Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 6274–6278, August 1989
Immunology

Structural analysis of the *HLA-DR, -DQ*, and *-DP* alleles on the celiac disease-associated *HLA-DR3* (*DRw17*) haplotype

(major histocompatibility complex/class II D-region human leukocyte antigens/gluten-sensitive enteropathy/second exon DNA sequence)

MARTIN F. KAGNOFF*†, JULIA I. HARWOOD*, TEODORICA L. BUGAWAN‡, AND HENRY A. ERLICH‡

*Department of Medicine, M-023-D, University of California at San Diego, La Jolla, CA 92093; and ‡Department of Human Genetics, Cetus Corporation, Emeryville, CA 94608

Communicated by J. Edwin Seegmiller, May 11, 1989 (received for review March 25, 1989)

ABSTRACT **Celiac disease is strongly associated with the HLA class II D-region serologic markers DR3 (DRw17) and DQw2. Moreover, by restriction fragment length polymorphism analysis, greater than 90% of *DR3* (*DRw17*), *DQw2* celiac disease patients have a polymorphic 4.0-kilobase *Rsa* I *DP B* gene DNA fragment. The present study sought to determine if there is a unique HLA class II D-region *A* or *B* gene structural variant on the *DR3* (*DRw17*) haplotype found in celiac disease. The polymorphic second exons of the coding *DRB*, *DQA* and *DQB*, and *DPA* and *DPB* genes in celiac disease patients with the *DR3* (*DRw17*) haplotype were sequenced after amplification by the polymerase chain reaction. To define the *DP B* genes associated with celiac disease, the second exons of the coding *DP B* genes from 27 celiac disease patients were amplified similarly and probed by using a panel of sequence specific oligonucleotides. The *HLA-DR*, *-DQ*, and *-DP A* and *B* gene second exon sequences of celiac disease patients were noted to be identical to sequences that can be found also, although at a significantly lower frequency, in unaffected individuals. This is compatible with a disease model wherein the HLA class II genes on the *DR3* (*DRw17*) haplotype are necessary, but not sufficient, for the phenotypic expression of celiac disease. Analysis of the *DP B* genes revealed a significant increase in the frequency of the alleles *DPB1* and *DPB3* in celiac disease. Furthermore, the increased frequency of the 4.0-kilobase *Rsa* I *DP B* gene restriction fragment length polymorphism in celiac disease can be accounted for by the overrepresentation in disease of the alleles *DPB1* and *DPB3*. The HLA-associated susceptibility to celiac disease appears to be multigenic, with specific, but structurally normal, allelic variants in the *DP* and *DQ/DR* subregions contributing to disease susceptibility.**

Celiac disease is characterized by small intestinal mucosal injury and the malabsorption of most nutrients. Disease is activated by the dietary ingestion of wheat gluten and similar proteins in rye, barley, and oats (1). Susceptibility to celiac disease is associated with genes that map to the HLA class II D region of the major histocompatibility complex on chromosome 6 (2).

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 the respective α and β chains of the class II α/β heterodimer present on the cell surface (3). HLA class II molecules are highly polymorphic. The greatest polymorphism occurs in the second exon sequences, which code for the amino-terminal extracellular domain (4). Amino acid residues in that domain interact with peptide fragments and with the T-cell receptor for antigen on class II-restricted T cells (5, 6). HLA class II molecules are present constitutively on cells of the immune system (e.g., B cells, macrophages, and dendritic cells) but can also be induced on other cell types, including intestinal epithelial cells (7, 8).

The *HLA-DP* and *HLA-DQ* subregions each contain a single expressed *A* and *B* gene (9, 10). In contrast, one or more *B* genes (i.e., *B1*, *B3*, or *B4*) are expressed in the *HLA-DR* subregion together with a single *A* gene. Whereas the expressed *HLA-DR*, *-DQ*, and *-DP B* genes and the *HLA-DQ A* gene are polymorphic, the *HLA-DP* and *-DR A* genes are relatively nonpolymorphic (i.e., two known alleles of *DP A* and no known variants of *DR A*) (9, 11). Thus, *HLA-DR* and *HLA-DP* allelic variants reflect mainly polymorphisms in the expressed *B* genes (3, 10).

