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Presidential Address to The American Association of Immunologists

Major Histocompatibility Complex Proteins and TCRs: Do They Really Go Together Like a Horse and Carriage?[1]

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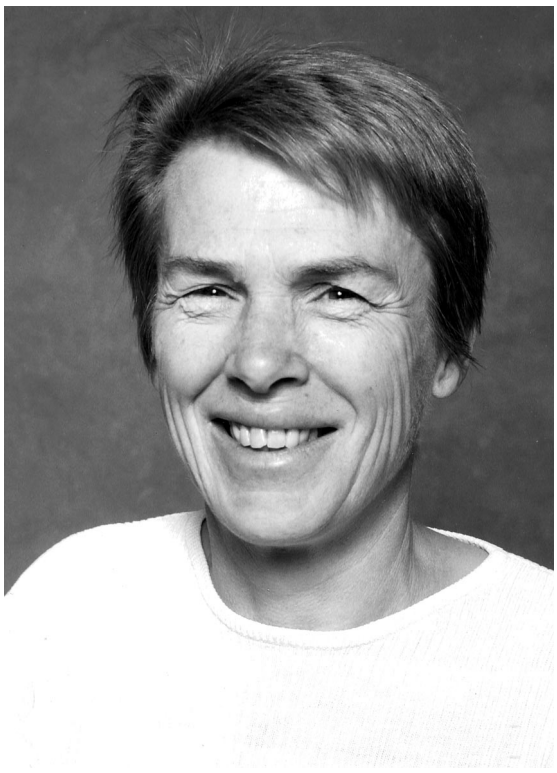
Early in the 20th century, scientists realized that grafts and transplanted tumors that differed from their hosts at the MHC were rapidly rejected. Later work showed that lymphocytes were unstoppably interested in MHC differences, so much so that Jerne, in a visionary article in the first issue of the *European Journal of Immunology* (1), proposed that lymphocyte receptors were selected evolutionarily to react with MHC proteins. His article continued with the proposal that lymphocytes would mutate their receptors during development in the thymus, such that, once mature, they could no longer react with the MHC of their hosts, but would retain the ability to react with other MHC alleles of their species. Jerne's ideas have been modified as our understanding of thymocyte development has increased. For example, it is now known that T cells rarely, if ever, mutate their α and β TCR genes (2, 3). Also, it is now recognized that thymocytes are selected in two ways for the reactivity of their TCRs, by positive selection for TCRs that react with self-MHC plus self-peptides with low but appreciable affinity (4–9), and by deletion if their TCRs react too well with self-MHC plus self-peptides (10–12).

Despite the modifications to Jerne's hypothesis, which were forced by these findings, immunologists clung to the idea that MHC proteins and the α- and β-chains of TCRs must coevolve to have some affinity for each other. There is quite a lot of evidence for the notion. $\alpha\beta$TCR$^+$ T cells do react with appreciable frequency in the absence of priming with foreign MHC proteins (13–16). The frequency of allo-MHC-reactive T cells in the unprimed population is much higher than for any other Ag except the superantigens. Such a high alloreactivity could be because of an evolutionarily conserved fit between TCRs and MHC. On the other hand, the germline repertoire of $\alpha\beta$ TCRs may actually be completely random and the high frequency with which TCRs react with MHC may be because of the fact that allogeneic MHC proteins, with the many host-derived peptides to which they are bound, actually comprise thousands of Ags, not one (17), an idea that is supported by the fact that many alloreactive T cells recognize both the allogeneic MHC protein and the peptide bound to it (18). Alternatively, high frequency reaction with foreign MHC may simply be because of positive selection in the thymus for low reaction with self-MHC (plus peptides) and heteroclitic cross reaction between self and foreign MHC.



Phillipa Marrack

To deal with the complication that positive selection almost certainly biases the repertoire of $\alpha\beta$ TCRs toward MHC reaction, several groups have tested the allo-MHC reactivity of TCRs from thymocytes that have not been positively and negatively selected or that have been formed by random combinations of TCR α- and β-chains (19, 20). The experiments suggested that the unselected $\alpha\beta$TCR repertoire had some intrinsic affinity for MHC. However,

