

MHC CLASS II-DERIVED PEPTIDES CAN BIND TO
CLASS II MOLECULES, INCLUDING SELF MOLECULES,
AND PREVENT ANTIGEN PRESENTATION

BY EDWARD F. ROSLONIEC,* LAURA J. VITEZ,*
SOREN BUUS,* AND JOHN H. FREED*†

*From the *Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206; and the †Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80262*

The discrimination of self from non-self is an integral function of the immune system. Both class I and class II molecules of the MHC play a central role in this discrimination in that these molecules serve as the self recognition structures for the TCR during the presentation of foreign antigens (1, 2). As antigen presentation structures, the class I and class II molecules selectively bind peptides that presumably are the result of intracellular degradation or "processing" (3-7). Although the TCR is capable of discriminating self from non-self, MHC molecules do not appear to make such a discrimination in that it is now clear that degradation of self proteins can also result in peptides that can be bound by autologous class II (8-10) and presumably by class I molecules.

Recent studies from a number of laboratories have suggested that class I-derived peptides can be bound by class I molecules. Examples of this include the binding of human class I (HLA-C)-derived peptides to murine class I (H-2) molecules (11, 12), the binding of H-2L^d-derived peptides to H-2D^k or H-2D^{dm1} (13, 14), and the binding of an HLA-A2-derived peptide to HLA-Aw69 (15, 16). Although each of these systems represents an artificial situation in which the class I molecule is exposed to a peptide that it would not normally encounter, they do demonstrate the feasibility of class I-derived peptides being bound by intact class I molecules. Furthermore, they raise the possibility that such binding could occur normally in the nonmanipulated animal.

We have extended these observations to the class II system in a manner that might better reflect naturally occurring antigen processing and presentation. Specifically, we asked whether class II molecules contain peptide sequences that are capable of binding to their own class II or other class II alleles. We have identified synthetic peptides based on the sequences from the polymorphic regions of the I-A^k molecule that bind to both I-A^k and I-A^d. These peptides competitively block the presentation of antigen to T cell hybrids of different peptide specificities, as well as com-

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petitively inhibit the direct binding of foreign peptides to the solubilized class II molecules. These data support the conclusions that self peptides can be presented by class II molecules (8, 17, 18) and also support speculations that degraded MHC may constitute some of the determinants recognized by auto- and alloreactive T cells (13, 15, 16, 19).

Materials and Methods

Cell Lines. Cell lines used in these studies were maintained *in vitro* in RPMI 1640 (Irvine Scientific, Irvine, CA) with 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, 20 $\mu\text{g}/\text{ml}$ gentamicin, 5×10^{-5} M 2-ME, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin (complete medium). The B cell lymphoma lines CH12.LX (CH12) (20) was used as a source of I-A^k-expressing APC and A20-1.11 (A20) (21, 22) cells were used as a source of I-A^d-expressing APC. The T cell hybrids used in these studies are either I-A^k restricted and specific for either the 34-45, 46-61, or 74-96 fragment of HEL (23), or I-A^d restricted and specific for the 323-339 fragment of OVA (24). HT-2 cells (25) were maintained by passage every other day in complete medium supplemented to 25% with an IL-2-containing supernatant.

Antigen Presentation Assay. Antigen presentation experiments were performed in 96-well microtiter plates in a total volume of 0.3 ml containing 10^5 APC, 10^5 T cell hybridoma cells, and 50 μl of a solution of trypsinized HEL or a synthetic peptide representing the OVA sequence 323-339. The amounts of the HEL and OVA antigens used were selected on the basis of antigen dose-response curves for each T cell hybrid and were 10-30 μg of HEL digest or 100 ng of OVA (323-339). Before their addition to the antigen presentation cultures the APC were fixed with 0.5% paraformaldehyde in PBS for 20 min and washed three times. The ability of a PMR peptide to competitively inhibit the presentation of the HEL and OVA antigenic peptides was assessed by direct addition of 2-400 μg of the inhibitory peptide to the antigen presentation culture. In some experiments T cell hybridomas or fixed APC were prepulsed with the inhibitory peptides for 4 h, washed, and added to the antigen presentation cultures. Cell cultures were maintained at 37°C in 5% humidified CO₂ for 20-24 h after which seven 80- μl twofold serial dilutions were made for determination of IL-2 titers. 4×10^3 HT-2 cells were added to each supernatant dilution, and after 16-20 h HT-2 cell viability was evaluated by visual inspection. IL-2 titers were defined as the reciprocal of the highest twofold serial dilution maintaining 90% viability of the HT-2 cells. Results are presented as units of IL-2 per milliliter of undiluted supernatant, as described by Kappler et al. (26). Since the IL-2 titer determinant is based on serial twofold dilutions, titers that differ by a factor of four or less are generally considered not significantly different.

