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REVIEW ARTICLE

Presentation of Antigenic Peptides by Products of the Major Histocompatibility Complex

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Abstract: Molecules encoded by the major histocompatibility complex (MHC) are polymorphic integral membrane proteins adapted to the presentation of peptide fragments of foreign antigens to antigen-specific T-cells. The diversity of infectious agents to which an immune response must be mounted poses a unique problem for receptor–ligand interactions; how can proteins whose polymorphism is necessarily limited bind an array of peptides almost infinite in its complexity? Both MHC class I and class II determinants have achieved this goal by harnessing a limited number of peptide side chains to anchor the epitope in place while exploiting conserved features of peptide structure, independent of their primary sequence. While class I molecules interact predominantly with the N- and C-termini of peptides, class II determinants form an extensive hydrogen bonding network along the length of the peptide backbone. Such a strategy ensures high-affinity binding, while selectively exposing the unique features of each ligand for recognition by the T-cell receptor. © 1998 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: MHC class I; MHC class II; peptide binding groove; anchor residues; hydrophobic pockets; peptide termini

The MHC consists of a tightly linked cluster of genes spanning 3000 kb on the short arm of chromosome 6 in the human and chromosome 17 in the mouse. Class I and class II proteins, encoded within this region, are members of the immunoglobulin supergene family and are by far the most polymorphic proteins so far identified among higher vertebrates [1]. Although over 100 class I alleles have been characterized among humans, each shares a common structure: a heavy chain of 45 kDa is folded into a short intracytoplasmic domain, a hydrophobic transmembrane portion and a major extracellular region arranged into three domains. The three-dimensional structure is maintained by two intrachain disulphide bonds and stabilized by a non-covalent association with β_2-microglobulin (β_2m), a non-MHC encoded protein, 12 kDa in size.

In contrast, class II determinants are composed of two chains, α (33 kDa) and β (28 kDa), both of which are integral membrane proteins and which associate non-covalently. Interestingly, the polymorphic regions of both class I and II molecules are localized within the membrane distal domains which fold in a remarkably similar fashion to form a groove composed of two α helices separated by eight strands of an anti-parallel β pleated sheet (Figure 1). While in class II determinants, α_1 and β_1 domain each contribute an α helix and four strands

Abbreviations: APC, antigen presenting cell; CDR, complementarity-determining region; CLIP, Class II associated invariant chain peptide; CTL, cytotoxic T lymphocyte; HEL, hen eggwhite lysozyme; MHC, major histocompatibility complex; TCR, T-cell receptor.

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of the β pleated sheet to this structure, α_1 and α_2 domains assume this role among class I molecules.

Although it had long been recognized that products of the MHC exert control over the immune response to a foreign protein, confirmation that they do so by binding appropriate peptide fragments and presenting them to T-cells awaited elucidation of their crystal structure. Crystallographic analysis of HLA-A2 [2] and HLA-Aw68 [3] clarified most aspects of their conformation but revealed a poorly resolved, electron-dense material occupying the groove, subsequently shown to represent endogenously bound peptides derived from diverse sources. The recent description of the crystal structure of HLA-DR1 has confirmed these findings to be equally applicable to class II molecules [4], but has highlighted something of a paradox: given that the affinity of the T-cell receptor (TCR) for an individual peptide–MHC complex has been shown to be extremely low [5–7], MHC determinants must compensate by binding peptides with comparatively high affinity. Nevertheless, in order to maximize their ability to present peptides from a vast array of potential pathogens, their binding must remain highly promiscuous. High affinity yet promiscuity of binding is a feature of MHC molecules unique among protein–ligand interactions, one that has been achieved differently among class I and II determinants by exploiting those aspects of peptide structure, independent of amino acid sequence.

Peptide Presentation by MHC Class I

A Universal Role for the N- and C-termini of Bound Peptides. Experiments by Schumacher and colleagues, using mutant RMA-S cells expressing empty class I molecules, revealed that class I binds predominantly to short peptides of only nine amino acids in length, even when they constitute a trace component in a mixture of longer peptides [8]. X-ray crystallography has since suggested a molecular basis for this marked preference: being closed at either end by conserved yet bulky residues such as Tyr84, Trp167, Tyr159 and Tyr171, the peptide-binding groove imposes rigid constraints on the length of the peptides bound. Interestingly, crystallographic analysis of HLA-B27 occupied by self-peptides, succeeded in resolving the N- and C-termini of the peptide ligands in spite of their heterogeneity. In contrast, the remainder of the groove was characterized by a lack of contiguous electron density. These findings suggested that all peptides, irrespective of their origin, were anchored similarly at their termini, thereby restricting their length, but adopted widely differing conformations in between [9].

