Association of HLA-DQw3 ($TA10^-$) With Type I Diabetes Occurs With DR3/4 but not DR1/4 Patients

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We have shown in previous studies that the $TA10^-$ subtype of HLA-DQw3 is significantly increased in HLA-DR4 type I (insulin-dependent) diabetic patients. Data presented in this article indicate that this association only occurs in heterozygous DR3/4 patients and not in DR1/4 patients. Because there is an interactive effect of both DR3/4 and DR1/4 in type I diabetes, the data indicate that the contribution of DR4 haplotypes varies depending on the haplotype borne on the homologous chromosome. In addition, the frequency of the B44-DR4 haplotype was shown to be decreased in DQw3 ($TA10^-$) diabetic subjects who were DR3/4 compared with those who were DR1/4 or DR4/x (a pooled group of patients with different DR alleles). This finding suggests that the decrease in the B44-DR4 haplotype in type I diabetes is not solely dependent on its linkage disequilibrium with the DQw3 ($TA10^+$) allele but suggests there is an additional effect exerted independently. *Diabetes* 37:926–29, 1988

The DQ locus allele DQw3, which is in strong linkage disequilibrium with both DR4 and DR5, can be serologically subtyped into two specificities. These subtypes are referred to as DQw3 ($TA10^+$) and DQw3 ($TA10^-$), so called after the original monoclonal antibody described by Maeda, which defined the TA10 specificity (1). Many allosera have been subsequently described as defining TA10, but none clearly define the $TA10^-$ DQw3 subtype. Therefore, the designation of the $TA10^-$ DQw3 subtype is made on the basis of a lack of reaction with TA10 antisera and positive reaction with sera designated as DQw3. We have previously shown that among type I diabetic subjects who are DR4 there is a strong association with DQw3 ($TA10^-$), the $TA10^+$ DQw3 subtype occurring significantly less frequently than that seen in the normal population (2). In addition we have shown that the $TA10^+$ DQw3 subtype in DR4 individuals is in positive linkage disequilibrium with the B locus allele B44 (2). This observation was interpreted as explaining the previously reported decrease of B44 in type I diabetes (3).

On examining data from a larger number of patients than reported in the initial study, we observed that type I diabetic subjects who were DQw3 ($TA10^+$) were rarely DR3/4. We therefore decided to study the distribution of DQw3 subtypes in diabetic subjects on the basis of subgrouping according to DR type. We subgrouped the patients into three groups (DR3/4, DR1/4, and DR4/x) because of the known excess of the heterozygote DR3/4 patients in type I diabetes (4,5), which we also observed in our group of patients, and because of an observed increase in DR1/4 heterozygotes after removal of DR3/4 patients from the analysis. The remaining heterogenous group of DR4 patients, designated DR4/x, were considered separately for comparison.

MATERIALS AND METHODS

All patients tested were Caucasians from the Royal Melbourne Hospital Diabetes Clinics defined as having type I diabetes by standard clinical criteria (6,7) and an absence of circulating C-peptide (postprandial concentration <0.10 nM). Data for normal control population frequencies were obtained from a local panel of 201 nondiabetic healthy individuals or, where stipulated, from the Ninth Histocompatibility Data Base (courtesy of E.D. Albert, Kinderpoliklinik der Universität München, Munich, FRG).

Peripheral-blood lymphocytes were isolated from heparinized blood by a standard gradient centrifugation technique (8). B-lymphocytes for DR and DQ typing were separated by a nylon-wool column method (9,10) with minor modifications. The nonadherent T-lymphocytes were used for typing for HLA-A and -B.

HLA-A/HLA-B typing was performed with a standard mi-

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TABLE 1
Interaction of DR3/4 and DR1/4 in type I diabetes

HLA type	Type I diabetic subjects			Control subjects		
	n	Frequency (%)	Allele frequency	*n*	Frequency (%)	Allele frequency
All subjects	258			201		
DR3*	146	56.6	0.341	61	30.3	0.165
DR4†	183	70.9	0.461	66	32.8	0.180
DR3/4	99	38.4		12	6.0	
Excluding DR3/4 subjects	159			189		
DR1	39	24.5	0.131	39	20.6	0.109
DR4	84	52.8	0.313	54	28.6	0.155
DR1/4	24	15.1		6	3.2	

*$P < 1 \times 10^{-5}$, †$P < 1 \times 10^{-6}$ for diabetic vs. control subjects.

crolymphocytotoxicity assay (11), and a similar method, but with prolonged incubation times, was used for DR and DQ typing (12). Positive and negative controls were included on the DR and DQ typing trays to assess the viability and degree of B-lymphocyte purity. A minimum of four allosera were used to define TA10.