T Cell Hybridomas. T cell hybridomas were made by fusing antigen-stimulated lymph node cells from B10.A(4R) mice to BW5147 cells with polyethylene glycol (PEG 1450, 1,300-1,600 MW; Eastman Kodak Co., Rochester, NY) as previously described (27). T cell hybrids h4Ly7.5, h4Ly50.5, and kLy1.1 are I-A^k restricted and specific for peptides HEL(34-45), HEL(46-61), and HEL(74-96), respectively (23). I-A^d-restricted T cell hybrids DO-11.10, 8DO-51.15, and 3DO-54.8 are all specific for the OVA peptide OVA(323-339) (24), although each T cell hybrid recognizes a different epitope on this peptide (Marrack, P., National Jewish Center for Immunology and Respiratory Medicine, personal communication).

Antigen. Trypsinized HEL was produced by initially dissolving 1.5 g of native HEL in 100 ml of 8 M urea, 0.2 M 2-ME and stirring overnight at room temperature. The reduced, denatured antigen was alkylated with 0.3 M iodoacetic acid while maintaining the pH near 8.0 by the addition of 2 M NaOH. The antigen was subsequently dialyzed against several changes of 5 mM Tris/HCl, pH 7.4, followed by 0.1 M (NH₄)HCO₃, and lyophilized. Trypsinization of the denatured HEL was done at 37°C in 0.1 M (NH₄)HCO₃ with two addi-

¹ *Abbreviations used in this paper:* HEL, hen egg lysozyme; HEL(46-61), a synthetic peptide corresponding to the sequence of lysozyme between residues 46 and 61; OVA, ovalbumin; OVA(323-339), a synthetic peptide corresponding to the sequence of ovalbumin between residues 323 and 339; PMR, polymorphic region.

tions of trypsin (1% wt/wt) (Trypsin-TPCK; Cooper Biomedical, Malvern, PA) over 24 h, followed by lyophilization.

Peptides representing the native OVA(323-339) and HEL(46-61) sequences and the seven polymorphic regions of the I-A^k molecule (Table I) were synthesized by the general solid phase method outlined by Merrifield et al. (28) on a peptide synthesizer (model 430A; Applied Biosystems, Foster City, CA) as described by Buus et al. (29). The synthetic peptides were purified by reverse-phase HPLC using a Vydac (Hesperia, CA) C₁₈ semi-preparative column and a gradient of 0-50% acetonitrile in 0.1% trifluoroacetic acid. The purified synthetic peptides were analyzed for amino acid composition and their amino acid sequences were determined; they were found to be >90% pure by these criteria.

I-A Binding Assay. I-A binding experiments were performed as described by Buus et al. (30). I-A^k and I-A^d were affinity purified from lysates of AKTB-1b and A20 lymphoma cells, respectively, as previously described (29). The antigenic peptides HEL (46-61) and OVA(323-339) were synthesized as described above with the addition of a tyrosine residue on the amino terminus for purposes of ¹²⁵I-labeling (31). Each binding assay consisted of 5-10 μg of I-A^k or I-A^d, 10 ng (0.5 μM) of ¹²⁵I-labeled peptide, and 10-1,000 μM of the competitive PMR peptides. After a 40-48-h incubation at room temperature the percent HEL(46-61) or OVA(323-339) peptide bound was determined by G-50 column chromatography (30).

