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Genetic and serological heterogeneity of the supertypic HLA-B locus specificities Bw4 and Bw6

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Abstract. Gene cloning and sequencing of the *HLA-B* locus split antigens B38 (B16.1) and B39 (B16.2) allowed localization of their subtypic as well as their public specificities HLA-Bw4 or -Bw6 to the α-helical region of the $\alpha 1$ domain flanked by the amino acid positions 74–83. Comparison of their amino acid sequences with those of other *HLA-B*-locus alleles established HLA-Bw6 to be distinguished by Ser at residue 77 and Asn at residue 80. In contrast, HLA-Bw4 is characterized by at least seven different patterns of amino acid exchanges at positions 77 and 80–83. Reactivity patterns of Bw4- or Bw6-specific monoclonal antibodies reveal two alloantigenic epitopes contributing to the HLA-Bw4 or -Bw6 specificity residing next to the region of highest diversity of the $\alpha 1$ domain.

Introduction

HLA class I antigens are polymorphic membrane glycoproteins with ubiquitous distribution on almost all nucleated cells. They bind peptides of processed antigens and present them to cytotoxic T lymphocytes (CTL; Germain 1986, Chen and Parham 1989). Initially, class I antigens controlled by the *HLA-A, B, C* loci were differentiated by the reactivity patterns mainly of pregnancy alloantisera with individual cells. Development and use of specific monoclonal antibodies, as well as T-cell responses and biochemical analyses, revealed extensive structural heterogeneity of these molecules. HLA-A, B, C antigens were thus shown to express a variety of different antigenic determinants. Like the antigens of the *H-2* system in the mouse (summarized in Klein 1986), some of the HLA antigens are unique and define particular *HLA* alleles as so-called private specificities, whereas others are termed supertypic or public specificities because they are shared by several allelic products of a single locus or even by molecules encoded by different *HLA* loci. The earliest known supertypic HLA determinants were the HLA-Bw4 and -Bw6 specificities (van Rood 1962). Either of them is expressed together with a particular private specificity on a given HLA-B molecule (Ayres and Cresswell 1976). While the HLA-Bw4 determinant is found in addition on the *HLA-A*-locus antigens A24, A23, and A32 (Kostyu et al. 1980), the HLA-Bw6 public specificity is also present on the product of the *HLA-C*-locus allele *Cw3* (Layet et al. 1985). Molecular variants of the Bw4 specificity have been reported based on the serological reactivity patterns of different Bw4-specific monoclonal antibodies (Müller et al. 1985a, Müller et al. 1982). Recent cloning and sequencing of a number of different *HLA* alleles (Parham et al. 1988), as well as determination of the X-ray crystallographic structure of the HLA-A2 antigen (Bjorkman et al. 1987), has facilitated localization of various alloantigenic epitopes detected by monoclonal antibodies or recognized by CTL. Comparison of the primary structure of HLA-B and -A molecules (Wan et al. 1986), as well as exon shuffling experiments with *HLA-B7* and *-B27* genes (Toubert et al. 1988), recently suggested that the Bw4 and Bw6 specificities map to the first external domain, in particular to the amino acid residues 79–83. To characterize further genetic and structural heterogeneity of Bw4 and Bw6, we have exploited their association with the HLA-B antigens B38 and B39 which as subtypes of the HLA-B16 antigen are highly cross-reactive, but differ in the expression of these supertypic specificities.

Materials and methods

Cells. Peripheral blood leucocytes (PBL) were obtained from healthy donors after Ficoll hypaque density gradient centrifugation. HLA serotyping was performed by routine cytotoxicity assays using local standardized antisera, as well as antibodies of the Tenth International Histocompatibility Workshop. PBL of donor S with the HLA phenotype

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A2, A33, B44, B39, Cw5, Bw4, Bw6 and of donor Z with the HLA phenotype A26, Bw64, B38, Cw8, Bw4, Bw6 were selected for cloning of the *B38* and *B39* genes. All Epstein-Barr virus transformed B-cell lines were obtained from the Tenth International Histocompatibility Workshop cell-line panel with well-characterized HLA phenotype and genotype, and grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). L(tk$^-$) cells, kindly provided by W. Summer (Yale University, New Haven, Connecticut), were maintained in Dulbecco's modified Eagle's medium (DMEM, Boehringer Mannheim, Mannheim, FRG) supplemented with 10% FCS.