RESULTS

DR3/4 and DR1/4 interactive effects. Two hundred fifty-eight type I diabetic subjects were DR and DQ typed. There was an increase in the frequency of DR3/4 heterozygotes in excess of that expected, given the frequency of the individual alleles DR3 and DR4 (Tables 1 and 2). When the 99 DR3/4 patients were excluded from the analysis, there appeared to be an increased co-occurrence of DR1/4 patients (observed 24, expected 13.0) given the residual individual frequencies of DR1 and DR4. These findings suggest interactive effects in two subgroups of patients, DR3/4 and DR1/4. There was no excess of putative DR4 homozygotes.

TA10 status of patient subgroups. One hundred nine DR4 and DQw3 type I diabetic subjects were typed for TA10 status. The patients were subgrouped according to the DR allele on the homologous chromosome (DR3/4, DR1/4, and DR4/x), and the distribution of TA10 was compared with that of normal control subjects. The number of patients who were DR2/4, DRw6/DR4, DR7/4, DRw8/DR4, DRw9/DR4, or DRw10/DR4 was too small to allow comparison of TA10 status in these individual groups, and hence, they were pooled to form the DR4/x group. Because nearly all DR5 haplotypes are DQw3 (TA10$^+$), 2 DR4/5 patients were excluded from the analysis, as were the DR4 homozygotes from the DR4/x group, to ensure comparability with the DR1/4 and DR3/4 groups of patients, i.e., with a second allele borne on a DQw3$^-$ haplotype. The pooling of DR4 patients with different DR alleles on the homologous chromosome does not imply they were a distinct homogeneous genetic subgroup; instead, they were included simply for comparison with the DR1/4 and DR3/4 patients. Table 3 shows the distribution of DQw3 subtypes among DR4 patients subgrouped according to the DR allele on the homologous chromosome (i.e., DR3/4, DR1/4, and DR4/x) and normal control subjects. The DQw3 (TA10$^-$) association previously described appeared confined to the DR3/4 group of patients. The DR1/4 and DR4/x patients showed a distribution of DQw3 subtypes similar to that seen in control subjects.

B locus association in patients subgrouped according to DR and TA10 status. Table 4 shows the distribution of B44 in the three subgroups of patients. As expected, the frequency of B44 was increased in the TA10$^+$ groups due to the linkage disequilibrium of B44, DR4, and DQw3 (TA10$^+$). The interesting observation, however, was the decreased frequency of B44 in the DR3/4 and DQw3 (TA10$^-$) group of 52 patients compared with the 24 patients who made up the other DQw3 (TA10$^-$) groups. The frequency of B44 appeared to be decreased in the DR3/4 and DQw3 (TA10$^-$) group to a greater extent than would be expected with TA10$^-$ status alone. It is unlikely that this apparent decrease was due to the presence of B44 on the homologous

TABLE 2
Observed and expected co-occurrence of alleles

Allele	Observed	Expected	*P*
DR3/4			
Diabetic	99	81.1	<.05*
Control	12	11.9	NS
DR1/4			
Diabetic	24	13.0	<.005
Control	6	6.4	NS

The expected number of DR3/4 and DR1/4 individuals was calculated for the patient and control groups based on individual allele frequencies. Individuals with either DR1, DR3, or DR4, but with no second DR allele, were considered homozygous.
*Calculated with $\chi^2 = (O - E)^2/E$, where O is the observed value and E is the expected value.

TABLE 3
TA10 status of DR4 and DQw3 type I diabetic subjects subgrouped according to DR allele on homologous chromosome

	n	TA10$^+$ (*n*)	TA10$^-$ (*n*)	TA10$^+$ (%)
DR1/4*†	20	7	13	35.0
DR4/x†‡	19	8	11	42.1
DR3/4*‡	55	3	52	5.5
DR4/y control§	583	180	403	30.9

*$P < .001$, †P NS, ‡$P < .001$.
§Data source was the Ninth International Histocompatibility Testing Workshop. DR4/y control subjects include DR1/4, DR3/4, and DR4/x.

