Arginine at Positions 13 or 70-71 in Pocket 4 of HLA-DRB1 Alleles Is Associated with Susceptibility to Tuberculoid Leprosy

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Summary

Evaluation of human histocompatibility leukocyte antigen (HLA) class II genes in 54 cases of tuberculoid leprosy (TL) and 44 controls has shown a positive association with HLA-DRB1 alleles that contain Arg^{13} or Arg^{70}-Arg^{71}. Among TL patients, 87% carry specific alleles of DRB1 Arg^{13} or Arg^{70}-Arg^{71} as compared to 43% among controls ($p = 5 \times 10^{-6}$) conferring a relative risk of 8.8. Thus, susceptibility to TL involves three critical amino acid positions of the β chain, the side chains of which, when modeled on the DR1 crystal structure, line a pocket (pocket 4) accommodating the side chain of a bound peptide. This study suggests that disease susceptibility may be determined by the independent contribution of polymorphic residues participating in the formation of a functional arrangement (i.e., pocket) within the binding cleft of an HLA molecule.

Leprosy is a chronic infectious disease caused by a relatively nontoxic microorganism, *Mycobacterium leprae*. In the natural history of *M. leprae* infection, >95% of individuals resist disease by establishing protective immunity and only the minority develops leprosy (1). Nevertheless, leprosy is a major world health problem affecting an estimated 10.6 million patients in the world of which two thirds (62%) are found in Southeast Asia and 34% in Africa (2). Once leprosy becomes established, there is a wide spectrum of clinical manifestations. Tuberculoid leprosy (TL)[1] and lepromatous leprosy (LL) are two opposite poles in the spectrum, representing stable disease forms with distinct immunopathological and clinical findings. Borderline leprosy is an intermediate, unstable form of the disease with clinical and pathological manifestations between the two poles (2).

TL is the localized, hyperactive form of leprosy. As in healthy, exposed individuals, the lepromin skin test is positive and there is a vigorous in vitro proliferation of PBL to mycobacterial antigens. However, in the skin lesions, very few if any acid-fast bacilli are present (3). At the other end of the spectrum, LL is the anergic, widespread form of leprosy. Macrophages loaded with acid-fast bacilli accumulate in the lesions, but the lepromin test is negative and PBL do not proliferate in vitro to *M. leprae* antigens (3). It appears that different immunopathogenic mechanisms operate in different disease forms. Since there is no association between disease forms and different *M. leprae* strains or exposure levels to *M. leprae*, the reaction of the host may determine the form of the disease that develops. Previous family and population studies have linked these host factors to HLA class II molecules.

In Asian-Indian families with leprosy, TL was associated with DR2 (4). This association has also been detected in sporadic TL patients from ethnically diverse populations, e.g., India, Japan. Thailand, and Korea (5–11), whereas an association with DR3 has been reported in two distinct populations from Venezuela and Surinam (12, 13). In regard to LL, although early reports suggested that the DR2 association involves only TL (5, 8, 12, 14), other subsequent reports do not confirm this finding (7, 9, 15–17). In all these studies, serological HLA typing was employed as the typing method, therefore, HLA differences at the mo-

[1] *Abbreviations used in this paper:* LL, lepromatous leprosy; rr, relative risk; TL, tuberculoid leprosy.

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lecular level between patients and controls could not be evaluated.

HLA molecules are receptor cell-surface glycoproteins that bind peptides and present them to the T cells (18, 19). This interaction causes stimulation of the T cell and activation of an immune response. There are class I and II HLA molecules with different domain organization but similar structure (20, 21). Polymorphic residues in both class I and II molecules are clustered within the peptide-binding region and are responsible for the different peptide specificities observed for different histocompatibility molecules. Both class I and II molecules have allele-specific binding motifs (22, 23). Peptides bound to class I are of defined length (8–10 residues); their motifs are characterized by a strong preference for a few side chains at some positions in the peptide and wide tolerance for many side chains at the other positions (24). Peptides bound to class II molecules are longer with no apparent restriction on peptide length (25, 26). The main chain atoms of the peptides form hydrogen bonds with HLA residues conserved in most class II alleles, and the side chains are accommodated by polymorphic pockets in the binding site. These pockets appear to determine the peptide specificity of different class II proteins (27). Polymorphic residues in the binding cleft of HLA class II molecules, therefore, control the binding of foreign peptides, and indirectly, the immune response to these peptides. The objective of this study was to use a DNA-based typing procedure to identify polymorphic residues within the binding cleft that may be associated with the disease, TL.