Celiac disease is strongly associated with serologic HLA markers on an extended *HLA-DR3* (*DRw17*) haplotype (12). The major D-region markers of this haplotype are *DR3* (*DRw17*) and *DQw2* (12–14). Eighty to 90% of celiac disease patients of Northern European ancestry have the DR3 (DRw17) and DQw2 serologic specificities, but those markers are also present in 20–30% of nonceliacs from the same geographic area (15). More telomeric, this extended *DR3* haplotype is marked by the SC01 complotype and the HLA class I specificity HLA-B8 (1, 12).

In Southern Europeans (e.g., Spaniards and Italians), the HLA-DR7 serologic specificity also has been associated with celiac disease (16, 17). Since *HLA-DR7* haplotypes, like *HLA-DR3* haplotypes, often have the *DQB2* allele that encodes the DQw2 serologic specificity (18), this suggested that the *DQB2* allele may contribute to disease susceptibility. However, patients with *DR7* usually are heterozygous for *HLA-DR5* or *HLA-DR3* on the complementary chromosome (16, 17). Further, a specific *DQ A* allele (*DQA4.1*) that is common to *DR3* and *DR5* haplotypes (19) also has been associated with celiac disease based on serologic, restriction fragment length polymorphism (RFLP), and oligonucleotide analysis (20, 21). The increased susceptibility to celiac disease in individuals with a *DR7* haplotype (i.e., having *DQB2* but lacking *DQA4.1*) associated with heterozygosity for an accompanying *DR5* haplotype (i.e., lacking *DQB2* but having *DQA4.1*) (22, 23) suggests that a DQ antigen encoded both by *DQA4.1* and *DQB2* contributes to disease susceptibility. This DQ antigen would be encoded in cis on *DR3* haplotypes and in trans on *DR5/DR7* haplotypes.

By using RFLP analysis, >95% of *DR3* (*DRw17*), *DQw2* celiac disease patients compared to 27% of *DR3* (*DRw17*), *DQw2* controls were shown to have a 4-kilobase (kb) *Rsa* I DP β-chain RFLP (24). In addition, celiac disease has been associated with a DP α-chain RFLP (24, 25). These *DP* subregion markers could be increased among celiac disease patients secondary to linkage disequilibrium with genes in the *DR/DQ* subregion, or *DP* genes could contribute indepen-

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.

Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.
†To whom reprint requests should be addressed.

dently to disease susceptibility. In this regard, there generally is a high degree of recombination (estimated to be 2–5%) between the *HLA-DP* and the *HLA-DR/DQ* subregions and therefore only weak linkage disequilibrium between genes in those subregions (26, 27). It may be that celiac disease selects for an extended *HLA* haplotype that includes *DR3, DQw2* and a particular *DP* allele marked by the 4.0-kb DP β-chain RFLP. Alternatively, the putative *DP*-linked susceptibility gene may be present on the other haplotype. In either case, HLA susceptibility to celiac disease could be multigenic, with genes in *DP* and *DQ/DR* determining disease susceptibility (24).

In the present study, we asked if there is a unique HLA class II *A* or *B* gene structural variant on the *DR3* (*DRw17*) haplotype associated with celiac disease. In further studies, we characterized the HLA class II *DP* subregion allelic variants associated with celiac disease.

MATERIALS AND METHODS

Patients. Peripheral blood lymphocytes from 27 unrelated celiac disease patients and 50 random healthy controls in California were used as a source of DNA. Twenty-five out of 27 patients were of Northern European Caucasian ancestry and 2 were of Italian ancestry. Epstein–Barr virus-transformed lymphoblastoid B-cell lines were developed from celiac disease peripheral blood lymphocytes as described (24). The diagnosis of celiac disease was based on clinical evidence of malabsorption, a small bowel biopsy compatible with celiac disease, clinical and/or biopsy improvement on a gluten-free diet, and clinical and/or biopsy abnormalities upon rechallenge with a gluten-containing diet (15). Patients were HLA-typed by a standard complement-dependent microcytotoxicity assay or by RFLP analysis with HLA class II cDNA probes (28).

