Biophysical and structural studies of TCRs and ligands: implications for T cell signaling

E Sally Ward* and Ayub Qadri

The availability of soluble αβ TCRs and the individual chains has now made it possible to carry out structural studies of these molecules and analyze their molecular interactions with peptide–MHC ligands. Recent X-ray crystallographic structures of TCR α and β chains have finally established their structural similarity with the Ig molecules. Kinetic measurements of the interaction between TCRs and their ligands have provided strong evidence in favour of an affinity/avidity model for T cell activation in the periphery as well as during development in the thymus.

Addresses
Cancer Immunobiology Center, Department of Microbiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-8576, USA
*e-mail: Sally@skylab.swmed.edu

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Abbreviations

APC	antigen-presenting cell
APL	altered peptide ligand
CDR	complementarity determining region
CTL	cytotoxic T lymphocyte
NMR	nuclear magnetic resonance
SEB	staphylococcal enterotoxin B
SEC	staphylococcal enterotoxin C
TCR	T cell receptor

Introduction

T cells recognize antigens as small peptide fragments bound to MHC molecules on the surface of APCs. The recognition of the MHC–peptide complex is mediated by a heterodimeric TCR composed of disulfide-linked α and β chains (γ and δ in γδ T cells). These heterodimers, in association with a cluster of proteins collectively termed the CD3 complex, form the signal-competent receptor on the surface of a T cell. Both α and β chains (as well as γ and δ in γδ TCRs) contain variable and constant domains and, as in Igs, the specificity of antigen recognition is solely dictated by the variable region. Structurally, TCRs have been thought to be very similar to Igs and this has been confirmed by recent X-ray crystallographic studies of individual α and β chains[1••,2••]. Peptides which bind to MHC molecules to form the ligands for the TCR can originate not only from foreign antigens but also from self-proteins and evidence supports the concept that self-MHC–self-peptide complexes serve as ligands for the selection of T cells in the thymus [3]. Thus, by their ability to bind foreign as well as self peptides, MHC–peptide ligands are not only involved in the activation of T cells in the periphery ensuring an effective immune response against a virus or a bacterium but they also play a key role in determining the fate of developing T cells during thymic selection. The extent of T cell activation in the periphery and positive/negative selection of T cells in the thymus have been thought to be a consequence of either differential conformational changes in the TCR complex in response to distinct MHC–peptide ligands or variations in affinities of the TCR for different MHC–peptide ligands, resulting in a variety of signaling outcomes [4,5]. With the availability of soluble MHC and TCR proteins we have now begun to understand the intricacies of the interactions between these two complex sets of molecules and how these interactions might determine quantitatively and qualitatively different outcomes of T cell signaling. In this review we discuss important structural features of the TCR chains and of their MHC ligands, together with recent studies on the influence of ligand structure on T cell development and signaling.