Results

Peptides Corresponding to the Polymorphic Regions of Class II Can Block Presentation of Antigenic Peptides to T Cell Hybridomas. Peptides corresponding to the seven polymorphic regions (PMR) of the α1 and β1 domains of the I-A^k molecule were synthesized according to the sequences shown in Table I; the *d* allelic sequences are included for comparison. The *k* and *d* alleles exhibit between one and five amino acid differences in these peptides. The synthetic peptides were chosen such that the polymorphic

TABLE I
Amino Acid Sequences of I-A^k and I-A^d Polymorphic Region Peptides

Peptide (residues)*	Haplotype	Sequence†
α-1 (7-20)	<i>k</i>	DHVGSYG I TVYQSP
	<i>d</i>	----V--T-----
α-2 (50-64)	<i>k</i>	PEFAQLRRFEPQGGL
	<i>d</i>	---G---IL-----
α-3 (64-81)	<i>k</i>	LQNIATGKHNLEILTKRS
	<i>d</i>	-----AE-----G-----
β-1 (4-19)	<i>k</i>	ERHFVHQFQPCYFTN
	<i>d</i>	-----V--KGE--Y--
β-2 (20-35)	<i>k</i>	GTQRIRLVIRYIYNRE
	<i>d</i>	-----T-----
β-3 (57-74)	<i>k</i>	DAEYWNKQ*Y*LERTRAE
	<i>d</i>	-----S-PEI-----
β-4 (80-95)	<i>k</i>	RHNYEKTETPTSLRRL
	<i>d</i>	-----GP--S-----

* Residue numbers are based on *d* haplotype numbering.

† Dashes indicate identity between the two sequences and asterisks indicate genetically deleted residues.

residues tend to be in the center of the peptides with the overall length of the peptides ranging from 14 to 18 amino acids each.

Each of the k haplotype PMR-derived peptides was tested for its ability to inhibit the presentation of tryptic fragments of hen egg lysozyme (HEL) to a set of T cell hybridomas, each of which is specific for a different HEL peptide (23) and restricted by the I-A^k molecule. As the results in Fig. 1 show, peptides corresponding to the first and third PMR of the A _{α} ^k chain (α^k -1 and α^k -3, respectively) inhibit, in a dose-dependent manner, the presentation of the HEL(34-45) peptide to the h4Ly7.5 T-cell hybridoma (*panel A*), of HEL(46-61) to the h4Ly50.5 hybridoma (*panel B*), and of HEL(74-96) to the kLy1.1 hybridoma (*panel C*). As shown in Fig. 1, the α^k -2 peptide showed no inhibitory activity. Similarly, all peptides corresponding to the A _{β} ^k chain PMR are noninhibitory (data not shown).

To ascertain the specificity of these inhibitions, the full set of peptides corresponding to the PMR of the A _{α} ^k and A _{β} ^k chains were tested for their ability to inhibit the presentation of the OVA(323-339) peptides to three I-A^d restricted T cell hybridomas. Although each of these hybridomas is specific for the OVA (323-339) peptide (3), each exhibits a different fine specificity for the antigen as determined by truncation and amino acid substitution studies (Marrack, P., personal communication). The data from these experiments, shown in Fig. 2, reveal the unanticipated result that the α^k -1 peptide is strongly inhibitory to the presentation of the OVA peptide