The floor of the peptide binding groove of class I molecules has been shown to be folded into six subsites or specificity pockets, designated A to F [3]. The A and F pockets at either end of the groove are highly conserved among different allelic forms of class I and, as such, are ideally suited to accommodating the single most conserved feature of all peptides: their charged termini [9–12]. This they achieve by means of a network of hydrogen bonds involving conserved tyrosine and threonine residues, the importance of which has been amply demonstrated by complementary experimental approaches. Latron and co-workers used site-directed mutagenesis to introduce mutations at the critical residues in either the A or F pockets of HLA-A2, replacing them with phenylalanine or valine, selected for their inability to form hydrogen bonds [13]. Although mutant class I molecules bearing an altered F pocket remained functionally competent, molecules receiving mutations in the A pocket were significantly compromised in their ability to present peptides from influenza virus matrix protein to antigen-specific cytotoxic T-lymphocytes (CTL). A loss of presentation capacity, approaching two orders of magnitude, suggested a fundamental role for these conserved residues in stabilizing peptide binding.

An alternative approach to studying peptide–MHC interactions has been adopted by Bouvier and Wiley that builds upon these findings; rather than targeting critical residues within the HLA-A2 molecule, they chemically modified either the N- or the C-terminus of the matrix protein epitope, thereby preventing the formation of hydrogen bonds [14]. Interestingly, the introduction of a methyl group at either end of the peptide significantly reduced the thermal stability of the resulting complex suggesting that both the A and F pockets play an equally crucial role in peptide binding. Consistent with these conclusions, peptides acid-eluted from class I molecules have been found to lack proline residues at the N-terminus, even though it is a common anchor residue at the P2 position of human class I molecules, such as HLA-B53, B51 and B7 [15] and the murine determinant, H-2L^d [16]. Since the side chain of proline is covalently bound to the α amino group, such peptides lack a charged terminus with which hydrogen bonds may form.

