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Research Articles

Immunological Self, Nonself Discrimination

JEAN-GERARD GUILLET, MING-ZONG LAI, THOMAS J. BRINER, SOREN BUUS, ALESSANDRO SETTE, HOWARD M. GREY, JOHN A. SMITH, MALCOLM L. GEFTER*

The ability of immunodominant peptides derived from several antigen systems to compete with each other for T cell activation was studied. Only peptides restricted by a given transplantation antigen are mutually competitive. There is a correlation between haplotype restriction, ability to bind to the appropriate transplantation antigen, and ability to inhibit activation of other T cells restricted by the same transplantation antigen. An exception was noted in that a peptide derived from an antigen, bacteriophage lambda cI repressor, binds to the I-E^d molecule in a specific way, yet is not I-E^d–restricted. Comparison of the sequence of the repressor peptide with that of other peptides able to bind to (and be restricted by) I-E^d and a polymorphic region of the I-E^d molecule itself revealed a significant degree of homology. Thus, peptides restricted by a given class II molecule appear to be homologous to a portion of the class II molecule itself. The repressor-derived peptide is identical at several polymorphic residues at this site, and this may account for the failure of I-E^d to act as a restriction element. Comparison of antigenic peptide sequences with transplantation antigen sequences suggests a model that provides a basis for explaining self, nonself discrimination as well as alloreactivity.

IN DIVERSE ANTIGENIC SYSTEMS, IMMUNOLOGICAL RECOGNITION of foreign proteins by T helper cells is directed to a limited number of sites within these antigens (*1–4*). The T cell recognition sites have been mapped by substituting protein fragments or synthetic peptides for the native antigen in a system in which T helper cell activation in vitro is measured in the presence of antigen-presenting cells. Our findings (*5*) and those of Babbitt *et al.* (*6, 7*) showed direct binding of these immunodominant peptides to class II transplantation antigens (present normally on the surface of antigen-presenting cells); these results suggest that at least one role for the class II molecule in T cell activation is to hold the peptide in a favorable conformation for recognition. In addition, these studies demonstrated that the absence of binding of a peptide to the class II molecule can be correlated with immune unresponsiveness for certain antigen-strain combinations. The binding of an immunodominant peptide to a class II molecule is a necessary, but not sufficient, condition to promote immunity. The absence of T cells

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able to recognize the bound antigen, that is, a hole in the repertoire, can also account for immune tolerance (*8*).

Studies from our laboratory have shown that peptides capable of being presented by a given class II molecule compete with each other with regard to T cell activation. Specifically, the immunodominant peptide derived from bacteriophage lambda repressor (cI) when presented by the I-A^d class II molecule, is able to stimulate many individual clones of T cell hybridomas. Peptides derived from the unrelated antigens staphylococcal nuclease and ovalbumin, which can also be presented by I-A^d (I-A^d–restricted), inhibit activation of cI-specific T cells. T cells recognizing the same cI peptide but in the context of I-E^k are not inhibited by these same I-A^d–restricted peptides (*9*). More interestingly, the peptides derived from ovalbumin and the nuclease could be transformed into peptides capable of stimulating cI-specific T cells, even if only a few amino acid residues were replaced. These results allowed us to formulate a model in which we postulate that all antigenic proteins have stretches of amino acids which, in peptide form, are able to fit a common peptide binding site on the class II molecule and that the T cell receptor must recognize all antigenic peptides in a similar conformation (and in the same class II molecule context) on a given class II molecule (*9*).

We have now extended these observations to another antigenic system and show that the inhibition observed for T cell activation is competitive and that the basis of the competition is likely to be a result of competition of peptides for direct binding to the class II molecule as opposed to competition of the already bound peptides for T cell activation.

