Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 8051–8055, October 1990
Immunology

Structural requirements for recognition of the HLA-Dw14 class II epitope: A key HLA determinant associated with rheumatoid arthritis

(major histocompatibility complex/disease susceptibility genes/retroviral vectors)

AKIKAZU HIRAIWA[†‡], KATSUO YAMANAKA[†], WILLIAM W. KWOK[†], ERIC M. MICKELSON[§], SUSAN MASEWICZ[§], JOHN A. HANSEN[§‡], SUSAN F. RADKA[¶], AND GERALD T. NEPOM[†,||,††]

[†]Virginia Mason Research Center, 1000 Seneca Street, Seattle, WA 98101; [§]Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104; [||]Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195; and [¶]Oncogen Corporation, 3005 First Avenue, Seattle, WA 98121

Communicated by Earl Benditt, July 20, 1990

ABSTRACT **Although HLA genes have been shown to be associated with certain diseases, the basis for this association is unknown. Recent studies, however, have documented patterns of nucleotide sequence variation among some HLA genes associated with a particular disease. For rheumatoid arthritis, HLA genes in most patients have a shared nucleotide sequence encoding a key structural element of an HLA class II polypeptide; this sequence element is critical for the interaction of the HLA molecule with antigenic peptides and with responding T cells, suggestive of a direct role for this sequence element in disease susceptibility. We describe the serological and cellular immunologic characteristics encoded by this rheumatoid arthritis-associated sequence element. Site-directed mutagenesis of the *DRB1* gene was used to define amino acids critical for antibody and T-cell recognition of this structural element, focusing on residues that distinguish the rheumatoid arthritis-associated alleles *Dw4* and *Dw14* from a closely related allele, *Dw10*, not associated with disease. Both the gain and loss of rheumatoid arthritis-associated epitopes were highly dependent on three residues within a discrete domain of the HLA-DR molecule. Recognition was most strongly influenced by the following amino acids (in order): 70 > 71 > 67. Some alloreactive T-cell clones were also influenced by amino acid variation in portions of the DR molecule lying outside the shared sequence element.**

The HLA-DR4^{+} family of human class II molecules is encoded by a series of related alleles at the *HLA-DRB1* locus (1–3). Two of these alleles, termed *DRB1*0401* (*Dw4*) and *DRB1*0404* (*Dw14*), are highly associated with rheumatoid arthritis and account for the serologic *HLA-DR4* association with that disease (4–6). Another related *DRB1* allele, termed *DRB1*0402* (*Dw10*), is not associated with rheumatoid arthritis (7, 8). The amino acid differences among class II molecules encoded by the *Dw4*, *Dw14*, and *Dw10* genes cluster in a linear sequence array spanning codons 67–74 of the *DRB1* locus (2). Structural studies that have focused on this region of sequence polymorphism have documented that alloreactive T cells distinguish among these alleles and apparently recognize the polypeptide product of this polymorphic segment as a discrete functional "epitope" (9, 10). The sequence for the HLA-Dw14 epitope is of particular interest, since the majority of rheumatoid arthritis patients who are not *HLA-DR4* carry different class II alleles that, although they differ in other areas of primary *DRB1* sequence, share the *Dw14*-related sequence corresponding to codon 67–74 (6). This has led to the "shared epitope hypothesis" in which a discrete portion of a single class II molecule (i.e., the Dw14 epitope) is the primary genetic component of the major histocompatibility complex accounting for disease association (11).

Structural models for the class II molecule (12), based on the HLA class I structure determined by crystallography (13), predict an important structural role for the *DRB1* sequence from codon 67 to codon 74. In this structural model, the most striking functional element of the class II molecule is an antigen binding groove, bounded on either side by a long α-helical loop contributed by the class II α and β chains, respectively. Amino acid side chains on these α-helices are predicted to either point "into" the groove and interact with antigen or point "up" and potentially interact with the T-cell receptor. Indeed, analysis of site-specific amino acid substitutions on the class I structure precisely mapped T-cell recognition elements in both alloreactive and syngeneic recognition to these key residues (14). The Dw14 epitope is predicted to occupy the middle portion of the β chain α-helix and is, therefore, very likely to control the major T-cell recognition properties of the molecule. Since this is the same structural epitope highly correlated with susceptibility to rheumatoid arthritis, an understanding of the structure–function relationships contributed by this epitope provides an opportunity to develop insights into the molecular requirements for genetically controlled T-cell recognition events in this disease.