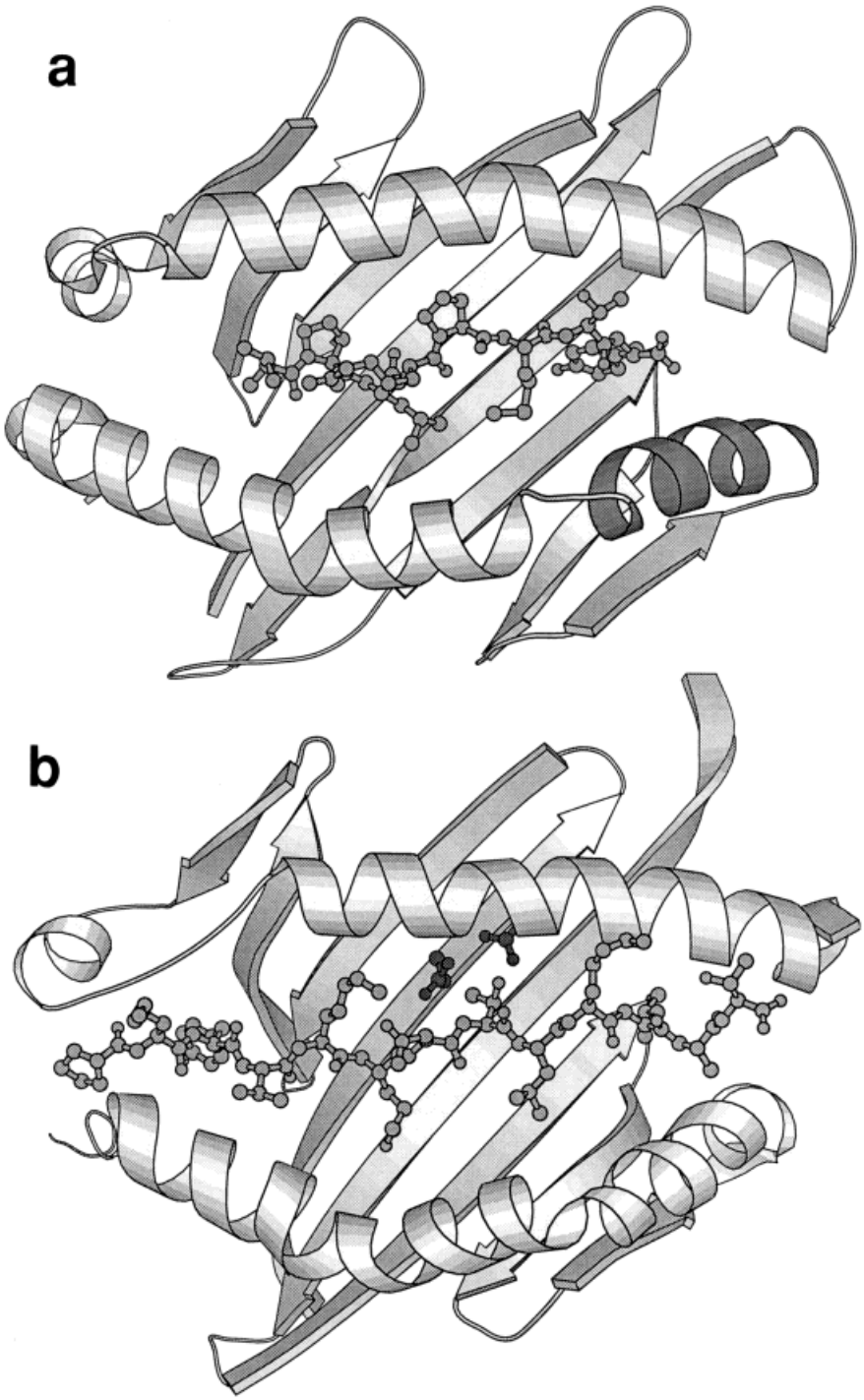

Figure 1 (a) Ribbon diagram showing the conformation of the binding groove of the human class I molecule HLA-B35*01 occupied by an octamer peptide from the HIV-1 Nef protein, represented in a ball and socket format. Darker shading of the α_2 α helix denotes the portion displaced upon binding of the peptide (see text for details). (b) Schematic diagram of the complex between the human class II molecule HLA-DR1 bound to a peptide from the influenza virus matrix protein. Reproduced from [30] with the kind permission of the author and publishers.

The presence of proline at the P1 position may, therefore, actively discriminate against the binding of such peptides, underlining the importance of a hydrogen bonding network responsible for anchoring the N-terminus in place.

The advent of novel techniques for the *in vitro* folding of soluble class I molecules has permitted their loading with a single species of peptide. X-ray crystallography of just such a complex between H-2K^b and an epitope from vesicular stomatitis virus nucleocapsid protein has enabled the conformation of bound peptide to be resolved along its entire length [17]. Direct visualization of the ends of the peptide has confirmed how the N-terminal nitrogen hydrogen bonds with the hydroxyl group of Tyr171 while the C-terminal oxygen atom forms a hydrogen bond with the hydroxyl group of Tyr84. This conformation is similar to that proposed for HLA-B27, suggesting the arrangement to be common to mouse and man.

In spite of the widespread applicability of this model, important exceptions to the rule have been described. In particular, studies of the non-classical class Ib molecule in the mouse, H2-M3, have shown the A pocket to be partially occluded due to the replacement of Trp167 and Tyr171 with leucine and phenylalanine respectively [18]. The bulky side chains of these residues point directly into the pocket, reducing it in size and displacing the N-terminal amino acid side chain into the adjacent B pocket. Although the integrity of the A pocket is necessarily compromised, it retains vestigial function, accommodating N-formyl groups via a hydrogen bonding network involving His9 and an ordered water molecule: indeed, this specificity readily explains the 10^4-fold higher affinity of H2-M3 for N-formylated peptides [18]. Importantly, the resulting displacement of nonamer peptides along the groove, dictates a non-canonical conformation at the C-terminus: while the penultimate amino acid occupies the F pocket, the C-terminal residue is found to project vertically upwards out of the groove in a manner analogous to that described for a decamer peptide from calreticulin bound to HLA-A2 [19]. While the binding of peptide termini is clearly important for the presentation of epitopes by class I determinants, it is, therefore, far from indispensable should other constraints apply. These findings suggest that further interactions must occur between the peptide and MHC molecule which may compensate for any inadequacies in the function of the A and F pockets.

Relative Contribution of the Backbone and Side Chains to Peptide Binding. Although early crystallographic studies of class I molecules failed to resolve the structure of peptides bound within the groove [2], the study of purified MHC determinants complexed with a single, well-characterized peptide has provided clear insights into the overall conformation such peptides adopt upon binding. These studies have concluded unanimously that rather than forming a helical structure within the peptide-binding groove, epitopes bind as an extended β strand [9,10,17,20]. By preventing intrapeptide hydrogen bonding, this extended conformation enables class I molecules to optimize the number of hydrogen bonds formed between residues lining the groove and the peptide backbone, the other feature common to all peptides. By exploiting universal aspects of peptide structure, class I determinants may therefore ensure high-affinity interactions with a wide variety of ligands, in a manner relatively independent of their precise primary sequence [21].

Nevertheless, a degree of allelic specificity is conferred upon this interaction by pockets B to F in the floor of the peptide-binding groove. Comparisons between HLA-A2 and Aw68, whose sequences differ at 13 amino acids, have revealed that 11 of these residues are confined to the specificity-determining pockets, one of the remaining two residues lying at the putative CD8-binding site of the α_3 domain [3]. Polymorphism localized predominately within these subsites may transform the overall shape and charge of parts of the groove or introduce subtle variations in the size, location and orientation of the pockets themselves. Such differences between class I isoforms are important since it is into these pockets that the side chains of critical amino acids may penetrate, anchoring the peptide in place. The combined attributes of the specificity pockets, together with the physicochemical properties of the residues with which they are lined, therefore impose certain constraints upon which peptides may form productive associations, selecting only sequences bearing a complementary binding motif [22,23].