Amplification of Genomic DNA by the Polymerase Chain Reaction (PCR). Genomic DNA was purified from peripheral blood lymphocytes or Epstein–Barr virus-transformed lymphoblastoid B-cell lines as described (15, 24). Genomic DNA was amplified by using PCR with *Thermus aquaticus* (*Taq*) DNA polymerase (29–31). Amplified genomic DNA containing the second exon of the coding *DP B* gene of each of the 27 patients was analyzed by dot blot hybridization with sequence-specific oligonucleotide probes as described (11). In addition, the second exons of the *DQA* and *DQB, DPA* and *DPB*, and *DRB* genes from three patients, each heterozygous at the *DR* locus, were amplified and sequenced. The HLA type of the 3 patients as determined by serology was B8, 14; DR1, 3; DQw1, w2: B8, 48; DR2, 3; DQw1, w2: B8, 44; DR3, 7; DQw2, w2. The oligonucleotide primers selected for PCR amplification contained nucleotide sequences at their 5′ ends that were not complementary to the genomic template but became incorporated into the amplified products to provide *Bam*HI and *Pst* I restriction sites for subsequent cloning into the vector M13mp18. *HLA-DQB* genes were amplified by using the primers GH28 and GH29 (32), *HLA-DQA* genes were amplified by using the primers GH26 and GH27 (33), *HLA-DRB* genes were amplified by using the primers GH46 and GH50 (32), *HLA-DPB* genes were amplified by using the primers DPϕ1 and DPϕ3 (11), and *HLA-DPA* genes were amplified by using the primers GH98 and GH99 (11). Eight to 10 separate clones from each PCR amplification were sequenced by the dideoxy chain-termination method with Sequenase (United States Biochemical) as recommended by the manufacturer. Multiple clones were sequenced to ensure detection of both alleles and any potential nucleotide misincorporation.

RESULTS

The second exon nucleotide sequences of *DRB, DQA* and *DQB*, and *DPA* and *DPB* alleles of three celiac disease patients, each heterozygous for the *HLA-DR3* (*DRw17*) haplotype, were compared to available *HLA-DR, -DQ*, and *-DP* second exon allelic sequences (4, 11, 19). As indicated in Table 1, sequences of the celiac disease patients were identical, in each case, to *DR, DQ*, and *DP* allelic sequences in unaffected individuals. The absence of class II sequences unique for celiac disease is consistent with the finding that putatively HLA identical siblings and monozygotic twins can be discordant for the phenotypic expression of this disease (16, 35).

HLA *DP B* Genes Associated with Celiac Disease. Each of the 3 celiac disease patients described above had the relatively rare allele *DPB1*. This prompted further characterization, using sequence-specific oligonucleotide probes, of the *DP B* genes present in a group of 27 celiac disease patients. As shown in Tables 2 and 3, the *DP B* alleles *DPB1* and *DPB3* were significantly increased in celiac disease patients compared to controls. The *DPB1* allele was present in 14/27 (52%) of celiac disease patients compared to 6/50 (12%) of controls ($P = 0.0002$). The *DPB3* allele was present in 9/27 (33%) of celiac disease patients compared to 6/50 (12%) of controls ($P = 0.02$). RFLP analysis of homozygous and heterozygous DP cell lines using *Rsa* I and a DP β-chain cDNA probe (24) revealed that the 4.0-kb DP β-chain genomic RFLP, which is increased in *DR3, DQw2* celiac disease patients relative to *DR3, DQw2* controls, correlates with the specificities DPw1, DPw3, and DPw5 (M.F.K., unpublished data). The increased frequency of the RFLP in celiac disease can be accounted for by the overrepresentation in disease of the *DPB1* and *DPB3* alleles.

β Chains on the Celiac Disease-Associated *DR3* (*DRw17*) Haplotype Share a Positively Charged Lysine Residue at a Proposed Peptide Contact Site. Analysis of the *DP B* alleles associated with celiac disease revealed that the *DPB1* and *DPB3* alleles each encode a positively charged lysine residue at position 69 (Table 4). We note also that *DPB4.2*, an allele reported to be increased in a population of Italian celiac disease patients (37) and *DPB4.1*, the second *DPB* allele present in many of the patients (Table 2), encode a lysine at position 69.