In this report, we have performed site-directed mutagenesis within the *DRB1* gene to define the structural characteristics of the Dw14 epitope. We report here the gain and loss of both serologic and cellular recognition patterns for this region and directly demonstrate the presence of a discrete class II epitope dependent on a specific set of amino acid substitutions at codons 67, 70, and 71.

MATERIALS AND METHODS

Cell Lines and Antibodies. Monoclonal antibodies (mAbs) used in this study were characterized by ELISAs on homozygous B-cell panels or have been described. mAb CCCL20, originally described as a DR"4+1" specificity also termed "MC1-like" (15), reacts with Dw14 but not Dw10-positive cells; DR-allospecific mAbs used as positive controls were mAb GS 359-13F10, specific for HLA-DR4 (16), and mAb SFR3DR5 is specific for HLA-DR5 (17). Negative control mAbs with irrelevant specificities were F9 or

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: mAb, monoclonal antibody; LCL, lymphoblastoid cell line.

[‡]Present address: First Department of Internal Medicine, Nagoya University School of Medicine, Japan.
[††]To whom reprint requests should be addressed.

RG719.1. All mAbs were used at concentrations empirically determined to be in "antibody excess," usually 1:80 dilution of ascites fluid, except for mAbs 359-13F10 and RG719.1, which were used directly from hybridoma culture supernatants.

Epstein–Barr virus-transformed human B-lymphoblastoid cell lines (LCLs) were obtained from the 10th International Histocompatibility Workshop Typing Panel (18) or were directly transformed from peripheral blood lymphocytes. LCL MAT (*DR3*, *DQw2*,) was maintained in RPMI 1640 medium supplemented with 10% (vol/vol) fetal calf serum prior to retroviral infection.

***HLA-DRB1*-Containing Retroviral Vectors.** HLA class II genes and mutagenized constructs were introduced into MAT LCL by using retroviral infection. The retroviral constructs contained a 5′ long terminal repeat from Moloney murine sarcoma virus, a neomycin-resistance gene under the transcriptional control of this long terminal repeat, and a 3′ long terminal repeat from Moloney murine leukemia virus. Transcription of the class II genes was initiated from a cytomegalovirus immediate early gene (CMV-IE) promoter sequence inserted 3′ of the neomycin-resistance gene. Vector DNA was transfected to Psi-II cells, an ecotropic packaging cell line, and introduced into PA317 cells, an amphotropic packaging cell line, followed by transfer to MAT grown in the presence of G418, to select for neomycin-resistant cells. Details of the construction of retroviral vectors, generation of recombinant virus-producing cell lines, and infection of LCLs by cocultivation have been described (19).

Class II cDNA for the wild-type *Dw14* and *DR5 DRB1* genes were originally cloned from cell lines BIN40 and FPAc32 and kindly provided by P. Gregersen (North Shore University Hospital, Manhasset, NY). Oligonucleotide-mediated mutagenesis of genes for Dw14β or DR5β was performed using M13 bacteriophage as described by Burke and Olson (20) with minor modifications: a 1.5-kilobase or a 1.3-kilobase *Hin*dIII–*Xba* I fragment including Dw14 or DR5 cDNA, respectively, was cloned from the expression vector into M13mp19 for mutagenesis. Mutagenic oligomers used (with substitutions indicated) were 5′-TGCTCCAGGATA-TCCTTCTGG-3′, for the Dw14βm67 mutant and Dw14βm67/m71 mutant; 5′-GCCCGCCTATCTTCAAG-GAGGTCC-3′, for the Dw14βm70 mutant; 5′-CGCGGC-CCGCTCCTGCTCCAG-3′, for the Dw14βm71 mutant; 5′-CCCGCCTCTGTTCCAGGAAGTC-3′, for the DR5βm70 mutant; and 5′-CCTCTGTTCCAGGAGATCCTTCTG-3′, for the DR5βm67/m70 mutant. These oligomers also have silent mutation changes compared to wild-type sequence to facilitate screening. Nucleotide sequencing of the DRβ coding region of each M13 construct was performed to confirm that the desired mutation was obtained. The translated amino acid sequences for each DRβ polypeptide are shown in Table 1. The *Hin*dIII–*Xba* I DNA fragment from each mutant phage was then reinserted into the expression vector.

Cytofluorometric Analyses. Approximately 5 × 10^5 cells were incubated with 15 μl of mAb for 1 hr at 4°C, washed with 2.5 ml of isotonic phosphate-buffered saline with 1% fetal calf serum, and then incubated with 15 μl of fluorescein isothiocyanate-labeled anti-immunoglobulin for another 1 hr at 4°C. After additional washing, cells were fixed with 2% (wt/vol) paraformaldehyde and analyzed on a Becton Dickinson FACS IV flow cytometer.