Over recent years, a number of groups have eluted endogenously bound peptides from purified class I determinants and subjected them to Edman degradation to define their precise sequence. While such studies have confirmed the immense variety of epitopes presented, they have defined binding motifs unique to each of the class I allotypes studied (reviewed in [24]). Many show the importance of the

amino acid at position 2 (P2) of the peptide as an anchor residue accommodated by the B pocket; analysis of peptides acid-eluted from HLA-B27, for example, has found arginine to be the only amino acid tolerated at this position. Although less stringent restrictions appear to apply to residues at other positions, P1 and P9 are predominately positively charged, while a marked preference for a hydrophobic residue is apparent at P3 [25]. In contrast, peptides extracted from the murine class I molecule, H-2D^b, reveal the principal anchor residue to lie at P5 which is dominated by asparagine, the side chain of which is predicted to fit optimally into the C pocket situated in the centre of the groove. This same position was found to be critical for peptide binding to H-2K^b, although amino acids with aromatic side chains (tyrosine or phenylalanine) were strongly favoured [26].

A comparison of the binding motifs described to date raises two important issues. Firstly, all peptides appear to employ their C-terminal residue as an anchor site irrespective of the restriction element, the use of the amino acids at this position being strictly limited to a selected group sharing aliphatic hydrocarbon side chains [24]. The universal nature of the anchor residue at the C-terminus may best be attributed to the conserved features of the F pocket into which it penetrates. Secondly, with a few notable exceptions [26,27], the majority of the contact between the bound epitope and the floor of the binding groove is polarized to the ends of the peptides; while the termini promote binding independent of the primary sequence, the anchor residues at P2 and P9 confer a limited degree of specificity on the interaction. This being so, the central portion of most bound peptides remains largely untethered, providing few constraints as to the conformation adopted within this region. This flexibility has important repercussions, both for the repertoire of peptides that may bind to class I and their subsequent recognition by T-cells.

Solvent Molecules Increase the Compatibility Between Peptides and MHC Determinants. The freedom of expression that bound peptides enjoy within their central portion, permits epitopes that do not conform precisely to length requirements to be presented, assuming, that is, they contain essential anchor residues. The problem of length that a decapeptide raises, may be resolved either by the introduction of a kink in its otherwise extended conformation [9], or by bulging of the peptide upwards out of the groove [11,20,28]. This upward displacement is facilitated either by a ridge across the floor of the groove, in the case of some murine class I molecules such as H-2D^b [29], or by water molecules which form a hydrogen-bonding bridge between the peptide and the β-pleated sheet below. By filling the underlying space, these solvent molecules help stabilize the bulging portion and improve the compatibility between the peptide and its restriction element [30].

Bound water molecules have been classified as fixed, semi-fixed or variable according to the role they play within the peptide-binding groove [15]. Fixed molecules are common to all alleles and therefore satisfy conserved hydrogen-bonding requirements: the A pockets of each class I molecule studied to data contain a water molecule responsible for establishing the hydrogen bond network with the N-terminus of those peptides capable of binding. In contrast, semi-fixed water molecules are more flexible in their location, vying for position with side chains derived either from the MHC or the antigenic peptide itself, while variable solvent molecules are recruited only as required to fill any space that may otherwise destabilize the complex.

Perhaps the most striking example of the role played by variable water molecules in extending the repertoire of peptides that may bind to class I determinants has been provided by a comparison of HLA-B53 complexed with two different epitopes, one derived from *Plasmodium falciparum*, the other from HIV-2 [15]. While the conformation of the two peptides was shown to be remarkably similar towards the anchor resides at either end, the structures adopted in between were manifestly distinct. In particular, radically different conformations of the side chains at P7 could be accommodated within the groove by the acquisition or displacement of a cluster of water molecules beneath the peptide, thereby providing some level of functional plasticity. That variable water molecules also serve to enhance the fit of anchor residues with their complementary pockets has been observed during a similar study of different peptides bound to H-2K^b [31]. As a polar molecule, water may therefore confer upon an otherwise quite rigid structure a surprising degree of malleability, without which the interaction of many peptides would prove sterically less favourable.

Interaction of the T-cell Receptor With Peptide–Class I Complexes. Since the ends of peptides

bound to class I are buried deep within conserved pockets of the binding groove, it is the central region that is efficiently displayed for perusal by antigen-specific T-cells owing to its upward displacement away from the floor of the groove. Consistent with the exquisite specificity of this recognition event, the central residues of five viral peptides have been found to adopt surprisingly different conformations when bound to the same class I molecule, providing unique antigenic identities which T-cells may readily distinguish [32].