Monoclonal antibodies. The monoclonal antibodies (mAbs) W6/32.HL (anti-HLA-ABCE heavy chain) and its inactive variant W6/32.HK were obtained from A. Ziegler (Institute of Immunology, Marburg, FRG; Barnstable et al. 1978). The antibody 4E (Yang et al. 1984) was kindly provided by S. Y. Yang (Sloan Kettering Institute, New York, USA). The Bw4-specific antibodies TÜ109 (Müller et al. 1985a) and TÜ48 (Müller et al. 1982) as well as the A2- and A28-specific mAb TÜ101 (Müller et al. 1983) from our laboratory have been described previously. The 2BC4 (Kandzia et al. 1981) and the SFR8-B6 antibodies (Radka et al. 1982) directed against HLA-Bw6 were generous gifts of E. Westphal (Institut für Hygiene, Kiel, FRG) and S. Radka (Duke University, Durham, USA), respectively. Further BW4- or BW6-specific monoclonal reagents, as well as A2- or A26-specific mAbs, were obtained during the Tenth International Histocompatibility Workshop.

Probes. HLA-B genes were isolated by using *HLA-A* and *-B* locus-specific probes derived from the *HLA-B7* cDNA and the *HLA-A2* gene as described by Koller and co-workers (Koller et al. 1984). The *HLA-B27* (Weiss et al. 1985) and *-B51* genes (Pohla et al. 1989) have been described previously. The *HLA-Cw3* gene (Sodoyer et al. 1984) was a gift of B. R. Jordan (CNRS, Marseille, France). The *HLA-A2* and *-B7* genes (Barbosa et al. 1982), as well as the *HLA-B7* cDNA probe (Sood et al. 1981), were kindly provided by S. Weissman (Yale University, New Haven, Connecticut).

DNA analysis. DNA extraction, restriction endonuclease mapping, and Southern blot hybridizations using specific ^{32}P multiprimed labeled probes under highly stringent conditions were carried out according to the procedures described earlier (Williams et al. 1987, Feinberg and Vogelstein 1983).

Isolation of genomic clones. Genomic DNA extracted from lymphocytes of donor S and Z was completely digested with *Eco* RI and electrophoresed on a preparative 0.7% agarose gel. DNA fragments (6 kbp long) eluted from the gels were cloned in the *Eco* RI site of lambda gt11 (Promega, Madison, Wisconsin) as described previously (Williams et al. 1987). The two independent partial genomic libraries were screened by hybridization with *HLA-B7* cDNA and *HLA-A* or *-B* locus-specific probes under highly stringent conditions. Positive clones hybridizing with *HLA-B7* cDNA and the *HLA-B* locus-specific probe were plaque purified.

Transfection of cloned genes. Cloned *HLA* genes were cotransfected with the thymidine kinase gene into mouse L(tk$^-$) cells by the calcium phosphate precipitation method as described earlier (Wigler et al. 1979). As controls, L(tk$^-$) cells were transfected with the thymidine kinase gene only. After selection in HAT medium (DMEM supplemented with 15 μg/ml hypoxanthine, 1 μg/ml aminopterin, and 5 μg/ml thymidine), colonies were picked and grown in mass culture.

Immunofluorescence analysis. Indirect immunofluorescence labeling of PBL and transfectants was performed with the use of poly- and monomorphic monoclonal HLA-specific antibodies and evaluated on fluorescence-activated cell sorter (FACS) IV as previously described (Müller et al. 1985b).

Radioimmunoassay. Surface HLA expression of transfectants was analyzed by a quantitative radioimmunoassay using the monomorphic monoclonal antibodies W6/32.HL and 4E, as well as the polymorphic monoclonal antibodies TÜ109 and 2BC4 as reported earlier (Schmidt et al. 1987).

Isoelectric focusing (IEF) analysis. Isoelectric focusing was carried out on HLA class I products immunoprecipitated with the monoclonal antibodies W6/32.HL or 4E from ^{35}S-labeled transfectants and PBL of donor S and Z according to the procedure described previously (Yang et al. 1984, Yang 1989). PBL were stimulated with phytohemagglutinin overnight, transfectants with mouse interferon for 24 h before labeling.

