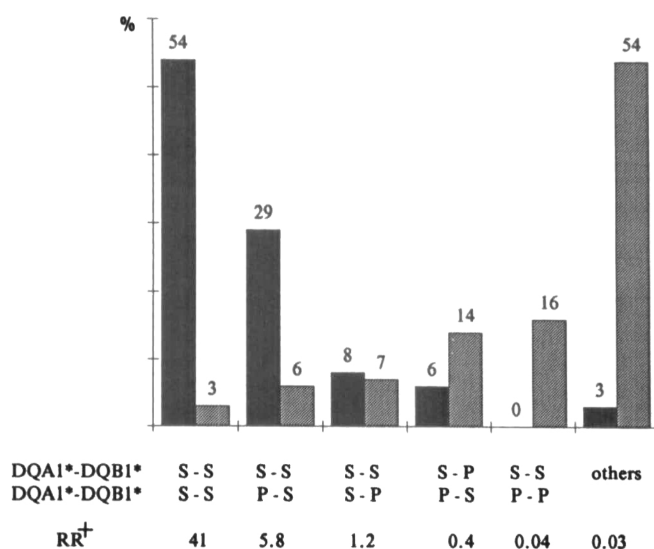

FIG. 1. Frequencies (%) of different HLA-DQ genotypes according to number of susceptibility (S) or protective (P) DQA1 and DQB1 alleles. Although 97% of diabetic (■) and 46% of control (▨) subjects possessed a genotype encoding at least 1 DQαβ susceptibility heterodimer (S-S) either in cis or trans (relative risk [RR] 32, $P < 10^{-7}$), the highest risk was observed for S-S/S-S genotypes exclusively encoding susceptibility heterodimers (RR 41, $P < 10^{-7}$). Genotypes unable to encode a DQαβ susceptibility heterodimer are summarized as *others*. Genotypes of the 4 patients in this group are shown in Table 4 (the last 4 genotypes). +, confidence intervals (CI) of each RR are as follows: RR 41, CI 17.05–95.09; RR 5.8, CI 2.61–12.73; RR 0.4, CI 0.15–1.00; RR 0.04, CI 0.009–0.17; RR 0.03, CI 0.01–0.06.

either the DQA1*0501-DQB1*0201 or the DQA1*0301-DQB1*0302 haplotype together with various other haplotypes.

The frequencies of the different genotypes show that the degree of susceptibility or resistance is related to the two haplotypes in a genotype (Fig. 1). Fifty-four percent of patients and 3% of control subjects had a genotype exclusively composed of DQA1 and DQB1 susceptibility alleles (S-S/S-S; RR 41, CI 17.05–95.9, $P < 10^{-7}$) encoding 4 types of susceptibility heterodimers (2 in cis and 2 in trans). Among patients with two susceptibility heterodimers, two categories were observed: genotypes composed of one DQA1 and two DQB1 susceptibility alleles (S-S/P-S) were increased among patients (29 vs. 6% in control subjects), whereas those composed of two DQA1 alleles and one DQB1 susceptibility allele (S-S/S-P) were found nearly equally among patients and control subjects (8 vs. 7%). Six percent of patients and 30% of control subjects possessed genotypes containing one DQA1 and one DQB1 susceptibility allele (encoding only one susceptibility heterodimer). Among the latter, all patients (6 vs. 14% in control subjects) carried these alleles on separate haplotypes (S-P/P-S genotypes) and none, compared with 16% of control subjects, on the same haplotype (S-S/P-P) ($P < 0.03$). This may indicate a particular role for the trans-encoded DQαβ heterodimers. Genotypes that are unable to encode an IDDM susceptibility DQ heterodimer neither in cis nor trans (summarized as "others" in Fig. 1) were highly significantly decreased among patients (3 vs. 54% in control subjects, RR 0.03, CI 0.01–0.06, $P < 10^{-7}$).

DQA1 and DQB1 combinations. To evaluate whether all DQαβ susceptibility heterodimers account for a similar risk of IDDM, the frequencies of the different DQA1-DQB1 combinations formed in cis and/or in trans were compared in patients and control subjects. Table 5 presents the most frequent DQA1-DQB1 combinations among patients sorted according to RR, from high to low. The DQA1*0501-DQB1*0302 combination, formed only by genes in trans, showed the highest RR (35.2, CI 12.88–96.78, $P < 10^{-7}$). Both DQA1*0301-DQB1*0201 (formed mainly in trans) and

TABLE 5
Distribution of the most frequent HLA DQA1-DQB1 combinations formed in cis and/or trans in patients and healthy nondiabetic control subjects

DQA1*-DQB1*	Insulin-dependent n	Insulin-dependent %	Control n	Control %	Relative risk	Confidence interval	P
0501–0302							
Cis	0		0				
Trans	46		2				
Cis or trans	46	40	2	2	35.3	12.88–96.78	10^{-7}
0301–0201							
Cis	5		1				
Trans	50		5				
Cis or trans	55	48	6	6	15.6	7.11–34.19	10^{-7}
0301–0302							
Cis	75		17				
Trans	9		0				
Cis or trans	84	73	17	16	14.5	7.85–26.5	10^{-7}
0501–0201							
Cis	71		20				
Trans	3		8				
Cis or trans	84	73	28	23	7.7	4.36–13.59	10^{-7}
0301–0501							
Cis	0		0				
Trans	12		3				
Cis or trans	12	10	3	3	4.1	1.22–13.75	0.02