Structure of the TCR and its ligand

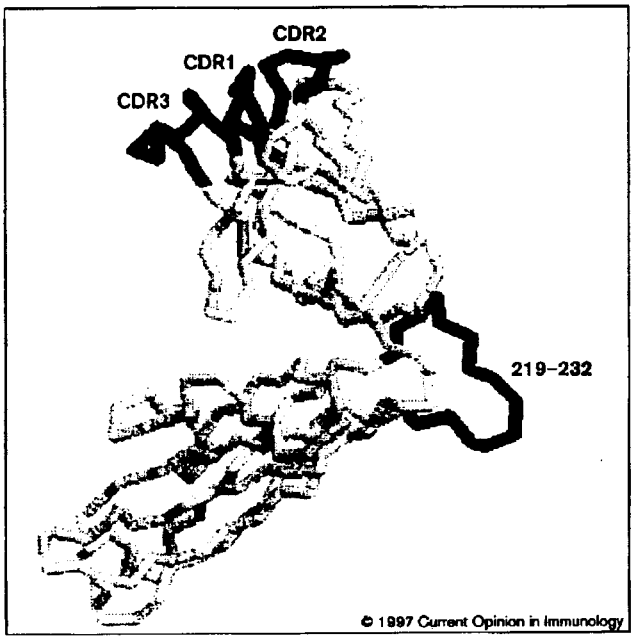
The receptor

Based on amino acid sequence homology, the structure of a TCR has long been considered to closely resemble that of an Ig. Attempts to determine the 3D structure of this heterodimeric recognition unit have been thwarted primarily due to inavailability of soluble and functional TCR molecules in suitable yield for use in crystallization or nuclear magnetic resonance (NMR) analyses. Crystal structures of a murine TCRβ chain and variable domain of an α chain have recently been reported, however [1••,2••]. The β chain was derived from a TCR recognizing a hemagglutinin peptide of an influenza virus presented by the murine I-E^d molecule. The 3D structure of this molecule shows two domains very much like the variable and constant domains of an Ig molecule [1••]. The general organization of the complementarity determining regions (CDRs), which are important for the recognition of ligand, is very similar to that of the hypervariable loops of Igs. The conformations of the Vβ loops, however, are distinct from those of Igs. Furthermore, the amino acid residues that pack the CDR1 and CDR2 loops are reasonably well conserved across murine Vβs. This conservation indicates that there may be canonical forms for Vβ CDRs. In turn, this is consistent with the earlier suggestions that these regions are involved in the interaction with relatively conserved residues on the α-helices of the MHC molecule, with the more diverse CDR3 loops interacting with peptide [6]. A closer look at the crystal structure, however, also reveals proximity of some amino acid residues of CDR1 and CDR2 to CDR3 which would argue that the division in interaction may not always be followed. The

latter might permit some flexibility in the orientation a TCR can have with respect to the MHC–peptide ligand.

Unlike the Ig variable and constant domains, the Vβ and Cβ are seen in close association with each other in the crystals (Fig. 1) and the Vβ–Cβ interaction encompasses approximately 800Å^2. If this feature also exists in native αβ TCRs, then it could result in increased rigidity in the region analogous to the Fab elbow. Bentley *et al.* [1••] have suggested that a conformational change brought about by the binding of the ligand to such a rigid structure might facilitate signal transduction. Whether or not the β chain maintains this rigid conformation when it is associated with the α chain can only be confirmed once the 3D structure of a complete TCR heterodimer has been determined (see Note added in proof). The Vβ–Cβ structure [1••] also reveals a solvent-exposed loop in the Cβ domain which might be involved in interaction with one of the CD3 proteins. Importantly, the crystal structure represents a biologically active conformation at least in terms of binding to various superantigens [7•] and the 3D structure of this Vβ complexed with staphylococcal enterotoxin C (SEC) shows a conformation of Vβ essentially identical [8] to the one reported by Bentley *et al.* [1••].

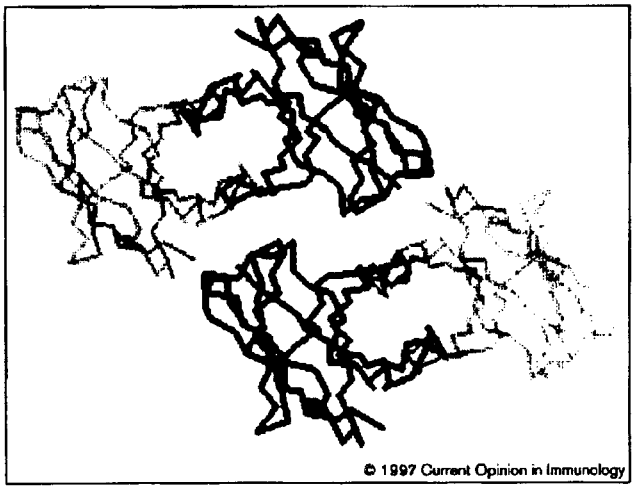
Figure 1



Three-dimensional structure (α-carbon trace) of the murine TCR Vβ 8.2-Cβ chain. CDR1,CDR2, CDR3 and the solvent-exposed loop (residues 219–232) are shown in black, with the remainder of the structure in grey.

The X-ray crystallographic structure of a murine Vα domain has also been recently described [2••]. This Vα domain is derived from a TCR which recognizes an encephalitogenic amino-terminal peptide of myelin basic protein in association with I-A^u [9]. The structure, although very similar to an Ig light chain variable region, shows a striking difference relative to Igs in the folding topology of the β strands. In the crystal structure there is a strand switch from one β sheet to another which results in the hydrogen bonding of the c" strand to the d strand instead of the c' strand [2••]. This strand switch removes a bulge on the surface of the domain which allows two Vα homodimers to pack as a dimer of dimers in the crystal form (Fig. 2). The structure of this molecule, in conjunction with the crystal structure of the β chain, has been used to model the 3D structure of the complete 1934.4 TCR, because this TCR also uses Vβ8.2 [2••]. The model suggests that an αβ TCR can undergo dimerization mediated primarily through Vα–Vα interactions.

