REVIEW

Human leukocyte antigen Bw4 and Bw6 epitopes recognized by antibodies and natural killer cells

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Purpose of review

To describe the structural basis of human leukocyte antigen (HLA) Bw4 and Bw6 epitopes that are recognized by antibodies and the KIR3DL1 natural killer cell receptor.

Recent findings

Molecular modeling and X-ray crystallography have refined our understanding of Bw4 and Bw6. These epitopes had been defined by comparison of HLA allele sequences and by site-directed mutagenesis. Anti-Bw4 and anti-Bw6 antibodies and KIR3DL1 receptors recognize HLA α-1 α-helix residues 77–83 in combination with other HLA regions. The variability of HLA sequences within the 77–83 region and at other sites indicates that the Bw4 epitope is complex. Adding complexity, HLA-bound peptides influence Bw4 and Bw6 epitopes. These structures are recognized by diverse antibodies and KIR3DL1 allotypes. This diversity allowed a Bw4^{+} patient to produce anti-Bw4 antibody without breaking self-tolerance.

Summary

Bw4 and Bw6 epitopes are best regarded as families of related structures that are recognized by a diverse array of antibodies and KIR3DL1 allotypes.

Keywords

antibody, Bw4, Bw6, human leukocyte antigen epitope, KIR3DL1

INTRODUCTION

Antibody to human leukocyte antigen (HLA) molecules is a major barrier to transplantation. People are immunized by allogeneic HLA molecules during transplantation, blood transfusion, or pregnancy. The resulting anti-HLA antibodies can react with 'private' epitopes, which are shared by very few other HLA allele products, or 'public' epitopes, which are encoded by many HLA alleles. Perhaps the most important public epitopes are Bw4 and Bw6, which were first described by Van Rood and Van Leeuwen [1]. Either the Bw4 or the Bw6 epitope is expressed by virtually all HLA-B molecules; Bw4 also is found on a few HLA-A proteins. Consequently, high-level patient antibody either to Bw4 or to Bw6 will preclude transplantation with the majority of available donor kidneys, pancreases, hearts, and lungs. Despite acting like a biallelic system within the HLA-B locus, Bw4 and Bw6 epitopes and their antibodies are complex. Analysis of closely related HLA-B alleles that differentially expressed Bw4 and Bw6 suggested that these epitopes are determined by amino acid residues 77, 80–83 on the α-1 α-helix [2]. Bw4 epitope heterogeneity is shown by the presence of amino acids N, D, or possibly S at residue 77 and isoleucine-alanine-leucine-arginine, threonine-leucine-leucine-arginine (TLLR), or threonine-alanine-leucine-arginine at residues 80–83.

SITE-DIRECTED MUTAGENESIS CONFIRMS BW4 AND BW6 EPITOPE HETEROGENEITY

Using site-directed mutagenesis, the Bw6^{+} HLA-B*07:02 residue 80–83 asparagine-leucine-arginine-glycine sequence was replaced with the TLLR sequence found in Bw4^{+} HLA-B*27:05. The variant molecule bound all six anti-Bw4 mouse mAb and all three anti-Bw4 human antibodies tested [3]. The same mutation destroyed the Bw6 epitope as

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KEY POINTS

- HLA Bw4 and Bw6 epitopes are centered on α-1 α-helix amino acid residues 77–83, which have diverse amino acid sequences.
- Bw4 and Bw6 epitopes also include other HLA structures and, in some cases, bound peptide.
- The NK cell KIR3DL1 receptor has diverse allotypes that differentially recognize Bw4^{+} HLA molecules and their bound peptides.
- The antibodies that recognize Bw4 and Bw6 are diverse.
- Epitope and antibody diversity allows Bw4^{+} or Bw6^{+} patients to produce antibody to Bw4 or Bw6 epitopes, respectively, without breaking self-tolerance.

detected by all three mouse mAb and three out of four human antibodies tested. Single amino acid substitutions at conserved residue 79 (R79G) or polymorphic residue 82 (R82L) abrogated binding by SFR8-B6 anti-Bw6 mAb. Modeling provided strong rationale for the experimental findings. HLA amino acids R79 and R82 are oriented outward with the potential to form highly energetic bonds with complementary antibody amino acids [4▪]. Even though antibody–antigen contact sites are large, a few amino acid side chains often provide most of the binding energy [5]. Substitution of residue 83 (G83R) abrogated binding by the BB7.6 anti-Bw6 mAb [3], probably by steric hindrance [4▪]. The HLA-B*07:02 R82L and G83R single amino acid substitutions each severely reduced binding by human anti-Bw6 alloantisera. Interestingly, the R82L and G83R single amino acid substitutions each conferred significant binding by three and five out of six anti-Bw4 mAb, respectively [3]. A major conclusion is that the Bw4 and Bw6 epitopes are heterogeneous and anti-Bw4 and anti-Bw6 antibodies form energetic contacts with particular amino acids in the 77–83 region.