Materials and Methods

Populations Tested and Statistical Analysis. 54 unrelated patients with sporadic TL were randomly selected from major hospitals in Delhi. These patients were from the north Indian states of Punjab, Haryana, Uttar, Pradesh, Bihar, and Delhi. None of them had a history of familial leprosy. The patients were classified according to the Ridley and Jopling criteria (28) by clinical examination and a review of their clinical histories. Skin-slit smear bacteriology and histopathological examination of skin biopsy specimens were performed in all patients. Preliminary results on the typing of some of the patients have been previously published (29). 44 unrelated healthy individuals constituted the control group. None of them had a family history of leprosy. They were all matched with patients for socioeconomic status and were living in the same area as the patients.

The chi square test with Yates' correction was used to evaluate the differences of the allelic frequencies between various groups. To correct for the multiple comparisons carried out, the apparent significance level was multiplied by the number of statistical tests (30). Fisher's exact test was used to determine the statistical significance of the differences between the subtypes of DR6-positive TL patients and controls because of the relatively low number of subjects involved in those cases. For haplotypes showing a statistically significant difference in frequency between patients and controls, relative risk was calculated as: $rr = [Pd\ (1-Pc)]/[1-Pd)\ Pc]$, where Pd and Pc are the frequencies of individuals positive for the allele(s) among patients and controls, respectively.

DNA Extraction. A standard phenol-chloroform extraction method (31) was used for the isolation of high molecular weight genomic DNA from whole blood samples and from a reference panel of B lymphoblastoid cell lines (32) that constituted the positive and negative controls for the amplification and hybridization reactions.

PCR Amplification of Genomic DNA. Locus and allele-specific amplifications of genomic DNA with the PCR were performed for DRB1-, DRB3-, DRB4-, DRB5-, and DQB1-associated alleles. The primers used in the PCR and the amplification conditions have been described earlier (31). Cycling was performed with a thermal cycler (model 9600; Perkin-Elmer Cetus Corp., Norwalk, CT). Amplified samples were electrophoresed in a 1% agarose gel containing ethidium bromide and were visualized under UV light.

Dot Blot and Hybridization. Amplified DNA was blotted on Hybond N+ nylon transfer membranes (Amersham Corp., Arlington Heights, IL). The membranes were hybridized with the probes in a hybridization incubator (Robbins Scientific Corp., Sunnyvale, CA). A panel of sequence-specific oligonucleotide probes (SSOP), described earlier (31), was used with some modifications. The oligonucleotide probes were labeled with digoxigenin-11-dUTP (Boehringer Mannheim Corp., Indianapolis, IN) using terminal deoxynucleotidyl transferase (Boehringer Mannheim) according to the manufacturer's instructions. The labels were detected by sheep anti–digoxigenin antibody fragments (Fab) conjugated to alkaline phosphatase (Boehringer-Mannheim) diluted at 150 mU/ml. Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (all from Sigma Chemical Co., St. Louis, MO) were used as the chromogenic substrates.

Results and Discussion

54 unrelated sporadic cases of TL and 44 healthy controls from northern India were typed for HLA class II genes (DRB1, B3, B4, B5, and DQB1) by amplification of DNA with the PCR and hybridization with SSOP. Frequencies of the HLA-DRB1 alleles in the control population were similar to those reported for other Asian-Indian populations (5, 33, 34). The number of individuals positive for each of the different DRB1* alleles in the two populations of TL patients and controls is shown in Table 1. Noticeably, none of the alleles as defined by DNA-based HLA typing shows a statistically significant difference between control and TL patients.

