I-A\textsuperscript{k} POLYMORPHISMS DEFINE A FUNCTIONALLY DOMINANT REGION FOR THE PRESENTATION OF HEN EGG LYSOZYME PEPTIDES

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The class II molecules of the MHC not only bind processed antigenic peptides but also interact with the TCR. This latter interaction is thought to be the basis for allele specific "restriction" of Ag presentation to T cells. The specificity of this interaction is likely due to amino acid differences in a small number of polymorphic or "hypervariable" regions located in the amino terminal domains of the \(\alpha\) and \(\beta\)-chains. We have explored the functional significance of these polymorphic regions in an I-A\textsuperscript{k}-restricted, hen egg lysozyme specific Ag presentation system in which the measurement of IL-2 production by T cell hybridomas was used as the indicator of TCR recognition of the I-A/Ag complex. Chimeric I-A molecules, in which \(\beta\) allelic residues were substituted in one or more of the polymorphic regions of the \(\alpha\)-chain or in which \(\alpha\) allelic residues were substituted in one or more of the polymorphic regions of the \(\beta\)-chain, were used to examine the contribution of each polymorphic region of the molecule to its function. The results obtained demonstrate that the regions between residues 69 to 76 of the \(\alpha\)-chain and the regions between residues 63 to 67 and 75 to 78 of the \(\beta\)-chain exert a dominant effect on the presentation of lysozyme peptides by I-A\textsuperscript{k} to the T cell hybridomas in our panel. These observations were confirmed and extended by the analysis of Ag presentation by seven serologically selected mutants, all of which have amino acid interchanges in or around the dominant polymorphic regions. The results suggest that the serologically selected mutants fail to present Ag not because they fail to bind the peptide Ag but because the amino acid substitutions destabilize the interaction between the Ia/peptide complex and the TCR. Use of the recently published hypothetical model for class II structure to interpret the Ag presentation results suggests that the dominant polymorphic regions lie across from one another near one end of the \(\alpha\)-helices that form the two walls of the proposed Ag-binding cleft located on the top surface of the class II molecule. Furthermore, the majority of the amino acids which have been changed in the serologically selected mutants have side chains which are postulated to point toward the exterior of the molecule and would, therefore, be potential contact residues for the TCR.

The class II molecules, I-A and I-E, are transmembrane glycoproteins encoded in the murine MHC. These molecules, collectively referred to as la Ag, are found on the surface of APC and function as recognition structures for the Ag-specific receptor on Th cells (1). It has been proposed that the activation of the Th cell is dependent on the simultaneous interaction of its TCR with elements of the la molecule that identify it as self as well as a processed antigenic peptide bound to the la molecule (2). It is this proposed dual role of serving as restriction element and presenter of Ag that makes the la molecule a central focus in the study of Th cell activation.

Ia molecules are heterodimers, with both the \(\alpha\)- and \(\beta\)-chain composed of two extracellular domains, a transmembrane segment, and a cytoplasmic tail (3). Sequence analysis of several I-A alleles has revealed that a considerable polymorphism exists in the amino terminal domain (first domain) (4–7), and that these polymorphic regions tend to cluster in three (\(\alpha\)-chain) or four (\(\beta\)-chain) areas. Given that Ia Ag are known to be the Ir genes responsible for haplotype differences in the ability to respond to immunogens, these polymorphic regions have come under increasing scrutiny as the source of the differential Ir gene effects.

Several different approaches have been used to determine the role of Ia polymorphic regions in the presentation of Ag. One method has been to use anti-Ia mAb as inhibitory probes of Ag presentation (8–10). Inasmuch as many anti-I-A and anti-I-E mAb demonstrate haplotype specificity, it is reasonable to suggest that this haplotype specificity is based on the differences in the amino acid sequence of each of the Ia alleles. When these mAb were used as inhibitory probes of Ag presentation, several different patterns of T cell recognition of Ag in the context of I-A (8, 10) and I-E (9) were observed. These experiments demonstrated that the simple combination of an
antigenic peptide and an Ia molecule can generate multiple functional epitopes on the I-A and I-E molecules. That these multiple epitopes are likely TCR interaction sites on the Ia molecules is supported by two different observations. First, recent experiments based on the competitive binding of antigenic peptides by both solubilized I-A and I-E have indicated that there is only one Ag binding site on the I-A and I-E molecules (11, 12). Second, when anti-I-A \( ^{\alpha} \) mAb were used as inhibitors of the direct binding of Ag to solubilized I-A \(^{\alpha} \), two mAb that are known to block the presentation of antigen were found not to block the direct binding of Ag to I-A \(^{\alpha} \) (10).