DNA sequencing. After restriction endonuclease mapping of recombinant clones, subcloning of appropriate fragments up to about 500 bp was performed into pUC13. Supercoil DNA sequencing according to Chen and Seeburg (1985) was carried out by the dideoxy chain termination method (Sanger et al. 1977) using Sequenase ™ (United States Biogenical Corporation, Fa. Renner, Dannstatt, FRG) or *Taq* polymerase (Stratagene, Heidelberg, FRG).

Results

Cloning of HLA-B38 and -B39 genes. HLA-B38(Bw4) and *HLA-Bw39(Bw6)* genes were obtained from partial genomic libraries of two German Caucasian individuals who were serologically typed heterozygous for the supertypic determinants Bw4/Bw6. IEF analysis of immunoprecipitated HLA class I antigens and comparison with previously established band patterns of HLA-A, -B, -C allelic products (Yang 1989) revealed that donor S expressed the *HLA-B*-locus subtypes *Bw44.2(Bw4) B39(B16.2; Bw6)*, whereas donor Z's genotype was *Bw64(Bw6)*, *B38(B16.1; Bw4;* data not shown). On the basis of Southern blot hybridization of *Eco* RI-digested genomic DNA with the *HLA-B* locus-specific probe, it appeared that *HLA-B* genes were included in 6-kb fragments of both donors exclusively. From each of the two partial genomic libraries established from these fragments, four recombinant lgt11 clones which strongly hybridized with the *HLA-B* locus-specific probe were selected for transfection of L(tk-) cells. The transfected cells were shown in radioactive binding assays with poly- and monomorphic monoclonal antibodies to express Bw4- or Bw6-positive HLA-B products (Table 1). No reactivity was seen with the monoclonal antibodies TÜ101 [anti-A2, A28; (Müller et al. 1983)] or Tenth International Histocompatibility workshop 2066 and 2067 mAbs specific for A2 or A26, ruling out the possibility that these clones encoded A2 or A26 of donor's S or Z lymphocytes (data not shown). The identity of the *HLA-B38* and *-B39*, as well as of the *HLA-Bw64* genes, was further established by IEF analysis of the expressed, immunoprecipitated HLA class I molecules from the transfectants in comparison with the respective products from the donor's lymphocyts (data not shown). Weak binding of 4E and W6/32.HL, but not of TÜ109 or 2BC4, on L(tk$^-$) cells transfected with clone S1.1.a

Table 1. Identification of the cloned genes after expression in mouse L cells by an indirect radioactive binding assay with the mAb W6/32.HL (common *HLA-ABCE*), 4E (*HLA-B* and *C*), 2BC4 (*HLA-Bw6*), Tü109 (*HLA-Bw4*).

Transfected cell line	Donor	mAb Reactivity on transfected cells				Expressed *HLA* gene
		W6/32.HL	4E	2BC4	TÜ109	
Z1a a3	Z	7579*	16734	27659	253	*B14*
Z8b a2	Z	3500	4485	5260	134	*B14*
Z5a b2	Z	11637	11896	228	2426	*B38*
S13b a3	S	14144	15701	20258	290	*B39*
S14a b1	S	8725	7221	11297	148	*B39*
S1.1.a	S	483	638	423	268	*B44*??
S2.1.c	S	830	986	340	260	*B44*??
BII3		432	258	472	291	none

* cpm (average of three triplicates).
BII3 is a cell line transfected with the thymidine kinase gene of herpes simplex, but not with an *HLA* gene.

or S2.1.c of donor Z suggested that these clones encoded HLA-Bw44.2.

