Presentation of a Self-peptide in Two Distinct Conformations by a Disease-associated HLA-B27 Subtype

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The MHC locus on human chromosome 6 harbors the most polymorphic genes in the human genome, and the large number of alleles in human populations has permitted key structural features of MHC class I and class II genes that influence susceptibility to several human autoimmune diseases to be delineated. In this issue, Hülsmeyer et al. adds a new twist to the already rich literature on MHC polymorphisms and human disease (1). The authors determined the crystal structures of two HLA-B27 subtypes (B*2705 and B*2709) with the same self-peptide (pVIPR, derived from vasoactive intestinal peptide type 1 receptor). These two subtypes are remarkable in that they differ only at one heavy chain residue but are differentially associated with susceptibility to ankylosing spondylitis (AS), a chronic inflammatory joint disease. The pVIPR peptide bound in a conventional conformation to both HLA-B27 subtypes but in a second unique conformation to the AS-associated B*2705 subtype. In the conventional binding mode, an arginine at position 5 of the peptide was solvent exposed and thus available for TCR recognition. In the second conformation, this arginine instead formed a salt bridge with a buried polymorphic residue located at the floor of the binding site (Asp 116) (Fig. 1). The drastically altered position of this central peptide residue also changed the conformation of the entire central peptide segment from positions 3 to 7 (1). A single, buried polymorphic MHC class I residue can thus affect the global conformation of a bound peptide.

HLA-B27 and Ankylosing Spondylitis. In 1973, a striking association between HLA-B27 and AS was reported (2, 3). Even though susceptibility to many other human diseases has since been shown to be associated with particular alleles of MHC class I and/or class II genes, it remains one of the strongest associations between a MHC gene and a chronic inflammatory disease. An extensive search failed to identify other genes in the MHC class I region with a tighter linkage to AS, leading to the conclusion that the HLA-B gene itself is responsible. HLA-B27 is associated with AS in different ethnic groups across the world, and the analysis of HLA-B27 subtypes in different populations laid the foundation for the structure-function study by Hülsmeyer et al. (1), since it resulted in the identification of two HLA-B27 subtypes (B*2706 and B*2709) that are not associated with susceptibility to the disease (4, 5). Among Caucasians, susceptibility to AS is associated with the B*2705 and B*2702 subtypes, whereas other subtypes are very rare. In contrast, the B*2706 subtype is the most prevalent among Indonesians (80%) but does not confer susceptibility to AS. This subtype is of interest, since it differs from the B*2704 subtype that confers susceptibility to AS in Asian populations at only two positions at the floor of the binding site, positions 114 and 116 (4). A study on the isolated Sardinian population resulted in the identification of the B*2709 subtype in 10 of 40 normal HLA-B27$^+$ subjects (25%) but none of the 35 HLA-B27$^+$ AS patients (5). This B27 subtype was studied by Hülsmeyer et al. (1) because it differs from the common AS-associated B*2705 subtype only at position 116. Most HLA-B27 subtypes, including the AS-associated B*2705 subtype, carry a negative charge (aspartic acid) at position 116, whereas tyrosine (B*2706) or histidine (B*2709) are present at this position in the subtypes not associated with susceptibility to AS. Both the 114 and 116 polymorphisms may contribute to the absence of an AS association for the B*2706 subtype, since the AS-associated B*2707 has a tyrosine at position 116.

Structural Features of HLA-B27 Critical for Peptide Binding. How does HLA-B27 confer susceptibility to AS? Many of the experimental approaches that are being used to approach this question are based on the important advances that resulted from the crystal structure of HLA-B27, reported in 1991 (6). The structure was determined with HLA-B27 molecules purified from a human EBV-transformed B cell line, and the binding site was thus occupied with a diverse set of self-peptides. Despite the heterogeneity of the bound peptide pool, the electron density in the binding site could be interpreted as nonameric peptides that were bound in a largely extended conformation. Particularly remarkable was the electron density at the P2 position, since it reached deep into a hydrophobic pocket that ended near a negatively charged Glu 45 (6). These crystallographic data were complemented by sequence analysis of eluted peptides: the identified peptides were nine amino acids in length, and