Figure 2



The tetramer of Vα domains observed in the X-ray crystallographic structure of a murine TCR Vα4.2 domain (α-carbon trace). The two domains that mediate dimerization of Vα domains are shown in black. In an αβ heterodimer, the Vβ domain would pack with Vα in a similar way as that seen for the Vα (grey)–Vα (black) interaction. Thus, replacement of the Vα domains in grey by Vβ domains leads to a proposed model for the dimerization of αβ heterodimers.

Dimerization of TCRs would, at first glance, be compatible with the 3D structure of HLA-DR. The latter has been shown to crystallize as a dimer of heterodimers [10] and interestingly the tetrameric model structure of the TCR shows significant complementarity to the HLA-DR tetramer [2••]. Due to the lack of planarity of the HLA-DR bound peptides, however, some reorientation of the TCRs in the tetramer model needs to be invoked to accommodate a good fit. Furthermore, as has been pointed out by others earlier [11,12], the presence of a tetrameric HLA-DR under physiological conditions would require binding of two identical peptides to the dimer of heterodimers. A mechanism that would drive

formation of such a molecular complex is not known at present. It is of interest as to whether the Vα tetramer is physiologically relevant. Clearly, at present there is no experimental evidence demonstrating TCR dimerization on the surface of a T cell. The fact that T cell activation by antibodies requires, in general, bivalent antibodies and the total lack of N-glycosylation sites at the dimerization interface [2••], however, argues in favour of dimerization. We favour a model in which binding of the MHC–peptide ligand to the TCR induces a conformational change which promotes dimerization of the TCR through Vα–Vα interaction. Thus, in this model, Vα-mediated dimerization is a transient event mediated by interaction with cognate ligand. The ability to induce this conformational change might also be influenced by the affinity (in particular, the off rate or the dissociation rate) of the MHC–peptide ligand for the TCR. Recently Sykulev *et al.* [13•] have also suggested that a monovalent MHC–peptide complex might induce a conformational change in the TCR which could strengthen the formation of a dimeric TCR. This type of model is consistent with the T cell activation model proposed by Janeway [4], in addition to being compatible with the concept that the half-life of TCR–MHC–peptide interaction may need to be long enough for the conformational change to be induced.

The formation of a stable complex through interaction between MHC–peptide ligand and a dimeric TCR would reduce the lateral mobility of the TCR–CD3 complexes and thereby increase aggregation of TCRs in the area of contact between a T cell and an APC. This would lead to more efficient signaling by facilitating the process of bringing the intracellular kinases and their substrates, the main players in T cell activation [14], into close proximity. Whether or not the TCR has the potential to form dimers of αβ heterodimers may be clearer once the crystal structure of a complete, functional soluble TCR is available (see Note added in proof). It should also be possible to engineer a TCR which is unable to form a dimer through Vα–Vα interaction and to express this engineered α chain as an αβ TCR on the surface of a T cell. Analysis of signaling in response to a variety of ligands in such a T cell could shed light on the functional significance of dimerization of the TCR.

The ligand

Despite close structural similarity with the Ig molecule the TCR sees its ligand in a very different form, namely a complex of a peptide bound to an MHC molecule on the surface of an APC. Not only do these peptides have a range of sizes and shapes but they also differ from each other in the manner by which they are generated and presented [15–17]. The ability of a small number of MHC molecules to bind a large array of antigenic peptides results in the generation of an extremely diverse set of MHC–peptide complexes which are presented to the T cells. The crystal structures of both MHC class I and class II have revealed that the peptide binds to a groove, or a cleft, the sides of which are made by α helices and the floor by a β sheet. A striking difference between the structures of the clefts of the two molecules is that the groove in the MHC class II is open whereas that of class I is closed at both ends [16]. Analysis of these 3D structures suggests that a very small number of peptide side chains are actually accessible for recognition by the TCR [16]. The ligand for the TCR, therefore, is mainly composed of residues contributed by the MHC molecule with the peptide residues forming the pivotal elements of specific recognition. This is consistent with the interaction of CDR1 and CDR2 with the α helices of MHC and the highly variable CDR3 with the peptide residues as shown in a number of experimental systems [6]. The recent 3D structure of human MHC class I HLA-B*3501 complexed with an HIV-1 peptide suggests a role also for MHC main chain atoms, in addition to the peptide residues, in determining the antigenic identity of the MHC–peptide complex [18•].