Generation of Alloproliferative T-Cell Clones and T-Cell Proliferation Assays. Alloproliferative T-cell clones 14B and EMM025 were derived from *in vitro* primings and expansions, in which stimulator cells were Dw14$^+$ and the responder cells were Dw4$^+$, as described (9). Analysis of LCL panels showed that among DR4$^+$ haplotypes, both clones recognize Dw14$^+$ stimulators, but not Dw4$^+$, Dw10$^+$, or Dw13$^+$ stimulators. In addition to Dw14$^+$ cells, clone 14B also responds to DR1(Dw1)$^+$ and DRw6(Dw16)$^+$ cells (see below).

T-cell clones 14B and EMM025 were screened for specific responses by assaying 1 × 10^4 responder cells for proliferative activity after incubation with 2.5 × 10^4 irradiated (10,000 rad; 1 rad = 0.01 Gy) stimulator cells from MAT transfected with Dw14β, Dw4β, DR5β, Dw14βm67, Dw14βm70, Dw14βm71, Dw14βm67/m71, DR5βm70, DR5βm67/70, or several other transformed cell lines in U-bottom microtiter plates (Linbro) for 66 hr. During the final 18 hr of incubation, 1 μCi of [^{3}H]thymidine (specific activity, 6.7 Ci/mmol; 1 Ci = 37 GBq) was added, and the final activity per culture was expressed as cpm incorporated (Δcpm = cpm of infected stimulator LCLs − cpm of uninfected stimulator LCLs).

Table 1. Amino acid sequences for residues 56–86 of the *DRB1* alleles analyzed in this study

Allele(s)	Sequence			
	60	70	80	
DR4,Dw14	PDAEY	WNSQKDLLEQ	RRAAVDTYCR	HNYGVV
DR1	.....	....	....	.....G
DRw6,Dw16	.....	....	....	.....G
DR4,Dw10	.....	......I..D	E.........	......
DR5	..E..	......F..D	....	.....G
DR4,Dw4	.....	....	K.........	.....G
DR3	.....	....	K.GR..N...	......

RESULTS

The HLA-Dw14 epitope was originally defined using T-cell alloreactive clone 14B, whose pattern of alloreactivity on a stimulator-cell panel corresponded precisely to an oligonucleotide hybridization pattern within the *Dw14 DRB1* gene (9). As shown in Table 1, this shared sequence, which spans a region from amino acid codons 56 to 85, is present in the *HLA-DR1*, *HLA-Dw16*, and *HLA-Dw14 DRB1* genes (21). As the epitope function of this sequence was first detected within the *Dw14* gene, it is commonly referred to as the Dw14 epitope. As indicated in Table 2, this reactivity pattern includes cells expressing DR1, Dw16, and Dw14. T-cell clone EMM025 is a similar, but not identical, alloreactive T-cell clone. EMM025 is stimulated by Dw14$^+$ cells but not by Dw4$^+$, Dw10$^+$, DR1$^+$, or Dw16$^+$ cells, likely implicating residues outside the codon 67–74 region in the epitope recognized by EMM025 (Table 2).

We screened a variety of HLA-DR-reactive mAbs on DR4$^+$ cell panels to identify a serologic pattern of recognition for the Dw14 epitope similar to the T-cell stimulation pattern. mAb CCCL20 had originally been described as a DR "4+1" specificity, sometimes termed "MC1-like" (15). This mAb was distributed as part of the 10th International Histocompatibility Workshop and determined by us to immunoprecipitate DR molecules from Dw14$^+$ LCLs but not from Dw10$^+$ LCLs. Immunofluorescent profiles generated with this antibody on a panel of homozygous LCLs are shown in Fig. 1. Gradations of reactivity were apparent that suggest a direct correlation with the T-cell-defined Dw14 epitope. Dw10$^+$ LCLs were negative for staining with CCCL20, whereas Dw14$^+$, Dw16$^+$, and DR1$^+$ cells all gave very strong staining patterns. Other cells, typing as *Dw4*, *DR5* (Fig. 1), and *DR3* (see Fig. 3) gave low to intermediate staining patterns.