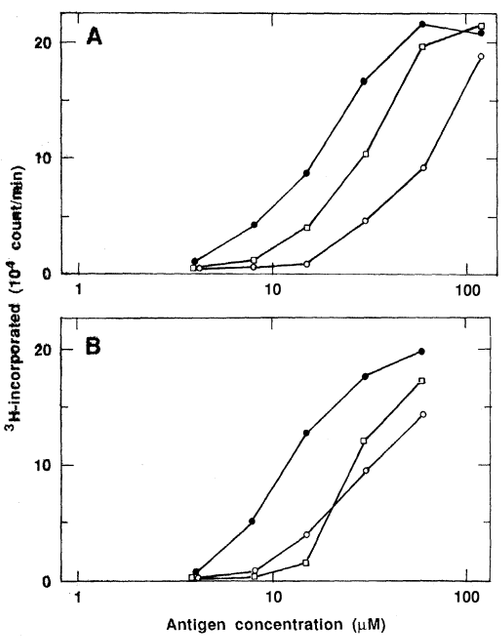

Fig. 1. Inhibition of 7B7.3 activation by related peptides. (**A**) The activity of 7B7.3 T cell hybridoma (5×10^4 cell per well) was measured in the presence of various concentrations of P15-26 either in RPMI-1640 with 10 percent fetal calf serum alone (●) or in the presence of 20 μ*M* P12-24 (□) or 60 μ*M* P12-24 (○). (**B**) The activity of T cell hybridomas 7B7.3 was measured in the presence of various concentrations of P15-26 either in RPMI-1640 with 10 percent fetal calf serum alone (●) or 30 μ*M* ovalbumin (P324-336) (□) or 60 μ*M* staphylococcal nuclease (P61-80) (○). A20 B cell lymphoma line (5×10^4 cells per well) was used as antigen-presenting cells. After 24 hours of culture, 50 μl of supernatant was harvested and then assayed for interleukin-2 concentration by following incorporation of [^{3}H]thymidine into the interleukin-2–dependent CTL-L cell line (10^4 cells per well). The indicated values represent the arithmetic mean of triplicate samples taken from the experiments carried out ab initio. The standard deviations were all less than 8 percent of the sample mean. T cell hybridomas were obtained as described (*9*). A20, the I-A^d–I-E^d postive A20.2J BALB/cBl lymphoma line which presents antigen in an MHC-restricted fashion to T cells, was a gift from Drs. J. Kappler and P. Marrack.

Table 1. Binding of ^{125}I-labeled cI P12-26 analog to class II molecules. The peptide P12-26 was modified by the addition of tyrosine residue to the NH_2-terminus to serve as an acceptor for the ^{125}I. Ia molecules were purified from Nonidet P-40 (NP-40) lysates of A20 ($H2^d$) or AKTB-1b ($H2^k$) cells by affinity chromatography using the monoclonal antibodies. MK-D6 (I-A^d–specific), 10-3-6 (I-A^k–specific) or 14-4-4 (I-$E^{d/k}$–specific) were coupled to Sepharose 4B beads (Pharmacia Fine Chemicals, Sweden) (*5*). The gel filtration assay for determining the degree of association between immunogenic peptides and Ia has been described (*38*). Briefly, 40 μ*M* of purified Ia in 1 percent solution of NP-40 and phosphate-buffered saline was mixed with 0.2 μ*M* of ^{125}I-labeled peptide (approximately 200,000 count/min for each experiment) and incubated for 48 hours at room temperature to allow for formation of the Ia-peptide complex. The Ia-peptide complexes were separated from free peptide by gel filtration, and the percentage of peptide bound to Ia was calculated as the ratio of the ^{125}I-labeled peptide in the void volume to the total ^{125}I-labeled peptide recovered.

Class II antigen	Percent of peptide bound
I-A^d	1.6 ± 0.8 (n = 5)
I-E^d	8.9 ± 2.2 (n = 7)*
I-A^k	0.3 ± 0.5 (n = 5)
I-E^k	2.3 ± 1.7 (n = 5)

*Level of binding is significantly different from the other three class II molecules at the >99 percent confidence level.

Furthermore, as already noted (*5, 6*), there is a correlation between the class II molecule restriction for a given peptide and the ability of that peptide to bind to that class II molecule in vitro. We now report a single exception to this correlation. It appears that a hole in the repertoire was found; that is, binding of a peptide was readily detected in vitro, but T cells specific for the bound peptide are apparently not present in vivo. Analysis of the basis for this exception allowed the formulation of a model to account for the biochemical basis of self, nonself discrimination.