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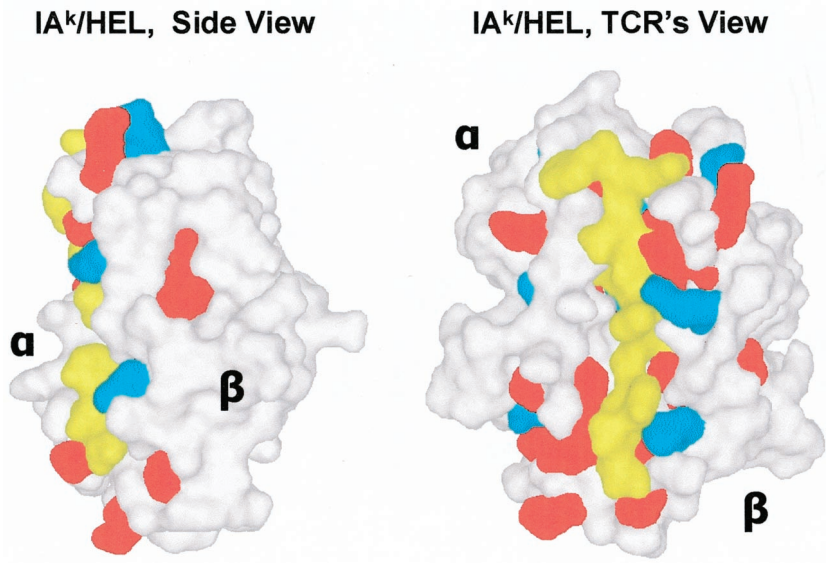

FIGURE 1. Most of the surface of MHC proteins exposed to TCRs is conserved between alleles. Shown are the side and top views of IA^k bound to a peptide from hen egg lysosome (HEL).[3] Residues in white are conserved between all mouse IE alleles. Residues in pale blue are conserved between almost all alleles and residues in red are variable between alleles. Amino acids of the bound peptide are shown in yellow. The structure shown is from Ref. 45.

neither experiment dealt with the problem of the many ligands offered by MHC plus host peptides. Others tackled this question by studying whether particular TCR α- or β-chains were particularly likely to be used in reactions with particular MHC proteins, class I vs class II (21–24) or MHC alleles (24–26). Again, in all cases evidence of preferential use was observed, suggesting some overall reactivity of particular TCR V regions for MHC. However, others have shown that receptors differing by only a single amino acid residue can be expressed on CD4$^+$ or CD8$^+$ T cells (27).

Therefore, with all of these results in hand, it was very disappointing when the x-ray crystallographically solved structures of $\alpha\beta$TCRs bound to MHC proteins began to appear with no sign from one structure to another of conserved interactions (28–34). Admittedly the structures show a generally conserved alignment, with the TCR α-chain CDR1 and CDR2 loops over the C-terminal end of the α helix of the class I MHC α2 domain/class II β1 domain and the TCR β-chain CDR1 and CDR2 loops over the C-terminal end of the α helix of the class I or class II αI domains (Fig. 1). However, the angle at which the TCR lies on MHC can vary by as much as 30° and the tilt of the TCR on MHC also varies, such that sometimes almost all of the TCR β-chain loops are lifted completely off the MHC. This variability in the geometry of TCR/MHC engagement precludes interactions that are conserved in all structures (34, 35). The people who worked out these structures have had to reluctantly conclude that TCR and MHC may not have some conserved fit with each other. The loose conservation of alignment may be because of ridges on the MHC protein at the C-terminal ends of the α helices of the class I MHC α2 domain/class II β1 domain and the class I or class II αI domains and an alignment forced by CD4 or CD8 as they join the TCR/MHC complex on the surfaces of the interacting cells (34, 35).

However, a result we obtained some years ago and have since rediscovered has led us to reconsider these results and propose a hypothesis that still allows for evolutionarily conserved fits between $\alpha\beta$ TCRs and MHC. The result in question comes from examination of the reactivities of T cells from mice in which all detectable class II MHC proteins are bound to a single peptide. To make such animals, a transgene coding for IAβ^b bound at its N-terminal end via a flexible peptide linker to a peptide that can bind to IA^b, is bred in to animals that are knocked out for the wild type IAβ^b chain, and for invariant chain (C2$^-$Ii$^-$). In the transgene-positive C2$^-$Ii$^-$ mice, all detectable class II proteins are bound to the introduced peptide (36).

We have now prepared two mice of this type. The peptides bound to IAβ^b in these mice, Ep and 2W1S, are shown in Table I. In one of the strains (C2$^-$Ii$^-$Ep), all detectable IA^b is bound to a peptide from the IEα chain (36). In the second strain (C2$^-$Ii$^-$2WIS), all detectable IA^b is bound to a peptide (2W1S) that differs from Ep in three amino acids. Previous work from our laboratory has shown that this peptide binds more tightly to IA^b than Ep does (37).