The binding interface of antibody with protein antigen is large and typically involves discontinuous stretches of amino acids [6]. Therefore, anti-Bw4 and anti-Bw6 antibodies could be expected to contact distinct HLA surfaces that overlap on HLA α-1 α-helix residues 77–83. SFR8-B6 anti-Bw6 mAb binding was abrogated by the B*07:02 A90D mutation on a loop connecting the α-1 α-helix and a β-strand (Fig. 1, top). In contrast, the BB7.6 anti-Bw6 mAb was affected by amino acid substitutions at residues 148 and 150 on the B*07:02 α-2 α-helix, strongly suggesting that BB7.6 straddles the HLA peptide binding groove to contact both α-helices (Fig. 1, bottom). Bw4 epitope heterogeneity was confirmed by El-Awar *et al.* [7]. Each of three mouse mAb bound to most Bw4^{+} proteins tested, with one or a few exceptions. Nonbinding proteins correlated with sequences outside the 77–83 region, including connecting loop residue 90 and α-2 α-helix residues. Similarly, Marrari *et al.* [8] described a human anti-Bw4 mAb that bound nearly all Bw4 members; nonbinding exceptions correlated with α-2 α-helix residues. Therefore, anti-Bw4 and anti-Bw6 antibodies contact the 77–83 region, but form distinct footprints on HLA molecules.

PRODUCTION OF ANTI-BW4 ANTIBODY IN A BW4+ TRANSPLANT CANDIDATE

My colleagues and I recently analyzed serum from a Bw4^{+} B*27:05 patient that recognized nearly all Bw4^{+} molecules tested, including HLA-A Bw4^{+} proteins [9▪]. Bw4 epitope specificity was suggested by reactivity with Bw4^{+} B*49:01, but not Bw6^{+} B*50:01, molecules that differ only at amino acid residues 77 and 80–83. However, the patient's antibody did not bind self B*27:05. B*51:01-transfected cells absorbed anti-Bw4 reactivity to both HLA-A and HLA-B molecules, leaving only relatively weak binding to a private epitope on HLA-B17, the presumptive immunogen. Although unusual, this case should not be surprising. The immune system can recognize foreign proteins that differ from self by a single amino acid [10]. Given the sequence heterogeneity in Bw4^{+} HLA amino acids 77–83 and the footprint of anti-Bw4 and anti-Bw6 antibodies outside this α-1 α-helix region, we expect that occasional Bw4^{+} and Bw6^{+} patients will produce antibody to nonself Bw4 and Bw6 epitopes, respectively.

NATURAL KILLER CELL RECEPTORS EXPLAIN BW4 EPITOPE CONSERVATION

Bw4 and Bw6 epitopes have been well conserved in primate evolution. The Bw4 epitope has been shuffled between HLA loci, possibly by gene conversion events, arguing that Bw4 has been retained by positive selection [11]. The biological importance of Bw4 was illuminated by the discovery of killer cell immunoglobulin-like receptors (KIR) [12–14]. These receptors control the activity of most mature NK lymphocytes and some T lymphocytes. KIR can be inhibitory or stimulatory. KIR with long cytoplasmic tails engage SHP-1 and SHP-2 phosphatases and inhibit NK cell activation [15]. KIR with a charged transmembrane amino acid engage the DAP12 adaptor molecule and stimulate NK cells [15]. A variety of extracellular ligands, including viral antigens, may crosslink and activate stimulatory KIR, but most of the physiologic ligands remain unknown