However, upon careful evaluation, there are certain differences between the two populations that are very instructive. The comparable frequency of alleles 1501 and 1502 within TL and control groups (32 vs 37% in TL patients and 14 vs 18% in control subjects) suggests that none of these two alleles has an advantage over the other in terms of disease susceptibility. Therefore, the single amino acid difference [V(1501) → G(1502)] at codon 86 does not play a major role in disease susceptibility and allows these two alleles to be considered as a single entity (DRB1*15). However, as shown in Table 2, the number of TL patients positive for DRB1*15 (1501 + 1502) is significantly higher as compared to the number of control individuals positive for the same alleles (TL [37 of 54] 69%; control [14 of 44] 32%; uncorrected $p = 0.0007$ and corrected for nine tests

Table 1. *HLA-DRB1 Typing of TL Patients and Controls*

Alleles	Patients (n = 54) n	%	Controls (n = 44) n	%	Corrected p
DRB1*0101	3*	6	4	9	NS‡
DRB1*0102	0	—	1	—	
DRB1*1501	17	32	6	14	NS
DRB1*1502	20	37	8	18	NS
DRB1*1602	1	—	1	—	
DRB1*0301	6	11	12	27	NS
DRB1*0401	1	—	2	—	
DRB1*0403	2	—	3	7	NS
DRB1*0406	1	—	0	—	
DRB1*0409	0	—	1	—	
DRB1*1101	4	7	3	7	NS
DRB1*1103	0	—	1	—	
DRB1*1104	1	—	1	—	
DRB1*1106	0	—	1	—	
DRB1*1201	0	—	2	—	
DRB1*1202	2	—	1	—	
DRB1*1301	4	7	8	18	NS
DRB1*1302	1	—	0	—	
DRB1*1401	1	—	0	—	
DRB1*1402	0	—	1	—	
DRB1*1403	0	—	1	—	
DRB1*1404	12	22	4	9	NS
DRB1*0701	9	17	15	34	NS
DRB1*0803	0	—	2	—	
DRB1*0901	2	—	1	—	
DRB1*1001	9	17	3	7	NS

*Number of individuals positive for a particular DRB1 allele.
‡Statistical analysis was performed in 10 out of 26 DRB1 alleles. The remaining 16 were not tested for statistical differences because two or fewer instances were observed in both control and TL patients.

Table 2. *Association of DRB1 Alleles with TL*

Alleles	Patients		Controls		rr	Corrected p
	n = 54	%	n = 44	%		
DRB1*15	37	69	14	32	4.7	0.0063*
DR6 positives	n = 16	%	n = 12	%		
DRB1*1301	4	25	8	67	0.17	0.033‡
DRB1*1404	12	75	4	33	6	0.033
DR2 negatives	n = 15	%	n = 27	%		
DRB1*1404	6	40	1	4	7.1	0.034§

*Statistical analysis was performed in 10 out of 26 DRB1 alleles. The remaining 16 were not tested for statistical differences because two or fewer instances were observed in both control and TL patients. The p value correction factor is therefore 9 (DRB1*1501 and DRB1*1502 were combined into one allele).
‡Fisher's exact test was used to determine statistical significance in this group.
§Statistical analysis was performed in 7 out of 18 alleles present in the DR2-negative individuals. The remaining 11 were not tested for statistical differences because two or fewer instances were observed in both control and TL patients. The p value correction factor is therefore 7.

p = 0.0063, rr = 4.7). This suggests the presence of epitope(s) on the DRB1*15 sequence that is important for TL susceptibility. Additionally, among DR6-positive individuals (DR6 includes DRB1*13 and DRB1*14 alleles), only two alleles occur with reasonable frequencies in our population, namely DRB1*1301 and DRB1*1404 (Table 2). It was observed that the frequencies of these two alleles in the TL patients and controls were characterized by an inverse relationship. Among TL patients (Table 2) there was an increased number of cases positive for DRB1*1404 (12 of 16, 75%) as compared to controls (4 of 12, 33%; p = 0.034) and a decreased number of TL cases positive for DRB1*1301 (4 of 16, 25%) as compared to controls (8 of 12, 67%; p = 0.034). DRB1*1404 was also increased among DR2-negative (DR2 includes DRB1*15 and DRB1*16 alleles) TL patients (6 of 15, 40%) as compared to DR2-negative controls (1 of 27, 4%); uncorrected p = 0.0048, and corrected for seven tests p = 0.034 with rr = 7.1 (Table 2). Since alleles DRB1*1301 and DRB1*1404 are splits of the serologic specificity DR6, this inverse relationship of the two subtypes may very well explain why, in previous serological studies, the importance of the DR6 specificity went undetected.