We wished to compare the repertoires of CD4$^+$ T cells in these mice with each other and with that of mice that lack class II MHC altogether (C2$^-$Ii$^-$). T cells in these mice cannot be primed by injection of simple proteins or peptides either because they lack class II altogether (C2$^-$Ii$^-$) or because all their available class II is occupied irreversibly with the transgenic covalent peptides (C2$^-$Ii$^-$Ep and C2$^-$Ii$^-$2W1S). In the past, we have dealt with this problem by making chimeras containing wild-type IA^b-presenting cells derived from a fetal liver graft but single peptide presenting thymus epithelium (38, 39). However, some cells in such mice might be positively selected in the thymus on wild-type cells derived from the fetal liver graft. Additionally, such mice, unlike true single peptide animals, are fully tolerant to IA^b bound to many mouse peptides, so their T cell repertoire does not truly represent the repertoire of single peptide animals.

To circumvent this priming problem we used dendritic cells from C2$^-$Ii$^-$ animals, infected with a retrovirus expressing IAβ^b bound to a single peptide, the 3K peptide shown in Table I. The two single peptide mice and C2$^-$Ii$^-$ mice were primed with these cells. Seven days later, T cells were harvested from the immunized animals, expanded by culture with IA^b/3K-presenting cells and then IL-2, and fused to BW$\alpha^-\beta^-$. Hybrids were screened for reactivity with tetramers bearing IA^b bound to the 3K peptide.

Table I. *Peptides used in these experiments*

Peptide Name	Sequence
Ep	ASFEAQGALANIAVDK
2W1S	ASFEAWGALANWAVDS
3K	ASFEAQKAKANKAVDK

[3] Abbreviation used in this paper: HEL, hen egg lysosome.

Positive hybrids were tested for their ability to react with spleen cells from mice of different MHC types, and for their ability to react with IA^b-3K adhered to plastic dishes. The results are shown in Table II.

Most of the hybrids reacted with IA^b bound to the 3K peptide. As we have previously shown for $CD4^+$ T cells from $C2^-Ii^-Ep$ animals, a very high proportion of the hybrids reacted with IA^b bound to the many mouse peptides with which it is associated in C57BL6 mice (36), presumably, a reflection of their positive selection and negative selection in the thymus on IA^b bound to a single peptide. None of the hybrids reacted with IA^b bound to Ep or 2W1S. A total of 38.5% of the $C2^-Ii^-Ep$ hybridomas and 71.4% of the $C2^-Ii^-2W1S$ reacted also with allogeneic MHC (presumably class II) bound to mouse peptides. This result is reminiscent of data we have previously reported for non-Ag-selected T cells from $C2^-Ii^-Ep$ mice (36). As we have previously shown, hybridomas made from nonselected $CD4^+$ T cells from C57BL6 mice only rarely react with allogeneic MHC (36). In a recent experiment, the frequency against the bank of allogeneic targets used here was 4%. Thus we believe that both MHC/Ag specific and the entire pools of $CD4^+$ T cells from single peptide animals are very likely to react with IA^b or allogeneic class II bound to one or more mouse peptides.

In 1996, when we first reported that $CD4^+$ T cells from single peptide mice were very likely to react with allogeneic MHC we thought the result was an unremarkable illustration of the ability of TCRs to react with configurations of MHC that are conserved between different MHC alleles. However, the fact that crystallographic solutions of TCRs bound to MHCs have failed to demonstrate any conserved interactions led us to reconsider these data.

To reconcile the various results we first looked carefully at the structure of MHC proteins. Fig. 1 shows the surface of class II as it appears to the TCR. Residues in white are those that are conserved in sequence between all known mouse class II IA proteins. About two-thirds of this surface of class II is completely conserved with a few other amino acids expressed in most class II proteins. Therefore, TCRs are exposed to many IA conserved amino acids as they interact with MHC and peptide.

Comparison of the actual configurations of these amino acids between structures of the same MHC protein (IE^k) bound to different peptides shows that the bound peptide changes the exposure and configuration of these conserved amino acids (Fig. 2). Comparison of different IA^s bound to different peptides (Fig. 3) demonstrates this same phenomenon. For example, the arginine that is present at position 70 on the β-chain of almost all IA proteins varies tremendously in configuration depending on the peptide bound.

Other investigators have noticed that the bound peptide changes the configuration of MHC. For example, recognition of class I proteins by certain mAbs depends upon the peptide with which the class I is engaged (40–42). Similar results have been observed for class II and peptides (43, 44).