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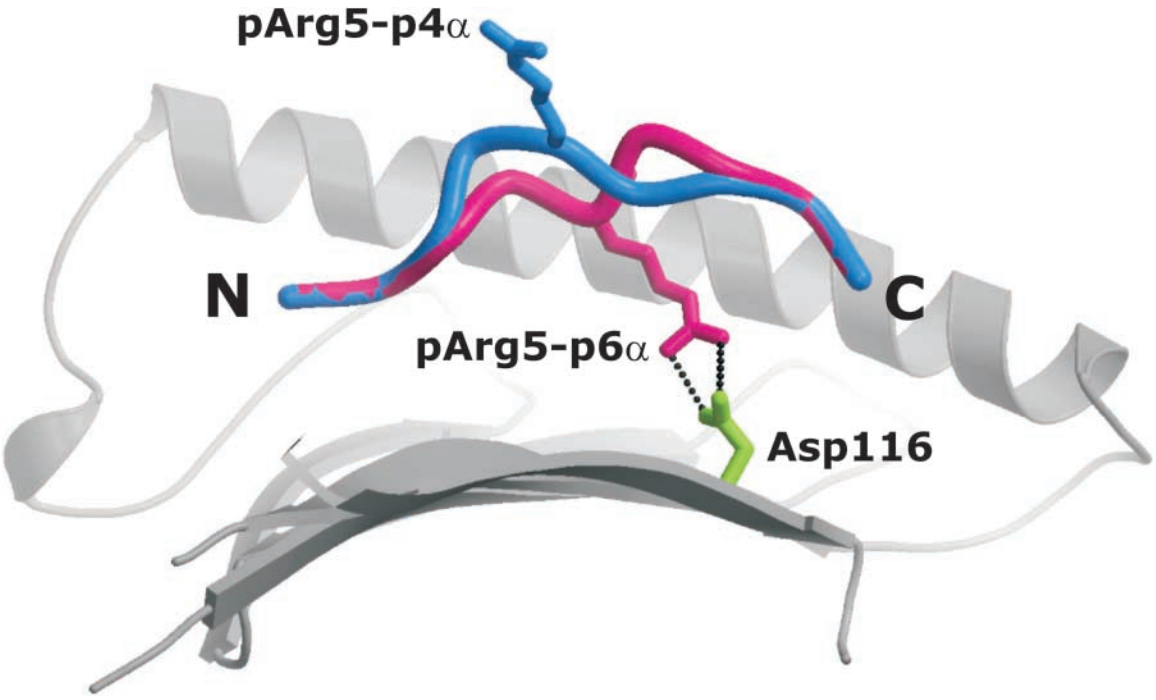

Figure 1. Two distinct conformations of the pVIPR peptide in the binding site of HLA-B27. Two HLA-B27 subtypes that are differentially associated with susceptibility to AS were crystallized with a self-peptide (pVIPR). The peptide bound in a conventional conformation (termed p4α, blue) to both B27 subtypes but also in a novel conformation (termed p6α, pink) to the AS-associated B*2705 subtype. In the novel conformation, the arginine at position 5 of the peptide formed a salt bridge with Asp 116 located on the floor of the peptide-binding site, the only residue that differs between the B*2705 and B*2709 subtypes. The heavy chain α1 helix and the floor of the peptide-binding site are shown (gray), whereas the α2 helix has been cut away to provide better visibility of the peptide.

remarkably, all of these peptides carried an arginine at position 2 (7). Alignment of these self-peptide sequences permitted definition of a HLA-B27 binding motif that has since been used extensively to identify peptides presented by this MHC molecule to CD8 T cells.

Conformation of Peptides Bound to HLA-B27. The HLA-A2 and HLA-B27 structures also revealed the general mechanism by which peptides are anchored in the binding site. Two clusters of conserved amino acids, one at each end of the peptide-binding groove, are positioned to contact polar atoms of the main chain of the first two and the last two amino acids of the peptide (6, 8). Although the position of the terminal peptide residues is thus fixed, a substantial degree of conformational flexibility is possible for the central peptide segment, as shown dramatically by the two conformations of pVIPR bound to HLA-B*2705 (Fig. 1). In both the conventional and unconventional pVIPR peptide conformations observed in the structure, the electron density for the main chain and side chains are virtually identical for the NH_2- and COOH-terminal segments (P1 Arg, P2 Arg, P8 His, and P9 Leu). However, each side chain in the central P3 to P7 segment is positioned differently in the two pVIPR peptide conformations. The interaction of P5 Arg with the 116 Asp at the floor of the peptide-binding site in the nonconventional conformation thereby drastically alters the shape and charge distribution in the central peptide segment accessible to the T cell receptor. Hülsmeyer et al. thus demonstrate for the first time that the same peptide can be bound by a MHC class I molecule in two grossly different conformations due to a polymorphic residue located on the floor of the binding site (1). Comparison of the structures of HLA-A2 with five different viral peptides had shown that the central peptide segment differed in its conformation and the positioning of the peptide side chains among all viral peptides and that this conformational flexibility contributes to the antigenic identity of each MHC class I–bound peptide (8). Similar findings have been made for murine MHC class I–peptide complexes, indicating that such conformational flexibility is a general property of peptide binding by MHC class I molecules (9). The conformation of the bound peptide is also dependent on peptide length, since the position of the peptide termini is typically fixed. The most dramatic example is the structure of a rat MHC class I molecule with a 13-residue peptide in which the central peptide segment bulges out of the binding site. This central peptide segment was highly flexible, since it assumed different conformations that were stabilized by crystal contacts in the two molecules of the asymmetric unit (10). Such grossly different peptide conformations are not likely to occur in MHC class II–peptide complexes, due to fundamental differences in the anchoring of peptides between MHC class I and class II proteins: conserved residues of the MHC class II binding site form hydrogen bonds along the length of the peptide backbone, and not only at the termini, and thus force the peptide into an extended conformation (11).