The identification of the DRB1*15 alleles that were positively associated with TL, the significantly increased numbers of TL patients positive for DRB1*1404 among DRB1*15 negative cases, and the inverse relationship of DRB1*1301 and DRB1*1404 alleles among DR6-positive control and TL individuals focused our attention on the amino acid residues that were different between the negatively and positively associated allelic forms. We reasoned that the most important amino acid positions for disease susceptibility would be those that differed in the negatively as compared to the positively associated alleles. These amino acid residues were found to be located at positions β^{13}, β^{37}, β^{70}, and β^{71} (Fig. 1). The two positively associated alleles DRB1*15 and DRB1*1404 were characterized by arginines at positions β^{13} and $\beta^{70/71}$, respectively. Additionally, it was noticed that the DRB1*1301 allele is characterized by S^{13}, D^{70}, and E^{71} which represent a negative charge, whereas DRB1*1404 is characterized by G^{13}, R^{70}, and R^{71}, which represent a positive charge. Disease association was therefore reexamined using the presence of arginine at these three positions and the absence of negatively charged amino acids in any of the same positions as criteria, regardless of allelic typing. Those alleles include DRB1*1501 (R^{13}, Q^{70}, A^{71}), DRB1*1502 (R^{13}, Q^{70}, A^{71}), DRB1*1401

	9	10	11	12	13	16	32	37	47	57	60	67	70	71	74	
DRB1*1301	E	Y	S	T	S	H	H	N	Y	D	Y	I	D	E	A	Negative Association among DR6 positive individuals.
DRB1*1404	-	-	-	-	G	Y	-	F	H	A	H	L	R	R	E	Positive Association among DR6 positive individuals.
DRB1*1501 or 1502	W	Q	P	K	R	-	Y	S	-	-	-	-	Q	A	-	Positive Association

Figure 1. Residues that differ among DR alleles that are positively and negatively associated with TL. Amino acid residues are indicated that are different between DRB1*1301 and DRB1*1404 or between DRB1*1301 and DRB1*15 allele. Residues at positions 13, 37, 70, and 71 were different in both comparisons. DRB1*1301 ($S^{13}N^{37}D^{70}E^{71}$) → DRB1*1404 ($G^{13}F^{37}R^{70}R^{71}$) or → DRB1*15 ($R^{13}S^{37}Q^{70}A^{71}$).

(S^{13}, R^{70}, R^{71}), DRB1*1404 (G^{13}, R^{70}, R^{71}), DRB1*0901 (F^{13}, R^{70}, R^{71}), and DRB1*1001 (F^{13}, R^{70}, R^{71}). As shown in table 3, the phenotype Arg^{13} positive or $Arg^{70/71}$ positive was overrepresented among patients as compared to controls (TL patients [47 of 54] 87%; controls [19 of 44] 43%; $p = 5 \times 10^{-6}$, rr 8.8). Characteristically, most of the patients were DRB1-Arg^{13} positive (37 of 54, 69%). Among those patients that lacked DRB1 alleles with Arg^{13}, there was a significant increase in DRB1 alleles that were positive for $Arg^{70/71}$ (TL patients [10 of 16] 63%; controls [5 of 29] 17%; $p = 0.003$).

When the criterion of association was expanded to include alleles with any positive charges at positions β^{13} or $\beta^{70/71}$, alleles DRB1*0401 (H^{13}, Q^{70}, K^{71}), DRB1*0403 (H^{13}, Q^{70}, R^{71}), DRB1*0406 (H^{13}, Q^{70}, R^{71}), and DRB1*0409 (H^{13}, Q^{70}, K^{71}) were also counted among those that could confer susceptibility. In addition to the DRB1*04 alleles, no others satisfied this criterion in our population. Statistical analysis showed that by including the DRB1*04 alleles within the group of alleles that confer susceptibility, the p value of "positive charge at positions β^{13} or $\beta^{70/71}$" (TL patients [49 of 54] 92%; controls [23 of 44] 52%; $p = 25 \times 10^{-6}$) did not show a better association as compared to the p value of "Arg^{13} or $Arg^{70/71}$" ($p = 5 \times 10^{-6}$) whereas rr was at a comparable level (8.9 vs 8.8) (Table 3). The frequency of all DRB1*04 alleles combined in the Asian-Indian population is between 7 and 10% (33, 34), which is comparable with that of DRB1*14 alleles. If these DRB1*04 alleles were positively associated with the disease there should have been an increased frequency, though not neccessarily statistically significant, of TL patients positive for DR4 as compared to controls. However, such an increased frequency was not observed (Table 1).

