

HLA-DQ rather than HLA-DR region might be involved in dominant nonsusceptibility to diabetes

(insulin-dependent diabetes mellitus/histocompatibility antigens)

G. STERKERS*[†], D. ZELISZEWSKI*, A. M. CHAUSSÉE[‡], I. DESCHAMPS[§], M. P. FONT[‡], C. FREIDEL[¶], J. HORS[‡], H. BETUEL[¶], J. DAUSSET[‡], AND J. P. LEVY*

*Laboratoire d'Immunologie et Virologie des Tumeurs, Institut National de la Santé et de la Recherche Médicale Unité 152, Centre National de la Recherche Scientifique Unité Associée 628, Hôpital Cochin, 27 rue du faubourg Saint Jacques, 75014 Paris, France; [†]Laboratoire d'Immunogénétique de la Transplantation, Institut National de la Santé et de la Recherche Médicale Unité 93, Centre Hayem, Hôpital Saint Louis, 75010 Paris, France; [‡]Hôpital Herold, 7 place Rhin et Danube, 75019 Paris, France; and [§]Centre Régional de Transfusion Sanguine de Lyon, 1 rue du Vercors, 69007 Lyon, France

Contributed by J. Dausset, May 2, 1988

ABSTRACT Since HLA-DRw15 (a subdivision of the HLA-DR2 specificity previously called DR2 long) is associated with dominant nonsusceptibility to insulin-dependent diabetes mellitus (IDDM), while HLA-DRw16 (another subdivision of HLA-DR2, previously called DR2 short) is positively associated with the disease, we looked for particular characteristics of HLA products encoded by the DR2 haplotypes of IDDM patients. The results show the following: (i) HLA-DQ molecules of HLA-DRw15-positive IDDM patients are different from those of HLA-DRw15-positive controls, suggesting that the HLA-DQ gene of DRw15 haplotypes is involved in a protective effect. (ii) HLA-DR and -DQ products of DRw16-positive IDDM are functionally indistinguishable from those of HLA-DRw16-positive controls. Furthermore, our data provide evidence that the residue at position 57 on the DQ β chain could play a crucial biological role in antigen presentation to T cells as far as the DRw16 haplotype is concerned. This observation fits with the recent observation of correlation between DQ β allelic polymorphism at position 57 and both susceptibility and resistance to IDDM.

An increased frequency of some HLA-DR specificities has been reported among patients with insulin-dependent diabetes mellitus (IDDM): DR3, DR4, and, to a lesser extent, DR1, DRw13, and DRw16 (the last being a subdivision of DR2 previously called DR2 short, AZH, or FJO)^{||} (1-8). In contrast, an extremely decreased frequency is observed for the DRw15 specificity (a subdivision of DR2 previously called DR2 long)^{||} (1, 9, 10). Because of an aberrant expression of class II molecules on the target tissue (11), and since HLA-D region cell surface products play an essential role in the presentation of antigens to T lymphocytes, it has been proposed that class II genes represent the basis of the disease susceptibility by allowing pathogenic recognition of an autoantigen (12). However, because of strong linkage disequilibrium between HLA-DR and -DQ genes, one cannot assess whether one or the other of these two genes is involved. Several reports have suggested that diabetes is more closely associated with gene polymorphism within the DQ than the DR region (6, 13-16). However, the exact location of the genes involved in negative or positive association with IDDM has not been determined. Moreover, the reasons for these negative or positive associations remain unknown.

Recent developments have made it possible to generate monospecific T-cell clones restricted by class II molecules. These reagents can be used as fine probes to analyze class II molecule polymorphism (17-19) and its biological role. We have therefore attempted to identify more precisely the HLA

functional polymorphism of HLA-DR2 (DRw15 or DRw16)-positive IDDM patients by using influenza-specific, HLA class II-restricted, cloned T cells from a DRw16 healthy homozygous individual. When possible, the relationship between the structure of an HLA molecule and its function was determined by comparing T-cell reactivity against a panel of HLA-phenotyped antigen-presenting cells (APC) from healthy donors and the available corresponding HLA class II sequences. The HLA class II polymorphism of IDDM patients was also characterized by their HLA-DR, -DQ, and -Dw specificities according to conventional typing and by restriction fragment length polymorphism (RFLP) analysis of the DQ region.