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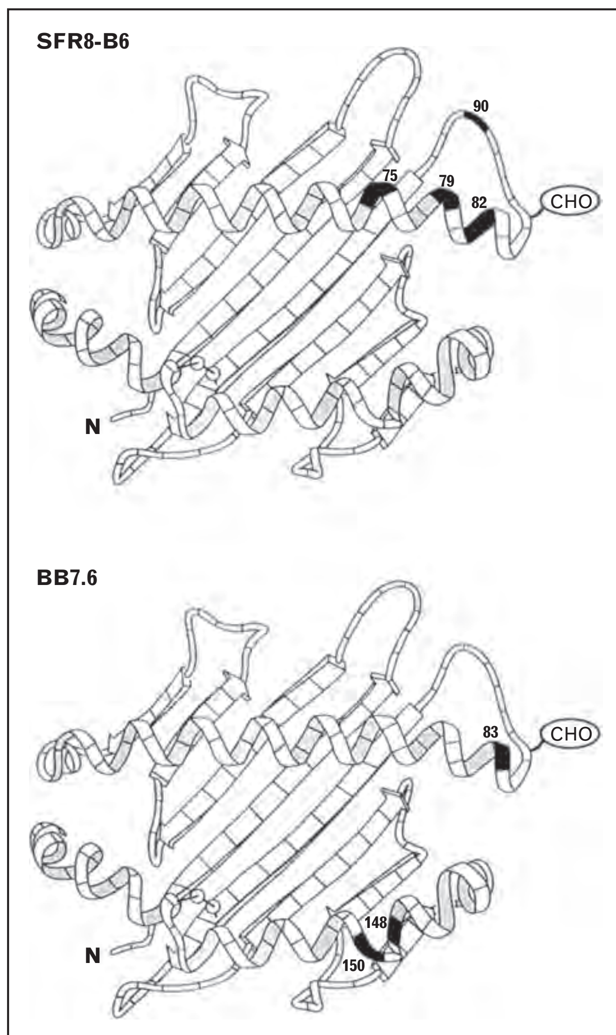

FIGURE 1. Ribbon diagram of the HLA α-carbon backbone, showing single B*07:02 amino acid replacements that substantially diminished SFR8-B6 mAb binding (top) or BB7.6 mAb binding (bottom). Reproduced from [3], copyright 1994, the American Association of Immunologists, Inc.

[15]. In contrast, many inhibitory KIR recognize HLA class I molecules. As will be reviewed below, the KIR3DL1 inhibitory receptor recognizes Bw4. Given that HLA–KIR interactions determine NK cell maturation and activation [15,16], it is not surprising that the Bw4 epitope has been preserved in primate evolution. However, this consideration does not explain why the Bw6 epitope also is conserved.

KIR3DL1 RECOGNIZES HETEROGENEOUS BW4 EPITOPES

Several groups have tested the ability of transfected HLA molecules to inhibit NK cells. Bw4$^+$ molecules inhibited KIR3DL1$^+$ NK cells in all studies [17–22]. The Bw4 epitope was implicated by comparison of closely related HLA-B alleles that differed exclusively in residues 77–83 [20,23]. However, Bw4$^+$ molecules were not equally potent and groups disagreed about which HLA-A and HLA-B molecules were most effective. As I will discuss below, these apparently incongruous findings are probably related to the fact that distinct KIR3DL1 allotypes differentially recognize specific Bw4$^+$ molecules. Collectively, these studies suggested that KIR3DL1 recognizes a subset of Bw4$^+$ proteins, possibly influenced by specific 77–83 amino acid sequences. Extending this concept, O'Connor *et al.* [24] found differential recognition of Bw4$^+$ proteins, assaying NK cell clones and recombinant KIR proteins. They reported a hierarchy of HLA ligand recognition: B*51:01 > B*38:01 > B*58:01 > B*27:05. This was surprising because the first three Bw4$^+$ proteins are identical at residues 77–83. Differential KIR3DL1 recognition of Bw4$^+$ proteins that share the same 77–83 region, documented in this report and a later study [24,25▪], strongly suggested that the KIR3DL1 contact site extends beyond the HLA α-1 α-helix.