Table 3. *Association of DRB1 Shared Epitopes with TL*

Epitopes	Patients		Controls		rr	p
	$n = 54$	%	$n = 44$	%		
R^{13} or $R^{70/71}$	47	87	19	43	8.8	5×10^{-6}
R^{13}	37	69	14	32	4.7	0.0003
Positive charge at β^{13} or $\beta^{70/71}$	49	90	23	52	8.9	25×10^{-6}
R^{13} negatives	$n = 16$	%	$n = 29$	%		
$R^{70/71}$	10	63	5	17	7.1	0.003

Therefore, the positive charge at positions β^{13} or $\beta^{70/71}$ did not improve the association with the disease, and it is unlikely that the alleles DRB1*0401, DRB1*0403, DRB1*0406, and DRB1*0409 are among those that confer susceptibility.

The absence of statistically significant differences in the numbers of individual DRB1 alleles between TL patients and controls in contrast to the highly statistically significant difference for Arg^{13}, $Arg^{70/71}$ in the same population is very likely due to the relatively small size of the population sample analyzed and to the typing method employed (DNA-based typing), which substantially increases the number of detected alleles. However, irrespective of the absence of statistically significant allelic differences, the DNA-based typing has provided amino acid sequence information, hence, eliminating the need to establish allelic associations before a valid association can be established at the amino acid level. Therefore, identification of particular amino acid(s)/epitopes present in more than one allele and predisposing to disease susceptibility may become apparent by studying populations, whose size can be smaller, as compared to the large size of a population that would be required if allelic associations were to be established. Rare alleles such as DRB1*1401 and DRB1*0901 that are represented in 1–3% of this population would only significantly increase in a population of over 1,000 individuals. DRB1*1001, which is a more frequent allele (4–6%) in this population (33, 34), already shows the tendency of an association ($p = 0.18$) yet does not reach significant levels. No definitive statement, therefore, can be made concerning the association of these three alleles with TL. However, the presence of $Arg^{70/71}$ in their DRβ chains suggests that these alleles may play a role in disease susceptibility.

The amino acid substitution at position 37 was not found to be significantly different between patients and controls.

To examine whether other HLA genes within the MHC that are in linkage disequilibrium with HLA-DRB1 alleles influence the association of TL, the distribution of the allelic forms of DRB3, DRB4, DRB5, and DQB1 genes was evaluated. No significant differences were found in the frequencies of these alleles between TL patients and controls (data not shown). Furthermore, no linkage disequilibrium was identified between Arg at position 13 or 70/71 of the DRB1 gene and other DRB or DQB genes.

It is noteworthy that from the crystal structure of a HLA-DR molecule the side chains of positions 13, 70, and 71 are in close proximity (19, 20), and that charge differences in the local environment may affect the binding of

peptides to the cleft or their recognition by TCR (Fig. 2 *A*). Reminiscent of this observation is the finding that in HLA-DR1, the side chains of residues 13, 70, and 71 line a pocket, pocket 4 (Fig. 2 *B*) that accommodates a Gln side chain from the hemagglutinin peptide (27). The exact crystallographic structure of other HLA-DR alleles with an Arg at positions 13 or 70 and 71 is not known. It is very likely, however, that the side chains of these amino acids will also contribute to the formation of this pocket.