MATERIALS AND METHODS

HLA Genotyping of Peripheral Blood Mononuclear Cells (PBM). PBM were isolated as previously described (17) from healthy donors and from four DR2 (DRw15 or DRw16)-positive diabetic patients and six of their first-degree relatives, allowing clearcut haplotype assignment. HLA serological phenotyping was performed with Ninth Histocompatibility Workshop and local reagents according to standard procedures (20). Cellular HLA-Dw typing was performed by testing PBM reactivities against a panel of homozygous typing cells as previously described (21).

Influenza-Specific Cloned Cell Lines. The influenza-specific cloned cell lines used in this work have been extensively characterized elsewhere (18). Their main characteristics are described in Table 1. They are influenza A/Texas-specific, HLA class II-restricted, proliferating T-cell lines obtained after cloning by a limiting dilution procedure as previously described (17).

Influenza Viruses. Influenza viruses were kindly provided by C. Hannoun (Institut Pasteur, Paris). Influenza A/Texas/77 and B/Singapore/222/79 viruses were grown in allantoic embryonated chicken eggs and partially UV-inactivated before use.

Culture Medium. Culture medium consisted of RPMI 1640 DM supplemented with 1% glutamine, 1% pyruvate, 1%

Abbreviations: IDDM, insulin-dependent diabetes mellitus; RFLP, restriction fragment length polymorphism; PBM, peripheral blood mononuclear cells; APC, antigen-presenting cells.

[†]To whom reprint requests should be addressed.

^{||}HLA-DRw15 and HLA-DRw16 are serological specificities according to the most recent international HLA (human leukocyte antigen) nomenclature, which was established during the Tenth Histocompatibility Workshop (New York, Nov. 13-17, 1987); they correspond to previous specificities HLA-DR2 long and HLA-DR2 short, respectively. HLA-Dw21 is a cellular specificity associated with HLA-DRw16/DQw1 and corresponds to the previous FJO and AZH specificities.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Table 1. Characteristics of the cell clones used in this study

Original donor genotype	Clone	Restricting molecule	Phenotype of efficient allogeneic APC
<i>DRw16/DQw1/Dw21</i>	IIIg	HLA-DQ	All DRw16/DQw1/Dw21 None DRw15/DQw1/Dw2 or Dw12 None DRw16-negative
<i>DRw16/DQw1/Dw21</i>	Ie	HLA-DR	All DRw16/DQw1/Dw21 or Dw22 None DRw15/DQw1/Dw2 or Dw12 None DRw16-negative

The two T-cell clones used in this paper were from an HLA-genotyped donor. The restricting molecule of each clone was determined by the ability of well-characterized HLA-specific monoclonal antibodies to inhibit its specific response. The correlation of these restrictions with HLA haplotypes was determined by the ability of HLA-phenotyped allogeneic APC to present the specific antigen to each clone. These results have been published (18, 19). In the previous study 14 DRw16/DQw1/Dw21 and 10 DRw15/DQw1/Dw2 or Dw12 allogeneic APC were tested.

nonessential amino acids, 20 μ M 2-mercaptoethanol, antibiotics, and 5% heat-inactivated human pooled type AB sera.

Antigen-Specific Proliferation of Cloned T Cells. Cloned T cells (1×10^4) were seeded into round-bottom microtiter plates with 1×10^5 allogeneic APC that had received 4000 rads (40 Gy) of γ irradiation and had been incubated with the influenza A/Texas virus, in 200 μ l of culture medium under 5% CO₂

humid atmosphere. On the third day of culture, 0.8 μ Ci (1 Ci = 37 GBq) of [³H]thymidine (Amersham) was added during the last 6 hr of culture. In each experiment cloned cells were cultured in parallel with APC previously infected with B/Singapore virus to monitor the specificity of the reaction. Results are expressed as mean counts per minute (cpm) \pm standard deviation (SD) of triplicate cultures.