SITE-DIRECTED MUTAGENESIS CONFIRMS KIR3DL1 BW4 EPITOPE HETEROGENEITY

To more precisely map the KIR3DL1 Bw4 epitope, Gumperz *et al.* [23] mutated residues 82 and 83 in Bw6$^+$ B*15:02 and Bw4$^+$ B*15:13. KIR3DL1 inhibition of NK cell-mediated cytotoxicity was weakly affected by mutation of residue 83, but was minimally affected by the residue 82 substitution [23]. KIR3DL1 recognition was confirmed and extended by Kurago *et al.* [26], who tested B*07:02 mutants that had substitutions matching Bw4$^+$ B*27:05. They found an inhibitory hierarchy on KIR3DL1$^+$ NK cell-mediated cytotoxicity of B*27:05 (most inhibitory) > B*07:02 (residue 80, 82 and 83 substitution) > B*07:02 (G83R substitution) > B*07:02 (R82L substitution) > B*07:02 (N80T substitution) = B*07:02. They also showed a similar inhibitory hierarchy of the HLA mutants on KIR3DL1$^+$ NK cell IFN-γ production [26]. Later, Sanjanwala *et al.* [25▪] mutated Bw4$^+$ B*15:13 and B*51:01 molecules and confirmed the importance of residue 83, with little or no contribution by residue 77 or 80. Surprisingly, the L82R mutation destroyed the KIR3DL1 epitope in B*51:01, but had no effect in the context of B*15:13, molecules that are identical in the 77–83 region [25▪]. In summary, R 83 is critical for the KIR3DL1 epitope, with an important contribution by L82. Thus, the Bw4 KIR3DL1 epitope appears similar to the Bw4 serological epitope, which usually requires R83 and often requires L82 [3].

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Mutagenesis work was complemented by a notable structural study. Vivian *et al.* [27■■] showed that KIR3DL1 and B*57:01 formed a huge (1740 Å^2) contact interface, which included largely invariant HLA residues. KIR3DL1 wrapped around HLA-B*57:01, contacting both α-helices, connecting loops near the α-1 α-helix, and even the β_2-microglobulin chain[27■■]. Of note, KIR3DL1 did not contact B*57:01 L82 [27■■]. Therefore, the previously described effects of the L82R substitution presumably were due to steric or charge hindrance or to conformational effects [25■,26]. Vivian *et al.* [27■■] also showed that B*57:01 I80A and R83A substitutions greatly reduced KIR3DL1-binding affinity. As expected, both T and I, the two amino acids found at position 80 of Bw4$^+$ molecules, allowed binding to KIR3DL1. This study confirmed a critical role for R83, but differed from prior reports [25■,26] in finding that HLA residue 80 is also important for KIR3DL1 binding. The difference between the studies likely is due to the amino acid replacements tested: T80N [26], I80N [25■], and I80A [27■■].

HETEROGENEOUS BW4 EPITOPES ARE RECOGNIZED BY HETEROGENEOUS KIR3DL1 ALLOTYPES

Parham *et al.* [28] and others demonstrated considerable KIR3DL1 allelic diversity. The most salient variability is that some alleles at this locus encode stimulatory receptors (KIR3DS1). Except for rare allotypes, KIR3DS1 proteins do not measurably bind HLA ligands *in vitro* [29], although there is genetic evidence of interaction between KIR3DS1 and Bw4$^+$ HLA-B alleles *in vivo* [30]. Even among KIR3DL1 molecules, allelic variation leads to differences in percentage expression by NK cells, NK cell surface density, and affinity or specificity for Bw4$^+$ ligands [28,31]. Thus, many of the apparently contradictory findings concerning which Bw4$^+$ molecules engage KIR3DL1 likely were due to the particular KIR3DL1 allotype(s) expressed on the NK cells tested. Confirming this hypothesis, O'Connor *et al.* [24] found that both KIR3DL1*001 and KIR3DL1*002 recognized B*51:01 well, but the former barely engaged B*27:05 and the latter barely engaged B*58:01. Thananchai *et al.* [32■] found that several KIR3DL1 allotypes bound to tetramers of Bw4$^+$ A*24:02 protein, but only KIR3DL1*005 bound both A*24:02 and B*57:01 tetramers.