Our finding suggests that the presence of Arg at positions 13 or 70/71 in pocket 4 of the DRB1 alleles confers susceptibility to TL. We propose that the presence of a positive charge generated by Arg at positions 13 primarily or 70 and 71 secondarily may influence a critical site within the binding groove of the DR chain that affects peptide binding and/or T cell interactions in the immune response associated with TL. In support of this hypothesis are a number of in vitro studies performed independently by other investigators (36–38) wherein positions 13, 70, and 71 were shown to be directly involved in antigen-specific T cell responses. These studies have examined the amino acid residues at various positions of the DRβ chain that are important for antigen-specific T cell recognition. By employing L cell transfectants expressing a single DR allele and site-directed mutagenesis it has been determined that among amino acid residues 9, 11, 13, 28, 30, and 37 that are localized to the floor of the binding groove, substitutions at position 13 more frequently eliminated T cell recognition than substitutions at any of the other five positions (36–38). Additionally, substitutions in the α-helix at positions 70, 71, and 74 could drastically affect T cell responses (36–38).

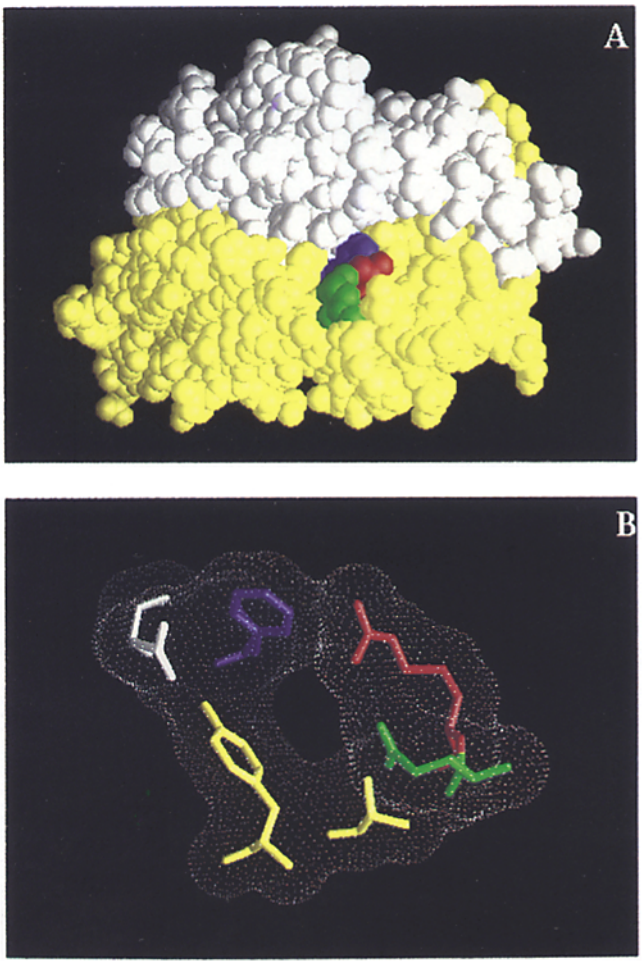

Figure 2. Presentation of amino acids forming pocket 4 of the DR1 crystal structure. (*A*) Crystal structure is shown as spherical model where α chain is shown in white and β chain in yellow. The pictures are generated using GRASP (35). The three residues β^{13} Phe (*blue*), β^{70} Gln (*green*), and β^{71} Arg (*red*) are shown on the structure. (*B*) Pocket formation is shown as dotted surface. The walls of the pocket are formed by residues β^{13} Phe (*blue*), β^{70} Gln (*green*), β^{71} Arg (*red*), β^{74} Ala (*yellow*), β^{78} Tyr (*yellow*), and α^{9} Gln (*white*). These residues are within 12 Å^2 distances.

Furthermore, studies on the association of MHC class II molecules with chronic autoimmune disorders have shown that amino acids at positions 67, 70, 71, and 74 of HLA-DRB1 are associated with susceptibility to rheumatoid arthritis. In rheumatoid arthritis, the DRB1 alleles associated with the disease involve amino acid substitutions at positions 70, 71, and 74 that change the local charge of the region affected by these amino acids to a more positive one (39, 40). Recently, characterization of peptides bound to the DRB1*04 alleles which are positively (DRB1*0404) and negatively (DRB1*0402) associated with rheumatoid arthritis revealed that both alleles have identical requirements at the anchor residue p1 and at p6 but a substantially different requirement at position p4 (41). DRB1*0404 binds peptides with a negative charge at p4 whereas the reverse is true for DRB1*0402 which binds peptides with a positive charge at the same position. Since these two HLA alleles are different in positions 67, 70, and 71 and positions 70 and 71 participate in the formation of pocket 4, these findings emphasize the importance of the structural characteristics of these HLA alleles at this region and strongly suggest that pocket 4 of these two HLA molecules determines the kind of peptides that will be accommodated.