The β chains encoded by the *DQB1, DRB1*, and *DRB3* loci on the celiac disease-associated *HLA-DR3* (*DRw17*) haplotype similarly have a positively charged lysine at the homologous position (position 71) (Table 5). In the case of DQ β chains, a positive charge at this position is unique for the DQw2 β chain (19). When superimposed on a hypothetical

Table 1. Second exon sequences in celiac disease patients correspond to sequences in nonceliacs

	Alleles at *DR, DQ*, and *DP* loci						
Patient	*DRB1**	*DRB3*†	*DRB4*‡	*DQB1*§	*DQA1*	*DPB*	*DPA*
VW	*w17 w15*	*52a*	*54a*	*2 w6*	*4.1 1.2*	*1 4.2*	*2 1*
LS	*w17 1*	*52a*	—	*2 w5*	*4.1 1.1*	*1 4.1*	*2 1*
AC	*w17 7*	*52a*	*53*	*2 2*	*4.1 2*¶	*1 1*	*2 2*

Nomenclature for alleles at *DRB1, DRB3*, and *DRB4* loci is as reported in ref. 34. Nomenclature for alleles at the *DQA1* locus is as reported in ref. 19. Nomenclature for coding *DPB* and *DBA* alleles is as reported in ref. 11.

*Alternative nomenclature: *w17* = *DR3*; *w15* = *DR2w2* (ref. 34).

†Alternative nomenclature: *52a* = *B3***0101* (ref. 34).

‡Alternative nomenclature: *53* = *B4***0101* (ref. 34); *54a* = DR2w2βIII. For 54a, it is not known if the locus is *DRB3* or *DRB4*.

§Alternative nomenclature: *w6* = *DQ1.2*; *w5* = *DQ1.1* (ref. 34).

¶*DQA1-4.1* (*DQA4.1*) codes for the DQ α chain on the *DR3* and *DR5* haplotypes, whereas *DQA1-2* (*DQA2*) codes for the DQ α chain on the *DR7* haplotype (19).

Table 2. Distribution of *DPB* allelic variants in celiac disease

Patient	*DPB* allele	4.0-kb *Rsa* I DP β RFLP	DR speci- ficity*	DQ specificity
AC	*1 1*	+	3;7	w2;w2
JB	*1 4.1*	+	3;5	w2;w3
MW	*1 4.1*	+	3;NT	w2;NT
LS	*1 4.1*	+	3;1	w2;w1
MS	*1 4.2*	+	3;7	w2;w2
VW	*1 4.2*	+	3;2	w2;w1
EM	*1 4.1*	+	3;6	w2;w1
JG	*1 3*	+	3;NT	w2;NT
CD	*1 3*	+	3;4	w2;w3
KO	*1 2.1*	+	3;3	w2;w2
HF	*1 13*	+	3;NT	w2;NT
BB	*1 15*	+	3;6	w2;w1
VS	*1 1*	+	3;3	w2;w2
MF	*1* New	+	3;3	w2;w2
BE	*3 4.1*	+	3;3	w2;w2
WC	*3 4.1*	+	3;7	w2;w2
WG	*3 4.1*	+	7;2	w2;w1
JC	*3 4.1*	+	3;w8	w2;NT
HS	*3 4.2*	+	3;3	w2;w2
CB	*3 4.2*	+	3;7	w2;w2
NJ	*3 14*	+	3;NT	w2;NT
DF	*4.2 4.1*	−	3;NT	w2;NT
MK	*4.2 4.1*	−	7;5	w2;w3
CA	*4.2* New	−	3;2	w2;NT
FR	*11 4.1*	+	3;7	w2;w2
TO	*2.2 4.1*	−	3;7	w2;w2
MD	*9 4.1*	+	3;3	w2;w2

NT, not tested; New, not *DPB1-15*.
*DR3 = Dw17.

model of the foreign antigen-binding site of class II histocompatibility molecules (36), residue 69 of DP β-chains and residue 71 of DR β-chains and DQ β-chains correspond to an identical position located on the α-helix of the β chain. This residue is thought to point into the processed antigen-binding site of the class II molecule (36).