In the structural models for HLA class I and class II conformation, residues 56–86 define a major α-helical loop of the molecule, predicted to interact with both antigen and T-cell receptors and form an integral part of the recognition complex contributed by the HLA molecule (12–14). Since the *Dw14*, *DR1*, and *Dw16* genes are identical in this α-helical loop and differ from the other *DRB1* alleles at one or more sites in this sequence, we investigated the minimal structural

Table 2. Stimulation of anti-Dw14 T-cell clones 14B and EMM025 by transfected LCLs

LCL	Codons that differ from *Dw14*	Δcpm EMM025	Δcpm 14B
Homozygous typing cells			
DR4,Dw10 (YAR)	67, 70, 71	302	202
DR5 (SWEIG)	58, 67, 70, 86	−101	208
DR4,Dw4 (PF)	71, 86	−133	−178
DR4,Dw14 (MT)		26,759	3524
DRw6,Dw16 (AMALA)	86	20	5301
DR1 (WT100)	86	−47	4832
None		637	766
Transfected MAT cell lines			
Dw14		29,388	5372
DR5	58, 67, 70, 86	15	703
DR5m70	58, 67, 86	−774	655
DR5m67/70	58, 86	−808	1039
None		222	685
Dw14		34,266	4009
Dw14m67	67	5,641	4008
Dw14m70	70	846	622
Dw14m71	71	185	1020
Dw14m67/71	67, 71	1,381	699
DR3 (wild-type)	71, 73, 74, 77	1,007	765
None		435	1253

Codons between codons 56 and 86 that differ from *Dw14* are indicated. Δcpm = (mean cpm in LCL culture and in T cells) − (mean cpm with stimulator LCL alone); samples were assayed in duplicate.

requirements that are sufficient for the CCCL20 serologic reactivity pattern, using gene transfer and mutagenesis.

Gain of the Dw14 Epitope. Dw14 or DR5 DRB1 cDNA was inserted into MAT-LCLs by retroviral-mediated gene transfer. As shown in Fig. 2, the serologic epitopes expressed by these transfectants recapitulated the expected profiles; namely, mAb CCCL20 reacted strongly with the *Dw14* transfectant and weakly with the *DR5* transfectant, whereas mAb SFR3DR5 reacted strongly with the *DR5* transfectant but not with the *Dw14* transfectant. Of the three residues that distinguish the *Dw14* from the *Dw10* gene (namely residues 67, 70, and 71), the *DR5 DRB1* allele differs in two, at codons 67 and 70. The contribution of each of these residues to reconstituting the Dw14 epitope was investigated by site-directed mutagenesis of the DR5 cDNA. Substitution to a glutamine

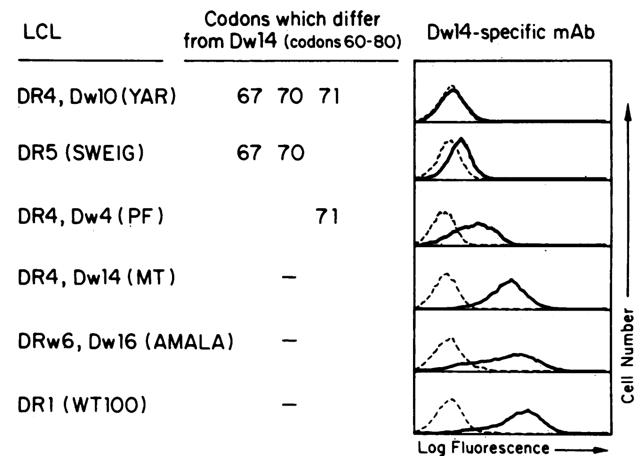

FIG. 1. Reactivity of mAb CCCL20 (Dw14-specific) with homozygous LCLs. Dashed line, staining with mAb F9 (negative control); solid line, staining with mAb CCCL20. *HLA-DR* specificities and sites of sequence polymorphisms within the *DRB1* locus are listed for each LCL tested.

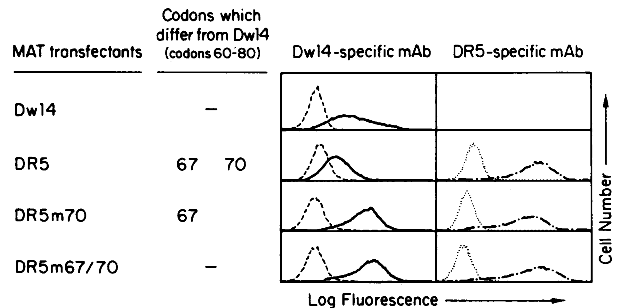

FIG. 2. Cytofluorometric analysis of the following LCL transfectants: *Dw14*, *DR5*, *DR5m70*, and *DR5m67/70* using mAb RG7119.1 negative control (. . . . .), mAb SFR3-DR5 (DR5-specific) (– · – · –), F9 (– – –), and CCCL20 (——).