HUMAN LEUKOCYTE ANTIGEN-BOUND PEPTIDES INFLUENCE BW4 EPITOPES

Given that Bw4 and Bw6 antibody binding sites can straddle the peptide binding groove, some of these antibodies are expected to contact HLA-bound peptides, especially peptide amino acid 8 (P8) which lies near HLA residues 80–83. As an example, Takamiya *et al.* [33] transfected B*51:01 into mouse RMA-S cells, which are genetically deficient in loading peptides into the HLA class I peptide binding groove. They incubated transfected RMA-S cells with β_2-microglobulin and B*51:01-binding peptides, allowing them to reconstitute fully formed B*51:01 on the cell surface. Tü109 anti-Bw4 mAb binding was favored by hydrophobic, bulky, or basic peptide amino acids 8 [33]. This peptide-sensitive mAb was not exquisitely peptide-specific and therefore could bind many Bw4$^+$ cell surface proteins containing a panoply of cellular-derived peptides. If an antibody was specific for a particular peptide amino acid 8, its binding could be difficult to detect *in vitro*. Such antibodies might be clinically significant. However, there is no clearly defined relationship between antibody-binding density and transplant rejection.

Similar to some antibodies, KIR3DL1 binding is influenced by HLA-bound peptides. Peruzzi *et al.* [34] found that B*27:05-binding peptides differed in their ability to protect transfected RMA-S cells from NK-mediated cytotoxicity. Protection was retained with peptides that had small peptide amino acid 8 side chains, but was lost with charged peptide amino acid 8 Glu or Lys residues, suggesting that steric or charge interference prevented effective contact between B*27:05 and the NK cell receptor (presumably KIR3DL1). Later experiments using virally transduced NK cells confirmed that KIR3DL1 was sensitive to the B*27:05-bound peptide on the surface of transfected RMA-S cells [35]. Thananchai *et al.* [32■] extended these observations and reported that KIR3DL1 binding to HLA tetramers was influenced by the KIR3DL1 allotype, the particular Bw4$^+$ HLA protein, and the HLA-bound peptide. For example, the KIR3DL1*001 allotype, but not the 005 allotype, bound Bw4$^+$ A*24:02 tetramers containing a HIV p17 peptide, but the opposite was true for A*24:02 tetramers containing a cytomegalovirus pp65 peptide [32■]. Fadda *et al.* [36■] found that KIR3DL1*001 binding affinities for B*57:01 and A*24:02 tetramers were strongly influenced by the HLA-associated peptides. In X-ray crystallography studies, Vivian *et al.* [27■■] found that KIR3DL1*001 made limited van der Walls contact with the peptide amino acid 8 side chain of a B*57:01-bound peptide. Affinity measurements of KIR3DL1*001-B*57:01 binding showed that several, but not all, tested peptide amino acids 8 were tolerated [27■■]. Using a combination of chimeric protein binding assay and X-ray crystallography, O'Connor *et al.* [37■■] recently showed that KIR3DL1 residues 166, 200,

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and 282 form a network of contacts involving HLA-bound peptide amino acids 8 and 9 and HLA residues 76, 77, and 80. Formation of this network is influenced by polymorphic KIR3DL1 residue 283. This network explains how NK cell activation is controlled by the interaction of KIR3DL1 allotypes, HLA Bw4 I80 and T80 allotypes, and specific HLA-bound peptides. Collectively, these studies show that KIR3DL1, as with some anti-Bw4 mAb, is not exquisitely peptide-specific and therefore binds $Bw4^+$ proteins that contain a large variety of cellular-derived peptides. It follows that normal $Bw4^+$ cells are recognized by KIR3DL1 and are protected from NK cell mediated attack. However, the HLA-bound peptides in virus-infected cells may be dominated by a limited repertoire of viral peptides and it is possible that these peptide-HLA complexes are not well recognized by KIR3DL1. As a result, this could render infected cells susceptible to NK cell mediated cytotoxicity, even if $Bw4^+$ proteins are expressed at the infected cell surface.

CONCLUSION

Bw4 and Bw6 epitopes are variable, because of amino acid sequence differences in the 77–83 region, other HLA regions, and HLA-bound peptides. Antibodies are quite diverse and can differentially recognize specific $Bw4^+$ or $Bw6^+$ molecules. Although not as diversified as antibodies, KIR3DL1 allotypes show significant variability, which affects Bw4 epitope fine specificity. Therefore, it is appropriate to think of the Bw4 epitope as a family of related structures. This means that anti-Bw4 antibodies and $KIR3DL1^+$ NK cells might not recognize all $Bw4^+$ molecules. Also, $Bw4^+$ patients might produce anti-Bw4 antibodies.

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Conflicts of interest

The author declares no conflicts of interest.

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▪▪ of outstanding interest

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Gives a structural explanation for how KIR3DL1-Bw4 recognition is influenced by variations in KIR, HLA, and HLA-bound peptides

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