Table 2. Antigen-specific proliferation assay using PBM from IDDM patients and their family members as APC

Exp.	Family	Panel	APC		Influenza virus strain present		[³ H]Thymidine incorporation, cpm	
			HLA genotype		A	B	Clone Ie	Clone IIIg
1	Autologous		<i>A3/B7/C-/DRw16/DQw1/Dw21</i>		+	-	<i>41,764 \pm 5379</i>	<i>33,197 \pm 2540</i>
			<i>A2/B39/C-/DRw16/DQw1/Dw21</i>		-	+	3,055 \pm 682	5,308 \pm 671
	1	IDDM DRPA	<i>A2/C-/B5/DRw15/DQw1/Dw12</i>		+	-	3,303 \pm 252	5,301 \pm 1148
			<i>A3/C-/B13/DR7/DQNT/DwNT</i>		-	+	3,397 \pm 400	2,556 \pm 347
2	Autologous		<i>A3/B7/C-/DRw16/DQw1/Dw21</i>		+	-	<i>12,198 \pm 2053</i>	<i>11,033 \pm 1871</i>
			<i>A2/B39/C-/DRw16/DQw1/Dw21</i>		-	+	3,477 \pm 1264	2,224 \pm 636
	2	IDDM DECA	<i>A2/Cw7/B15/DRw15/DQw3/Dw-</i>		+	-	2,517 \pm 1203	4,494 \pm 1973
			<i>A3/Cw3/B7/DRw13/DQw1/Dw19</i>		-	+	2,115 \pm 854	4,721 \pm 2232
3	Autologous		<i>A2/Cw7/B15/DRw15/DQw3/Dw-</i>		+	-	2,424 \pm 929	4,603 \pm 1180
			<i>A3/Cw3/B7/DRw13/DQw1/Dw19</i>		-	+	2,384 \pm 696	8,490 \pm 3296
	2	Sister DECR	<i>A2/Cw7/B15/DRw15/DQw3/Dw-</i>		+	-	3,939 \pm 631	1,830 \pm 178
			<i>A1/Cw7/B8/DR3/DQw1/Dw3</i>		-	+	4,272 \pm 1051	1,825 \pm 322
3	Autologous		<i>A3/B7/C-/DRw16/DQw1/Dw22</i>		+	-	<i>41,459 \pm 1471</i>	<i>19,240 \pm 3335</i>
			<i>A2/B39/C-/DRw16/DQw1/Dw22</i>		-	+	1,189 \pm 359	494 \pm 27
	3	IDDM CEFR	<i>A2/C5/B2/DRw16/DQw1/Dw22</i>		+	-	<i>40,656 \pm 8392</i>	<i>25,899 \pm 3211</i>
			<i>A28/C5/B14/DR1/DQw1/Dw1</i>		-	+	1,411 \pm 299	643 \pm 109
4	Autologous		<i>A2/C5/B12/DRw16/DQw1/Dw22</i>		+	-	<i>58,987 \pm 1057</i>	<i>26,449 \pm 205</i>
			<i>A28/C5/B14/DR1/DQw1/Dw1</i>		-	+	844 \pm 106	1,312 \pm 653
	4	IDDM PEJF	<i>A2/C5/B12/DRw16/DQw1/Dw22</i>		+	-	<i>51,907 \pm 698</i>	<i>16,928 \pm 679</i>
			<i>A9/C-/B5/DR5/DQNT/DwNT</i>		-	+	1,068 \pm 174	843 \pm 530
4	Autologous		<i>A28/C5/B14/DR1/DQw1/Dw1</i>		+	-	573 \pm 479	836 \pm 244
			<i>A9/C3/B18/DR5/DQNT/DwNT</i>		-	+	1,033 \pm 578	1,296 \pm 595
	4	IDDM PEJF	<i>A3/B7/C-/DRw16/DQw1/Dw22</i>		+	-	<i>23,202 \pm 2168</i>	<i>32,328 \pm 1583</i>
			<i>A2/B39/C-/DRw16/DQw1/Dw22</i>		-	+	2,882 \pm 341	3,763 \pm 460
4	IDDM PEJF	<i>A1/C-/B17/DRw16/DQw1/DDw22</i>		+	-	<i>18,997 \pm 979</i>	<i>42,345 \pm 2725</i>	
		<i>A2/C-/B38/DR3/DQNT/Dw3</i>		-	+	2,945 \pm 35	5,089 \pm 794	