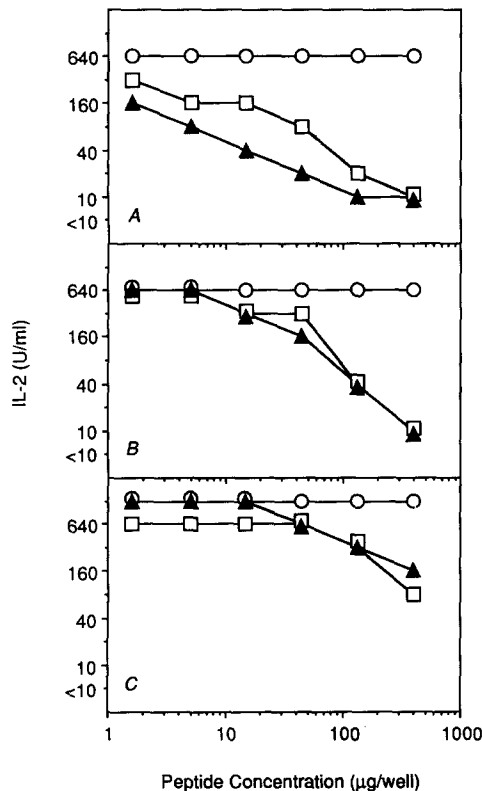


FIGURE 1. Inhibition of I-A^k-restricted antigen presentation by I-A^k PMR-derived peptides. Synthetic peptides based on I-A^k PMR sequences α^k -1 (□), α^k -2 (○), α^k -3 (▲) were tested for their ability to inhibit the presentation of HEL tryptic peptides HEL(34-45), HEL(46-61), and HEL(74-96) to I-A^k-restricted T cell hybrids h4Ly7.5 (A), h4Ly50.5 (B), and kLy1.1 (C), respectively. Before the antigen presentation assays, APC (CH-12) were fixed with 0.5% paraformaldehyde. The HEL antigenic peptides and the inhibitory α^k peptides were present for the duration of the 24-h antigen presentation culture. IL-2 production was assessed by the ability of twofold dilutions of culture supernatants to support the growth of the IL-2-dependent cell line, HT-2.

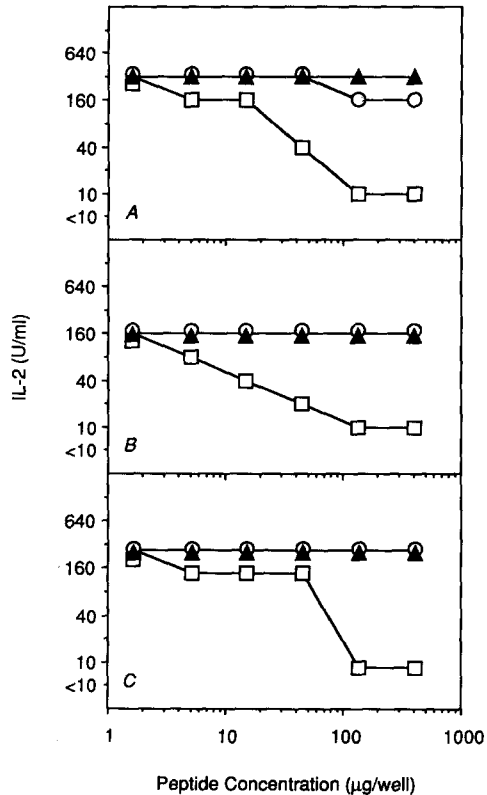


FIGURE 2. Inhibition of I-A^k-restricted antigen presentation by I-A^k PMR-derived peptides. Synthetic peptides α^k -1 (\square), α^k -2 (\circ), α^k -3 (\blacktriangle) were tested for their ability to inhibit the presentation of OVA(323-339) to I-A^k-restricted T cell hybrids DO-11.10 (A), 8DO-51.15 (B), and 3DO-54.8 (C). Before the antigen presentation assays, APC (A20) were fixed with 0.5% paraformaldehyde. The OVA peptide and the inhibitory α^k peptides were present for the duration of the 24-h antigen presentation culture. IL-2 production was assessed as described in Fig. 1.

to all three hybridomas. The peptides corresponding to the PMR of the A β^k chain were noninhibitory at all doses tested (data not shown).