The idea that the bound peptide affects the conformation of MHC bears on the observation that, even from wild-type mice, alloreactive T cells are often peptide specific. This result is often interpreted as an indication that the receptors on alloreactive T cells must engage amino acid residues of both the allogeneic MHC and the bound peptide, a conclusion that is supported by the x-ray-solved structures of a few such combinations. However, peptide specificity does not prove peptide engagement. In some cases alloreactive TCRs may actually bind only to MHC residues pushed in to a certain configuration by a particular peptide or set of peptides.

$CD4^+$ T cells from single peptide mice will have been exposed to only one of the possible configurations of IA^b controlled by the single peptide engaged in these mice. Consequently they will not be tolerant to any of the other configurations of IA^b allowed by engagement of IA^b by other peptides. This lack of deletion, combined with positive selection of thymocytes that can react with low avidity with the configuration of MHC that is present, plus the expressed peptide, which allows the very high frequency with which these T cells react with IA^b from wild type mice. The same lack of deletion allows the appearance of T cells bearing receptors that can react with other conserved configurations of MHC, that is, allogeneic IA proteins.

Table II. *Many $CD4^+$ T cells from single peptide mice are alloreactive*

	IL-2 (U/ml) Secreted in Response to							
	Spleen cells bearing H2 of the indicated haplotype							
	b	li^-	d	f	k	q	s	Plate-bound IAb-3K
Yae5-4.6	9.7	23.6	<0.3	<0.3	<0.3		<0.3	<5
Yae-15.8	254.4	134.6	<0.3	<0.3	<0.3		<0.3	40
Yae5-35.13.11	<0.3	<0.3	<0.3	<0.3	<0.3		<0.3	80
Yae5-62.8	2260.6	1745.5	<0.3	20.5	1001.5	>640	2676.5	40
Yae5-78.12	<0.3	<0.3	<0.3	<0.3	<0.3		<0.3	<5
Yae5-97	9693.5	308.2	<0.3	<0.3	<0.3		<0.3	640
Yae5-100.5	2342.8	156.1	<0.3	<0.3	<0.3		<0.3	160
Yae5-143.3	865.3	449.3	<0.3	<0.3	<0.3		<0.3	40
Yae6-13.2	534.4	393.6	<0.3	<0.3	<0.3		1.6	20
Yae9-14.1	47.5	<0.3	<0.3	<0.3	<0.3		<0.3	80
Yae10-20.3	7.2	<0.3	126.3	<0.3	<0.3		<0.3	20
Yae10-99	1200.6	1102.8	<0.3	<0.3	12.4		<0.3	20
Yae10-106.3	165.2	14	12.7	<0.3	<0.3		247.9	<5
2W1S4-21	2322	640	<0.3	<0.3	<0.3		<0.3	640
2W1S4-43	806.3	312	22.8	<0.3	<0.3	<2.5	<0.3	1280
2W1S12-20.4	856.6	715.5	<0.3	<0.3	<0.3	320	<0.3	40
2W1s12-30.4	<0.3	<0.3	0.5	<0.3	18		<0.3	<5
2W1S12-95.3	<0.3	<0.3	<0.3	<0.3	<0.3		<0.3	<5
2W1S12-118.6	60.9	67.3	1.2	<0.3	<0.3		<0.3	<5
2W1S12-146.3	34.8	134.8	14	<0.3	<0.3		<0.3	<5