T cells (1×10^4) from Ie or IIIg clones were stimulated by HLA-genotyped autologous or allogeneic irradiated APC (1×10^5). Allogeneic APC were from DRw15-positive (family 1 and 2) or DRw16-positive IDDM patients or members of their families. NT, not tested; Dw- is a cellular specificity not identified so far. The cells were cultured in the presence of the influenza virus specifically recognized by these clones, A/Texas (A), or in the presence of an irrelevant antigen, the B/Singapore influenza virus (B). [³H]Thymidine incorporation was determined on the third day of culture. Results are expressed as mean \pm SD; positive results are in italics.

RFLP. Genomic DNA, extracted from PBM according to a method described elsewhere (7), was digested by the restriction enzymes that are indicated in the legend of Fig. 1 and as directed by the suppliers (New England Biolabs). The restriction fragments were separated in agarose gels by electrophoresis, then transferred onto hybridization membranes and hybridized with ^{32}P -labeled DQ β -gene-specific probes furnished by Larhammar and coworkers (Uppsala, Sweden). The hybridization was performed for 40 hr at 42°C with hybridization buffer containing radiolabeled probe at 10 ng/ml. Membranes were washed at room temperature with 0.30 M NaCl/0.03 M sodium citrate for 10 min and twice at 65°C in 0.30 M NaCl/0.03 M sodium citrate/0.5% NaDodSO $_4$ for 10 min. Membranes were then exposed to x-ray films.

RESULTS

DQ Molecules Borne by Two DRw15 IDDM Patients Are Different from Those of DRw15 Controls. A subdivision of DR2 (DRw15)^{||} is correlated with a dominant nonsusceptibility to IDDM, but this protection is not complete since exceptionally IDDM patients are DRw15. Among our panel of IDDM individuals, two are DRw15. DR products from these diabetics do not differ from those of controls as determined by serology. However, as shown in Table 2, the haplotype of one IDDM patient, DECA (family 2), associates DRw15 and DQw3 according to the genotypic analysis of this family. As shown in Fig. 1, this DQw3 specificity is confirmed by RFLP analysis by the presence of a 7.9-kilobase-pair (kb) band characteristic of DQw3 after digestion with *Hind*III enzyme. To our knowledge, the DQw3 specificity has never been reported to be associated with DRw15 on the same haplotype in healthy populations. Indeed, data from 10 DRw15 healthy controls previously published by us (18, 19) and all homozygous typing cells used for the Tenth Histocompatibility Workshop always have DRw15 associated with DQw1. By contrast, IDDM patient DRPA (family 1) associates, as usual, DRw15 with DQw1 (Table 2). However, the DQ gene RFLP pattern of this patient shows a 2.6-, a 6.9-, and

a 16-kb band with *Eco*RV, *Hind*III, and *Eco*RI enzymes, respectively. This pattern is correlated with HLA-DR1, -DRw16, -DRw10, and -DRw14 but not with -DRw15 in healthy populations tested so far (refs. 7, 22, and 23 and results from the Tenth Histocompatibility Workshop). These results indicate that the DQ products of these two patients bearing DRw15 are different from those of DRw15 healthy individuals.