TABLE 4
Distribution of B44 in type I diabetic subjects subgrouped according to DR and TA10 type

	TA10 status	*n*	B44$^+$ (*n*)	B44$^-$ (*n*)	B44$^+$ (%)
DR1/4	+	7	4	3	57.1
	−*	13	5	8	38.5
DR4/x	+	8	7	1	87.5
	−†	11	3	8	27.3
DR3/4	+	3	2	1	66.6
	−*†‡	52	3	49	5.5
DR4/y	+	180	77	103	42.8
control§	−‡	403	99	304	24.6

*†$P < .005$, by χ^2-test with Yates' connection; ‡$P < .0005$.
§Data source was the Ninth International Histocompatibility Testing Workshop.

chromosome in the other groups because B44 is in negative linkage disequilibrium with DR1, and the only other DR allele apart from DR4 that is in positive linkage disequilibrium with B44 is DR7. (There were only 8 DR4/7 patients, and 4 were TA10$^+$). Therefore, there appears to have been a decrease in the B44-DR4 haplotype in DR3/4 diabetic subjects independent of the decrease in DQw3 (TA10$^+$).

DISCUSSION

We have previously shown with human allosera that the TA10$^-$ subtype of DQw3 is significantly increased in Caucasian DR4 type I diabetic subjects (2). This association has also been shown at the DNA level with a DQβ probe and the enzyme *Bam*HI. A 3.7-kilobase band appears to correlate closely with the presence of the serologically defined TA10 specificity (13) and is significantly decreased in frequency in type I diabetic subjects (14), whereas the allelic 12-kilobase band is increased in frequency.

Our data from this study show that the association of DR4 and DQw3 (TA10$^-$) with type I diabetes is confined to the DR3/4 subgroup of patients, with DR1/4 and DR4/x patients showing a distribution of DQw3 subtypes similar to that seen in DR4 control subjects. Note, however, that the DR4/x group of patients is heterogeneous with respect to the DR allele on the homologous chromosome. We could not draw any conclusions concerning the TA10 status of the DR4 haplotypes occurring with DR2, DRw6, DR7, DRw8, DRw9, and DRw10 on the other haplotype, due to either small numbers of these alleles or their total absence in the patient group.

These data showing different DR4 haplotype associations, based on DQ serology, suggest that the effect of DR4 haplotype gene(s) on type I diabetes susceptibility is influenced by HLA genes present on the homologous chromosome in at least two genetic subgroups of patients. At the functional level, this indicates a role for trans-gene interaction possibly at the DQ locus in DR3/4 individuals. This interpretation is compatible with data of Nepom et al. (15), who have shown that hybrid molecules consisting of DQw2 α-chains from the DR3 haplotype and DQw3 β-chains from the DR4 haplotype are present in DR3/4 diabetic subjects and their HLA-identical unaffected siblings. What gene-interactive effects occur in DR1/4 type I diabetic individuals is not clear. The serological data do not permit a discrimination at DR or DQ between the diabetic DR4 haplotypes associated with DR1 and those seen in the general population. However, the contribution of DR4 haplotypes in DR1/4 diabetic subjects is obviously different from that seen in the DR3/4 group of patients.

These data also demonstrate that the previously described decrease of the B44-DR4 haplotype in type I diabetes (3) is confined to the DR3/4 subgroup of patients who are DQw3 (TA10$^-$). This decrease is significantly greater than would be expected if the observed decrease simply reflected linkage disequilibrium of B44-DR4 with DQw3 (TA10$^+$). Also, the decrease in B44-DR4 is not the result of a compensating decrease due to an increase in the frequency of Bw62-DR4, because the frequency of that haplotype in DR3/4 diabetic subjects was not higher than the frequency observed in the other patient subgroups (data not shown). The data suggest that B44-DR4 is not a risk haplotype when associated with DR3 on the homologous chromosome, and in fact, in DR3 individuals it would appear to confer a protective advantage.

In summary, there is evidence, based on DQw3 serology, for distinct genetic subgroups involving DR4 haplotypes in type I diabetes. This evidence suggests that interacting genes may be operative in DR3/4 and DR1/4 diabetic patients and that the contribution of the DR4 haplotype varies depending on the haplotype contained on the homologous chromosome.

We are attempting to establish whether genetic subgrouping of patients by haplotype provides associations with clinical parameters or markers of immune function that may be implicated in the etiology of type I diabetes.

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