

## Structural Limitations to the Mimetic HLA Epitope Hypothesis

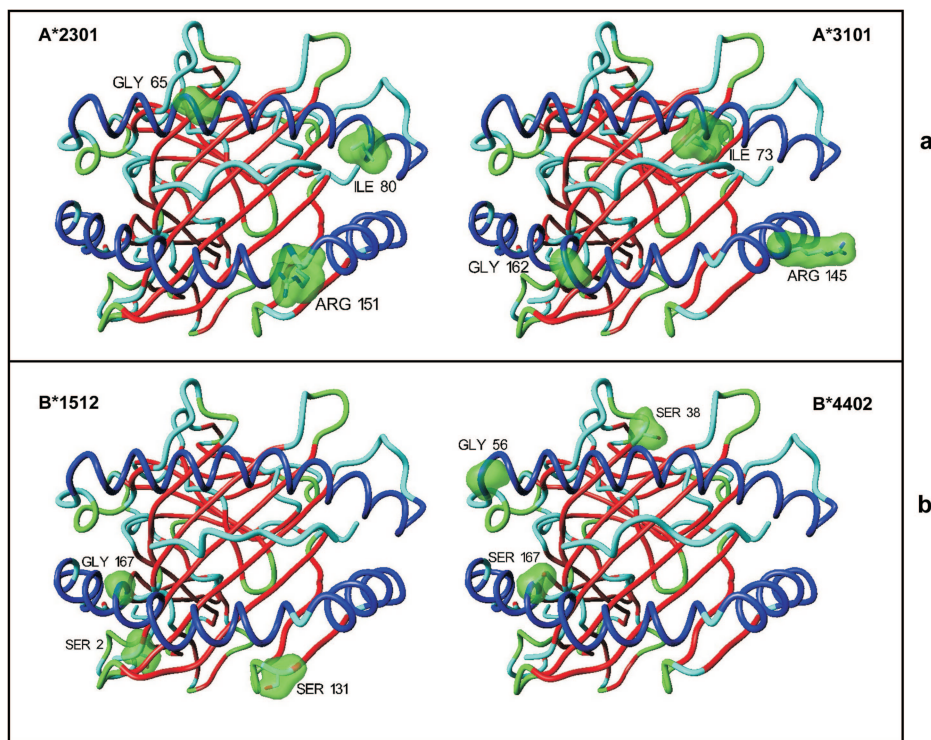
Sasaki et al. (1) described a novel approach to define immunogenic B-cell epitopes on human leukocyte antigen (HLA) based on the concept of primary and mimetic epitopes consisting of the same three amino-acids, sterically positioned at similar distances from each other that define dimensionally equivalent areas, recognized by the same antibody. They use this concept to explain the pattern of unexpected HLA antibody crossreactivity.

Although the concept of primary and mimetic epitopes is an interesting explanation for the pattern of antibody binding to HLA, we believe that it fails to fully accommodate knowledge of the structural basis of alloantibody/HLA interactions. For example, the authors suggest that the reactivity of mAb 3 against HLA-A\*2301 is due

to the primary epitope 065G/080I/151R, which is equivalent to the mimetic epitope 073I/145R/162G found on HLA-A\*3101, -A\*3301 and -A\*3303. Both epitopes span the peptide binding groove but the amino-acid composition between the epitopes' equivalent positions and within the area they define is different (Fig. 1a). The same applies for mAb 1, where the primary epitope (002S/131S/167G) is located on the side of HLA-B\*1512, whereas the mimetic epitope (038S/056G/167S) on HLA-B\*4402 covers parts of both alpha-helices and the peptide-binding groove (Fig. 1b). Therefore, primary and mimetic epitopes may have disparate structural characteristics and differing degrees of exposure of their corresponding critical amino-acids. The latter can be seen in Figure 1(b) where for the primary epitope 002S/131S/167G present on HLA-B\*1512, two amino-

acids are readily accessible on the upper surface of the molecule, but access to the third (serine at position 2), is limited by an overlying loop of the beta-pleated sheet. In the mimetic epitope 038S/056G/167S of HLA-B\*4402, the corresponding serine at position-167 is part of the alpha-helix and readily accessible from the surface. Accordingly, it is unlikely that an antibody that binds the primary epitope will also bind effectively to the mimetic epitope given their differing structural characteristics.

In the report of Sasaki et al., two additional points need clarification. In the analysis of mAb 3 reactivity, the authors exclude the putative mimetic epitope 017R/065G/080I because residue 017R is located in a "sterically disadvantaged" position. However, position 17 is part of a turn between two strands of the beta-pleated sheet and is



**FIGURE 1.** Structural disparity between primary and mimetic epitopes. (a) shows the putative three amino-acid primary epitope 065G/080I/151R present on HLA-A\*2301 and the corresponding mimetic epitope 073I/145R/162G, comprising the same three amino-acids, present on HLA-A\*3101 considered to form two structurally homologous antibody binding sites. The amino-acid residue composition and structural characteristics of the molecular surface bordered by the three amino-acids defining the primary and mimetic epitopes differ making it unlikely that the same antibody binds effectively to both. Similarly, the serine at position-2 of the primary epitope 002S/131S/167G, present on HLA-B\*1512, is only accessible from the side/bottom of the peptide-binding groove, whereas the corresponding serine at position-167 of the mimetic epitope 038S/056G/167S, found on HLA-B\*4402, is part of the alpha-helix and readily accessible from the top (b). The green clouds depict the molecular surface of the amino-acid residues comprising the relevant primary and mimetic epitopes.

readily accessible to alloantibody. Additionally, the authors exclude, without sufficient justification, primary and mimetic epitopes that include a nonpolymorphic amino-acid residue. Moreover, exclusion of the monomorphic positions from the outset would have a marked effect on both the nature and the number of primary/mimetic epitope pairs identified.

Analysis of the structural basis of antibody-antigen interactions and characterization of functional epitopes on the protein surface has shown that the affinity and the specificity of antibody-antigen interaction is determined predominantly by a small number of residues contributing most of the binding free energy, termed "hot-spots" (2, 3). Accurate prediction of functional epitopes to elucidate HLA immunogenicity is now becoming possible and should take into account variables that include not only the position and accessibility of their amino-acid residues, but

also their side chain hydrophobicity and electrostatic properties (4, 5).

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## Structural Limitations to the Mimetic HLA Epitope Hypothesis

In response to the letter of Kosmoliaptsis et al., we wish to clarify that the objective of our article (1) was to provide an explanation for the unexpected binding of monoclonal antibodies and extra antibodies produced in transplantation. Primary and mimetic epitopes, defined by three identical amino acids similar distances apart, may account for the "specificity" or "antigenic determinants" of the antibody binding. Nonpolymorphic positions and surrounding amino acids are excluded because they are not likely to define the specificity of reactivity.

Our observations on mAb 2 support the idea that surrounding the amino acids do not have an effect on the specificity but rather the affinity of binding. mAb 2 is specific for HLA A\*1102, A\*1101, and A\*4301. Note that the antibody binds to A\*1102 and A\*1101 with

similar affinities as reflected by their MFI. Conversely, the same antibody binds A\*4301 at approximately 50% of the MFI. A\*1102 and A\*1101 only differ in their amino acid sequence at a single position (position 19), whereas A\*4301 has different amino acids at 18 positions when compared with A\*1101 (2), suggesting that the amino acids adjacent to determinants do affect the "affinity" of the antibody binding. We concur with the letter that while defining affinities of the antibody, the hydrophobicity and electrostatic properties of the adjacent amino acids in the antibody binding "pocket" should be considered.

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