DR and DQ Products of DRw16 IDDM Patients Are Functionally Indistinguishable from Those of DRw16 Controls. Despite serological cross-reactivity between HLA class II molecules encoded by HLA-DRw15 and DRw16 haplotypes, their DR and DQ products are not identical as determined by the reactivity of influenza A/Texas-specific, HLA-DR- or -DQ-restricted T-cell clones. Indeed, two T-cell clones, Ie and IIIg, DR- and DQ-restricted, respectively, from a DRw16/DQw1/Dw21 homozygous individual, exclusively recognize their specific viral antigen when presented by HLA-DRw16/DQw1/Dw21-positive APC, as fully documented elsewhere (18, 19) and recalled in Table 1. These two clones were used to functionally define class II molecules of DRw15- and DRw16-positive IDDM patients. As shown in Table 2, DRw16 APC from IDDM patients or members of their families (families 3 and 4) could induce a specific proliferation of these clones when cultured in the presence of the influenza A/Texas virus. As control no proliferation could be observed in the presence of an irrelevant antigen, the influenza B/Singapore virus. Similar results have been obtained with 10 out of 10 HLA-DRw16 APC from a panel of unrelated healthy individuals (18). These results indicate that the HLA-DR and -DQ products of the DRw16-positive IDDM patients are functionally indistinguishable from those of DRw16-positive healthy controls. This is confirmed by cellular HLA-Dw typing (Table 2). The HLA-DRw16 IDDM patients investigated were Dw21, as DRw16 healthy Caucasoid subjects usually are (18, 21, 24). Moreover, RFLP analysis shows the bands characteristic of the DQw1 gene associated with DRw16 in both the healthy individuals and the DRw16 patients—i.e., the 2.6-, 6.9-, and 16-kb bands with

Enzyme and fragment size (Kb)	Eco RV			Hind III		Eco RI			
	2.6(*)	5.2(†)	14(‡)	6.9(§)	7.9(¶)	16(*)	2.75(†)	12(†)	24(‡)
HLA phenotype									
Control healthy individuals									
A11 DRw16/DQw1/Dw21	■				■		■		
A11 DRw15/DQw1/Dw2		■					■	■	
A11 DRw15/DQw1/Dw12			■						■
IDDM patients									
CEFR: DRw16/DQw1/Dw21	■				■		■		
PEJF: DRw16/DQw1/Dw21	■				■		■		
DRPA: DRw15/DQw1/Dw(12)	■						■		
DECA: DRw15/DQw3/Dw-					■				

FIG. 1. RFLP patterns of IDDM patients when a cDNA probe specific for DQ β genes was used. DNA samples from healthy or IDDM individuals were digested with *Eco*RV, *Hind*III, or *Eco*RI restriction enzyme. The restriction fragments were separated by electrophoresis on agarose gels and then hybridized with a DQ β gene-specific ^{32}P -labeled probe. Black rectangles indicate fragments binding the probe. Only informative fragments are shown—i.e., fragments characteristic for their association with some DR or Dw specificities in healthy populations. Characterizations are as follows: (*), fragments characteristic of a cluster including DQw1 genes from DR1, DRw16, DRw10, and DRw14 haplotypes; (†), fragments characteristic of a cluster including DQw1 genes from DRw15/Dw2 and DRw13 haplotypes; (‡), fragments characteristic of DQw1 genes from the DRw15/Dw12 haplotype; (§), fragment characteristic of all DQw1 genes; (¶), fragment characteristic of all DQw3 genes. Only the DRw15 or DRw16 haplotype of each individual has been taken into account, according to the genotypic analysis of the families (see Table 2). Control healthy individuals refers to a series of nine DRw15/Dw2 or Dw12 or DRw16/Dw21 healthy Caucasoid donors. Each pattern associated with Dw21, Dw2, and Dw12 (7, 22, 23) is shown in this figure.