The TCR responsible for such discernment, is a heterodimeric glycoprotein composed of disulphide-linked α and β chains, both of 40–50 kDa in the mouse, depending on the extent of glycosylation. As members of the immunoglobulin supergene family, the extracellular portions of both chains are folded into a number of immunoglobulin-like domains [33,34]. The ability to recognize a universe of different antigenic peptides is conferred upon the TCR by its membrane distal portion, which is highly diverse. Such diversity is encoded within genes constructed from non-contiguous DNA sequences, each selected at random from an extensive pool of gene segments. For the α-chain, for instance, variable (V) and junctional (J) genes are selected from a repertoire of 50 Vα and 50 Jα gene segments and are juxtaposed to encode the primary amino acid sequence. An additional diversity gene (D) contributes to the structure of the β chain and may be selected from one of two possible gene segments, each of which may be read in any reading frame, thereby substantially increasing variability of the final gene product. The junctions between gene segments, known as N-regions, are also the site of additional diversity since nucleotides may be randomly inserted or deleted: taken together, these mechanisms for the generation of diversity permit upwards of 10^9 different specificities to be conferred on the resulting TCR [35].

The combining site of the TCR has a number of loops projecting from its surface which represent sites of greatest diversity; as such, it is these complementarity-determining regions (CDRs) that make direct contact with its ligand, the peptide–MHC complex. Of the CDRs, only CDR3 is encoded within a portion of the recombined gene spanning the junction between V and J, for the α chain, or J and D in the case of the β chain, these junctions being the sites of N-region addition. The resulting hypervariable nature of CDR3 has led to the hypothesis that CDR1 and 2 are positioned over the α helices of the peptide-binding groove, while CDR3 of both the α and β chains are poised to interact with the most diverse component of the bipartite ligand, the antigenic peptide itself [36]. This notion has received support from studies of site-directed mutagenesis of residues within CDR3 which were found to abolish peptide recognition. The recent crystallization of both human and murine TCRs bound to their specific peptide–class I complexes has, however, revealed some deficiencies in this model [37].

Resolution of the crystal structure of the human A6 TCR has revealed how CDRα3 and CDRβ3 meet to form a large hydrophobic pocket, approximately 10 Å deep, positioned directly over its cognate antigen, the Tax peptide of HTLV-1 bound to HLA-A2 [38]. A tyrosine residue at position 5 of the peptide projects upwards out of the groove and is accommodated within this pocket in a manner analogous to anchor residues bound to the MHC. In addition to contacting the peptide, however, portions of CDRβ3 and CDRα1–3 interact with the α helices of the A2 binding groove. Furthermore, contrary to early expectations, CDRα1 was found to lie directly over the N-terminus of the peptide, while CDRβ1 was located over the C-terminus, a conformation remarkably similar to that adopted by the murine 2C TCR bound to a complex between H-2K^b and the dEV8 peptide [39].

Perhaps the most striking finding to emerge from these studies was the way in which the long dimensions of the TCR and MHC were tilted by 20–30° with respect to one another, resulting in a diagonal orientation for the TCR. This conformation appears to allow engagement while precluding contact between the TCR and the N-terminal regions of the two α helices: since these portions of the molecule represent regional high points within the three-dimensional structure, they would otherwise sterically hinder the interaction. That this pattern of TCR binding may be universal has been elegantly demonstrated by Sun and colleagues [40] who investigated the specificity of 59 CTL clones specific for H-2K^b. While engagement of the TCR was reproducibly disrupted by mutations in the C-terminal regions of the α helices, similar modifications of the N-terminal regions were consistently tolerated.

An interesting corollary emanates from recent structural studies of the TCR, namely, the surprising dearth of residues from the antigenic peptide involved in its ligation. This finding has revived long-standing interest in whether conformational

changes in the MHC, induced upon peptide binding, may contribute to the antigenic identity of an epitope. Indirect evidence that such changes occur has been provided by the characterization of monoclonal antibodies capable of distinguishing specific peptide–class I complexes, even though they bind to the α helix of the α_2 domain, at a site external to the peptide-binding groove [41,42]. Perhaps the most compelling evidence, however, has been provided by resolution of the crystal structure of HLA-B35 bound to an octomer derived from HIV-1, Nef75–82 [43]. Being shorter than the optimal nine residues, the C-terminal amino acid of this epitope fails to interact with the F pocket in the conventional manner. In order to maintain the network of hydrogen bonds with Thr143, Lys146 and Trp147, however, the N-terminal portion of the α_2 helix (residues 138–149, shaded darker in Figure 1(a)) was found to be displaced compared with other class I alleles, the extent of this conformational change almost certainly affecting engagement of the TCR. That the TCR itself may undergo a reciprocal conformational change upon ligation has been inferred from studies of an alloreactive TCR specific for H-2K^b [44]. Unlike the 2C TCR, restricted to antigen recognition in the context of the same class I molecule, this receptor does not possess a deep hydrophobic pocket at the junction between CDRα3 and CDRβ3, into which the side chains of bound peptide may penetrate. Furthermore, models of this TCR bound to H-2K^b reveal that the CDRβ3 loop would collide with the peptide–MHC complex in a sterically unfavourable manner unless a suitable conformational change were to occur. That such structural changes contribute to T-cell signal transduction therefore remains a viable possibility.