at codon 70 (DR5m70) completely reconstituted the activity of the MAT transfectant with mAb CCCL20 (Fig. 2). The "double mutant" in which both codons 67 and 70 contain Dw14-like residues also displayed strong reactivity with mAb CCCL20. The immunodominant portion of this serologic epitope, therefore, appears to be dependent primarily on the region around residues 70 and 71. Interestingly, the DR5-reactive mAb was equally reactive with all MAT transfectants containing a modified or unmodified DR5 cDNA. This indicates that the DR5 alloepitope is distinct from the region of the Dw14 epitope, as the *DR5m70* and *DR5m67/70* transfectants expressed both the *Dw14* and the *DR5* allospecificities on the same molecule.

Loss of the Dw14 Epitope. To more directly evaluate the relative contributions of residues 67, 70, and 71 to the Dw14 epitope, we performed site-directed mutagenesis directly on the Dw14 cDNA, substituting *Dw10*-like residues at each of these positions to create a series of "intermediate phenotypes" distinguishing rheumatoid arthritis-susceptible from nonsusceptible alleles. Mutagenized and wild-type Dw14 cDNA were introduced into MAT LCLs and analyzed by indirect immunofluorescence. The HLA-DR4-specific antibody GS 359-13F10 reacted with all transfected lines, indicating a high level of expression of the *DR4* gene product in all cases (Fig. 3). However, different reactivity patterns were observed when these transfectants were stained with the mAb CCCL20. Although the wild-type *Dw14* transfectant was strongly positive by immunofluorescence, substitution at residue 70 or 71 or at both residues 67 and 71 resulted in markedly diminished reactivity with mAb CCCL20; substitution of residue 67 alone led to an intermediate loss of

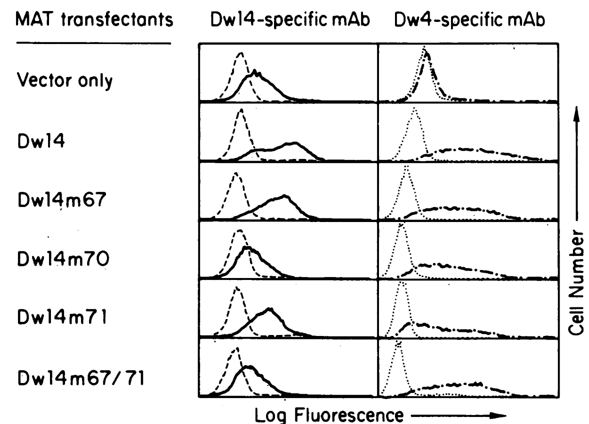

FIG. 3. Cytofluorometric analysis of the following DR3-LCL transfectants; vector only, *Dw14*, *Dw14m67*, *Dw14m70*, *Dw14m71*, and *Dw14m67/71* using rat mAb RG719.1 negative control (. . . . .), rat mAb 359-13F10 (DR4-specific) (– · – · –), F9 (– – –), and CCCL20 (——).

reactivity with CCCL20 (Fig. 3). The greatest decrease in reactivity was seen with the substitution of an aspartic acid for a glutamine at codon 70.

Serologically defined class II epitopes are usually thought to represent primary structural polymorphisms of the class II molecule itself, independent of peptide binding interactions or other functional aspects of class II interactions. T-cell recognition epitopes, on the other hand, are potentially much more complex, since direct peptide binding interactions, and the conformational changes they may induce, potentially play a major role in triggering T-cell recognition. We, therefore, compared the serologic definition of the Dw14 epitope with reactivity patterns generated by using the transfected MAT LCL as the stimulator cell for Dw14-reactive allospecific T-cell clones 14B and EMM025.

Allospecific T-Cell Recognition of the Dw14 Epitope. The LCL transfected with wild-type Dw14 cDNA stimulated both 14B and EMM025 T-cell clones (Table 2). Mutagenesis of the *Dw14* gene by substitution of *Dw10*-like residues at codon 70 or 71 led to a complete loss of alloreactivity for both clones (Table 2). Mutagenesis at residue 67 led to diminished reactivity with clone EMM025 but no inhibition of 14B reactivity. None of the *DR5* wild-type or mutagenized transfectants with substitutions at codon 67 or 70 stimulated either clone (Table 2).