Inhibition of antigen-specific T cell activation by nonstimulatory peptide analogs. The T cell hybridoma 7B7.3 was derived from a BALB/c mouse immunized with cI, and it can be stimulated with the peptide P15-26 (residues 15 to 26 of cI) in the context of I-A^d. The T cell hybridoma 8I, derived from the A/J strain, recognizes the same peptide, but in the context of I-E^k. Neither T cell can be stimulated with a homologous peptide analog P12-24 (residues 12 to 24 of cI). Other T cells, however, derived from BALB/c mice, can recognize P12-24 in the context of I-A^d. This suggests that P12-24 can bind to the I-A^d molecule, but presumably cannot stimulate 7B7.3 because it lacks a specific T cell interaction residue (an epitope). Since both peptides share 11 of 13 residues, we expected to be able to observe inhibition of P15-26 dependent 7B7.3 activation when the peptide P12-24 was also included in the cultures. This is indeed the case (Fig. 1).

In the case of 7B7.3, P12-24 inhibited activation by P15-26 in a dose-dependent manner. The potency of the inhibition depends on the concentration of the inhibitor. P12-24 changed the apparent affinity of 7B7.3 for P15-26 (Fig. 1); the 50 percent stimulation point of the P15-26 antigen dose response curve is at a concentration of 20.7 ± 1.2 μ*M* when P15-26 is cultured with 7B7.3 alone and at concentrations of 29.8 ± 2.0 μ*M* and 70.3 ± 4.9 μ*M* when P15-26 is cocultured with P12-24 at 20 μ*M* and 60 μ*M*, respectively. The inhibitory effect can be reversed by increasing the concentration of P15-26 in culture. This finding offers argument against nonspecific inhibition of T cell stimulation by toxic factors. Furthermore, the same peptide, P12-24, had no statistically significant effect on the interleukin-2 (IL-2) response of hybridoma 8I, a P12-26 responsive, I-E^k–restricted T cell hybridoma. We have demonstrat-

ed that the residues of P12-26 required for interaction with I-A^d and I-E^k are different (*10*). The absence of inhibition of the I-E^k–restricted T cells by P12-24 may therefore be due to the inability of that peptide to bind at all to the I-E^k molecules.

Inhibition of repressor-specific and ovalbumin-specific T cell activation by other I-A^d restricted peptides. As suggested previously (*9*), if there were only one peptide binding site on each class II molecule, we would expect that any immunodominant peptide restricted by a given class II molecule would act as a competitive inhibitor of the other's ability to stimulate specific T cells. Accordingly, we examined the competitive ability of peptides derived from staphylococcal nuclease, residues 61 to 80 (P61-80); and from ovalbumin, residues 324 to 336 (P324-336) to inhibit cI P15-26–dependent activation of the hybridoma 7B7.3. Each of these peptides has been shown in their respective cases to be I-A^d–restricted and to be immunodominant for their respective antigens (*2, 11*). These peptides do inhibit 7B7.3 activation (Fig. 1). The degree of inhibition is dependent on the ratio of activator to inhibitor in each case. Specificity is seen in that the same peptides are without significant effect on the I-E^k–restricted, P12-26–specific T cell, 8I (*12*).

The T cell hybridoma DO-11.10 is ovalbumin-specific, and I-A^d–restricted. It responds well to the peptide P323-339 derived from ovalbumin. It responds less well to the truncated analog P324-336. Since inhibition is apparently competitive the latter peptide was used as a stimulator. As potential inhibitors, the cI peptide P12-26 and a peptide influenza hemagglutinin site 2 (P126-138) (I-A^d–restricted) were used. Neither of these peptides can stimulate DO-11.10 on their own. These nonstimulatory peptides act as inhibitors for ovalbumin-specific T cell activation (Fig. 2). As a control, the influenza hemagglutinin-derived peptide P111-120, which is I-E^d–restricted, is without effect (*13*).

The binding of repressor peptide P12-26 to class II molecules in vitro. In order to gain further insight into the mechanism of competitive inhibition observed between peptides restricted by the same class II molecule, we began a study of peptide binding to class II molecules in vitro. Accordingly, the P12-26 peptide was labeled with ^{125}I and tested for its ability to bind to various class II molecules. The peptide could bind to class II molecules isolated from the d and k haplotypes (see Table 1). The I-A^d and I-E^k molecules are restricting elements for the peptide, whereas the I-A^k and I-E^d are not. It is surprising that the peptide binds most tightly to the I-E^d molecule despite the fact that we have never observed this molecule to act as a restricting element for P12-26–specific T cells derived from BALB/c mice (see below).