The simplest explanation for this observation is that this latter type of mAb binding to the class II molecule blocks access of the TCR to contact residues on the class II molecule itself or on the bound peptide. Thus, although the structure of the class II molecule clearly influences both peptide binding as well as interaction with the TCR, the differential inhibition obtained with those mAb that do not block peptide binding most likely derives from the fact that TCR with the same general specificity for peptide and class II molecule clearly have different fine specificities and, consequently, interact with the same peptide-class II complex in different ways (13).

Several groups have recently taken the molecular biological approach to understanding the role of polymorphic regions in the structure and function of the I-A molecule (14–16). In one approach, a series of recombinant A\( ^{\alpha} \) genes were constructed by exchanging either the whole \( \beta 1 \) domain or half of the \( \beta 1 \) domain among the A\( ^{\alpha} \), A\( ^{\beta} \), and A\( ^{\gamma} \) genes (14). These genes were then transfected with haplotype matched or mismatched A genes, and once expressed, tested for their ability to present Ag to T cell hybrids of each haplotype restriction. Based on these experiments it was concluded that the polymorphisms in both the A\( ^{\alpha} \) and A\( ^{\beta} \)-chain contributed to the recognition of Ia by T cells and that the entire \( \beta 1 \) domain contributed to this interaction. In addition to the functional aspects, the ability or inability of these mismatched-recombinant I-A molecules to assemble is dependent on the polymorphic residues in the amino terminal portion of the first domain (17). Although these data supported the hypothesis that the polymorphic regions of the A\( ^{\alpha} \)-chain play an important role in the function of class II molecules, this approach did not allow any distinction as to the individual contributions of each polymorphic region.

Recently, two laboratories have begun to examine the contribution of each A\( ^{\alpha} \) and A\( ^{\beta} \)-polymorphic region to the structure of the I-A \(^{\alpha} \) molecule (15, 16). By systematically replacing the \( k \) haplotype polymorphic region resi- dues with corresponding \( d \) haplotype (A\( ^{d} \)) (16) or b haplotype (A\( ^{b} \)) residues (15), serologically dominant regions of the A\( ^{\alpha} \)- and the A\( ^{\beta} \)-chains have been identified. In some instances, the replacement of \( k \) sequences with \( d \) sequences (A\( ^{d} \)) or b sequences (A\( ^{b} \)) resulted in the acquisition of new or b specific mAb binding sites. Here, we report the capacity of these "chimeric" I-A molecules to function in Ag presentation. We have examined the ability of these A\( ^{d} \) and A\( ^{b} \) chimeric I-A molecules to present a variety of HEL \(^{\alpha} \) peptides to a panel of HEL-specific, I-A\( ^{\alpha} \)-restricted T cell hybrids, each expressing a different TCR. For both the A\( ^{d} \)- and the A\( ^{b} \)-chains, we have identified polymorphic regions that exert a dominant influence on the presentation of all HEL peptides examined, as well as in subdominant regions that have little or no influence on this Ag presentation system. In addition, APC expressing mutant I-A \(^{\alpha} \) molecules with known amino acid substitutions were also tested with this panel of T cell hybrids in an effort to relate specific residues with I-A \(^{\alpha} \) function.

Finally, we have related these data to the recently proposed structural model of class II molecules in an attempt to advance our understanding of the structural-functional aspects of I-A.