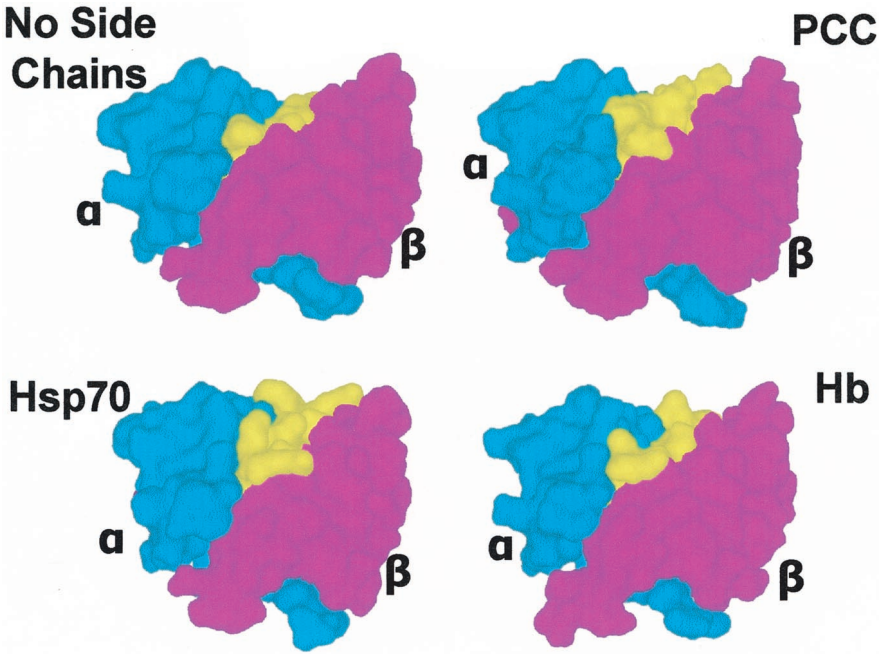

FIGURE 2. The bound peptide affects the exposure and configuration of MHC amino acids with which TCRs can interact. Shown are the top views of IE^k to three different peptides or modeled bound to a peptide with no side chains (polyglycine). Amino acids of the α- and β-chain of IE^k are in cyan and purple respectively. Amino acids of the bound peptide are in yellow. Data are from Ref. 46.

These ideas bear on the failure of the x-ray-solved structures to illustrate conserved interactions between TCRs and MHC. In normal mice and human beings all configurations of the conserved MHC residues will be present, expressed by the proteins bound to some of the many peptides that are engaged in such animals. Therefore, T cells that can react with appreciable affinity with these configurations will be deleted. Thus powerful illustrations of TCRs reacting with conserved MHC residues are automatically absent from mature T cell repertoires and will not be apparent in structures derived from T cells from normal animals interacting with MHC plus Ag peptides. However, such T cells will be available in the single peptide mice because only the T cells that react with the IA configuration present in these mice will have been deleted.

Finally, if TCRs that react well with conserved MHC configurations are absent from the mature T cell repertoire, what are the evolutionary pressures that lead to coevolution of TCR/MHC binding? We suggest that the mature TCR repertoire, on which natural selection can act, does contain remnants of the conserved interactions, expressed as a few TCR amino acids that react with a few of the conserved MHC residues. However, the amino acids that manage this vary from one TCR/MHC pair to another. So far, too few structures have been solved to allow such interactions to become apparent.

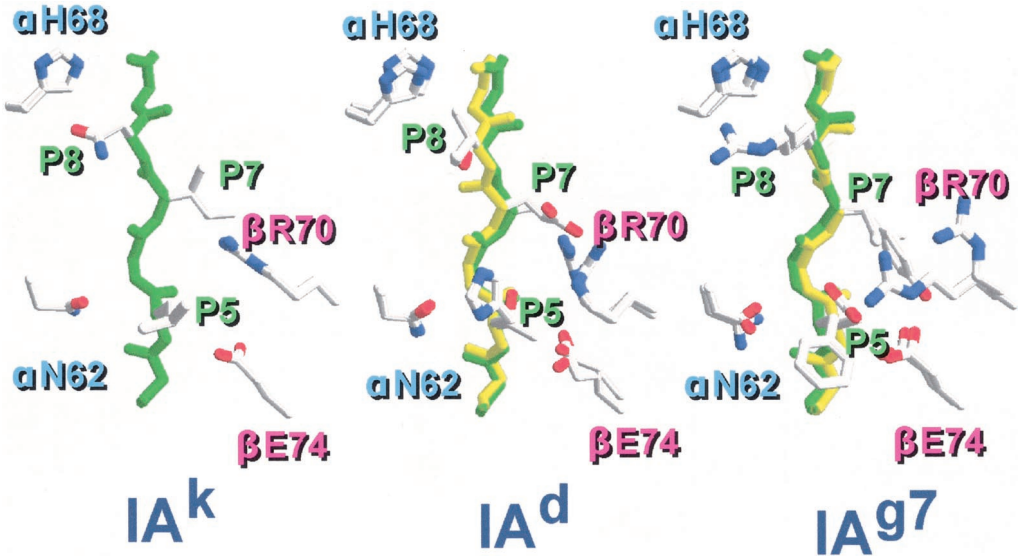

FIGURE 3. The bound peptide can have dramatic effects on the configuration of MHC amino acids with which TCRs can interact. The figure shows the configurations of 5 peptides bound to IA proteins: HEL_{50-62} bound to IA^k (45); chicken $OVA_{323-339}$ and influenza hemagglutinin 126–138 bound to IA^d (47); and HEL_{11-25} and glutamic acid dehydrogenase 207–220 bound to IA^{g7} (48, 49). Also shown are the configurations with each of the peptides of three amino acids that are conserved in all mouse IA alleles, αN62, αH68 and βE74 and the configuration with each of the peptides of one amino acid that is conserved in all mouse IA alleles except IA^s, βR70. The α carbon backbone of the peptide is shown in green or yellow.

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