Prepulsing the APCs but not the T Cell Hybridomas with PMR Peptides Blocks the Presentation of HEL Peptides. To establish whether the inhibition of class II-mediated antigen presentation by PMR peptides is homologous to that previously reported for the class I-mediated processes (15, 16), either the APCs or the HEL-specific T cell hybridomas were prepulsed with the A α^k chain PMR peptides. In contrast to the results reported for the class I-specific system in which inhibition of cytotoxicity could be blocked by prepulsing the CTL with the appropriate class I PMR peptide (16), we found that prepulsing the I-A^k-restricted HEL-specific peptides had no effect on the subsequent presentation of HEL peptides to the hybridomas (Fig. 3 A). However, as shown in Fig. 3 B, prepulsing of the APC with the α^k -1 and α^k -3 peptides produced a degree of inhibition of antigen presentation comparable to that seen in the direct competition experiments (see Fig. 1), whereas the α^k -2 peptide again had no effect on the presentation of the HEL peptides.

Synthetic Peptides Corresponding to the First and Third PMR of the A α^k Chain Bind Directly to the I-A^k Molecule. The most obvious explanation for the inhibition of antigen presentation observed when prepulsing the APC but not the T cell hybridomas with the PMR peptides is that the A α^k PMR peptides bind to the I-A^k molecule itself, therefore preventing the binding of the HEL peptides. This possibility was tested

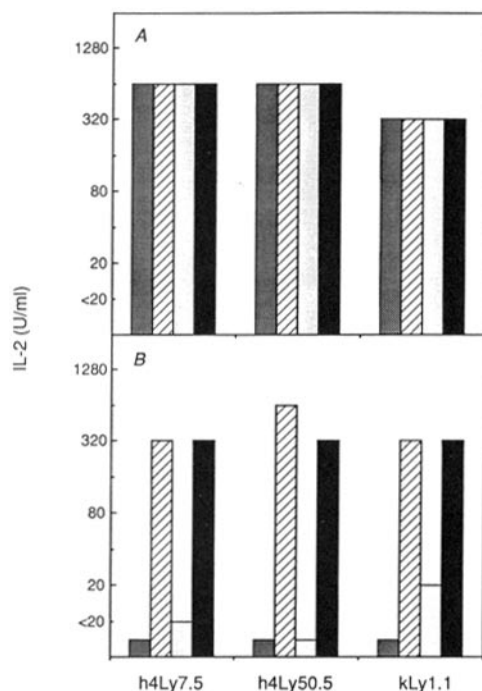


FIGURE 3. Determination of mode of antigen presentation inhibition by pre-pulsing T-cell hybrids and APC with I-A^k PMR-derived peptides. T cell hybrids (A) or fixed CH-12 APC (B) were prepulsed with either α^k-1 (▨), or α^k-2 (▩), or α^k-3 (▧), or no peptide (■) for 4 h and then washed before use in antigen presentation cultures. In the antigen presentation cultures prepulsed T cell hybrids were mixed with normal fixed APC (A) or prepulsed APC were mixed with normal T cell hybrids (B). HEL antigenic peptides were then added to the presentation cultures and were present throughout the 24 hour culture period. IL-2 production was assessed as described in Fig. 1.

by determining the ability of the PMR peptides to compete with ¹²⁵I-labeled HEL(46-61) peptide for binding to purified I-A^k molecules. As shown in Fig. 4 A, the α^k-1 and α^k-3 peptides blocked the binding of the ¹²⁵I-labeled HEL(46-61) indicator peptide although not quite as efficiently as the autologous HEL(46-61) peptide. Consistent with the results of the antigen presentation-inhibition experiments (Fig. 1), the α^k-2 peptide did not alter the binding of the ¹²⁵I-HEL(46-61).

Direct binding competitions were also carried out between ¹²⁵I-OVA(323-339) and the PMR peptides of the A α^k chain for binding to purified I-A^d molecule. The OVA(323-339) peptide, an immunodominant peptide in the H-2^d haplotype, binds strongly to the I-A^d molecule (3, 29, 30, 32). Nevertheless, the α^k-1 peptide successfully competes on a mole for mole basis with the ¹²⁵I-OVA(323-339) peptide for binding to purified I-A^d molecules, as shown in Fig. 4 B. Modest inhibition of binding of the ¹²⁵I-OVA(323-339) peptide is also obtained using the highest concentration of the α^k-3 peptide (see Fig. 4 B). These results closely parallel the antigen presentation results in Fig. 2 in which the α^k-1 peptide strongly inhibits presentation of the OVA(323-339) peptide to all three hybridomas tested and the α^k-3 peptide is weakly inhibitory to the presentation of antigen to the DO-11.10 hybridoma. In both the I-A^d and the I-A^k binding experiments the G-50 column chromatography data gave no indication of the existence of a PMR peptide-antigenic peptide complex (data not shown).