**MATERIALS AND METHODS**

*Cell lines.* All cell lines were maintained in vitro in RPMI 1640 ( Irvine Scientific, Irvine, CA) with 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, 20 \( \mu \)g/ml gentamicin, 5 \( \times \)10 \(^{-5} \) M 2-ME, 100 U/ml penicillin, and 100 \( \mu \)g/ml streptomycin (complete medium). The B cell lymphoma lines CH12.LX (CH12) (18) or TAD (19) were used as a source of wild type I-A \(^{\alpha} \)-expressing APC. The APC lines expressing the chimeric A\( ^{d} \)-chains were produced by cotransfecting chimeric A\( ^{d} \)-cDNA with wild type A\( ^{b} \)-cDNA and a herpes simplex virus tk gene into L cells (24-27) as previously described (15). The APC lines expressing the chimeric A\( ^{b} \)-chains were produced by cotransfecting the chimeric A\( ^{b} \)-cDNA with wild type A\( ^{\alpha} \)-cDNA and the pSR\( \beta \)neo neomycin resistance gene into M12.C3 cells (H-2\(^{a} \)) as previously described (18). Inasmuch as M12.C3 cells synthesize the A\( ^{\alpha} \)-chain, all chimeric A\( ^{\alpha} \)-APC were selected for expression of the A\( ^{\alpha} \)-chain with synthetic A\( ^{\alpha} \)-cDNA. The mAb A\( ^{d} \)- or A\( ^{b} \)-specific cell lines have been previously described (20). The mutant I-A \(^{\alpha} \)-cell line A19 was generously provided by Dr. L. Glimcher (Harvard University, Boston, MA). T cell hybrids were produced by immunization of B10.A(4R) mice (H-2\(^{a} \)) with either native HEL or a tryptic digest thereof and fusion of the stimulated lymph node cells with BW5147 cells as previously described (10). The IL-2 dependent cell line HT-2 (21) was maintained by passage every other day in complete medium supplemented to 25% with an IL-2-containing supernatant.

*Abbreviation used in this paper:* HEL, hen egg lysozyme.
is based on serial twofold dilutions, titers that differ by less than a factor of four are not considered significantly different.

**Immunofluorescence and flow cytometry.** I-A expression by chimeric and mutant APC was measured by direct immunofluorescence and flow cytometry before their use in Ag presentation experiments. APC (5 × 10⁴) were incubated with an appropriate dilution of fluorescein-conjugated anti-I-A mAb directed to either the A-α-chain (mAb H116-32 or 39J) (26) or the A-β-chain [10–3.8 (26) or with an anti-I-E-β-directed mAb, 1:4–45S (27), as a negative control, in 100 μl of PBS containing 0.1% azide for 30 min on ice. Cells were then washed three times with PBS, and fluorescence was measured by an Epics-C flow cytometer (Coulter, Hialeah, FL) or an Ortho 50H Cytofluorograph (Ortho Pharmaceutical, Raritan, NJ) using a 3-decade logarithmic scale.

**RESULTS**

**Determination of role of A-α-chain polymorphic regions in Ag presentation.** Chimeric I-A molecules were constructed by Landais et al. (15) and Buerstedde et al. (16) on the premise that regions of allelic polymorphism (polymorphic regions) play an important role in the function of class II molecules. Chimeric A-α-chains were produced by “cassette shuffling” segments of Aα- and Aβ-cDNA, producing all possible permutations of the three k and b allelic polymorphic regions (15). Each chimeric gene was transfected with a wild type Aβ-chain and the resulting cell lines were named for the genotype of the A-α-chain polymorphic regions. For example, the cell line “kkb” contains k haplotype amino acids in the first and third polymorphic regions and b haplotype sequences in the second polymorphic region (Fig. 1). In all instances nonpolymorphic residues are of the k haplotype.