DISCUSSION

HLA class II gene second exon nucleotide sequences on the *DR3* (*DRw17*) haplotype associated with celiac disease are identical to allelic variants also present, although at a significantly lower frequency, in unaffected individuals. This finding is compatible with a model in which HLA class II genes are necessary, but not sufficient, for the phenotypic expression of disease. In support of the latter, approximately 60% of HLA identical siblings and 25% of monozygotic twins are discordant for celiac disease (16, 35). Our findings in celiac disease parallel those in diseases associated with *HLA-DR* or *HLA-DQ* genes on the various *DR4* haplotypes (e.g., insulin-dependent diabetes mellitus, rheumatoid arthritis, and pemphigus vulgaris), which also lack class II gene sequences unique to patients (19, 38–40).

Table 3. Frequency of *DPB1* and *DPB3* alleles in celiac disease and control populations

DPB allele	Frequency of individuals with allele: Celiac disease	Random controls*	Relative risk	P†
1	14/27 (52%)	6/50 (12%)	7.9	0.0002
3	9/27 (33%)	6/50 (12%)	3.7	0.02

*The frequency of *DPB1* and *DPB3* in a panel of 100 DP-typed cell lines was identical to that in the California population of random controls reported herein (T.L.B. and H.A.E., unpublished data).
†Fisher's exact test.

There is a significant overrepresentation of the relatively rare *DPB* alleles *DPB1* and *DPB3* in our celiac disease patients. Moreover, this increase in the alleles *DPB1* and *DPB3* accounts for the increased frequency in this disease of a DP β-chain RFLP (24). The patients reported herein are mostly of Northern European Caucasian ancestry. In this regard, we note that a population of Italian celiac disease patients had an increase in the alleles *DPB4.2* and *DPB3* but not in *DPB1* (37). However, Italian celiac disease populations also differ markedly from Northern European celiacs in their distribution of *DR* alleles (17). Thus, with two exceptions, each of our patients had a *DR3* (*DRw17*) haplotype, whereas Italian celiac populations have been characterized by a significant increase in heterozygosity for *DR5/DR7* haplotypes (17).

Several points regarding the relative contribution of *DQ* and *DP* gene products to celiac disease susceptibility warrant comment. As regards *DQ* gene products, a specific DQw2 α/β heterodimer appears to be necessary, in most individuals, for celiac disease susceptibility. In support of this, each of our patients had the DQw2 specificity. Further, both *DR3* (*DRw17*) and *DR7* haplotypes, which are overrepresented in celiacs of Northern European or Italian and Spanish ancestry, respectively, carry the DQw2 specificity. Moreover, the same *DQA4.1/DQB2*-encoded α/β heterodimer carrying the DQw2 specificity that is encoded in cis on *DR3* (*DRw17*) haplotypes can be encoded in trans by the heterozygous *DR5/DR7* haplotype, which is overrepresented in the Italian and Spanish celiacs (16, 17). In addition, we note that celiac disease is unusual among black populations in the United States and that the *DR3* haplotype present in blacks often lacks the DQw2 specificity (41). As regards *DP* genes, susceptibility to celiac disease is significantly increased in individuals with DQw2 who also have the *HLA-DP* alleles *DPB1* or *DPB3*. The *DP* subregion may contribute to celiac disease through a direct effect of those genes or an effect of genes linked to the expressed *DPB* locus. In the case of the *DPB3* allele, no linkage disequilibrium has been noted with the *DR/DQ* subregion (T.L.B. and H.A.E., unpublished data), whereas the *DPB1* allele may show moderate linkage disequilibrium with *DR3* haplotypes (ref. 42; M.F.K. and H.A.E., unpublished data).

The antigen-binding site of the HLA class II histocompatibility molecules has been modeled based on the 3-

Table 4. HLA-DP β-chain amino acid residues that comprise postulated peptide contact sites in the antigen-binding groove

DPB allele	Position of amino acid in DP β chain: 9	11	13	26	28	35	36	55	59	65	68	69	72	76	80	84
1	Y	G	Q	E	Y	Y	A	A	W	I	E	K	V	V	N	D
3	—	L	—	—	—	F	V	D	—	L	—	—	—	—	—	—
4.2	F	—	—	—	—	F	V	D	—	—	—	—	—	M	—	G
4.1	F	—	—	—	—	F	—	—	—	—	—	—	—	M	—	G
2.1	F	—	—	—	—	F	V	D	—	—	—	E	—	M	—	G

The peptide contact sites in the antigen-binding groove are from the model of the class II antigen-binding site proposed by Brown *et al.* (36). —, Identical to amino acid in *DPB1* allele. Amino acids are identified by the single-letter code.

