HLA-DQ POLYMORPHISM ASSOCIATED WITH RESISTANCE
TO TYPE I DIABETES DETECTED WITH MONOCLONAL
ANTIBODIES, ISOELECTRIC POINT DIFFERENCES,
AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM

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Recently (1), we described a new genetic system that is HLA-DQ-related. Two alleles, 2B3 and TA10, can be recognized serologically. The 2B3 specificity detected with the IIB3 mAb was previously described (2) as a DQw1-related specificity. The TA10 specificity is DQw3-related and was first described by Maeda (3) with the TA10 mAb. TA10 can also be detected with alloantisera (4). Recently, an absolute correlation of the TA10 specificity with a RFLP using a DQβ probe has been described (5).

The 2B3 and TA10 determinants are in linkage disequilibrium with HLA-DR specificities. Both specificities are positively associated with DR4 but never occur together on the same haplotype (1). DR3$^+$, DQw2$^+$ haplotypes carry neither 2B3 nor TA10.

We wanted to know the molecular localization of these epitopes. Bontrop et al. (6) saw a polymorphism similar to 2B3/TA10 in a study where DQ molecules isolated from DR4 homozygous typing cells (HTC) focused in different positions. Furthermore, Tilanus et al. (7) studied RFLP obtained after digestion with various restriction enzymes and hybridization with a DQβ probe. Within a group of DR4 HTCs, specific fragments were observed, the presence of which closely associated with both the 2B3/TA10 polymorphism as well as the IEF experiments. The pertinent data showing the nearly perfect correlation between the mAbs, IEF, and RFLP will be summarized in this paper. The 2B3/TA10 polymorphism is apparently independent from HLA-D, at least in DR4 haplotypes (6, 7). Furthermore, it is genetically determined and linked to HLA, as shown by family segregation studies (1). The segregation of specific DNA fragments with 2B3 and TA10 was also shown in a family where the mother was DR3 homozygous, negative for 2B3 and TA10, and lacked the specific DNA fragments in RFLP (7). The father was DR4 homozygous and positive for both

2B3 and TA10. Two specific fragments could be shown in his RFLP; one was inherited by three children that were DR4$^+$, TA10$^+$, and the other segregated to the other two children that were DR4$^+$, 2B3$^+$. The latter two children happened to be diabetic patients.

This observation, together with other reports, suggested that other markers (i.e., DQβ) in addition to DR4 may influence disease susceptibility, notably to type I diabetes (5, 8). Here we present data concerning the DQ-related TA10/2B3 polymorphism and type I diabetes mellitus, as well as rheumatoid arthritis.

Materials and Methods

Cells.   14 EBV-transformed cell lines from DR4 homozygous typing cells with HLA-Dw4, Dw10, Dw13, Dw14, and Dw15 specificities were used as described previously (7).

64 children with type I diabetes mellitus attending the outpatient clinic for diabetes at the Sophia Children's Hospital in Rotterdam were studied. Blood from 116 patients with rheumatoid arthritis was made available by Dr. Westedt and Dr. Cats from the Department of Rheumatology of the University Hospital, Leiden.

A group of 297 healthy individuals served as control population for the 2B3 and TA10 specificities. In this group, 14 DR3$^+$,DR4$^+$ individuals were observed. In more recently DR typed controls, another 24 DR3$^+$,DR4$^+$ individuals were also typed for 2B3 and TA10. All patients and controls were of Dutch caucasoid origin.

Serological Typings.   HLA-DR and DQ typings were performed by two-color fluorescence (TCF) (9) or propidium iodide (PI) technique (10) with a set of highly selected class II antisera. The 2B3 and TA10 specificities were determined with the mAbs IIB3 and TA10, respectively (2, 3).

Biochemical Techniques.   Southern blot analyses with the DQβ cDNA probe were performed as described previously (7). Two-dimensional gel electrophoresis was conducted as described by Goyert et al. (11). HLA-DQ molecules were isolated with the DQ-specific mAb SPV-L3 (12).