Sequences of B38 and B39 genes. The exons encoding the first three extracellular domains of the *B38* and *B39* genes were sequenced (Fig. 1). The leader peptide of both genes was identical to that of *B7* (Biro et al. 1983). *HLA-B38* was found to differ from *HLA-B39* in only seven nucleotide substitutions confined to exon 2 encoding the α1 domain. From an overall comparison of their translated protein sequences with other known HLA class I proteins (Fig. 2), *HLA-B39* shows identity with *HLA-B14* in the α1 domain, whereas *HLA-B38* differs from *HLA-B14* at six positions between the residues 74–83. Within this region, *HLA-B38* is completely identical to *HLA-B58* (Ways et al. 1985), *B-51* and *B-52* (Hayashi et al. 1989). Within exon 3, *HLA-B38* and *-B39* exhibit identical unique sequences at the codons 106, 107, 158, 167, and 168 in comparison with other known *HLA-A, -B, C* genes. At codons 113 and/or 114, *HLA-B38* and *-B39* share nucleotides with *HLA-B13* (Zemmour et al. 1988), *-B58* (Ways et al. 1985), *-B60, -B40** (Ways et al. 1987), *-B14, -B65, -B42, -B18* (Parham et al. 1988), *-B7.1*, and *B7.2* (Orr et al. 1979, López de Castro et al. 1979). The nucleotide sequence at codon 116 of *HLA-B38* and *-B39* is also identical to *HLA-B14* and *-B65* (Parham et al. 1988). At position 131, *HLA-B38* and *-B39* differ from the consensus sequence (Parham et al. 1988) in a further nucleotide exchange producing serine instead of arginine, as demonstrated already in another group of HLA-B molecules (Parham et al. 1988). In all other positions of exon 3, the cloned genes were identical to the consensus sequence shared by many *HLA-B* alleles (Parham et al. 1988).

Genetic polymorphism of the Bw4 and Bw6 specificities. The sequence differences between HLA-B38 and -B39 were related to protein data of other HLA class I molecules to define the genetic basis of the serological polymorphism of the Bw4 and Bw6 and of the HLA-B16-related subtypic specificities. The seven nucleotide exchanges between the Bw4-positive *HLA-B38* and the Bw6-positive *HLA-B39* genes lead to six amino acid substitutions between positions 74 and 83 (Figs. 1 and 2). At amino acid position 77, as well as at positions 80–83, HLA-B39 is identical to all other Bw6-positive HLA-B molecules, as well as HLA-Cw3 which also carries the

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Exon 2 (α1-domain)
                                                                     60
HLA-B38   GGCTCCCACT CCATGAGGTA TTTCTACACC TCCGTGTCCC GGCCCGGCCG CGGGGAGCCC
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    120
HLA-B38   CGCTTCATCT CAGTGGGCTA CGTGGACGAC ACGCAGTTCG TGAGGTTCGA CAGCGACGCC
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    180
HLA-B38   GCGAGTCCGA GAGAGGAGCC GCGGGCGCCG TGGATAGAGC AGGAGGGGCC GGAATATTGG
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    240
HLA-B38   GACCGGAACA CACAGATCTG CAAGACCAAC ACACAGACTT ACCGAGAGAA CCTGCGGATC
HLA-B39   ---------- ---------- ---------- ---------G ---------G --------A-

HLA-B38   GCGCTCCGCT ACTACAACCA GAGCGAGGCC
HLA-B39   CT--G-G--- ---------- ----------

Exon 3 (α2-domain)
                                                                     60
HLA-B38   GGGTCTCACA CCCTCCAGAG GATGTACGGC TGCGACGTGG GGCCGGAACG GCGCCTCCTC
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    120
HLA-B38   CGCGGGCATA ACCAGTTCGC CTACGACGGC AAGGATTACA TCGCCCTGAA CGAGGACCTG
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    180
HLA-B38   AGCTCCTGGA CCGCCGCGGA CACCGCGGCT CAGATCACCC AGCGCAAGTG GGAGGCGGCC
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    240
HLA-B38   CGTGTGGCGG AGCAGCTGAG AACCTACCTG GAGGGCACGT GCGTGGAGTG CGTCCGCAGA
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------

HLA-B38   TACCTGGAGA ACGGGAAGGA GACGCTGCAG CGCGCG
HLA-B39   ---------- ---------- ---------- ------

Exon 4 (α3-domain)
                                                                     60
HLA-B38   GACCCCCCAA AGACACATGT GACCCACCAC CCCATCTCTG ACCATGAGGC CACCCTGAGG
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    120
HLA-B38   TGCTGGGCCC TGGGCTTCTA CCCTGCGGAG ATCACACTGA CCTGGCAGCG GGATGGCGAG
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    180
HLA-B38   GACCAAACTC AGGACACCGA GCTTGTGGAG ACCAGACCAG CAGGAGACAG AACCTTCCAG
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------
                                                                    240
HLA-B38   AAGTGGGCAG CTGTGGTGGT GCCTTCTGGA GAAGAGCAGA GATACACATG CCATGTACAG
HLA-B39   ---------- ---------- ---------- ---------- ---------- ----------

HLA-B38   CATGAGGGGC TGCCGAAGCC CCTCACCCTG AGATGG
HLA-B39   ---------- ---------- ---------- ------
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Fig. 1. DNA sequence of the second and third exons of the *HLA-B38* and *HLA-B39* genes.