Table 5. HLA-DR and -DQ β-chain amino acid residues that comprise postulated peptide contact sites in the antigen-binding groove

			Position in class II β chain															
Subregion	Locus	Allele	9	11	13	28	30	37	38	57	61	67	70	71	74	78	82	86
DR	*DRB1*	*3(w17)*	E	S	S	D	Y	N	V	D	W	L	Q	K	R	Y	N	V
	DRB3	*52a**	—	R	—	—	—	F	L	V	—	—	—	—	—	—	—	G
DQ	*DQB1*	*w2*	Y	F	G	S	S	I	—	A	—	I	R	—	A	V	—	E

The peptide contact sites in the antigen-binding groove are from the model of class II antigen-binding site proposed by Brown *et al.* (36). —, Identical to amino acid encoded by the *DRB1w17* (*DR3*) allele. Amino acids are identified by the single-letter code.
**DR52a* = *DRB3*0101* (34).

dimensional x-ray crystallographic structure of an HLA class I molecule (36, 43, 44). In this model, residues on the α-helices and β-sheets encoded by second exon sequences of the *A* and *B* genes form the walls and floor, respectively, of a putative peptide-binding groove. Position 71 of the DQ β chain and position 69 of the DP β chain correspond to an identical position on the α-helix formed by the β chain. The residue at this position points into the antigen-binding groove (36) and, together with adjacent residues on the floor of the β sheet and nearby residues on the α helix, can function as a peptide-binding site. We note that the positively charged lysine at position 71 is unique to the β chain of DQw2, relative to all other *DQB* gene products. A positively charged lysine residue is also present at the corresponding position (residue 69) of each DP β chain associated with celiac disease (i.e., encoded by *DPB1* and *DPB3* as reported herein; *DPB4.2* in Italian celiac disease patients). Similarly, *DPB4.1*, present as the second *DP* allele in many of our patients (Table 2), encodes a positively charged lysine at the corresponding position.

The likely importance of the lysine residue at position 69 as regards the *DP B* gene susceptibility alleles is supported by comparing the *DPB2.1* and *DPB4.2* alleles (Table 4). Thus, the only difference between *DPB2.1* and *DPB4.2* gene products is the substitution of a negatively charged glutamic acid residue in *DPB2.1* for a positively charged lysine residue in the *DPB4.2* gene product (11). The *DPB2.1* allele was present in 16% of our control subjects and has been found in as many as 20–47% of control subjects (refs. 45 and 46; H.A.E., unpublished data). *DPB2.1* was underrepresented in our patients (i.e., present in one patient who also had *DPB1*; negative relative risk 5.9, $P < 0.05$). In contrast, *DPB4.2* tended to be increased in our patients and was significantly increased in Italian celiacs (37). Unlike celiac disease, *DPw2* (*DPB2.1*) is overrepresented in pauciarticular juvenile rheumatoid arthritis (45–47).

Recent studies suggest that the electrostatic charge in the antigen-binding site of the class II molecule can be an important factor in governing the interaction between a peptide, a major histocompatibility complex molecule, and T cells (48). Nonetheless, the mechanism by which a positively charged residue at position 69 of the DP β chain or the corresponding position 71 of the DQ or DR β chain may contribute to disease susceptibility is not known. Further, *DP* and *DR* alleles that are not associated with celiac disease susceptibility may also encode a positively charged residue at the same position. Thus, other residues determining the framework in which the positively charged lysine residue occurs likely are important also in determining the disease susceptibility allele.

We thank Ms. M. Kreuger for technical assistance and Ms. A. Jackson for preparation of the manuscript. This work was supported by National Institutes of Health Grant DK35108.