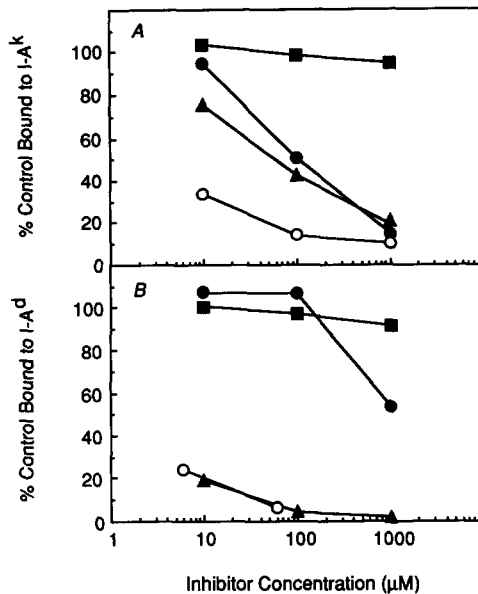


FIGURE 4. Inhibition of antigenic peptide binding by I-A^k and I-A^l with peptides derived from the PMR of I-A^k. Detergent solubilized, affinity purified I-A^k and I-A^l were tested for their ability to bind ¹²⁵I-HEL(46-61) (A) and ¹²⁵I-OVA(323-339) (B), respectively, in the presence of varying concentrations of the α^k-1(▲), α^k-2(■), α^k-3(●) peptides or homologous unlabeled HEL(46-61) (O, A) and OVA(323-339) (O, B) peptides. After 48-h incubations of I-A and peptides, the percent of labeled peptide bound to the I-A molecule was determined by fractionation over a G-50 Sephadex column. The percent of labeled peptide bound in the absence of peptide inhibitors was 14.3% for I-A^k and HEL(46-61) and 16.8% for I-A^l and OVA(323-339).

Discussion

A number of laboratories, using techniques from immunology, biochemistry, and x-ray crystallography, have produced data that suggest that the molecular basis for "MHC-restricted" presentation of antigen to T cells is the direct physical binding of proteolytic fragments of the antigen to the appropriate MHC molecule ("restriction element"), followed by the recognition of this complex by the appropriate antigen-specific, MHC-restricted TCR. While this model is a currently accepted paradigm for antigen-specific T cell recognition, a number of questions remain about other physiologically relevant and presumably related forms of T cell recognition, such as TCR-mediated positive and negative selection in the thymus and recognition of alloantigens by peripheral T cells. A significant portion of the uncertainty surrounding these latter processes derives from uncertainty over the exact ligand for the TCR, i.e., does the TCR recognize only MHC with bound peptide or can MHC molecules devoid of peptide also be recognized? If such "empty" MHC molecules exist and are recognized, in which compartments do they exist and what is the physical basis for their recognition by TCR that normally recognize a complex between peptide and MHC? Alternatively, if MHC molecules always have a peptide in the antigen binding cleft, what proportion of MHC molecules are occupied by self peptides, and is there a subset of self peptides, such as MHC-derived peptides, that are preferentially bound? Clearly, the answers to these questions will have a major impact on the issues of thymic selection and alloreactivity.