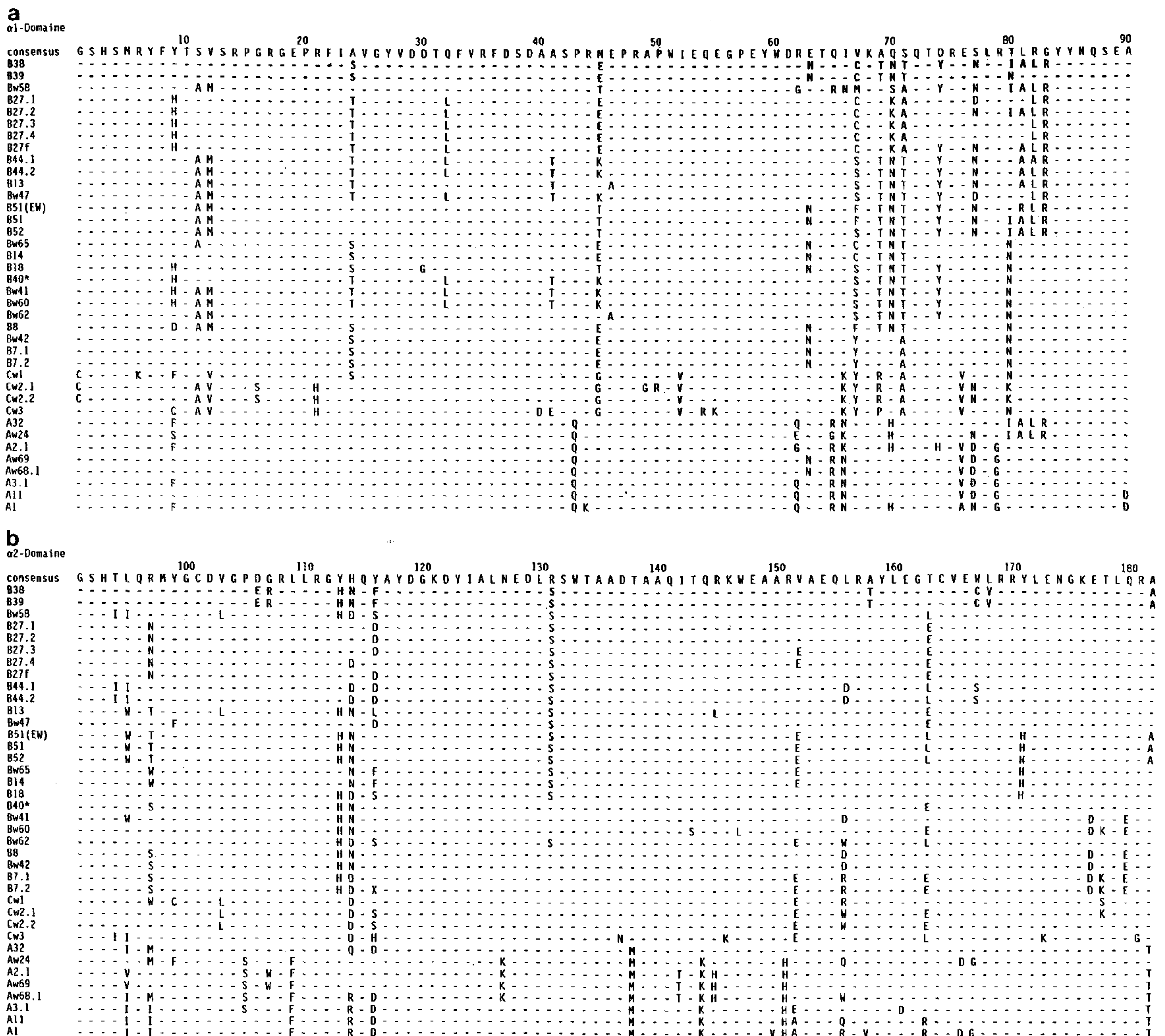

Fig. 2. Amino acid sequence comparison of HLA-B38 and HLA-B39 with other HLA-A, B, C molecules in the α1 and α2 domain. The *dashed* indicate identity with the consensus sequence derived from the sequences shown. The sequences were obtained from the following references: B27.1 (Weiss et al. 1985) and variants (Vega et al. 1986); B44.1 (Kottmann et al. 1986); B44.2 (Parham et al. 1988); B13 and B47 (Yang 1989); A1, Bw65, B14, B18, B41, B42 (Parham et al. 1988); Bw60, B40* (Ways et al. 1987); B8 (Parham et al. 1988); B7.1 and B7.2 (Cowan et al. 1987, López de Castro et al. 1979); Bw58 (Ways et al. 1985); A2.1 (Koller and Orr 1985); A24 (N'Guyen et al. 1985); A3.1 (Strachan et al. 1984); A11 (Cowan et al. 1987); A32 (Wan et al. 1986); Cw3 (Sodoyer et al. 1984); Cw1, Cw2 (Güssow et al. 1987); B51 and B52 (Hayashi et al. 1989); B51EW and Bw62 (Pohla et al. 1989); Aw69 and Aw68 (Holmes et al. 1987).

Bw6 determinant. The sequence LRG at positions 81–83 is also shared by all known HLA-A allelic products. In contrast, HLA-B38 is homologous to HLA-B58, -B51, and -B52 at amino acid position 77, as well as in the sequence IALR at positions 80–83. All other Bw4-positive HLA-B as well as -A allelic products are identical only in arginine at residue 83 and differ at positions 77 and 80–82. Thus, Bw4-positive HLA class I molecules may carry TLLR, TRLR, TALR, TAAR at positions 80–83 and asparagine, serine, or aspartatic acid at residue 77. Arginine at position 79 is shared by all HLA-B and -C molecules, but not by Bw4/Bw6-negative HLA-A molecules. The change of tyrosine in HLA-B38 to aspartic acid in HLA-B39 at position 74 is also found in other HLA-B or -C molecules independent of their expression of the Bw4 or Bw6 specificities and implies a charge difference of these molecules.

Serological polymorphism of the HLA-Bw4 or -Bw6 specificities. To correlate genetic with serological heterogeneity of the HLA-Bw4 and -Bw6 specificities, transfectants carrying defined *HLA* class I genes, as well