T Cell Receptor Recognition of HLA-B27–Peptide Complexes. The study also provides evidence that the alternative pVIPR peptide conformation affects T cell receptor recognition. One of 39 CTL lines recognized pVIPR only in the context of B*2705, but not B*2709, and this line may therefore recognize only the alternative peptide conformation. In addition, 15.4% of the CTL lines responded more vigorously when the peptide was presented by B*2705 rather than B*2709, even though the peptide bound with higher affinity to B*2709. Previous studies had demonstrated a higher frequency of pVIPR-reactive CD8 T cells in subjects with the B*2705 than the B*2709 allele, again suggesting that the alternative peptide conformation is recognized by T cells (12). Since the data presented in the current study were based on polyclonal cell lines in which CD8 T cells reactive with either peptide conformation may be present, the frequency of CD8 T cells that recognize the alternative peptide conformation may be underestimated. Particularly valuable would be the analysis of CD8 T cell clones, since such an analysis would demonstrate what fraction of CD8 T cells recognize the conventional versus the alternative conformation of pVIPR. This issue could also be addressed using tetramers of B*2705 and B*2709 with bound pVIPR. A particularly interesting question with respect to the pathogenesis of AS is whether the abundance of the peptide in the conventional versus the alternative conformation differs between subpopulations of antigen-presenting cells and depends on their activation/differentiation state.

HLA-B27 and Human Disease. Sequence analysis of peptides bound to B*2705 and B*2709 has demonstrated that the polymorphic 116 residue also influences the repertoire of peptides that is bound. B*2705 shared 79% of its peptide repertoire with B*2709, and all B*2705 ligands that were not bound by B*2709 had COOH-terminal basic or tyrosine residues (13). This polymorphism may, therefore, influence susceptibility to AS and reactive arthritis by two mechanisms: differential binding of peptides or induction of alternative peptide conformations, as described by Hülsmeyer et al. (1). Further work is thus required to determine which of these mechanisms is responsible for the differential association of these closely related B27 alleles with susceptibility to AS.

How common are peptides that bind to HLA-B27 in is this novel conformation? The minimal requirements for such a peptide are an arginine at P5 that can form a salt bridge with Asp 116, an arginine at the P2 anchor position, and the presence of an aliphatic residue at the P9 position (for example, xRxxRxxxL; preferences at secondary anchor residues not taken into consideration). In the structure of B*2705 with pVIPR, the P9 leucine side chain is too short to engage Asp 116, permitting the formation of a bidendate salt bridge between this MHC residue and P5 arginine of the peptide. This peptide conformation would probably not be possible with an arginine or lysine at the P9 anchor position and may also be disfavored when a bulkier aromatic group rather than leucine is present at P9. Thus, a relatively small subset of peptides that bind to HLA-B27 may assume such a conformation, since restrictions are placed both on the P5 position (1 of 20 possible side chains) and the P9 position (a small subset of permissible side chains). A search that also incorporated other features of the B27-binding motif indicated that such peptides are present in human proteins and in antigens from bacteria known to cause reactive arthritis.

HLA-B27 not only confers susceptibility to AS but also to reactive arthritis, a condition that can result following infection with *Chlamydia trachomatis*, *Salmonella*, *Shigella*, *Yersinia*, or *Campylobacter*. Also, ~20–40% of HLA-B27–positive patients with reactive arthritis progress to AS after 10–20 yr (14). HLA-B27–restricted CD8 T cell clones that specifically lyse *Yersinia*- or *Salmonella*-infected target cells have been isolated from the synovial fluid of patients with reactive arthritis (15), but it is not known which bacterial peptide(s) are critical in the pathogenesis and whether T cell recognition of self-peptides is involved in the chronic inflammatory stage of the disease.

Previous work on disease-associated MHC polymorphisms has provided a number of examples in which key MHC residues determine the peptide binding preferences and thus the repertoire of self-peptides that can be presented. For example, the DQ β57 polymorphism that is associated with susceptibility to type 1 diabetes determines the charge of the P9 pocket of the peptide binding site and thus has a drastic effect on the peptide repertoire that can be presented (16, 17). The work by Hülsmeyer et al. demonstrates a novel potential mechanism by which a disease-associated MHC polymorphism can affect peptide presentation: the induction of an alternative peptide conformation that changes the peptide surface accessible for T cell receptor recognition.

Fig. 1 was kindly provided by Dr. M. Hülsmeyer and colleagues.

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