Commentary

Motifs and Supermotifs for MHC Class II Binding Peptides

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In the last few years, our understanding of the mechanisms involved in the interaction between major histocompatibility complex (MHC) class II molecules and antigenic peptides has greatly increased. The determination of the x-ray structure of a human class II molecule (1, 2), the selection of large, class II–bound, peptide repertoires using M13 peptide display libraries (3, 4), and the characterization of class II–eluted, naturally processed peptides (5–9) has elucidated the structural requirements for peptide binding to class II molecules.

The peptide binding groove of class II molecules is open at both ends (1, 2), thus allowing class II–bound peptides to extend beyond both termini. As a result, these peptides are longer than those bound by class I molecules and exhibit considerable length variation, typically 12–24 residues (5–9). Class II molecules form many conserved hydrogen bonds with the peptide main chain, thus forcing class II–bound peptides into a similar conformation despite differences in their primary sequence. In addition, class II molecules make several contacts with peptide side chains to increase the overall binding affinity of the bound peptides. Side chain contacts differ between different alleles, and determine the peptide sequence specificity or "motif" for each class II allele. Recently, the motifs of various human class II (DR) molecules have been identified by the characterization of large, DR-bound peptide pools selected from M13 peptide display libraries (4, 10). These motifs consist of several peptide positions where amino acids with similar side chains occur with increased frequencies (anchor positions).

The anchor position closest to the NH_2-terminus (anchor position 1) is essential for a high affinity interaction between peptides and all DR molecules tested so far. The position 1 anchor accepts only aromatic or large aliphatic peptide side chains that interact with a deep pocket in the DR binding groove, built by both the invariant DR α-chain and a fairly conserved part of the DR β-chain (1, 2). Other major, but less essential, anchor positions are found at peptide positions 4, 6, and 9. The peptide side chains in these positions interact with shallow pockets shaped by clusters of polymorphic residues of the DR β-chain and determine allele-specific peptide binding.

Of similar importance for peptide–class II interaction is the presence or absence of peptide side chains which can interfere with peptide binding (inhibitory residues). Studies on designer peptide libraries and natural peptide sequences have indicated both position- and allele-specific properties of inhibitory residues (10–13). Interestingly, inhibitory residues are found more frequently at anchor positions, since these are the major contact sites for the peptide side chains with the class II molecule. The capacity of a given peptide to bind a certain MHC class II molecule therefore, is the result of attracting and repelling forces between peptide side chains and residues lining the MHC binding site.

Several promiscuous peptides, capable of binding to many different class II alleles, have been identified (14–16). Promiscuous peptides should either contain overlapping class II binding motifs or, in case only one binding frame is used, they should use anchors that are conserved among DR ligands and should lack allele-specific contact sites that could prevent binding to other class II molecules. Such a "supermotif" has now been found both by Malcherek et al. (17) and Sette et al. (18) in the class II–associated invariant chain peptide (CLIP) described in this issue of *The Journal of Experimental Medicine*.

The invariant chain (Ii) has an important role in the regulation and function of class II molecules (19). It promotes effective association and folding of class II α and β subunits, induces their transport out of the endoplasmic reticulum, and inhibits class II peptide binding before entry into endosomal compartments. The ability of Ii to interact with class II and interfere with peptide loading has been mapped to Ii exon 3, which encodes amino acids 83–107 corresponding to the CLIP peptide (20). In principle, CLIP could bind, as part of Ii, to a conserved site of the class II molecule, altering its conformation and therefore preventing the binding of antigenic peptides, or it could behave as a promiscuous peptide by binding directly to the peptide binding sites of different class II alleles.

The two papers by Malcherek et al. (17) and Sette et al. (18) of this issue indicate that CLIP indeed possesses the characteristics of a promiscuous peptide binding the class II binding groove (Fig. 1). By synthetic peptide chemistry, Malcherek et al. (17) demonstrated that CLIP uses the same peptide frame to bind to the grooves of the two very different class II molecules, DRB1*0101 and DRB1*0301 (21, 22). CLIP uses a methionine as position 1 anchor. The usage of an aliphatic rather than an aromatic residue at the first anchor position allows CLIP to bind to all DR alleles independent of the polymorphic 86 position of the DRβ chain, which excludes large aromatic residues (Tyr and Trp) in some DR alleles (12, 23). Malcherek et al. (17) further indicate that CLIP lacks particular allele-specific contact sites at the major anchor and inhibitory positions 4 and 6 (Fig. 1). Indeed, Ala_{94} at position 4 and Pro_{96} at position 6 appear to be fairly well accepted by all DR alleles tested so far (24). With the excep-

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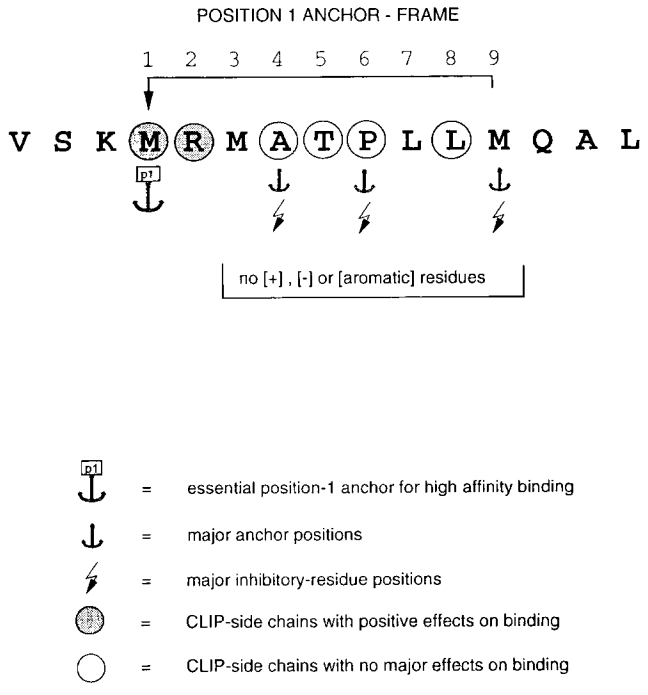

Figure 1. Anchor and inhibitory residue positions in the CLIP peptide.

tion of the Arg_{92} at relative position 2 (Fig. 1), CLIP has also avoided charged and large aromatic residues. There is sufficient evidence that both types of amino acids can interfere with peptide-binding to many class II molecules (10, 13). Thus, CLIP appears to have evolved as a promiscuous peptide by using a position 1 anchor and side chains in positions 2–9 which are generally accepted by all DR alleles.

The fact, however, that CLIP uses some allele-specific anchor residues, e.g., Met at position 9 for DR1 or Arg and Leu at positions 2 and 7 for DRB1*0401 (Fig. 1), support data from Sette et al. (18) showing major variations in the binding affinity of CLIP to different class II molecules. Furthermore, a better understanding of the peptide motifs recognized by DQ and DP molecules is necessary to generalize Malcherek's observation. In conclusion, Malcherek et al. (17) and Sette et al. (18) provide compelling, although not absolutely conclusive proof, that CLIP is indeed a universal class II ligand in accordance with the emerging principles of peptide–class II interaction. It needs to be shown, however, whether CLIP binds in a promiscuous mode also when it is part of the Ii chain and whether other Ii regions, besides CLIP, play a role in its interaction with the class II $\alpha\beta$ dimer (20).

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