In order to determine whether the binding of P12-26 is specific for the I-E^d molecule, we examined its ability to compete for binding with a myoglobin-derived peptide, known to be restricted by (and to bind to) I-E^d. The binding is indeed specific (Table 2). P12-26 also competes with other immunodominant peptides for binding to their respective class II molecules. Competition, however, is not observed for the lysozyme-derived peptide restricted by I-A^k, a class II molecule not bound by P12-26 (Table 1). In fact, P12-26 binds best to the I-E^d molecule, as shown by its relative binding ability as well as its relative competitive ability.

In view of the unexpected result, indicating that P12-26 binds well and specifically to the I-E^d molecule, we reexamined the question as to whether or not there were T cells restricted by I-E^d in the BALB/c mouse immunized with the NH_2-terminal domain of cI. Of more than 300 hybrids specific for P12-26 recovered from 15 cI-immune mice, none were shown to be restricted by the I-E^d molecule in that they could be stimulated by antigen in the presence of L cells expressing I-A^d (*14, 15*), but could not be shown to be stimulated by antigen in the presence of L cells expressing I-E^d. In contrast, of 80 hybrids specific for myoglobin (P135-147), 78 were shown to be restricted to I-E^d as assayed on L cells expressing I-E^d. Furthermore, unfractionated lymph node–derived T cells from P12-26 immune mice showed significant proliferation (as indicated by thymidine incorporation, 68,000 count/min) when cultured in a standard lymph node proliferation assay (*16*) with 10 μ*M* P12-26. However, the lymph node cells failed to show significant proliferation (1250 count/min) above cultures with no antigen added when the monoclonal antibody MKD6, specific for the I-A^d molecule, was added to identical cultures with 10 μ*M* P12-26. The proliferation of these same cultures was not inhibited (75,000 count/min) by a monoclonal antibody, 34-1-4S, which is specific for I-E^d and which inhibits the stimulation of a hemocyanin-specific I-E^d–restricted T cell hybridoma. Thus, there appears to be in cI-immune mice an absence of T cells able to recognize P12-26 in the context of the I-E^d molecule; there is apparently a hole in the repertoire with respect to P12-26 and the I-E^d molecule.

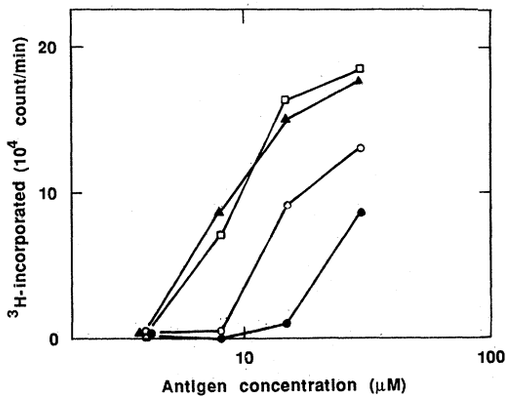

Fig. 2. Inhibition of activation of DO-11.0 T cell hybridomas. DO-11.10 was cultured with various concentrations of ovalbumin (P324-336) and A20 presenting cells in either medium alone (□) or in the presence of influenza hemagglutinin P111-120 (▲), or P12-26 (○), or influenza hemagglutinin site 2 (P126-138) (●), each at 50 μ*M*. When the cells had been in culture for 24 hours, stimulation was determined by quantitating the interleukin-2 released, as described in Fig. 1. The indicated values represent the arithmetic mean of triplicate samples (as explained in Fig. 1). The standard deviations were all less than 9.5 percent of the sample mean. DO-11.10 is an ovalbumin I-A^d–restricted T cell hybridoma (*11*).

Competition at the level of the I-a molecule. The immunodominant peptides derived from both staphylococcal nuclease and

Table 2. Capacity of cI P12-26 to inhibit binding of isotopically labeled peptides to various class II molecules. For the inhibition assay a dose-range of unlabeled cI P12-26 peptide was added to the incubation mixture of Ia and ^{125}I-labeled peptide (600, 120, 24 and 4.8 μ*M*). The degree of association between Ia and labeled peptide was determined by gel filtration as described in Table 1. The amount of peptide bound in the absence of inhibitors is 10.6 percent for ovalbumin (323 to 339)/I-A^d; 6.5 percent for myoglobin(132 to 153)/I-E^d; 21.5 percent for lysozyme(46 to 61)/I-A^k; 2.5 percent for cytochrome c(88 to 104)/I-E^k. The concentration of inhibitory peptide required to obtain a 50 percent inhibition of binding was calculated. Each experiment has been repeated three times.