Peptide Presentation by MHC Class II

Until recently, an equivalent role for class II molecules in the presentation of peptides to CD4$^+$ helper T-cells had been largely inferred from the evident similarity between the crystal structure of MHC class I [2,3], and the predicted topography of the membrane-distal portion of class II determinants [45] (Figure 1). Substantial weight was afforded such assumptions by experiments using equilibrium dialysis to demonstrate physical association between purified I-A^k and a known I-A^k-restricted peptide from hen eggwhite lysozyme (HEL) [46]. Extention of these findings to a panel of peptides revealed that epitopes exhibit a marked preference for their restriction element, showing inferior binding to class II molecules of an inappropriate haplotype [47]. The recent elucidation of the crystal structure of HLA-DR1 has, however, unequivocally confirmed the ability of such molecules to bind and present peptide antigens [4]. Many of the insights such studies have provided have helped explain some of the anomalies of class II function *in vivo*.

Structure of the Class II Binding Groove. The most notable difference between the structure of class I and class II determinants is undoubtedly the open nature of the class II binding groove. The C-terminal end of the α_1 helical region of HLA-DR1 has been shown to bear an arginine residue at a critical position (Argα76) occupied by tyrosine in class I determinants (Tyr84). This single amino acid change causes the class II α chain to bend 2 Å lower towards the floor of the peptide binding groove. Furthermore, a conserved salt bridge, which normally obscures the opposite end of the class I-binding cleft, is constitutively absent from class II determinants, leaving either end of the groove free of obstruction [4]. The most significant implication for peptide binding, is that the stringent constraints of length imposed upon class I-restricted epitopes, fail to apply to peptides binding MHC class II. Consequently, although the peptide binding groove is capable of accommodating only 15 amino acids, peptides up to 24 residues in length are commonly eluted from functional class II molecules [48]. Interestingly, this additional length, which has been shown to hang outside the groove at either end [49,50], may be far from redundant, occasionally serving to enhance T-cell recognition in a manner which currently remains ill defined [51]. Indeed, studies of an epitope from the measles virus fusion protein, F51–70, have revealed that at least three of the five known TCR contact residues lie outside the core sequence accommodated within the groove of its restriction element, HLA-DR1 [52].

Heterogeneity in the size of bound peptides appears to be a general feature of class II ligands. Peptides acid-eluted from I-A^d and I-E^d, for example, range in length from 13 to 17 amino acids [53], while peptides purified from HLA-DR1 may vary in size from 13 to 25 residues [54]. Importantly, this heterogeneity may apply equally to a single T-cell epitope which may be presented as a nested set of peptides: while all such peptides may share a core

sequence, the demonstrable lack of congruity at their N- and C-termini results in ragged ends [54,55]. When eluted from I-A^b, for instance, a self-epitope derived from the class II E_α chain was found to be presented as three co-dominant species: 52–68, 52–66 and 54–67 [56]. Similar heterogeneity in length surrounding a common core sequence has been reported for an I-E^k-restricted epitope of *Drosophila melanogaster* cytochrome *c* after feeding antigen-presenting cells (APC) with the native antigen [57]. That natural variation in the length of peptides spanning an epitope may have functional consequences has been elegantly demonstrated by experiments involving $CD4^-$ variants of T-cell hybridomas specific for HEL. While cells responded to the core sequence, 52–61, by tyrosine phosphorylation of CD3ζ, they failed to translate this signal into IL-2 secretion unless the peptide was extended at the C-terminus to encompass naturally occurring tryptophans at positions 62 and 63. Whether or not ligation of the TCR was productive, appeared, therefore, to be a function of residues flanking the core sequence [58].

The other major implication for peptide binding that emanates from the open structure of the class II binding groove is that the N- and C-termini of peptides are no longer available as universal structures capable of contributing to the affinity of the interaction. Consequently, the conserved tyrosine and phenylalanine residues lying in the A and F pockets of class I determinants are constitutively absent from class II molecules. Although these differences clearly reduce the options available to class II determinants for ensuring high-affinity peptide binding, the crystal structure of HLA-DR1 has provided clues as to how this goal may be achieved.