The pioneering studies of Babbitt et al. (33) demonstrated that self peptides can be bound by class II MHC molecules, and the more recent studies by Lorenz and Allen (8) have provided compelling evidence that this process can occur in vivo, at

least for hemoglobin or peptides derived from it. Based on these observations and the related observations that peptides derived from one class I molecule can be bound and presented by another class I molecule (11-13, 15), we decided to explore whether a given class II MHC molecule could bind peptides derived from its own sequence. If this should prove to be the case, it would raise the possibility that what had previously been interpreted as the binding of TCR to an "empty" class II molecule was in fact the binding of TCR to a class II molecule that had bound a proteolytic fragment derived from its own sequence.

The experiments reported here demonstrate unequivocally that the class II molecule I-A^k can bind synthetic peptides (α^k -1 and α^k -3) that consist of sequences derived from the I-A^k molecule itself. This binding was measured by the functional inhibition of the presentation of immunodominant HEL peptides to HEL-specific T cells by the synthetic peptides and by the inhibition of direct binding of the HEL(46-61) peptide to the I-A^k molecule by the same peptides that caused inhibition of antigen presentation. Of equal importance however are the results demonstrating that an I-A^k-derived peptide (α^k -1) can bind to the I-A^d molecule. These observations raise the possibility that the processing and presentation of polymorphic MHC molecules or perhaps of any polymorphic molecule may play a role in the generation of alloantigenic determinants. In addition, the direct binding data suggest that the affinity of interaction between the I-A^k and I-A^d molecules and the I-A^k-derived peptides can be quite high, perhaps on the order of 10^{-5} M based on the reported $K_d = \sim 2 \times 10^{-6}$ M for the binding of HEL(46-61) to the I-A^k molecule (33).

Our results are consistent with the recent data of Murphy et al. (34), which these authors prefer to interpret as the serological detection of an I-E-derived peptide bound to the I-A^b molecule. Assuming that the preferred interpretation of Murphy et al. is correct, this would constitute another example of a self class II-derived peptide binding to a self class II molecule although of a different isotype than the bound peptide. The work of Murphy et al. takes on added significance because the complex appears to be expressed in the thymic medulla but not in the thymic cortex, raising the possibility that it could play a role in negative selection of potentially autoreactive cells in the thymus (34).

Our results contrast with the results of Clayberger et al. (16) in which an HLA-A2-derived peptide (A2.56-69, containing residues 56 to 69 and corresponding approximately to the α^k -3 PMR peptide of the I-A^k molecule) was found to modulate the response of alloreactive CTL. Surprisingly this single peptide exhibited either inhibitory or stimulatory activity when used with a CTL clone (A2/B17) specific for an epitope shared between HLA-A2 and HLA-B17. Pretreatment of HLA-Aw69 expressing cells with the A2.56-69 peptide sensitized them for lysis by the A2/B17 clone. The authors interpret this result as an indication that the binding of the A2.56-69 peptide by the HLA-Aw69 molecule generated an epitope normally associated with the HLA-A2 or B17 molecule. This interpretation is consistent with the interpretation we have made of our results, except that Clayberger et al. designed their readout to detect the generation of an epitope through the binding of the MHC peptide rather than disruption of an indicator epitope consisting of foreign peptide plus MHC molecule.

The second function of this peptide is to inhibit the lysis of HLA-A2 or HLA-B17

expressing targets by the A2/B17 CTL clone. Had this inhibition been the result of peptide competition for the A2 or B17 binding cleft, then this result would have been analogous to the results reported here. However, the Clayberger results differ in one important regard: the inhibition of cytolysis is obtained by pretreating the T cell *and not the target cells* with the peptide. This result implies that the A2.56-69 peptide is interacting directly with the TCR and preventing the successful interaction of TCR with the complex of HLA-A2 (or HLA-B17) and some self peptide. It seems unlikely that a peptide consisting of only 14 amino acids could bind to the TCR with sufficient affinity to prevent the competitive binding of the class I molecule/peptide complex—a protein/protein interaction that most likely involves contacts spread over the entire top surface of the class I molecule (35) and entails a potential contact surface of 600–700 Å² (36). However, at the present time no other straight-forward explanation exists for the observation.