^{125}I-labeled peptide	Class II molecule bound	Concentration (μ*M*) of P12-26 required for 50 percent inhibition
Ovalbumin (P323-339)	I-A^d	300
Myoglobin (P132-153)	I-E^d	4
Lysozyme (Hen) (P46-61)	I-A^k	>2500†
Cytochrome c (pigeon) (P88-104)	I-E^k	300

*Numbers in parentheses refer to the amino acid residue positions in the parent molecule. †No inhibition of binding was detected at 2500 μ*M* P12-26.

1	2	3	4	5	6	7	8	9	10	11	12	13	SOURCE
Leu	Glu	Leu	Phe	Arg	Lys	Asp	Ile	Ala	Ala	Lys	Tyr	Lys	Myoglobin (34)
Val	Glu	Asn	Ala	Lys	Lys	Ile	Glu	Val	Glu	Phe	Asp	Lys	Nuclease (2)
Leu	Glu	Asp	Ala	Arg	Arg	Leu	Lys	Ala	Ile	Tyr	Glu	Lys	λ Repressor cI (9)
Leu	Glu	Asp	Ala	Arg	Ala	Ser	Val	Asp	Thr	Tyr	Cys	Arg	$I\text{-}E_\beta^d$ (20)
Leu	Glu	Gln	Lys	Arg	Ala	Glu	Val	Asp	Thr	Val	Cys	Arg	$I\text{-}E_\beta^k$ (20)
(Leu)	Glu	Arg	Phe	Glu	Ile	Phe	Pro	Lys	Glu				Influenza (11)
Leu	Glu	Arg	Phe		Ile	Tyr	Asn	Arg	Glu				$I\text{-}E_\beta^d$ (20)
Leu	Val	Arg	Tyr		Phe	Tyr	Asn	Leu	Glu				$I\text{-}E_\beta^k$ (20)

Fig. 3. Sequences of peptides restricted by I-E^d. Myoglobin (P135-147) recognized by I-E^d–restricted T cell clones; nuclease from *Staphylococcal aureus* (P66-78) recognized by H-2^d–restricted T cell clones; cI protein from λ repressor (P12-24); I-E_β^d sequence (P69-81); I-E_β^k sequence (P69-81); hemagglutinin from influenza virus (P111-120) recognized by I-E^d–restricted T cell hybridomas; I-E_β^d sequence (P28-36); I-E_β^k sequence (P28-36). Numbers in parentheses represent the amino acid residue positions in either the immunogen or the Ia molecule. The Leu in parentheses means that leucine and phenylalanine at the first position of peptide 111 to 120 from hemagglutinin are equivalent for stimulation of the hemagglutinin-specific I-E^d–restricted T cell clone. The underlined residues show identity between the antigens and I-E_β^d protein regions. All peptides were synthesized by the solid-phase method of Merrifield (*37*). The amino acid composition and the sequence analysis of the synthesized peptides correspond to the expected compositions. The amino acid composition of the peptides was determined by amino acid analysis with a Beckman 6300 analyzer (Beckman Instruments). The purity of the peptides as determined by sequence or high-performance liquid chromatography (or both) is 93 to 94 percent.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	SOURCE
						Ala	Val	His	Ala	Ala	His	Ala	Glu	Ile	Asn	Glu	Ala	Gly	Arg	Ovalbumin (10)
Glu	Tyr	Val	Arg	Tyr	Asp	Ser	Asp	Val	Gly	Glu	His	Arg	Ala	Val	Thr	Glu	Leu	Gly	Arg	$I\text{-}A_\beta^b$ (27)
Lys	Tyr	Leu	Glu	Phe	Ile	Ser	Glu	Ala	Ile	Ile	His	Val	Leu	His	Ser	Arg				Myoglobin (31)

Fig. 4. Comparison of partial myoglobin and ovalbumin sequence with class II antigen sequence. Ovalbumin (P326-339) recognized by DO-11.10; I-A_β^b sequence (P42-55); myoglobin (P102-118). Numbers in parentheses represent the amino acid residue position in the native molecule.