Table 2. Serological characterization of HLA-Bw4/Bw6 on transfectants and Tenth International Histocompatibility Workshop B-cell lines and correlation with amino acid sequence data of the $\alpha 1$ domain at residues 77 and 80–83.

Cells	HLA phenotype	Amino acid sequence		Bw4 and Bw6 correlated reactivity of different monoclonal antibodies			
		77	80–83	Anti-Bw4		Anti-Bw6	
				TÜ48	TÜ109	2BC4	SFR8.B6
Transfectants							
Z5	B38(Bw4)	N	IALR	++	+	–	–
HII3	B27.1(Bw4)	D	TLLR	++	+	–	–
III1	B51(EW)(Bw4)	N	TRLR	++	+	–	–
S13b	B39(Bw6)	S	NLRG	–	–	++	++
Z1a	Bw64(Bw6)	S	NLRG***	–	–	++	++
DII	B7(Bw6)	S	NLRG	–	–	++	++
GII3	Cw3(Bw6)	S	NLRG	–	–	–	++
CII3	A2	D	TLRG	–	–	–	–
B-cell lines							
WS 9050	B44.1**(Bw4)	N	TAAR***	+	–	–	–
WS 9001	A24.1(Bw4)	N	IALR***	++	–	–	–
WS 9035	A32(Bw4)	S	IALR***	++	–	–	–

Binding activity in an indirect immunofluorescence assay: ++, strong binding; +, weak binding; –, no reactivity; **, IEF defined variants; ***, presumed sequence data according to published gene sequences derived from other cell lines.

as B-cell lines with serologically and immunochemically well-characterized HLA class I antigens, were analyzed for their reactivity with various Bw4- and Bw6-specific monoclonal antibodies (Table 2). Comparison of available protein sequence data with binding patterns of monoclonal reagents revealed that the mAb TÜ48 (Müller et al. 1982) reacted with all tested cells expressing the HLA-Bw4 determinant. Sequence variability in positions 77 and 80–82 appeared to correlate with variability in binding activity of TÜ48. However, the mAb TÜ109 (Müller et al. 1985a) only recognized $Bw4^+$ HLA-B antigens except B44.1. This antibody did not react with HLA-A molecules in spite of shared amino acid sequences at positions 77 and 80–83. The Bw6-specific antibody 2BC4 (Kandzia et al. 1981) reacted only with the transfected cell lines expressing $Bw6^+$ HLA-B molecules, whereas the antibody SFR8-B6 (Radka et al. 1982) also recognized HLA-Cw3.