DISCUSSION

The *HLA-DRB1* locus is highly polymorphic, with an estimated 50–80 alleles, each encoding distinct HLA-DR polypeptides. Comparisons of nucleotide sequences among the various *DRB1* alleles indicate that small clusters of polymorphic residues are found at homologous positions in different alleles (21). This results in stretches of non-unique polymorphic residues linked together to form distinct allelic patterns. This observation has considerable functional and evolutionary importance, because some of these stretches of polymorphic residues can function as distinct epitopes for immunologic recognition. This has been directly demonstrated for the "Dw14 epitope," in which a stretch of sequence homology among the *Dw14*, *DR1*, and *Dw16* genes corresponds precisely to the ability to stimulate the alloreactive T-cell clone 14B (9). The minimal boundaries of this shared epitope encompass residues 67–74 of the β chain, defined by failure of $Dw10^+$ and $Dw13^+$ LCL, respectively, to stimulate clone 14B. Thus, although other residues in the *Dw14*, *DR1*, and *Dw16* genes are quite different, a limited region of sequence identity is sufficient to account for one specific form of functional identity.

The observation that a discrete Dw14 epitope is found on multiple *DRB1* alleles is important in understanding HLA-associated disease. The *HLA-Dw14* gene is highly associated with adult and juvenile polyarticular rheumatoid arthritis and represents a significant fraction, ≈30%, of the HLA-associated genetic risk in that disease, second only to the contribution of the *HLA-Dw4* gene (6, 8, 22). The *Dw4* gene is identical to the *Dw14* gene except for a conservative Arg → Lys substitution at residue 71 and a Val → Gly substitution at residue 86. Together *Dw4* and *Dw14* are present in ≈73% of patients with rheumatoid arthritis. Remarkably, most rheumatoid arthritis patients who are neither *Dw4* nor *Dw14* nevertheless carry an allele that shares the Dw14 epitope, usually *DR1* (6, 7). Coupled with the observation that the *Dw10* allele is not associated with rheumatoid arthritis, the *DRB1* sequences corresponding to the Dw14 epitope appear to be primary disease-associated elements that accounts for much of the HLA class II contribution to genetic susceptibility for rheumatoid arthritis.

In the present study, we report a detailed analysis of the structural basis for the Dw14 epitope, using a combination of site-directed mutagenesis and retroviral-mediated gene transfer. The serologic recognition of the Dw14 epitope, defined by mAb CCCL20, was highly discriminatory, focused on polymorphisms at residues 70 and 71 of the *DRB1*-encoded polypeptide, with a lesser contribution of residue 67. This serologic recognition was uninfluenced by polymorphisms in "contextual" residues that differ between the class II background associated with *DR1*, *DRw6*, *DR5m67/70*, or *DR4*, each of which carried the Dw14 epitope. T-cell reactivity of the Dw14 epitope was evaluated in this study by the reactivity patterns of two allospecific clones, 14B and EMM025. T-cell recognition of the Dw14 epitope was also highly dependent on residues 70 and 71. However, subtle differences among the clones and antibodies used in this study were observed that indicate that different structural contexts associated with the Dw14 epitope contribute to potentially important differences in recognition. For example, T-cell clone 14B, in contrast to mAb CCCL20, did not react with the *DR5m67/70* transfectant. This appears to indicate that stimulation of clone 14B is dependent on additional residues associated with DR4(Dw14) not found in DR5 polypeptides and outside the serologically defined epitope. One likely interpretation of this finding is that alloreactive clone 14B may be dependent on specific peptide interactions with the class II molecule in association with the Dw14 epitope and that the DR5 "context" does not permit appropriate peptide conformation. Alternatively, additional polymorphic residues associated with DR5, such as at residue 58, may be contributing to T-cell, but not serologic, interaction sites. The pattern of reactivity with clone EMM025 presents an analogous situation, in which the Dw14 epitope is dependent on residues 70 and 71, but where T-cell stimulation is lacking on either a *DR5(m67/70) DR1* (WT100 LCL) or a *DRw6* (AMALA LCL) background context. As before, the failure of the *DRw6 (Dw16)* or *DR1 DRB1* allele to support stimulation of EMM025 could be due to either a failure of major histocompatibility complex–peptide interaction or to the contribution of additional polymorphic residues that distinguish *Dw16*, *DR1*, and *Dw14*.