Statistical Analysis.   Associations between the TA10 and 2B3 specificities and type I diabetes or rheumatoid arthritis were calculated with the Woolf-Haldane method (13).

Results and Discussion

Serological and Biochemical Studies.   The 2B3/TA10 polymorphism was investigated in a group of DR4$^+$ HTCs including Dw4, Dw10, Dw13, Dw14, and Dw15 specificities. TA10 was detected on some Dw4 and some Dw13 HTCs, which is in agreement with earlier observations (14). 2B3 was present on the majority of HTCs. Two HTCs appeared to carry both the TA10 and 2B3 alleles, indicating heterozygosity for this DQ-related system.

11 of these DR4 HTCs were analyzed at the product level. HLA-DQ molecules were isolated with a monomorphic anti-DQ mAb SPV-L3 (12). HLA-DQ molecules appeared to focus in three different positions due to β chain pI differences (6).

The genomic DNAs isolated from the 14 DR4 HTCs were also studied for RFLP as obtained after digestion with various restriction enzymes and hybridization with a DQβ probe (7). When the enzyme Eco RI was used, we saw two polymorphic fragments of 15 and 20 kb within the RFLP obtained with the DR4 cells.

Table I shows that all 2B3$^+$ HTCs contained the DQβ2 or DQβ3 chain (as shown by IEF), as well as the 20-kb fragment in RFLP, whereas the presence of

TABLE I

Serologically Defined TA10 and 2B3 Specificities of DR4⁺ HTCs Compared with Protein Charge Heterogeneity and the TA10/2B3 Characteristic DNA Fragments

HTC		mAb		DQβ chain pI			Eco RI–DQβ	
		TA10	2B3	β1	β2	β3	15 kb	20 kb
BSM	Dw4	–	+	–	+	–	–	+
LRM	Dw4	+	–	+	–	–	+	–
MWM	Dw4	+	–	+	–	–	+	–
WEN	Dw4	+	+	+	+	–	+	+
JSM	Dw10	+	+	–	+	–	–	+
2046	Dw10	–	+	–	+	–	–	+
PEA	Dw13	+	–	+	–	–	+	–
EDW	Dw13	–	+	NT			–	+
LS40	Dw14	–	+	–	+	–	–	+
MT	Dw14	–	+	–	+	–	–	+
KY	Dw14	–	+	NT			–	+
Chop	Dw14	–	+	NT			–	+
YT2	Dw15	–	+	–	–	+	–	+
YOT	Dw15	–	+	–	–	+	–	+

NT, Not tested; +, present; –, absent.

TA10 is closely associated with the presence of DQβ1 chains and the presence of a 15-kb fragment. The only exception was an HTC that only had the DQβ2 chain and the 20-kb fragment in RFLP, but was clearly heterozygous for the serologically defined 2B3 and TA10 specificities on four different typing occasions (6, 7).

Both variants of this 2B3/TA10-related polymorphism were observed within the groups of Dw4 and Dw13 HTCs. Furthermore, one Dw4 HTC was heterozygous for 2B3/TA10, as detected by serology, by two-dimensional gel electrophoresis, and by Southern blot analyses indicating that this polymorphism is independent of HLA-D.

2B3/TA10 and Disease Susceptibility.   Previous studies (5, 8), as well as our observations in a family with two diabetic patients, did suggest that DR4-2B3 and not DR4-TA10 might be associated with susceptibility to type I diabetes. Therefore, we tested type I diabetes patients and controls for 2B3 and TA10 in addition to HLA-DR, and looked for an association with 2B3 and/or TA10. The data were analyzed taking into account the known DR phenotypes (DR2, 3, and 4) associated with type I diabetes (15). Because only 1 patient out of 65 was DR2⁺, we did not include either this patient or DR2⁺ controls in the analysis. As shown in Table II, there was a very significant (relative risk <0.10) negative association of TA10 with this disease. This was not only the case in DR4⁺, but also in DR4⁻ individuals. The only exception was the group of individuals with the DR4/x phenotype, suggesting that at least in DR4⁺ individuals, 2B3 might be associated with susceptibility, rather than TA10 with resistance to type I diabetes. Table III shows that this was not the case. We did not observe a significant association of 2B3 with diabetes in either DR4⁻ or DR4⁺ individuals, with the exception of DR3/4 individuals. The latter, however, is also explained by the negative association with TA10, as shown in Table II. Therefore, the simplest interpretation of the data shown in Table II and III is that TA10 is associated with resistance to type I diabetes, at least in DR2⁻ individuals. The