1. Kagnoff, M. F. (1988) in *Immunology and Allergy Clinics of North America*, ed. Kagnoff, M. F. (Saunders, Philadelphia), Vol. 8, pp. 505–520.
2. Tiwani, J. L. & Terasaki, P. I. (1985) *HLA and Disease Associations* (Springer, New York).
3. Korman, A. J., Boss, J. M., Spies, T., Sorrentino, R., Okada, K. & Stominger, J. L. (1985) *Immunol. Rev.* **85**, 45–86.
4. Todd, J. A., Bell, J. I. & McDevitt, H. O. (1987) *Nature (London)* **329**, 599–604.
5. Sette, A., Buus, S., Colon, S., Smith, J. A., Miles, C. & Grey, H. (1987) *Nature (London)* **328**, 395–399.
6. Cairns, J. S., Curtsinger, J. M., Dahl, C. A., Freeman, S., Alter, B. J. & Bach, F. H. (1985) *Nature (London)* **317**, 166–168.
7. Doar, A. S., Fuggle, S. V., Fabre, J. W., Ting, A. & Morris, P. J. (1984) *Transplantation* **38**, 293–298.
8. Spencer, J., Finn, T. & Isaacson, P. G. (1986) *Gut* **27**, 153–157.
9. Trowsdale, J., Young, J. A. T., Kelley, A. P., Austin, P. J., Carson, S., Meunier, H., So, A., Erlich, H. A., Spielman, R. S., Bodmer, J. & Bodmer, W. F. (1985) *Immunol. Rev.* **85**, 5–43.
10. Spies, T., Sorrentino, R., Boss, J. M., Okada, K. & Strominger, J. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5165–5169.
11. Bugawan, T. L., Horn, G. T., Long, C. M., Mickelson, E., Hansen, J. A., Ferrara, G. B., Angelini, G. & Erlich, H. A. (1988) *J. Immunol.* **141**, 4024–4030.
12. Alper, C. A., Fleischnick, E., Awdeh, Z., Katz, A. J. & Yunis, E. J. (1987) *J. Clin. Invest.* **79**, 251–256.
13. Tosi, R., Vismara, D., Tanigaki, N., Ferrara, G. B., Cicimarra, F., Buffolano, W., Follo, D. & Auricchio, S. (1983) *Clin. Immunol. Immunopathol.* **28**, 395–404.
14. Corrazza, G. R., Tabacchi, P., Frisoni, M., Prati, C. & Gasbarrini, G. (1985) *Gut* **26**, 1210–1213.
15. Howell, M. D., Austin, R. K., Kelleher, D., Nepom, G. T. & Kagnoff, M. F. (1986) *J. Exp. Med.* **164**, 333–338.
16. Mearin, M. L., Biemond, I., Pena, A. S., Polanco, I., Vasquez, G., Schreuder, Th. M., de Vries, R. P. B. & van Rood, J. J. (1983) *Gut* **24**, 532–537.
17. Morellini, M., Trabace, S., Mazzilli, M. C., Lulli, P., Cappellacci, S., Bonamico, M., Margarit, I. & Gandini, E. (1988) *Dis. Markers* **6**, 23–28.
18. Karr, R. W., Gregersen, P. K., Obata, F., Goldberg, D., Maccari, J., Alber, C. & Silver, J. (1986) *J. Immunol.* **137**, 2886–2890.
19. Horn, G. T., Bugawan, T. L., Long, C. M. & Erlich, H. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6012–6016.
20. Roep, B. O., Buntrop, R. E., Pena, A. S., van Eggermund, M. C. J. A., van Rood, J. J. & Giphart, M. J. (1988) *Hum. Immunol.* **23**, 271–280.
21. Sollid, L. M., Markussen, G., Ek, J., Gjende, H., Vartdal, F. & Thorsby, E. (1989) *J. Exp. Med.* **169**, 345–350.
22. Schiffenbauer, J., Didier, D. K., Klearman, M., Rice, K., Shuman, S., Tieber, V. L., Kittlesen, D. J. & Schwartz, B. D. (1987) *J. Immunol.* **139**, 228–233.
23. Duquesnoy, R. J., Zeevi, A. & Trucco, M. (1985) in *Molecular and Cellular Biology of Histocompatability Antigens*, eds. Schachter, B., Brodsky, F., Crosswill, C., Kostyu, D. & Sheehy, M. (Am. Soc. Histocompat. Immunogenet., New York).
24. Howell, M. D., Smith, J. R., Austin, R. K., Kelleher, D., Nepom, G. T., Volk, B. & Kagnoff, M. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 222–226.
25. Niven, M. J., Caffrey, C., Sachs, J. A., Gassell, P. G., Gallagher, R. B., Kumar, P. & Hitman, G. A. (1987) *Lancet* **i**, 805.
26. Sanchez-Perez, M. & Shaw, S. (1985) in *Human Class II Histocompatibility Antigens: Theoretical and Practical Aspects* (Springer, New York), pp. 83–108.
27. Termijtelen, A., Kahn, P. M., Shaw, S. & Van Rood, J. J. (1983) *Immunogenetics* **18**, 503–512.