The prominent role of residue 70 in these studies, its accessibility to mAb binding, and the radical nature of the allelic polymorphism at this position distinguishing *Dw14* and *Dw4* (Glu-70) from *Dw10* (Asp-70) indicate the importance of this site in immunologic recognition and in susceptibility to rheumatoid arthritis. The observation that discrete clusters of polymorphic sites within a class II sequence can function as homologous epitopes on different class II backgrounds provides direct experimental support for what has been termed the shared epitope hypothesis (11, 23). This hypothesis postulates that the functional units of class II interactions may be specific epitopes themselves rather than the entire allele. Different alleles with shared epitopes may, therefore, share important functional properties. Our experiments directly demonstrate the existence of shared epitopes and include the creation of an "artificial" shared epitope by mutagenesis on a *DR5* class II background. In an evolutionary time span, such shared epitopes appear to be "mobile epitopes" in that they have been apparently been shuffled among different *DRB1* alleles representing otherwise unrelated specificities, such as *HLA-DR4*, *DR1*, and *DRw6*.

The residues critical for Dw14 epitope function, codons 70 and 71 and to a lesser extent codon 67, are reminiscent of similar observations made in the murine model of a spontaneous class II mutation, the bm12 mouse. $H\text{-}2^{bm12}$ differs from the parental $H\text{-}2^{b}$ by three amino acid substitutions in the $A\beta$ gene corresponding to amino acids 67, 70, and 71 (24). These substitutions are apparently sufficient to trigger alloreactivity and also regulate a class II gene-controlled response to exogenous antigens, such as beef insulin and the acetylcholine receptor (24, 25). Due to the latter, the parental but not the bm12 mutant strain is susceptible to experimentally

induced myasthenia gravis (25). Site-directed mutagenesis of each of the three sites in the Aβ molecule has, like the present study, demonstrated loss of T-cell reactivity by alterations at each residue. This is consistent with the proposal that the important epitope encompasses multiple sites and not a single residue (26), although residue 70 may be immunodominant for allorecognition (27), analogous to our findings in DRβ. In other examples of murine and human class II structural studies, as well, it is clear that limited variation may have major functional consequences (28–30). For example, mutagenesis of the human DQβ chain at various polymorphic sites demonstrates multiple levels of interaction between polymorphic class II α and β chains, as well as with predicted peptide and T-cell recognition elements, in that single amino acid substitutions are sufficient in many cases to disrupt T-cell recognition but appear to operate by complex intermolecular interactions strongly influenced by both α and β chains (30). In the present study, since DRα is nonpolymorphic on all human haplotypes, the functional substitutions introduced by mutagenesis can be assumed to be due to β alterations alone, although, as noted above, the relative contributions of such substitutions to peptide interactions on the one hand and to T-cell receptor interactions on the other are not yet clear.

In the crystallographic structure of the HLA class I molecule A2, a discrete antigen (peptide) binding site can be visualized, bounded on two sides by long α-helical loops encoded by the α1 and α2 exons of the class I gene (13). Based on homologies with this structure, modeling of the HLA class II molecule predicts that residues 56–86 of the β chain will form one of these long α-helical loops (12). The specific orientation of the helix will dictate whether individual residues on the loop are positioned toward the putative peptide binding site, toward the putative T-cell receptor interaction sites, or elsewhere. In our studies, the successful "epitope shuffling" of the Dw14 recognition components by mutagenesis of residues clustered around codon 70 is consistent with the structural prediction that these residues form a discrete immunodominant site fairly independent of other background contextual residues. Since residues 70 and 71 were critical for serologic and cellular recognition of the epitope, the side chains at these positions in the *Dw14* sequence (Gln-70 and Arg-71) can be predicted to be oriented in a way that would permit such intermolecular interactions. Detailed understanding of the basis for these interactions should be accessible through the use of the site-specific transfectants described here in detailed peptide binding and T-cell receptor mutagenesis studies.

We thank Dr. Peter Gregersen for providing class II cDNA used in these studies and Nan Knitter-Jack and Phillip Thurtle for their excellent technical assistance. This work was supported by Grants AR37296 and AR39153 from the National Institutes of Health.