APC bearing these α-chain chimeric I-A molecules were tested for their ability to present antigen to a panel of HEL-specific, I-Ak-restricted T cell hybridomas. Although the relative contribution of each polymorphic region to the T cell’s recognition of I-A and Ag varies among the T cell hybrids, there was a definite hierarchy among the three α-chain polymorphic regions in their influence on Ag presentation. As shown in Table I, changing the third polymorphic region of the A-α-chain (region III, Fig. 1) had the most profound effect on the presentation of HEL peptides to I-Ak-restricted T cell hybridomas. None of the A-α-chimeric molecules containing b haplotype residues in the third polymorphic region (cell line “kkb,” “kbk,” “kkb”) present Ag to any of the T cell hybridomas. Conversely, maintaining the k haplotype residues in only the third polymorphic region (cell line “kkb”) is all that is required for Ag presentation to five T cell hybridomas, although some of their responses are marginal (Table I). Substituting the second polymorphic region has less of an effect than changing the third polymorphic region. Five of the T cell hybridomas do not recognize the chimeric “kkb” molecule; however, maintaining the k haplotype in the second polymorphic region alone (“kbk”) was not sufficient for recognition by any of the T cell hybridomas (Table I). When the first polymorphic region sequence is changed to the b haplotype (cell line “kkk”), there is little or no effect on the presentation of HEL peptides to the T cell hybrids. While only one T cell hybrid (kLyll.6.1) did not respond to the “kkk” APC, seven of the remaining 12 T cell hybridomas respond to a level comparable or greater than to the “kkk” wild type control. None of the T cell hybridomas used recognizes antigen in the context of mixed molecules AαAβ, AβAα, or I-Ab molecules (data not shown). Finally, there is no clear relationship between HEL peptide specificity and a T cell hybrid’s recognition of the Aα-chimeric I-A molecules.

To determine if the decreased recognition of the “kbk” and “kkb” APC is related to the concentration of Ag, Ag titrations were performed with selected T cell hybridomas. As shown in Figure 2, increasing the antigen concentration to a maximum solubility of 30 mg/ml did not change the conclusions drawn from the data in Table I. The recognition of the AαAβ, Aαk, and Aβk molecules and Ag generally improves with increasing antigen concentration, with the exception of one of the four T cell hybrids selected. Although the T cell hybrid kLyll.6.1 responds very well to antigen in the context of I-Ak (Fig. 2c), even at 30 mg/ml of antigen it fails to respond to the AαAβ molecule (Fig. 2a). In contrast, the Ag presentation ability of the “kbk” chimeric APC is not affected by increasing the antigen concentration (Fig. 2b). None of the T cell hybridomas recognizes Ag in the context of this chimeric I-A molecule, even at 30 mg/ml.

One possible explanation for the lack of antigen presentation by three of the Aα-chimeric I-A molecules (“kkb,” “kkb,” and “kkb”) is that their cell surface expression of I-A is much lower than the rest of the chimeric APC. Analysis of I-A expression by immunofluorescence at the time the Ag presentation experiments were performed indicates that this is not a viable explanation. Cell lines “kbk” and “kkb” express wild type levels of I-A (mean log channel fluorescence of 470 and 461, respectively, compared to “kkk” wild type level of 482). The cell surface expression of I-A by the nonpresenting line “bkb” is 90 channels lower than the wild type, but is virtually identical to that of “kbk,” a good APC line, and is still 200 channels above the negative control values.

**T cell hybrid recognition of chimeric A-α-chain I-A molecules.** The role of the polymorphic regions of the Aα-chain in Ag presentation was also examined with the same panel of T cell hybridomas. The four polymorphic regions4 of the Aα-chain (Fig. 1) were systematically

4 When all allelic Aα-sequences are compared, four regions of polymorphism (8 to 14, 26 to 28, 61 to 67, and 85 to 89) are discernible. However, the Aα- and Aβ-chains differ by only a single amino acid in the 26 to 28 region and differ by two in the 75 to 78 region. Thus, for the purposes of this paper we number the the Aα-polymorphic regions as follows: first 9 to 14, second = 63 to 67 (Aα-numbering), third = 75 to 78 and fourth = 85 to 89. These correspond respectively to the “A,” “B,” “C” and “D” regions of Buerstedde et al. (15).
switched to those of the d haplotype by site directed mutagenesis (16). Transfected cells expressing the chimeric I-A molecules were named for the genotype of the individual polymorphic regions as described above for the chimeric A,-chains. Each chimeric A,-chain was expressed with a wild type Amd chain to get it expressed on the cell surface. As described above for the chimeric A,-molecules, the effect of increasing Ag concentration on the function of the chimeric A,-molecules was also assessed. With increases in antigen concentration the response of three of the four T cell hybrids to the "kkdk" APC improves in comparison to the data shown in Table II (3 mg/ml of Ag). As shown in Figure 2, increasing the Ag concentration improves the T-cell recognition of the A,‘kkk,kk-chain (Fig. 2d), although functionally it is still less effective than the wild type I-A* (Fig. 2f) or the "kkdk" chimeric APC (Fig. 2e). In contrast the response of only one T cell hybrid to the "kkdk" chimeric APC improves with increasing Ag. Two T cell hybrids (kLy2.9 and kLy10.2) achieved their apparent maximum response at 3 mg/ml of Ag, and the fourth one (h4Ly7.5) does not respond to either the