ovalbumin are able to inhibit T cell activation of a cI-specific T cell hybridoma. The degree of inhibition observed is dependent on the ratio of stimulator to competitor, and therefore the inhibition with regard to the activator appears to be competitive in nature. Similarly, I-A^d–restricted peptides are shown to inhibit the activation of an ovalbumin-specific T cell hybridoma. In order to observe inhibition of the P12-26–specific T cells 7B7.3, we found it necessary to use the weakly stimulatory truncated analog P15-26 as an activator. Similarly, for the ovalbumin-specific T cell DO-11.10, we used a weakly stimulating peptide analog of ovalbumin as an activator. We presume that the failure to observe competition in other systems and for other cI-specific T cells which we tried is due to the difficulties in achieving a high enough concentration of inhibitor in vitro, relative to the concentration of the activator. In these cases where competition cannot be demonstrated as the concentration of the nonstimulatory peptide is increased, the point of nonspecific inhibition or toxicity is reached before any specific inhibition is seen. This could be due either to too high an apparent affinity of the activator peptide for the class II molecule (which results in the activator peptide being the most stimulatory within a set of related peptides derived from the immunogen) or to the enhancement of apparent affinity due to the interaction of bound peptide with the T cell receptor in a trimolecular complex (between Ia molecule, peptide, and T cell receptor) (*17, 18*) or to both.

Competition between related antigens for antigen presentation has been observed previously. It appears, for example, that poly(Glu,Lys) and dinitrophenyl-poly(L-Lys) both compete for a saturable common site on the peritoneal macrophages from strain 2 guinea pigs (*19*). More recently Rock and Benacerraf (*20*) using poly(Glu,Ala,Tyr)-specific T cell hybrids found that poly(Glu,Tyr), which does not stimulate the T cells, can compete with poly(Glu, Ala,Tyr) at the level of presenting cells. They extended their observation when they used poly(Glu,Lys,Leu)-specific T cell hybridomas and pointed out the blocking effect of poly(Glu,Leu) upon stimulation by poly(Glu,Lys,Phe) (*21*). Recently Buus and Werdelin (*22*) demonstrated that the decapeptide angiotensin I can compete with angiotensin III for presentation to guinea pig T cells by paraformaldehyde-treated antigen-presenting cells.

Lakey *et al.* (*23*) have presented data extending these results to peptide antigens in the cytochrome c system. The authors indicated that competition can occur at the level of the T cell receptor itself as well as at the level of the presenting cells. The possibility that antagonist analogs can compete with antigen for binding to the T cell receptor was investigated by Rao *et al.* (*24*). They demonstrated that, in competition for antigen binding to a *p*-azophenylarsonate reactive T cell clone, the *p*-azophenylarsonate analogs can bind, but fail to trigger the cell in a manner similar to an antagonist, as observed in hormone receptor interactions.

Our results are novel in that we demonstrate competition between unrelated peptides where the only commonality is their class II restriction. We cannot at present prove that this competition is due solely to competition for binding to the class II molecule; that explanation alone, however, can account for the data.

Antigenic peptides as analogs of self. Previously we noticed what appeared to be a fortuitous homology between the immunodominant peptides derived from cI and staphylococcal nuclease (*9*). Since the cI peptide binds to both I-A^d and I-E^d, and the nuclease peptide can apparently be restricted by both class II molecules and therefore presumably also binds to both, we compared their sequences to that of other peptides restricted by either I-A^dor I-E^d. The I-E^d–restricted, immunodominant peptide derived from sperm whale myoglobin bears a homology to both the repressor and the nuclease peptides at positions 1, 2, 5, 6, 9, and 13 (Fig. 3). Therefore these homologies may account, in some way, for their common restriction or binding ability (or both) to the I-E^d molecule (see Fig. 3).