1. Nepom, G. T., Nepom, B. S., Antonelli, P., Mickelson, E., Silver, J., Goyert, S. M. & Hansen, J. A. (1983) *J. Exp. Med.* **159**, 394–404.
2. Gregersen, P., Shen, M., Song, Q., Merryman, P., Degar, S., Seki, T., Maccari, J., Goldberg, D., Murphy, H., Schwenzer, J., Wang, C., Winchester, R., Nepom, G. & Silver, J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2642–2646.
3. Lanchbury, J. S. S., Nepom, G. T., Radka, S. F., Dejelo, C., Marshall, W. H., Perrier, P., Henry, A.-M., Knitter-Jack, N., Nepom, B. S., Kamoun, M., Zmijewski, C. M., Braun, W. E., Knowles, R. W. & Monos, D. (1989) in *Immunobiology of HLA*, ed. Dupont, B. (Springer, New York), Vol. 1, pp. 388–392.
4. Stastny, P. (1978) *N. Engl. J. Med.* **298**, 869–871.
5. Nepom, G. T., Seyfried, C. E., Holbeck, S. L., Wilske, K. R. & Nepom, B. S. (1986) *Lancet* **ii**, 1002–1005.
6. Nepom, G. T., Byers, P., Seyfried, C., Healey, L. A., Wilske, K. R., Stage, D. & Nepom, B. S. (1989) *Arthritis Rheum.* **32**, 15–21.
7. Winchester, R. J. (1986) *Clin. Aspects Autoimmun.* **1**, 9–26.
8. Nepom, G. T., Hansen, J. A. & Nepom, B. (1986) *J. Clin. Immunol.* **7**, 1–7.
9. Seyfried, C. E., Mickelson, E., Hansen, J. A. & Nepom, G. T. (1988) *Hum. Immunol.* **21**, 289–299.
10. Weyand, C. M. & Goronzy, J. J. (1989) *Hum. Immunol.* **24**, 133–143.
11. Gregersen, P. K., Silver, J. & Winchester, R. J. (1987) *Arthritis Rheum.* **30**, 1205–1213.
12. Brown, J. H., Jardetzky, T., Saper, M. A., Samraoui, B., Bjorkman, P. J. & Wiley, D. C. (1988) *Nature (London)* **332**, 845–850.
13. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 506–512.
14. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329**, 512–518.
15. Dejelo, C. L., Braun, W. E., Zachary, A. A., Teresi, G. A., Smerglia, A. R. & Clark, L. V. (1986) *Hum. Immunol.* **17**, 135–136.
16. Alber, C. A., Watts, R., Klone, E. P., Drover, S., Marshall, W. H., Radka, S. F. & Karr, R. W. (1989) *J. Immunol.* **143**, 2248–2255.
17. Radka, S. F., Machamer, C., Cresswell, P., Kostyu, D. D., Ward, F. E. & Amos, D. B. (1983) *J. Immunol.* **130**, 1863–1866.
18. Yang, S. Y., Milford, E., Hammerling, U. & Dupont, B. (1989) in *Immunobiology of HLA*, ed. Dupont, B. (Springer, New York), Vol. 1, pp. 11–19.
19. Kwok, W. W., Schwarz, D., Nepom, B., Thurtle, P., Hock, R. & Nepom, G. T. (1988) *J. Immunol.* **141**, 3123.
20. Burke, D. T. & Olson, M. V. (1986) *DNA* **5**, 325–332.
21. Gregersen, P. K., Todd, J. A., Erlich, H. A., Long, E., Servenius, B., Choi, E., Kao, H. T. & Lee, J. S. (1989) in *Immunobiology of HLA*, ed. Dupont, B. (Springer, New York), Vol. 1, pp. 1027–1031.
22. Nepom, B., Nepom, G. T., Schaller, J., Mickelson, E., Antonelli, P. & Hansen, J. A. (1984) *J. Clin. Invest.* **74**, 287–291.
23. Silver, J. & Goyert, S. M. (1985) in *Human Class II Histocompatibility Antigens*, eds. Moller, E., Solheim, B. & Ferrone, S. (Springer, Berlin), pp. 32–48.
24. Mengle-Gaw, L., Conner, S., McDevitt, H. O. & Fathman, C. G. (1984) *J. Exp. Med.* **160**, 1184–1194.
25. Hansen, T. H. & Tse, H. Y. (1987) *Crit. Rev. Immunol.* **7**, 169–229.
26. Ronchese, F., Brown, M. A. & Germain, R. N. (1987) *J. Immunol.* **139**, 629.
27. Bill, J., Ronchese, F., Germain, R. & Palmer, E. (1989) *J. Exp. Med.* **170**, 739–750.
28. Griffith, I. J., Choi, E. M. & Glimcher, L. H. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1090–1093.
29. Buerstedde, J.-M., Nilson, A. E., Chase, C. G., Bell, M. P., Beck, B. N., Pease, L. R. & McKean, D. J. (1989) *J. Exp. Med.* **169**, 1645–1654.
30. Kwok, W. W., Mickelson, E., Masewicz, S., Milner, E., Hansen, J. & Nepom, G. T. (1990) *J. Exp. Med.* **171**, 85–95.