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**FIGURE 2.** Effect of Ag concentration on the ability of chimeric I-A molecules to present Ag. Ag presentation ability of transfected L cells expressing chimeric A,-molecules "kkb" (a), "kkk" (b), and the wild type "kkkk" (c) and transfected M12.C3 cells (B lymphoma) expressing chimeric A, molecules "kkdk" (d), "kkkd" (e) and the wild type "kkkk" (f), was tested with T-cell hybrids h4Ly7.5 (w), kLy7.11 (E), kLy7.11 (E), kLyl1.10 (O), kLy10.2 (A), and kLy2.9 (A).
**FUNCTIONALLY DOMINANT REGION OF I-A<sup>k</sup> IN Ag PRESENTATION**

### TABLE II

**T cell hybridoma responses to HEL peptides presented by cells bearing I-A<sup>k</sup> with chimeric A<sub>r</sub>-chains**

<table>
<thead>
<tr>
<th>T Cell Hybrid</th>
<th>Peptide Specificity</th>
<th>APC (β-chain genotype)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IL-2 (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>kkkk&lt;sup&gt;b&lt;/sup&gt;</td>
<td>kdkk</td>
</tr>
<tr>
<td>h4Ly7.5</td>
<td>34-45</td>
<td>&gt;1280</td>
<td>–</td>
</tr>
<tr>
<td>h4Ly50.5</td>
<td>46-61</td>
<td>&gt;1280</td>
<td>–</td>
</tr>
<tr>
<td>kLy4.3</td>
<td>46-61</td>
<td>640</td>
<td>160</td>
</tr>
<tr>
<td>kLy10.2</td>
<td>46-61</td>
<td>1280</td>
<td>&gt;1280</td>
</tr>
<tr>
<td>kLy7.11</td>
<td>46-61</td>
<td>&gt;1280</td>
<td>–</td>
</tr>
<tr>
<td>kLy11.10</td>
<td>46-61</td>
<td>&gt;1280</td>
<td>–</td>
</tr>
<tr>
<td>kLy11.11</td>
<td>74-96</td>
<td>1280</td>
<td>–</td>
</tr>
<tr>
<td>kLy2.9</td>
<td>74-96</td>
<td>&gt;1280</td>
<td>–</td>
</tr>
<tr>
<td>kLy11.3</td>
<td>74-96</td>
<td>1280</td>
<td>–</td>
</tr>
<tr>
<td>kLy11.6.1</td>
<td>74-96</td>
<td>160</td>
<td>–</td>
</tr>
<tr>
<td>kLy18.4.1</td>
<td>Tryptic&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>kLy6.10</td>
<td>Tryptic&lt;sup&gt;c&lt;/sup&gt;</td>
<td>160</td>
<td>–</td>
</tr>
<tr>
<td>kLy11.12</td>
<td>Tryptic&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>h4Ly18.2.1</td>
<td></td>
<td>&gt;1280</td>
<td>–</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ag presentation experiments were performed as described in Materials and Methods. A concentration of 3 mg/ml of Ag was used in all experiments.

<sup>b</sup> The four letters represent the allelic form of each region of the A<sub>r</sub>-chain in which k differs from d: first letter = positions 9 to 17; second letter = positions 63 to 67 (A<sup>α</sup> numbering); third letter = positions 75 to 78; and fourth letter = positions 85 to 89. All chimeric A<sub>r</sub>-chains shown are paired with A<sup>α</sup>-chains.

<sup>c</sup> HEL(74-96) peptide specificities not yet confirmed with synthetic peptide.

<sup>d</sup> Responds to an unidentified HEL tryptic fragment.

<sup>e</sup> Responds to native HEL after processing by APC; does not respond to tryptic digest of HEL.