Mechanisms of Peptide Binding to MHC Class II. The finding of a single, large hydrophobic pocket at one end of the HLA-DR1 binding groove has suggested the involvement of anchor residues in tethering the peptide, in a manner analogous to class I determinants. That this pocket is composed of residues from the invariant α chain and a relatively conserved region of the DRβ chain identifies this pocket as being universally important in promoting peptide binding. Remarkably, although the many diverse approaches to defining allele-specific binding motifs have frequently yielded conflicting results, they have unanimously affirmed the importance of this major pocket in binding a critical aromatic or hydrophobic anchor residue lying towards the N-terminus. Studies of an HLA-DR1-restricted epitope from haemagglutinin (HA306–318), for instance, have revealed how only the tyrosine residue at position 308 is critical for binding. Indeed, a polyalanine peptide onto which this residue and a lysine at position 315 have been grafted, exhibit identical binding characteristics to the wild-type epitope [59], suggesting that other subsites are of limited importance. Using an entirely different approach, Hammer and colleagues have reached similar conclusions by screening purified DR1 for binding to a random peptide library expressed on the coat protein of filamentous M13 bacteriophage. The binding motif inferred from these experiments revealed an absolute requirement for an aromatic residue towards the N-terminus, designated position 1 (P1) of the core sequence or the index residue (i) [60]. That these findings are of physiological relevance has been endorsed by studies of naturally processed peptides acid-eluted from class II determinants (reviewed in [48,61]). This suggests that the P1 residue serves a function similar to the C-terminal amino acid of class I-restricted epitopes: a universal anchor residue.

The surprising indifference of known epitopes to the substitution of most residues to alanine, with the exception of the P1 anchor [59], suggests that hydrogen-bonding with the peptide backbone may be important in stabilizing peptide–class II interactions. Such a possibility has been investigated by introducing point mutations into the binding groove of HLA-DR7 which are known to abrogate presentation of HA307–319. Substitution of particular residues within the haemagglutinin epitope was, however, found to restore presentation, enabling an accurate picture of the orientation and conformation of the bound peptide to be constructed [62]. The extended conformation advocated by this model has since been verified by X-ray crystallography of purified peptide–HLA-DR1 complexes [50], but has revealed that the peptide is additionally twisted within the groove in a polyproline II-like conformation. The resulting left-handed helical twist encompasses the central nine amino acid residues of the bound peptide and helps maximize opportunities for hydrogen bonding between the peptide main chain and non-polymorphic residues within the groove of class II determinants. Indeed, a number of asparagine residues, evenly spaced along the sides of the DR1 binding cleft, interact with the

helical repeat of the peptide, constraining its conformation [63]. That many of these residues are conserved between HLA-DR, DQ and their murine homologues, suggests a universal pattern of peptide binding, independent of both its primary sequence and polymorphism within the MHC. The recent elucidation of the crystal structure of I-E^k covalently bound to epitopes from haemoglobin and murine heat shock protein 70 has added substantial weight to this assumption [64,65]. These observations suggest that MHC class II molecules have evolved a strategy for hydrogen bonding quite distinct from that of class I: rather than employing conserved residues at the end of the groove to anchor the peptide termini in place, class II determinants make use of conserved residues distributed along the length of the groove permitting multiple interactions with the peptide backbone [66].

Although high-affinity binding may be achieved by a combination of the P1 anchor residue and hydrogen bonding to the peptide main chain, the restriction specificity of an epitope appears to be a function of secondary anchor residues at positions 4, 6 and 9, of which P6 is perhaps the most important. Peptides binding to DRB1*0101 bear residues with small side chains at this position, notably alanine and glycine. Binding to DRB*0401, on the other hand, is governed by threonine and serine at P6, which share essential hydroxyl groups, while interactions with DRB1*1101 require positively charged amino acids at this site such as arginine and lysine [61]. The corresponding pattern of indentations and pockets within the floor of the groove resembles the structure of class I molecules but is more extensive, there being at least two further pockets, each accommodating a single peptide side chain. Just as the size of the binding groove of class I molecules places restrictions on the length of peptides presented, the distance between these hydrophobic pockets in the floor of class II imposes constraints on the repertoire of peptides that may bind: by being tethered at multiple sites along the peptide, the propensity for bulging outwards as a strategy for accommodating extra length is also severely compromised [4]. Nevertheless, at least some degree of flexibility has been demonstrated by Kropshofer and colleagues who studied a self-peptide eluted from HLA-DR1 [67]. This epitope displayed a two-residue contact motif involving hydrophobic amino acids at P1 and P9. Interestingly, binding was not significantly impaired by varying the position of the P9 anchor by one amino acid in either direction.