Another illustration of the importance of this region is the reported association of chronic beryllium disease with certain DPB1 alleles. A change at position 69 of the DPB1 gene from a lysine to a glutamic acid has been reported to be associated with chronic beryllium disease, a lung disorder related to beryllium exposure, and characterized by the accumulation in the lung of beryllium-specific $CD4^+$ MHC class II–restricted T lymphocytes (42). It is noteworthy that position 69 of the DPβ chain corresponds to position 71 of the DRβ chain and that the amino acids involved cause a major charge shift from positive (R) to negative (E).

The importance of a similar region in class I molecules has been previously indicated by other studies (43) that involve virus-specific, K^b-restricted cytotoxic effector cells responding to K^b mutants. The bm 1 mutant with altered amino acids at positions 152, 155, and 156 is the only one that affects the virus-specific cytotoxic effector cells so that characteristically, all clones lose their reactivity (43). It is interesting that none of the other bm mutants affects the cytotoxic cells so uniformly. These positions in the class I

molecule correspond to positions 66, 69, and 70 of the DRβ chain. Thus, both class I and II molecules appear to influence T cell recognition by changes in amino acids located in equivalent portions of the HLA molecule (class I-α^{156}/class II-β^{70}).

These independently reported observations using in vitro functional analysis of T cell clones and HLA disease association studies are, therefore, consistent with our conclusions that specific positions on the HLA β chains define a critical site that appears to play a more pronounced role in T cell recognition and activation. This effect may be the result of more than one amino acid substitution. A particular site within the groove may be influenced by several amino acid positions. Indeed, the formation of pockets as illustrated in the crystal of DR1-influenza peptide suggests that the binding of a foreign peptide is influenced by a number of side chains lining a particular pocket on the HLA molecule. Characteristically, pocket 4 is formed by the side chains of amino acids α^9 β^{13}, β^{70}, β^{71}, β^{74}, and β^{78}. Substitutions on any of these positions that would affect the local charge such as Arg at position 13 or Arg at positions 70 and 71 may both have the same effect as they will probably accommodate the binding of a negatively charged residue of the foreign peptide. This may indeed be the case as it has been reported that HLA-DRw17 (DRB1*0301), which has arginine instead of alanine at position β74, prefers aspartate or glutamate at the position that corresponds to hemagglutinin glutamine 311 (44, 45). The presence of Arg at 74 may also explain the reported association of TL with DR3 in two ethnic groups (12, 13). When these polymorphic positions that form the pocket involve substitutions that result in potent charge modifications, the binding of a peptide or of a TCR may be affected, provided the introduced modifications result in a net gaining or losing charge. However the apparent lack of association with DRB1*04 alleles, which have a histidine at β^{13}, suggests that the mere presence of a positive charge in this region may not be adequate and that arginine is instrumental in terms of selecting the appropriate peptide for binding.

In our study involving TL patients, the association with HLA-DR alleles including DR2 was confirmed. Furthermore, it appears that the associations of DRβ Arg^{13} or $Arg^{70/71}$ with TL may be specific for this form of the disease. A recent study examining TL and control individuals from the same geographical area as our population (46) by HLA oligotyping, reported the increased frequency of DRB1*15 alleles but did not detect the inverse relationship of DRB1*1301 and DRB1*1404 within the DR6-positive individuals. This may be related to the rather small number of cases (28 TL patients) who were examined.

In TL, it is likely that peptide(s) originating from *M. leprae* binds preferentially to HLA allelic forms characterized by Arg at positions 13 or 70-71 and stimulates particular T cell clones that result in a detrimental immune response of TL. Identification of peptide motifs that bind the different allelic forms associated with the disease would contribute significantly to the search for *M. leprae* antigenic determinants that initiate this response, and possibly for self-protein and peptides that become the targets. Our findings suggest that the disease-associated alleles will be characterized by a binding motif that includes a negatively charged amino acid at position 4 (numbering is based on the hemagglutinin peptide that binds DR1 [27]).

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