Table 3. DQ β chain sequences and clone IIIg reactivities

APC haplotype	DQ β chain sequence				
	10	20	30	40	50
DQw1.1	R D S P E D F V Y Q	F K G L C Y F T N G	T E R V R G V T R H	I Y N R E E Y V R F	D S D V G V Y R A V
DQw1.2 F M L . . . Y A
DQw1.12	. . P L .	. . A M Y . . . Y D
DQw1.21(AZH)
DQw1.18 L A
DQw1.19 L A

The DQ β first domain amino acid sequences are from the following sources: DR1/DQw1.1, B-cell line 45.1 (16); DR2/DQw1.2, cell line PGF; DR2/DQw1.12, cell line BGE (26); DR2/DQw1.21(AZH), cell line AZH (26); DRw13/DQw1.18, cell line WDV (16); DRw13/DQw1.19, cell line Daudi (16). The second number in the DQw designations (for example 2 in DQw1.2) refers to the Dw cellular typing number associated with the DQ allele. The numbers above the sequence refer to the amino acid positions. A dot indicates identity with the DQw1.1 sequence and a blank indicates that no sequence information is available.

EcoRV, *HindIII*, and *EcoRI*, respectively (Fig. 1). This DQ pattern has always been found in DRw16/DQw1 Caucasoid healthy controls (7, 22, 23). Conversely, no T-cell clone reactivity was observed with DRw15-positive IDDM APC (Table 2), indicating that DR and DQ products from DRw15 and DRw16 haplotypes are not functionally related among type I diabetic patients, as previously demonstrated by us with healthy controls (18, 19).

Functional Role of the Residue at Position 57 on DQ β Chain. We examined the amino acids of HLA class II molecules that could be functionally implicated in T-cell reactivity by looking for a correlation between the reactivity of the clones and the known HLA gene sequences. Deduced amino acid sequences of DQ β chain first domain of DR1/DQw1/Dw1 (DQw1.1), DRw15/DQw1/Dw2 (DQw1.2), DRw15/DQw1/Dw12 (DQw1.12), DRw16/DQw1/Dw21 [DQw1.21(AZH)], DRw13/DQw1/Dw18 (DQw1.18), and DRw13/DQw1/Dw19 (DQw1.19) haplotypes have been published (16, 25, 26). They are shown in Table 3. The reactivity of the DQ-restricted clone IIIg from a DRw16/DQw1/Dw21 homozygous individual has been determined by testing its reactivity against a large panel of healthy donor APC. Results against APC of various DQw1 haplotypes, indicated in Table 3, suggest that the residue in position 57 could play a crucial role in antigen presentation. Indeed, a specific proliferation of this clone was observed with DQw1.21 (AZH)-positive but not DQw1.1-positive APC; in these two the DQ β chains differ only by the amino acid at position 57.

DISCUSSION

With the improved definition of HLA polymorphism by the use of RFLP analysis, DQ-specific monoclonal antibodies, and gene sequences, it has been suggested that HLA-DQ rather than DR genes are involved in the susceptibility to IDDM associated with DR3 or DR4 specificities (6, 13–15). More recently, class II sequence analysis from haplotypes associated positively or negatively with IDDM has shown that DQ β alleles that do not encode aspartate at position 57 are to some extent significantly increased in IDDM, while "aspartate-positive" DQ β alleles are neutral or negatively associated with the disease (16). This suggested that the amino acid at position 57 might determine a critical function of the DQ molecule in IDDM. However, it is not understood why the DQ β aspartate-positive HLA-DRw15 haplotypes are associated with much stronger protection against IDDM than other DQ β aspartate-positive alleles. Moreover, DQ β allele sequences from DR7-DQw2 and DR3-DQw2 haplotypes are identical, while they are neutral and strongly associated with IDDM, respectively. Thus, further studies are required to definitely localize HLA-D region(s) involved in protection against or susceptibility to diabetes. In the present report, we

show that DRw15 patients express DQ products that differ from those of DRw15 haplotypes of healthy individuals. This was clear for one of them who was DQw3, never previously observed, to our knowledge, in healthy donors. The second patient was DRw15, but RFLP studies of DQ genes clearly demonstrated that his DQw1 product was different from the products of DRw15/DQw1 healthy donors described so far. Unfortunately no other DRw15 patient was available, since IDDM is exceptional among HLA-DRw15 subjects. However, these results strongly suggest that the DQw1 molecule characteristic of the DRw15 haplotype, or a product of a closely linked gene, protects against diabetes. Note also that both DQ and DR molecules from these two DRw15 patients were also different from those of DRw16 subjects, as shown by the functional studies with our T-cell clones.