Degeneracy of Peptide Binding to MHC Class II. The surprisingly limited contribution made to binding affinity by allele-specific anchor residues results in a high degree of degeneracy in peptide binding to class II determinants, which finds no parallel among class I-restricted peptides. A number of well-characterized epitopes, including HA307–319 [68], have been found to be presented in the context of a wide variety of different class II allotypes [69]. Paradoxically, such promiscuous peptides bind with remarkably high affinity in spite of their lack of specificity [68]. By far the most intriguing example of degeneracy must, however, be attributed to CLIP, a nested set of peptides derived from the invariant chain of class II molecules, based around the core sequence 91–99. These peptides have been shown to obscure the binding groove during the early phases of biosynthesis, thereby inhibiting peptide binding until class II intersects the endocytic pathway of antigen processing, where foreign peptides are abundant [70,71]. Since CLIP must perform this function for class II molecules irrespective of their haplotype, it binds indiscriminately to all peptide binding grooves studied to date [72]. The requirement for promiscuity of binding must, however, be tempered by a propensity for displacement of CLIP in favour of antigenic epitopes. This conflict of interests has been resolved by a 'supermotif' which is universally permissive for binding to class II, while incorporating bulky amino acid side chains capable of sterically hindering the interaction, thereby lowering its affinity.

The supermotif exhibited by CLIP consists of a methionine at P1, acting as the principal anchor residue, and inoffensive alanine and proline residues at P4 and P6 respectively, whose unremarkable side chains fail to confer any degree of allele-specificity, thereby reducing the negative impact of allele-specific contacts to a minimum [73]. In addition to these anchor residues, methionines at positions 93 and 99 (P3 and P9 respectively) are oriented downwards into the groove. That these residues subtly interfere with binding has been shown by replacing them with L-alanine: far from decreasing affinity for class II, such substitutions were found to enhance the ability of CLIP to compete with cognate antigen for presentation to T-cell hybridomas [74]. The suboptimal interaction caused by bulky methionines may, therefore, en-

sure that exchange of CLIP for foreign peptide is energetically favourable.

That CLIP is so tolerant of side-chain substitutions suggests that hydrogen bonding to the peptide backbone is especially important. Resolution of the crystal structure of HLA-DR3 bound to CLIP has revealed that the peptide binds in a manner similar to foreign epitopes, adopting a polyproline II-like conformation, in order to maximize contacts between non-polymorphic MHC residues and the peptide main chain [75]. The importance of these interactions has been elegantly demonstrated by Weenink and colleagues [76] who generated a set of analogues of CLIP by replacing each residue of the core sequence in turn with D-alanine. These analogues were designed to introduce sterochemical disruptions to the peptide backbone while compromising the integrity of side-chain interactions as little as possible: importantly, all such analogues were found to be entirely incapable of competing with antigenic peptide for presentation to T-cells.

Although it has long been assumed that peptide enjoys a largely passive role in the formation of functional complexes, recent observations have suggested that it has a far more dynamic part to play in ensuring its own presentation. Class II molecules from both mice and humans exist in one of two states which may be distinguished by their mobility in SDS-polyacrylamide gel electrophoresis. Floppy molecules have a characteristic propensity to dissociate into monomeric α and β chains, while the compact state remains stable in SDS. Interestingly, mutant APC have been characterized bearing a defect in the antigen-processing pathway, known to prevent foreign peptides from displacing CLIP [70]. These cells express only floppy molecules, suggesting that antigenic peptides permit the adoption of a stable long-lived structure, something the low affinity of CLIP is unable to achieve. These predictions have recently been addressed by Sadegh-Nasseri and colleagues [77], who have shown that purified I-E^k molecules transferred from conditions of low pH to a neutral environment in the absence of exogenously added peptide lack a compact dimeric structure. In contrast, brief exposure to an I-E^k restricted peptide during neutralization secured a stable conformation. These observations raise the possibility that peptide itself may direct the conformational maturation of MHC class II by acting as a portion of the class II molecule required for the completion of protein folding [78,79]. This propensity of some peptides to catalyse correct folding has recently been shown to extend the half-life of the class II molecules to which they bind [80]: far from remaining passive, therefore, foreign peptides actively co-operate with class II determinants to secure their presentation to the T-cell repertoire, thus aiding and abetting destruction of the very pathogens from which they derive.

CONCLUSION

Recent findings have greatly increased our understanding of the principles that govern binding of peptides to both MHC class I and class II determinants. Furthermore, co-crystallization of individual TCRs, bound to such complexes, has begun to resolve long-standing questions concerning the way in which the TCR discriminates between potential ligands, thereby retaining a high degree of specificity. The rewards of such understanding should not be underestimated: the identification of dominant epitopes from infectious agents by screening amino acid sequences for binding motifs, may provide candidates for vaccine development. Similar approaches applied to self-proteins may help elucidate pathogenic epitopes involved in autoimmune disease, while the design of peptides capable of interfering with ligation of the TCR holds promise as a strategy for immune intervention [81,82]. Only in the light of such detailed knowledge may we begin to unlock the enduring mysteries of the immune response while enabling the nature and extent of its impact to be refined.

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