TABLE II

2B3/TA10 and Type I Diabetes: Association of TA10 with Resistance

DR pheno-type	TA10 patients		TA10 controls		Relative risk*	χ^2	Significance (p)
	+	−	+	−			
4⁻	0	21	38	100	0.06	7.26	0.005
3/4	0	23	10‡	28‡	0.06	6.97	0.005
4/−§	0	8	6	10	0.09	4.12	0.05
4/x‖	4	8	15	24	0.84	0.08	NS

* See reference 13.
‡ The control panel contained 14 DR3/4 individuals, 4 of which were TA10⁺, but it was extended with 24 DR3/4 individuals.
§ Indicates a blank, most probably DR4 homozygotes.
‖ x, DR non-2; non-3; non-4.

TABLE III

2B3/TA10 and Type 1 Diabetes: No Association of 2B3 with Susceptibility

DR phenotype	2B3 patients		2B3 controls		Relative risk	χ^2	Significance (p)
	+	−	+	−			
4⁻	9	12	64	73	0.9	0.10	NS
3/4	23	0	28	10	17.3	6.97	0.005
4/−	8	0	14	2	2.9	0.76	NS
4/x	11	1	33	6	1.5	0.21	NS

See footnotes for Table II.

absence of a significant association of TA10 in DR4/x individuals suggests that on certain haplotypes (i.e., DR non-2, non-3, non-4) TA10 may not provide resistance to type I diabetes in the presence of DR4 on the other haplotype. In other words, this might indicate a certain interaction between (products of) haplotypes, such as complementation. This hypothesis may be answered by family studies.

That DR4 is indeed also associated with susceptibility in TA10⁺ individuals can be seen from the TA10⁺ rows of Table II; 4 out of 4 TA10⁺ patients, as compared with 25 out of 63 (see note) TA10⁺ controls, were positive for DR4 (relative risk 13.6; p, 0.0025). In conclusion, in addition to the known association of HLA-DR2 with resistance to type I diabetes (15, 16), the TA10 allele of HLA-DQβ appears to be a new marker for resistance to this disease. This indicates that not only HLA-DR but also other genes in the HLA-D region may influence the development of type I diabetes, as already suggested previously (5, 15–18). From a previous study (19) we concluded that such genes have to be situated telomeric from HLA-DP. Therefore, HLA-DQ might not only be a marker for but rather be the actual resistance gene.

In contrast to these significant effects of the 2B3/TA10 DQ polymorphism on the development of type I diabetes, no association was observed in a group of 116 rheumatoid arthritis patients (data not shown). This clearly indicates that different HLA-linked genes confer susceptibility or resistance to these two HLA-DR4-associated diseases, confirming previous observations (20).

Summary

A new HLA-DQ-related genetic system with two alleles, 2B3 and TA10, defined serologically by mAbs and alloantisera, showed an almost perfect correlation with charge differences on DQβ molecules, as well as with two polymorphic DNA fragments hybridizing with a DQβ probe and various restriction enzymes on a panel of 14 DR4$^+$ homozygous typing cells. It was therefore concluded that the serologically defined alleles 2B3 and TA10 are coded by the DQβ gene and situated on the HLA-DQβ chain. This 2B3/TA10 polymorphism is independent of HLA-D and segregates with HLA in families. The TA10 allele appears to be a new marker for resistance to type I diabetes, which is independent from the known resistance marker DR2, whereas no association was observed between this DQβ polymorphism and rheumatoid arthritis.

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