Discussion

Primary structure of the HLA-B split antigens B38 and B39 was determined to identify the amino acid substitutions responsible for the Bw4 and Bw6 specificities. HLA-B38 and -B39 are serologically difficult to distinguish by their private epitopes, but are clearly dissimilar in the Bw4 determinants. Previous comparison of HLA sequences (Parham et al. 1988, Wan et al. 1986) and exon shuffling experiments (Toubert et al. 1988) suggested positions 79–83 of the $\alpha 1$ domain to generate the Bw4 and Bw6 determinants. In this study, amino acid substitutions at positions 77, as well as at positions 80–83, are shown to contribute to the serologically defined Bw4 and Bw6 specificities and to correlate with reactivity patterns of monoclonal antibodies and alloantisera revealing heterogeneity and different epitopes of these antigens.

As has been shown for other serologically highly cross-reactive subtypes of HLA antigens, HLA-B38 and -B39 are very similar in their amino acid sequences, revealing only six amino acid differences within a limited region of high polymorphism in the $\alpha 1$ domain. When compared with other defined HLA molecules (Parham et al. 1988), B39 and B38 reveal unique sequences only in the $\alpha 2$ domain. According to the X-ray crystallographic structure of HLA-A2, the specific positions 106 and 107 are possibly situated on a loop outside the antigen binding site, and amino acid 167 at the $\alpha 2$-helical region is most likely involved in peptide binding. Positions 158 and 168 probably interact with the T-cell receptor (Bjorkman et al. 1987). The complete identity of exon 2 and shared substitutions of exon 3 indicate that *HLA-B39* and *-B14* could be derived from a common ancestor by a process involving intrallelic exchange events and point mutations, as has been suggested for other *HLA* alleles (Parham et al. 1988). No serological cross-reactivity between B14 and B39, with the exception for the Bw6 epitope, has been observed; thus, the corresponding allotypic reagents may preferentially be directed against epitopes located on the

α2 domain. Since *HLA-B38* shares nucleotides with *HLA-B58* (Ways et al. 1985), *-B51*, and *-B52* (Hayashi et al. 1989) at codons 74–83, it could have been generated in one step from *B39* by a single exchange event in which for example *HLA-B58, -B51*, or *-B52* donated another DNA segment encompassing codons 74–83. *HLA-B39* which shares the entire α1 domain with *HLA-B14*, could thus be the older of the two genes. Alternatively, *HLA-B38, -B39*, and *-B14* could have diverged simultaneously from a common ancestor by several steps of exchange events or point substitutions.

As the DNA segment flanked by codons 74 and 83 is the only region of the polymorphic α1 and α2 domains in which *HLA-B38* and *-B39* are different from each other, both subtypic epitopes, as well as the public determinants Bw4 or Bw6 on these molecules must be encoded within this area. The amino acid sequence SLRNLRG of HLA-B39 between residues 77 and 83 correlates completely with Bw6 of all other known HLA-B and -C antigens. According to the amino acid sequences, Bw6 is distinguishable from other HLA-A, -B, -C molecules only by the simultaneous presence of serine at position 77 and asparagine at position 80. In comparison, the HLA-Bw4 specificity could be related in HLA-B38 to the amino acid substitution asparagine at position 77 and the sequence IALR at residues 80–83. Similar substitutions, most likely generated by interlocus exchange processes, have already been found in the HLA-A molecules A32, and A24 carrying the Bw4 determinant (Wan et al. 1986), and were also shown to code for Bw4 in the HLA-B58 (Ways et al. 1985), -B51, -B52 (Hayashi et al. 1989), and -B27.2 (Vega et al. 1986) molecules. The same polymorphic Bw4-related substitutions have also been observed in chimpanzees' *B*-locus antigens (Mayer et al. 1988, Lawlor et al. 1988) and therefore may predate divergence of human and chimpanzees MHC products. In other HLA-B molecules, alternative sequences between residues 79 to 83 were previously claimed to also be associated with the Bw4 determinant (Wan et al. 1986). Except for B44.1, these HLA-B molecules share the amino acids leucine and arginine at residues 82 and 83 with HLA-B38, but differ from Bw4$^-$ HLA-A or Bw6$^+$ HLA-B antigens at positions 77 and 80–81. Thus, in contrast to HLA-Bw6, at least seven different patterns of the amino acid sequence between positions 77 and 83 could be related to HLA-Bw4. Rearrangement of small elements of sequences through gene conversion or intra-allelic exchange events, of which an example could be NLR found at positions 77–79 in Bw4 and at residues 80–82 in Bw6, in addition to point mutations, most likely have been involved in the generation of amino acid sequence variation associated with these public HLA-B specificities.