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DQA1*0301-DQB1*0302 (formed mainly in cis) showed similar values (RR 15.6, CI 7.11–34.19 and RR 14.9, CI 7.85–26.5, $P < 10^{-7}$, respectively). The DQA1*0501-DQB1*0201 combination, predominantly formed in cis, was relatively frequent among control subjects, and therefore had a lower RR (7.7, CI 4.36–13.59). In patients, the two trans DQ heterodimers (DQA1*0501-DQB1*0302 and DQA1*0301-DQB1*0201) were mainly encoded by the DR3-DQA1*0501-DQB1*0201/DR4-DQA1*0301-DQB1*0302 genotype, although each can also be encoded by several other genotypes either in cis or trans (Table 4). Another DQ combination, DQA1*0301-DQB1*0501, formed in trans position by DQA1*0301-DQB1*0302/DQA1*0101-DQB1*0501 (i.e., DR4-DQw8/DR1) and DQA1*0301-DQB1*0301/DQA1*0101-DQB1*0501 (i.e., DR4-DQw7/DR1) genotypes (Table 4), was also slightly increased among patients (10 vs. 3% in control subjects, RR 4.1, CI 1.22–13.5, $P < 0.02$). After exclusion of DR3-DQA1*0501-DQB1*0201/DR4-DQA1*0301-DQB1*0302 subjects, this trans combination was significantly associated with IDDM ($P < 0.001$).

DISCUSSION

In this study, extensive oligotyping for DQA1 and DQB1 alleles in 115 IDDM patients and 108 healthy nondiabetic control subjects was performed, and the analysis of both DQA1-DQB1 haplotypes and genotypes allowed us to confirm that IDDM susceptibility is most strongly associated with the expressed DQαβ susceptibility heterodimers (DQα $Arg52^+$/DQβ $Asp57^-$) rather than a single DQα or DQβ chain. Indeed, 97% of patients vs. 46% of control subjects possess a genotype encoding at least one such DQ heterodimer (RR 32, CI 14.25–71.86). Moreover, the degree of susceptibility appeared to depend on the number of such heterodimers that a subject can form according to his or her DQA1-DQB1 genotype, i.e., a dose effect. As expected, the highest risk of developing IDDM was in individuals with the S-S/S-S genotype (RR 41, CI 17.05–95.9) followed by those with three susceptibility alleles. However, the susceptibility conferred by the latter is modulated by the presence of protective DQA1 or DQB1 alleles. In S-S/P-S genotype, the susceptibility conferred by the S-S haplotype was partially neutralized by the presence of a protective DQA1 allele, whereas it was completely neutralized by the presence of a protective DQB1 allele in S-S/S-P genotype (Fig. 1). This suggests that the role, in terms of susceptibility or resistance, of DQB1 alleles is more important than that of DQA1 alleles. Among the patients with one DQA1 and one DQB1 susceptibility allele, none had an S-S/P-P genotype; all carried these alleles on separate haplotypes (S-P/P-S genotype), suggesting a particular role for the DQ heterodimer encoded in the trans position. Four patients had genotypes unable to encode a DQαβ susceptibility heterodimer, suggesting a heterogenity in the mechanism of disease susceptibility, although these patients showed no difference in the clinical manifestation of the disease. From a practical point of view, individuals can be classified in six categories with different RR (Fig. 1), if their genotypes are known.

Among S-S/S-S genotypes, only DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302 (i.e., DR3-DQw2/DR4-DQw8) was highly significantly increased in patients confirming previous studies (4). This observation raised the question whether all susceptibility DQ heterodimers encoded in cis and/or trans account for similar risk of IDDM. The distribution of the different DQ combinations, formed in cis and/or trans, showed a restricted number of DQ combinations significantly associated with IDDM, conferring susceptibility of varying strength (Table 5), a similar conclusion has recently been reported by Ronningen et al. (4). This suggests the probable implication of amino acids other than Asp57 of the DQβ chain and Arg52 of the DQα chain in IDDM susceptibility. The strongest IDDM association was with DQA1*0501-DQB1*0302 combination only formed by genes in trans position, followed by DQA1*0301-DQB1*0201, DQA1*0301-DQB1*0302, and DQA1*0501-DQB1*0201. Therefore, four different susceptibility heterodimers are correlated significantly with IDDM. Both DR3-DQA1*0501-DQB1*0201, DR4-DQA1*0301-DQB1*0302 homozygous and DR3-DQA1*0501-DQB1*0201/DR4-DQA1*0301-DQB1*0302 heterozygous genotypes encode for only susceptibility heterodimers. Homozygotes encode for only one type of susceptibility heterodimers, whereas heterozygotes encode for four different types of susceptibility heterodimers. In the latter, this simultaneous expression of a diversity of susceptibility molecules may explain their excess among IDDM patients. Moreover, the observation that no excess of genotypes (S-S/S-P, S-P/S-S, S-P/P-S, or S-S/P-P) able to form only one or two of these heterodimers (in cis and/or trans) was increased among patients supports the necessity of the simultaneous expression to have the highest risk to develop IDDM.

Assuming a role for HLA molecules in the thymic selection of T-cell repertoire (26,27), a diversity of susceptibility molecules could lead to the positive selection (or alternatively escape the negative selection) of pathogenetic T-cell clones, creating an additive effect. Whether these different susceptibility heterodimers bind the same or different peptides is unestablished. Besides their interest for possible disease mechanisms, our observations emphasize the need for DQα and β-chain typing for the assessment of the risk of IDDM.

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