IDDM is increased in DRw16 populations, but there is no information concerning the polymorphism of class II products in HLA-DRw16 IDDM as compared to healthy populations. From our study of the DR and DQ functional polymorphism, both DR and DQ products from DRw16/DQw1 IDDM patients appear functionally identical to those of HLA-DRw16/DQw1 healthy subjects. Indeed, (i) both DR and DQ molecules of DRw16 IDDM patients react, as do DRw16 Caucasoid controls (18), with T-cell clones strictly restricted by DR or DQ molecules encoded by the DRw16/DQw1 haplotype of a healthy individual; (ii) they type as Dw21 as usual for DRw16/DQw1 (FJO or AZH) Caucasoid donors; (iii) their pattern of DQ-associated RFLP is that of normal DRw16/DQw1/Dw21 (FJO or AZH) subjects. Because the HLA-DRw16 specificity is associated with IDDM, this suggests that the DRw16/DQw1/Dw21 haplotype encodes HLA class II molecules that contribute to the susceptibility to the disease rather than distinct products in DRw16-positive diabetics as compared with DRw16 controls. This is compatible with the recent observation that DR and DQ sequences found in DR3 and DR4 patients are also found in healthy controls (16).

The polymorphic HLA class II molecule residues that could play a functional role in T-cell recognition were investigated by comparing the pattern of T-cell reactivity against a panel of allogeneic cells and the sequences of HLA class II molecules expressed on these cells. Our results are evidence for the functional implication of the residue at position 57 on the DQ β chain. Indeed a DQ-restricted T-cell clone from a DRw16/DQw1.21 (FJO or AZH) individual could be activated by DQw1.21 (AZH) but not by DQw1.1 APC, the two DQ molecules being identical except for the valine/serine difference at position 57. DQ β polymorphism at position 57 has been reported to be associated with both susceptibility and resistance to IDDM (16). Hence, the demonstration that this amino acid could play a role in viral antigen presentation to T cells reinforces the idea that it might

Table 3. (Continued)

DQB chain sequence (cont.)					DQ-restricted clone IIIg reactivity*	
57	60	70	80	90		
T P Q G R P V A E Y		W N S Q K E V L E G	A R A S V D R V C R	H N T E V A Y R G A	L Q R	-
..... D	T . . E L . T F	-
..... D D I . . R	T . . E L . T F	-
..... S	+
..... D	T	-
.....	 R	T . . E L . T G . . G	-

*DQ-restricted clone IIIg reactivity with APC bearing the corresponding DQB alleles has been determined as described for Table 2. Induction (+) or noninduction (-) of specific proliferation by each APC is indicated.

participate in T-cell-dependent autoimmune response against the target tissue in IDDM.

In conclusion, our study provides evidence that DQ- rather than DR-region-encoded products of DRw15 individuals play a role in protection against IDDM. On the other hand, both DR and DQ products from the DRw16/DQw1/Dw21 haplotype are functionally identical in IDDM and healthy populations. Finally, we demonstrate that the amino acid at position 57 on the DQB chain could play a crucial functional role in physiological T-cell responses to viral antigens in HLA-DRw16 populations.

The authors thank Dr. C. Hannoun for his generous gift of the viruses and we thank Ms. H. Touitou and D. Hirtzlin for typing the manuscript.