Given that the cI peptide is homologous to other I-E^d–restricted peptides, we searched further for the explanation as to why there

		SOURCE
Ile Ser Glu Ala Ile Ile His Val Leu His Ser Arg		Myoglobin (31)
Ile Ser Gln Ala Val - His Ala Ala His Ala Glu Ile Asn Glu		Ovalbumin (10)
Leu Glu Asp Ala	Arg Arg Leu Lys Ala Ile Tyr Glu Lys	λ Repressor cI (9)
Val Glu Asn Ala	Lys Lys Ile Glu Val Glu Phe Asp Lys	Nuclease (2)
Arg Glu Glu Ala Tyr His Ala Ala Asp Ile Lys Asp		Ragweed (35)

Fig. 5. Sequences of peptides restricted by I-A^d. Myoglobin (P106-118); ovalbumin (P323-336); λ repressor cI (P12-26); nuclease from *Staphylococcus aureus* (P66-80); ragweed allergen (P54-65). Numbers in parentheses represent the amino acid residue position in the immunogen molecule. The dash in the ovalbumin sequence represents a deletion at that location compared to the peptide above. The alignment of myoglobin and ovalbumin is different from that in Fig. 4. Some of the T cell clones that are myoglobin I-A^d–restricted may recognize the allo–I-A^b differently from the way that ovalbumin I-A^d–restricted T cell clones are recognized.

	SOURCE
Ile Ala Tyr Leu Lys Gln Ala Thr Lys	Cytochrome c (35)
Leu Ala Tyr Ile Tyr Ala Asp Gly Lys	Nuclease (2)
Leu Lys Ala Ile Tyr Glu Lys Lys Lys	λ Repressor cI (9)
Ile Thr Ala Ser Val Asn Cys Ala Lys	Lysozyme (12)

Fig. 6. Sequences of peptides restricted by I-E^k. Moth cytochrome (P95-103); nuclease from *Staphylococcus aureus* (P89-97); cI protein from λ repressor (P18-26); hen egg-white lysozyme (P88-96). Numbers in parentheses represent the amino acid residue positions in the immunogen molecule.

were apparently no T cells able to recognize the cI peptide in the context of the I-E^d molecule. To this end, we compared the sequences of the I-E^d–restricted peptides to that of the I-E^d molecule itself (*25*). We found that in the third hypervariable region of the $E_\beta{}^d$ chain (residues 69 to 81), residues 1, 2, 5, and 13 as aligned were homologous to the peptides restricted by the I-E^d molecule (Fig. 3). What is striking is that the cI peptide is identical to the I-$E_\beta{}^d$ molecule at positions 1, 2, 3, 4, 5, and 11, and homologous at 13. Furthermore, in comparing the sequences of the I-$E_\beta{}^d$ molecule with that of the I-$E_\beta{}^k$ molecule in this region, positions 3, 4, 7, and 11 are the only polymorphic ones. The cI peptide is identical to the I-E^d molecule at three of these polymorphic residues and the other I-E^d–restricted peptides are not. This identity might therefore account for the presumed hole in the repertoire.

Although our analysis is based on only three peptides and one class II molecule, it would appear that the homologies observed are unlikely to be fortuitous. It is therefore not unreasonable to propose that the basis of selection of an immunodominant peptide within an antigen for T cell recognition rests on its ability to bind to a class II molecule and that the chemical requirements for such binding rest on a homology with a segment of the class II molecule itself. This line of reasoning suggests that there is an internal complementarity, consisting of an internal ligand and an internal receptor associated with particular domains, perhaps segregated on each chain of the class II molecule and that the internal ligand for binding is, in part, encoded by the polymorphic region. The same might be expected to be true for the internal receptor. The immunodominant peptide would then be bound to the class II molecule, displacing the internal ligand and taking on equivalent geometry. Foreign ligands (immunodominant peptides) would then be seen by T cells as analogs of the internal, self ligand. The comparison of the internal ligand with an external ligand then would form the basis of self, nonself discrimination. The chemical basis of the presumed ligand-receptor interaction is, of course, at present unknown. We prefer the idea that the residues forming the recognizable homology motifs in the various antigenic peptides are not necessarily contact residues between the ligand and the receptor [aggretopes as described in (*26*)], but are rather chemical elements forming a framework which is "permissive" for the ligand to assume a "shape" able to be bound by the T cell receptor.

In the case of the cI peptide described above, we argue that the foreign ligand is indistinguishable (at the polymorphic sites) from the self class II molecule and the "hole in the repertoire" is brought about by self-tolerance. This notion is of course not new (*27, 28*); however, in this article we provide molecular evidence lending credence to that notion.