### TABLE III

**T cell hybridoma responses to HEL peptides presented by cells bearing I-A<sup<k</sup> molecules with serologically selected mutant A<sub>r</sub>-chains**

<table>
<thead>
<tr>
<th>T-Hybrid</th>
<th>HEL Peptide Specificity</th>
<th>IL-2 (U/ml)</th>
<th>A&lt;sub&gt;r&lt;/sub&gt; Mutant APC&lt;sup&gt;a&lt;/sup&gt; (position: interchange)</th>
<th>Wild Type I-A&lt;sup&lt;k&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>h4Ly7.5</td>
<td>34-45</td>
<td>&gt;1280</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
<td>160</td>
</tr>
<tr>
<td>h4Ly50.5</td>
<td>46-61</td>
<td>&gt;1280</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1020</td>
</tr>
<tr>
<td>kL4.10</td>
<td>46-61</td>
<td>–</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80</td>
</tr>
<tr>
<td>kL7.4</td>
<td>46-61</td>
<td>–</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
<td>640</td>
</tr>
<tr>
<td>kL11.10</td>
<td>46-61</td>
<td>–</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
<td>640</td>
</tr>
<tr>
<td>kL1.1</td>
<td>74-96</td>
<td>1280</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1280</td>
</tr>
<tr>
<td>kL2.9</td>
<td>74-96</td>
<td>&gt;1280</td>
<td>–&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;1280</td>
</tr>
<tr>
<td>kL11.3</td>
<td>74-96</td>
<td>40</td>
<td>640</td>
<td>640</td>
</tr>
<tr>
<td>kL11.6.1</td>
<td>74-96</td>
<td>–</td>
<td>–</td>
<td>320</td>
</tr>
<tr>
<td>kL6.10</td>
<td>Tryptic&lt;sup&gt;c&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>80</td>
</tr>
<tr>
<td>kL11.12</td>
<td>Tryptic&lt;sup&gt;c&lt;/sup&gt;</td>
<td>320</td>
<td>1280</td>
<td>1280</td>
</tr>
<tr>
<td>h4Ly18.2.1</td>
<td></td>
<td>398</td>
<td>747</td>
<td>702</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ag presentation experiments were performed as described in Materials and Methods. A concentration of 3 mg/ml of Ag was used in all experiments.

<sup>b</sup> HE(74-96) peptide specificities not yet confirmed with synthetic peptide.

<sup>c</sup> Responds to an unidentified HEL tryptic fragment.

<sup>d</sup> Responds to native HEL after processing by APC; does not respond to tryptic digest of HEL.

In a nonconservative change [Glu → Lys] within the third polymorphic region, it is generally not disruptive to antigen presentation (Table III), as 11 of the 12 T cell hybrids tested responded to HEL in the context of this mutant I-A<sub>r</sub>-chain.

The effects on Ag presentation of a series of mutations in the A<sub>r</sub>-chain were also examined (Table IV). These mutations, all single amino acid changes in the first domain of the A<sub>r</sub>-chain (20), range from conservative substitutions (cell line F16) to nonconservative charge substitutions (cell lines G1, K5, and A19) (Table IV). As might be predicted, a conservative amino acid substitution occurring outside of the second polymorphic region has little or no effect on the ability of the I-A<sup<k</sup> molecule to present antigen (Glu → Asp at residue 59, cell line F16,}
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Table IV). However, two mutant APC, each containing a nonconservative charge substitution located between the second and third polymorphic regions and near the second polymorphic region of the \(\alpha\)-chain (Glu \(\rightarrow\) Lys at residue 69, cell line A19, and Arg \(\rightarrow\) Gln at residue 70, cell line K5), have drastically altered function as measured by T cell hybrid recognition of I-A and Ag. These two APC present Ag to only three of 12 and five of 12 T cell hybrids tested, respectively. Surprisingly, two different mutations at the same residue within the second polymorphic region (residue 64, cell lines G1 and LD3) do not have a major impact on the ability of the mutant I-A\(^b\) molecules to present Ag. Only 4 of the 12 T cell hybrids have difficulty recognizing Ag and the mutant I-A\(^b\) on the G1 and the LD3 mutant APC (Table IV).