Variable amino acid sequences are likely to also account for the serological differences of the HLA-Bw4 and -Bw6 specificities (Müller et al. 1985a, Müller et al. 1982, Layet et al. 1985). The observed weak correlation coefficients of Bw4- versus Bw6-specific alloantisera and monoclonal antibodies (Arnaiz-Villena et al. 1989) appear to reflect the genetic variability of these public specificities. Reactivity patterns of monoclonal antibodies on transfectants or B-cell lines allow differentiation of two alloantigenic epitopes associated with Bw4 formed by the α helix of the α1 domain between residues 77 and 83. One epitope which is suggested to contact the T-cell receptor according to the X-ray crystallographic structure of HLA-A2 is shared by all Bw4$^+$ HLA-B antigens, as well as by the HLA-A molecules A24, and A32, and is recognized by the Bw4-specific antibody TÜ48. This epitope has also been defined by alloantisera (Kostyu et al. 1980). Binding of TÜ48 most likely involves interaction, particularly with the amino acids leucine and asparagine, at residues 82 and 83 which are shared by all Bw4$^+$ HLA-A and -B antigens. The other Bw4 epitope is only present on HLA-B molecules and is discriminated by antibodies like TÜ109. Reactivity of this antibody may rely on a particular protein conformation induced at the α-helical region by different amino acids at residues 77, 80, 81, and 82. This is suggested by selective binding of TÜ109 to Bw4$^+$ HLA-B, but not -A, molecules inspite of amino acid sequence variation, and could also explain the lack of reactivity with HLA-Bw44.1, exhibiting a specific Bw4-related amino acid sequence N, TAAR at these positions. According to the differential reactivity pattern of the antibodies SFR8-B6 and 2BC4 on transfectants, the Bw6 specificity could also include two epitopes. One is most likely recognized by the antibody SFR8-B6 directed against Bw6$^+$ HLA-B and -C molecules, whereas the other one may be differentiated by 2BC4 selectively binding to HLA-B antigens. SFR8-B6 appears to interact predominantly with amino acid residues 80–83, whereas 2BC4 seems to react particularly with amino acids at positions 80–77. Conformational changes of the 2BC4-defined epitope in HLA-Cw3 through amino acid substitutions in the immediate neighboring positions (i. e., exchange of valine in HLA-Cw3 for glutamic acid at position 76 in HLA-B molecules) could explain lack of reactivity of 2BC4 with HLA-Cw3. Thus, both public specificities HLA-Bw4 and -Bw6 appear to be characterized by one epitope distant from amino acid segment 62–76 with greatest sequence diversity of the α1 domain, as well as by another epitope next to this area. Reactivity of the mAbs TÜ109 and 2BC4 indicate that the three-dimensional structure of the latter epitope may be influenced by amino acid substitutions in the region of high diversity. The subtypic epitope of HLA-B38 and -B39, which can be differentiated from the Bw4 or Bw6 specificity by specific alloantisera, is most likely formed by the amino acid exchange at position 74. It seems to be spatially distinct from the public specificities Bw4 and Bw6 in these molecules, whereas in other HLA-B antigens

such as the B27 variants, overlapping of public and private epitopes, may occur, as is suggested by the reactivity pattern of specific mAbs like TM1 (Thurau et al. 1989). Therefore, recognition of this epitope by Bw4- or Bw6-specific antisera may depend on similar conformation, but not neccessarily on identity of the amino acid sequence.

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