The I-E^d–restricted immunodominant peptide derived from influenza hemagglutinin bears little homology to the other peptides described above (except at positions 1 and 2) (Fig. 3). This peptide, however, does bear a striking homology to another part of the I-$E_\beta{}^d$ molecule itself. In this case, the residues of the I-$E_\beta{}^d$ molecule used for comparison are taken from the second hypervariable region of the $E_\beta{}^d$ chain (*25*). As aligned, positions 1, 2, 3, 4, 6, and 10 are identical, and positions 7 and 9 are homologous. The influenza peptide bears an insertion with respect to the I-$E_\beta{}^d$ molecule at residue 5, and this residue has been shown to be an epitope for T cell recognition (*13*). Experiments similar to those described in Table 2 indicate that the second and third hypervariable regions of the beta chain are involved in a single binding site in that the P12-26 of the repressor protein, homologous to the third hypervariable region, and the influenza peptide described above compete with each other for binding to the I-E^d molecule (*29*).

If our hypothesis has some basis in fact, then we might expect that alloreactivity is a result of T cell recognition of the internal ligand of a foreign class II molecule. If the internal ligand is composed of a number of polymorphic residues, then a given T cell could not distinguish between a foreign ligand bound to a self class II molecule and a foreign class II molecule bound with its own internal ligand. If there were places of identity between the two ligands, alloreactivity would result. Given the results above, namely, that there is homology between all ligands, foreign internal ligands could be readily considered as analogs of self and therefore chemically equivalent to self plus X (where X is an antigen). Each polymorphic residue of the foreign ligand would represent, in principle, a different foreign antigen in the context of self. Hence, a large

percentage of T cells would be able to respond to a single alloreactive class II molecule (*30*).

We examined the limited molecular data available relating T cell reactivity of a foreign antigen in the context of self class II to an alloreaction. The sequence of the I-A^d–restricted, immunodominant peptide of ovalbumin (as described in this article) is given in Fig. 4. It is aligned with a polymorphic region (residues 42 to 55) of the $A_\beta{}^d$ chain (*31*). It bears an identity at positions 12, 17, 19, and 20 as aligned. The single residue that is polymorphic in this region is position 12 being histidine in the I-$A_\beta{}^b$ molecule (and ovalbumin) and tyrosine in the I-$A_\beta{}^d$ molecule (the restricting element) and tyrosine also in the I-$A_\beta{}^k$. The ovalbumin-specific T cell hybridoma, DO-11.10, studied in this article, which recognizes ovalbumin in the context of I-A^d, shows an alloreaction with I-A^b but not I-A^k. Furthermore, this region was shown by Germain and colleagues to control the alloreaction of DO-11.10 (*32*), and the histidine residue 12 was shown by McConnell and colleagues to be essential for recognition of another ovalbumin-specific T cell (*33*). Thus, we argue that the DO-11.10 T cell cannot distinguish self plus X (where X is the ovalbumin peptide) and allo (where allo is I-$A_\beta{}^b$). For reasons to be explored further, we do not believe that this argument is complicated by the genetic origin of DO-11.10 (*34*).

The ovalbumin peptide described here appears to contain two regions of "permissive" residues (allowing it to bind to class II molecules). One region would appear to involve positions 17 to 20 (T cells like DO-11.10 require this region). The peptide deleted for these residues still can presumably bind to I-A^d since it can stimulate T cells like DO-54.8 (*11, 33*). Recent evidence suggests that residues in the region preceding position 12 also can form a "permissive" framework for I-A^d binding (*35*). T cells recognizing a peptide derived from sperm whale myoglobin in the context of I-A^d also show an alloreaction with I-A^b (*35, 36*). Myoglobin shows a homology with the I-A^b molecule at positions 2, 5, 7, 8, and 12 (Fig. 4). Again it appears that the histidine residue at position 12 might be responsible for the similarity between myoglobin in the context of self, I-A^d, and allo, I-A^b.

In consideration of the above, we might not always expect to find an obvious homology motif among peptides restricted by the same class II molecule. There are likely to be motifs associated with each ligand-like domain (Figs. 3 and 4). In Fig. 5, we present a compilation of data that appears to indicate that there might be three motifs associated with ligands for the I-A^d molecule. The alignments in Fig. 6 indicate the possibility of two motifs for the I-E^k molecule.

For assessing the generality of these observations, we will have to await the availability of more molecular data. It is "remarkable" that any homologies of structure are visible at the primary sequence level. The three-dimensional structure of the class II molecule, once known, should aid in this analysis.

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