**DISCUSSION**

Mutant and chimeric class II molecules present a unique system for the study of the structure-function relationships of I-A molecules. While earlier studies with mutants in the HEL model system (28) suffered from the lack of detailed knowledge of the structural basis for the mutations and of the overall structure of MHC molecules themselves, they did allow one to begin to enumerate the number of determinants generated by the interaction between an Ag and a class II molecule. The last 3 yr have seen an explosion in the knowledge of the sequences of the mutants (15, 20), of MHC structure (29, 30), and of the interaction of peptides with class II molecules (31, 32). The infusion of this ancillary data clearly allows a more focused discussion of the present results than would have been possible previously.

The working model for our analysis is based on the recently proposed molecular model for class II molecules (33) that is based on the x-ray diffraction-derived structure of a class I molecule (29). A top view of this model is presented in Figure 3. The model shows a series of eight strands of \(\beta\)-pleated sheet, four each contributed by the \(\alpha\), and \(\alpha\)-chains. These form the floor of the proposed Ag binding cleft (30, 33). Lying on top of these are two extended \(\alpha\)-helical regions, one each contributed by the A, and \(\alpha\)-chains, which form the walls of the putative Ag-binding cleft and which are proposed to make contact with the TCR (30, 33). Figure 3 shows the approximate locations of the polymorphic residues comprising the six polymorphic regions that were examined in these studies (Fig. 3A), and the approximate locations of the residues which are altered in the serologically selected mutants (Fig. 3B). The following analysis is based on two assumptions: 1) that the proposed structure for class II molecules is reasonably accurate, and 2) that substitutions, either of single amino acids or of entire polymorphic regions (that contain two to five amino acid differences each), cause only localized perturbations of the proposed structure, i.e., there are no significant long-range conformational changes. Although the latter assumption is unproven, recent serologic studies of mutant and chimeric I-A molecules (16, 34) suggest that it is a reasonable one. In addition, it is also a reasonable assumption given the proposed structure of the class II molecule, i.e., the existence of stretches of identical residues between the polymorphic regions or point mutations which serve to stabilize the \(\alpha\)-helical structures and anchor them to the "floor" of the \(\beta\)-pleated sheet.

The polymorphic region interchanges that have the most profound effect on Ag presentation, viz., the \(b\) for \(k\) substitution of the third polymorphic region of the \(\alpha\)-chain and the \(d\) for \(k\) substitution of the second and third polymorphic regions of the \(\beta\)-chain, occur in locations that lie across from one another on the \(\alpha\)-helical walls in the right hand portion of the proposed Ag-binding cleft (see Fig. 3). These observations, coupled with the observation that substitution of \(b\) allelic residues in the first and second polymorphic regions of the A,\(^b\)-chain or \(d\) allelic residues in the fourth polymorphic regions of the A,\(^b\)-chain cause significantly less disruption of the ability to present Ag, argues that the right-hand section of the I-A\(^b\) molecule, as viewed in Figure 3, plays the dominant role in the presentation of HEL fragments to T cell hybridomas specific for these peptides.

This conclusion is supported by other studies using the chimeric A,\(^b\)-APC in an insulin-specific, I-A\(^b\)-restricted
FUNCTIONALLY DOMINANT REGION OF I-A\(^b\) IN Ag PRESENTATION

Figure 3. Hypothetical model (32) of the top surface of the I-A molecule. The model shows nearly the entire first domain of the A\(_{\alpha}\)- and A\(_{\beta}\)-chains with the approximate amino termini as indicated. Each domain consists of four \(\beta\)-strands which together form the floor of the postulated Ag-binding cleft. The second half of the first domain of the A\(_{\alpha}\)-chain forms the \(\alpha\)-helix in the upper portion whereas the comparable region of the A\(_{\beta}\)-chain forms the lower helix. These \(\alpha\)-helices form the walls of the proposed antigen binding cleft. The stippled areas represent regions in which the class II sequence diverges sufficiently from the class I sequence upon which the model is based such that it is impossible to predict the class II structure. A. When comparing the \(k\) and \(b\) haplotype sequences, the three polymorphic regions of the A\(_{\alpha}\)-chain include residues 11 and 15 (first), residues 53, 56, 57, and 59 (second) and residues 69, 70, 75, and 76 (third). The four polymorphic regions of the A\(_{\beta}\)-chain, as determined by differences in the \(k\) and \(d\) haplotypes, include residues 9, 12, 13, 14, and 17 (first, not shown), 63, 65, 66, and 67 (second), 75 and 78 (third) and 85, 86, and 89 (fourth). B. The residue numbers of each chain indicate the position of the serologically selected, substitution mutations described in Table III (A\(_{\alpha}\)) and Table IV (A\(_{\beta}\)). The encircled numbers indicate the predicted location of the side chains of the appropriate amino acid residues (modified from Ref. 32).