We do not ascribe any significance to the observation that two of our three α chain-derived peptides bound to the I-A^k molecule, while none of the four β chain-derived peptides bound. The peptides used in the present study were chosen to include most of the polymorphic residues of the $\alpha 1$ and $\beta 1$ domains in such a way that the polymorphic residues are located toward the center of a given synthetic peptide. This arbitrary choice of peptide design may have coincidentally selected α chain peptides that could bind while producing β chain peptides in which the minimal binding sequences have been disrupted. A much more comprehensive set of overlapping peptides would have to be synthesized for each chain before one could conclude that the α and β chains differ significantly in their content of sequences that could be bound by the I-A^k molecule.

Our studies also provide some limited insight into the mechanism of allotypic recognition although they fall short of providing a definitive answer. The demonstrable binding of class II-derived peptides to class II molecules suggests that alloreactivity could consist, at least in part, of the reaction to class II molecules that have bound class II or MHC-derived peptides. This model is satisfying in the physicochemical sense because it requires that the TCR always bind an MHC molecule with an occupied cleft whether it is displaying antigen-specific reactivity or alloreactivity rather than the binding of molecules with an occupied cleft in the first case and “empty” molecules in the second. However, because of the high frequency of alloreactive T cells for a given class II specificity, it seems unlikely that *only* class II-derived peptides are involved in this process. The genetic studies of alloreactivity carried out to date do not allow the discrimination between binding of a class II-derived peptide from the same allele as the class II “restriction element” and the binding of a nonpolymorphic, non-MHC-encoded self peptide. Resolution of this issue would require the isolation and structural characterization of the endogenous peptides bound by a class II molecules on APC isolated from spleen and lymph nodes of intact animals. While this experiment has not been reported, Buus et al. (37) have examined the set of peptides bound by the I-A^d and I-E^d molecules of the A20 B cell lymphoma grown in tissue culture. This early characterization revealed a marked heterogeneity in the size and hydrophobicity of bound peptides but provided no further characterization. Nonetheless, this preliminary characterization tends to suggest that the endogenous peptides that are bound by class II molecules are derived from more than a single molecule or even a restricted set of molecules. However, until detailed se-

quence characterization is carried out on the endogenously bound peptides, it will be unclear how many proteins contribute peptides to the bound set and whether a subset of these proteins, e.g., the MHC molecules, contribute a disproportionate share of peptides.

The results obtained with the α^k -1 and α^k -3 peptides in the present study suggest that they fulfill the basic prerequisite for antigenicity, i.e., they can be bound by a class II molecule with reasonably high affinity. We are currently in the process of evaluating the immunogenicity of these peptides and the role they may play in auto-reactivity and alloreactivity.

Summary

Seven synthetic peptides corresponding to the polymorphic regions of the α and β chains of the I-A^k molecule were examined for their ability to inhibit the presentation of foreign antigens to antigen-specific, I-A-restricted T cell hybridomas. Two of the peptides, representing the sequences found in the first and third polymorphic regions (PMR) of the A α^k chain (α^k -1 and α^k -3) were capable of inhibiting the presentation of three different HEL-derived peptide antigens to their appropriate T cells. In addition, the α^k -1 peptide inhibited the presentation of the OVA(323-339) immunodominant peptide to the I-A^d-restricted T cell hybridomas specific for it. Prepulsing experiments demonstrated that the PMR peptides were interacting with the APC and not with the T cell hybridomas. These observations were confirmed and extended by the demonstration that the α^k -1 and α^k -3 peptides blocked the direct binding of HEL(46-61) to purified I-A^k and that the α^k -1 peptide blocked the binding of OVA(323-339) to I-A^d. The binding competition experiments suggest that the α^k -1 peptide binds to the I-A^k molecule from which it was derived with a $K_d \sim 10^{-5}$ M, while the α^k -3 peptide binds slightly less well. These combined data, suggesting that class II-derived peptides can bind to MHC class II molecules, including the autologous molecule from which they are derived, have important implications for the molecular basis of alloreactivity and autoreactivity. Further, they suggest a possible mechanism by which selecting elements, involving only MHC molecules, may be generated in the thymus.

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