28. Carlsson, B., Wallin, J., Böhme, J. & Möller, E. (1987) *Hum. Immunol.* **20**, 95–113.
29. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Ainheim, N. (1985) *Science* **230**, 1350–1354.
30. Mullis, K. B. & Faloona, F. A. (1987) *Methods Enzymol.* **155**, 335–350.
31. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
32. Scharf, S. J., Long, C. & Erlich, H. A. (1988) *Hum. Immunol.* **22**, 61–69.
33. Scharf, S. J., Horn, G. T. & Erlich, H. A. (1986) *Science* **233**, 1067–1078.
34. Nomenclature Committee on Leukocyte Antigens (1988) *Immunogenetics* **28**, 391–398.
35. Polanco, I., Biemond, I., Van Leeuwen, A., Schreuder, I., Kahn, M. P., Guerrero, J., D'Amaro, J., Vasquez, C., van Rood, J. J. & Pena, A. S. (1981) in *The Genetics of Coeliac Disease*, ed. McConnell, R. B. (MTP, Lancaster, U.K.), pp. 211–230.
36. Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J. & Wiley, D. C. (1988) *Nature (London)* **332**, 845–850.
37. Bugawan, T. L., Angelini, G., Larrick, J., Auricchio, S., Ferrara, G. B. & Erlich, H. A. (1989) *Nature (London)* **339**, 470–473.
38. Todd, J. A., Acha-Orbea, H., Bell, J. I., Chao, N., Fronek, Z., Jacob, C. O., McDermott, M., Sinha, A. A., Timmerman, L., Steinman, L. & McDevitt, H. O. (1988) *Science* **240**, 1003–1009.
39. Scharf, S. J., Friedmann, A., Brautbar, C., Szafer, F., Steinman, L., Horn, G., Gyllensten, U. & Erlich, H. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3504–3508.
40. Sinha, A. A., Brautbar, C., Szafer, F., Friedmann, A., Tzfoni, E., Todd, J. A., Steinman, L. & McDevitt, H. O. (1988) *Science* **239**, 1026–1029.
41. Hurley, C. K., Gregersen, P., Steiner, M., Bell, J., Hartzman, R., Nepom, G., Silver, J. & Johnson, A. H. (1988) *J. Immunol.* **140**, 885–892.
42. Termijtelen, A. & van Rood, J. J. (1981) *Tissue Antigens* **17**, 57–63.
43. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 506–512.
44. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 512–518.
45. Hoffman, R. W., Shaw, S., Francis, L. C., Larson, M. G., Petersen, R. A., Chylack, L. T. & Glass, D. N. (1986) *Arthritis Rheum.* **29**, 1057–1062.
46. Odum, N., Morling, N., Friis, J., Heilmann, C., Hyldig-Neilsen, J. J., Jakobsen, B. K., Pedersen, F. K., Platz, P., Ryder, L. P. & Svejgaard, A. (1986) *Tissue Antigens* **28**, 245–250.
47. Begovich, A. B., Bugawan, T. L., Nepom, B. S., Nepom, G. T. & Erlich, H. A. (1989) *J. Cell. Biochem. Suppl.* **13A**, 217 (abstr.).
48. Paterson, Y. (1989) in *The Immune Response to Structurally Defined Proteins: The Lysozyme Model*, eds. Smith-Gill, S. & Sercarz, E. (Adenine Press, Guilderland, NY), pp. 177–189.