presentation system, in allosecreative recognition of the chimeric A\(_{\alpha}\)-APC, and through the use of anti-I-A\(^b\) mAb inhibition of Ag presentation. The third polymorphic region of the A\(_{\beta}\)-chain is also dominant in the I-A\(^b\)-restricted recognition of insulin,\(^6\) although its dominance is less pronounced than in the I-A\(^b\)-restricted presentation. The A\(_{\beta}\)-polymorphic region dependence of a small panel of I-A\(^b\)- and I-A\(^b\)-restricted allosecreative T cells is variable, similar to the I-A\(^b\)-restricted recognition of Ag. Allosecreative T cells dependent on the second polymorphic region of the A\(_{\alpha}\)-chain alone, dependent on several polymorphic regions, as well as some independent of the second, third, and fourth polymorphic regions have all been described in this system (34). Finally, the inhibition of Ag presentation and/or peptide binding by mAb directed against epitopes that map to the third polymorphic region of A\(_{\alpha}\) (15) or the second polymorphic region of A\(_{\beta}\) (16) supports the proposed functional relevance of the right-hand portion of the antigen binding cleft in HEL peptide presentation (10, 35).

The regions of the class II molecule that are functionally dominant in the HEL system are also regions of serologic immunodominance (15, 16). Substitution of \(b\) allelic residues in the third polymorphic region of the A\(_{\alpha}\)-chain results in the loss of the two A\(_{\alpha}\)-defined serologic epitopes (15), and \(d\) residue substitutions in the second polymorphic region results in the loss of two of the three A\(_{\beta}\)-defined epitopes (16). Despite the correspondence between the dominant regions defined by mAb and the functionally dominant regions described in this report and elsewhere (34) (see footnote 5), there are differences between the manner in which the TCR and mAb detect the class II molecule. In general, the mAb-defined epitopes appear to be more localized than those defined by Ag presentation. Thus, whereas the third polymorphic region of the A\(_{\alpha}\)-chain appears to control a major portion of the serology of the I-A\(^b\) molecule, individual TCR appear to be sensitive to changes at more that one polymorphic region of the I-A\(^b\) molecule. For example, several of the T cell hybrids in Table II are sensitive to changes in the second, third, and fourth polymorphic regions of the A\(_{\beta}\)-chain even though the second and fourth polymorphic regions are at opposite ends of the \(\beta\)-chain \(\alpha\)-helix, approximately 25 Å apart. Similar results have recently been reported for the recognition of mutant MHC class I molecules by mAb and by allosecreative CTL (36).

Neither the present study nor the use of mAb to inhibit Ag presentation allows one to discriminate between the possibilities: 1) that the effects observed are a reflection of disrupting the interaction of the TCR with the I-A\(^b\) molecule or with the bound peptide, 2) that the effects are due to disruption of the ability of I-A\(^b\) to bind the peptide Ag, or 3) a combination of the above. However, as the data presented in Tables I and II demonstrate, the complete failure of chimeric molecules with the third polymorphic region interchanges in the A\(_{\alpha}\)-chain and the drastically reduced capacity of those with the second and third polymorphic region interchanges in the A\(_{\beta}\)-chain to present Ag raises the possibility that HEL peptides may bind in this region in wild type I-A\(^b\). This possibility is supported by our observation that mAb directed to the second polymorphic region of the A\(_{\alpha}\)-chain (20) block binding of HEL(46-61) to wild type I-A\(^b\). However, Brown et al. (33) have postulated that the HEL(46-61) peptide binds in the left-hand portion of the cleft. Direct binding experiments using the chimeric I-A molecules used in the current experiments will discriminate between these two possible binding locations within the Ag-binding cleft. Such studies are currently in progress.

The proposed model for class II structure also allows a
FUNCTIONALLY DOMINANT REGION OF I-A<sup>α</sup> IN Ag PRESENTATION