1. Tiwari, J. L. & Terasaki, P. I., eds. (1985) *HLA and Disease Associations* (Springer, New York).
2. Svejgaard, A., Platz, P. & Ryder, L. P. (1983) *Immunol. Rev.* **70**, 193-218.
3. Sachs, J. A., Cudworth, A. G., Jaraquemada, D., Gorsuch, A. N. & Festenstein, H. (1980) *Diabetologia* **18**, 41-43.
4. Rotter, J. (1981) *Am. J. Hum. Genet.* **33**, 835-843.
5. Barbosa, J., Bach, F. H. & Rich, S. S. (1982) *Clin. Genet.* **21**, 25-29.
6. Böhme, J., Carlsson, B., Wallin, J., Möller, E., Persson, B., Peterson, P. A. & Rask, L. (1986) *J. Immunol.* **137**, 941-947.
7. Cohen, N., Brautbar, C., Font, M. P., Dausset, J. & Cohen, D. (1986) *Immunogenetics* **23**, 84-89.
8. Segall, M., Noreen, H., Schluender, L., Swenson, M., Barbosa, J. & Bach, F. H. (1986) *Hum. Immunol.* **17**, 61-68.
9. Svejgaard, A., Platz, P. & Ryder, L. P. (1980) in *Histocompatibility Testing: 1980*, eds. Terasaki, P. I. (Univ. of California, Los Angeles), pp. 638-656.

10. Bach, F. H., Rich, S. S., Barbosa, J. & Segall, M. (1985) *Hum. Immunol.* **12**, 59-64.
11. Botazzo, G. F., Dean, B. M., McNally, J. M., MacKay, E. H., Swift, P. G. F. & Gamble, D. R. (1985) *N. Engl. J. Med.* **313**, 353-360.
12. Botazzo, G. F., Pujol-Borrel, R., Hanafusa, T. & Feldmann, M. (1983) *Lancet* **ii**, 1115-1119.
13. Tait, B. D. & Boyle, A. J. (1986) *Tissue Antigens* **28**, 65-71.
14. Schreuder, G. M., Tilanus, M. G., Bontrop, R. E., Bruining, G. J., Giphart, M. J., Van Rood, J. J. & de Vries, R. R. (1986) *J. Exp. Med.* **164**, 938-943.
15. Cohen-Haguenauer, O., Robbins, E., Masset, C., Busson, M., Deschamps, I., Hors, J., Lalouel, J. M., Dausset, J. & Cohen, D. (1985) *Proc. Natl. Acad. Sci. USA* **81**, 3335-3339.
16. Todd, J. A., Bell, J. I. & Mc Devitt, H. O. (1987) *Nature (London)* **329**, 599-604.
17. Sterkers, G., Henin, Y., Lepage, V., Fradelizzi, D., Hannoun, C. & Levy, J. P. (1984) *Eur. J. Immunol.* **14**, 125-132.
18. Zeliszewski, D., Sterkers, G., Freidel, A. C., Gebuhrer, L., Betuel, H. & Levy, J. P. (1986) *Immunogenetics* **23**, 385-392.
19. Zeliszewski, D., Sterkers, G., Brautbar, C., Betuel, H., Layrisse, Z. & Levy, J. P. (1987) *Immunogenetics* **26**, 107-110.
20. Betuel, H., Gebuhrer, L., Lambert, J., Freidel, A. C. & Farré, A. (1983) *Hum. Immunol.* **8**, 227-237.
21. Freidel, A. C., Betuel, H., Gebuhrer, L., Farré, A. & Lambert, L. (1987) *Tissue Antigens* **29**, 129-140.
22. Cohen, D., Cohen, O., Marcadet, A., Massart, C., Lathrop, M., Deschamps, I., Hors, J., Schuller, E. & Dausset, J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1774-1778.
23. Font, M. P., Gebuhrer, L., Betuel, H., Freidel, C., Dausset, J. & Cohen, D. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3361-3365.
24. Reinsmoen, N. L., Layrisse, Z., Betuel, H., Freidel, A. C. & Bach, F. H. (1984) *Hum. Immunol.* **11**, 105-115.
25. Tonnelle, C., De Mars, R. & Long, E. O. (1985) *EMBO J.* **4**, 2839-2847.
26. Lee, B. S. M., Bell, J. I., Rust, N. A. & Mc Devitt, H. O. (1987) *Immunogenetics* **26**, 85-91.