As discussed above, the X-ray crystallographic analysis of HLA-DR showed this molecule to crystallize not as a monomer but as a dimer of αβ heterodimers. These dimers have been found in the crystals of HLA-DR homogenously complexed with a single peptide as well as in HLA-DR–superantigen complexes [19,20]. Recently, dimeric I-E^k molecules have been reported to exist on the surface of a murine B cell lymphoma [21]. The physiological relevance of this dimeric I-E^k was revealed by the ability of a monoclonal antibody recognizing specifically the dimeric I-E^k to inhibit the T cell response to a low-affinity antigenic peptide [21,22]. These studies were strengthened by a study from Boniface *et al.* [23] demonstrating that a chemically cross-linked (dimeric) I-E molecule in solution could activate a T cell. They also showed that the off rate of the MHC–TCR interaction was 10–40 fold lower for this complex when compared with a monomer. These results suggest that dimeric MHC molecules could stabilize the MHC–TCR complex by increasing the avidity of the interaction. Abastado *et al.* [24•] have also demonstrated that a soluble dimeric MHC class I molecule can activate a T cell *in vitro*.

Recently, 3D structures of I-E molecules with covalently bound single peptides were reported by Fremont *et al.* [25•]. These structures reveal a cluster of acidic amino acids in the binding groove which reiterates the role of pH in the generation, binding or exchange of peptides to the MHC class II molecules. In the crystal form, the I-E^k molecules pack as dimers of αβ dimers that have a different geometry and orientation when compared with the human homologue, HLA-DR. The physiological relevance of these I-E dimers is, however, unclear because the peptide linker between MHC and the antigenic peptide forms part of the dimer interface. Clearly, more studies are required to conclusively determine the

physiological significance of the dimeric MHC class II molecules observed in crystals.

Interaction between the TCR and its ligand

The orientation

The precise interaction between an MHC–peptide complex and the TCR and the orientation of this interaction cannot at present be accurately defined due to inavailability of a 3D structure of a tripartite complex (see Note added in proof). Mutagenesis studies carried out in a number of experimental systems, however, have shown that CDRs 1 and 2 are involved in binding to the α helices of the MHC molecule whereas CDR3 is critical for the recognition of the peptide [6]. Recent structural analysis of TCR–ligand interactions in a system in which the TCR was directed against a photoreactive peptide bound to H-2K^d also assigns a role to the Vβ encoded c and c' strand, in addition to CDR3 loops of both Vα and Vβ, in the recognition of MHC–peptide complex [26•]. These mutagenesis studies also suggest that TCR and MHC might not always interact in the same orientation.

In an elegant study, Jorgenson *et al.* [27] mapped the TCR contact residues by variant peptide immunization of single chain transgenic mice. This study once again highlighted the importance of Vα and Vβ CDR3s in peptide recognition and led to a proposed topology for the TCR–MHC interaction in which CDRs 1 and 2 of Vα position over the α helix of MHC class II (I-E^k in this study) β chain whereas those of Vβ lie over the α helix of the α chain (Fig. 3a). A similar rotational orientation favouring the interaction of Vα with DRβ has been recently proposed by Brawley and Concannon [28•]. Because HLA-DR is a human homologue of I-E, the question arises whether the similarity in orientation between the two systems is a consequence of structural similarities. An extensive study analyzing the interaction of a TCR with an MHC molecule by Hong *et al.* [29] suggested a different orientation, although CDR3 residues of both Vα and Vβ still played a central role in recognizing the peptide. This study was carried out with a TCR which recognizes a peptide from the protein conalbumin bound to the self-MHC molecule I-A^k and was also alloreactive to a number of non-self MHC molecules. By mutating the TCR as well as the MHC molecule this analysis showed that CDR3s of both Vα and Vβ were important for all the recognition events by this TCR. Further, the MHC interaction site was mapped to the amino-terminal half of the TCR α chain and this led to a proposed orientation in which CDR1 and/or CDR2 of the TCRα chain lie over the MHC class IIα chain (Fig. 3b). More recently this group has employed the strategy described by Jorgenson *et al.* [27] and has presented data that reinforces their earlier proposed orientation [30•]. In fact, they argue that this is a general model for the orientation of the TCR of CD4+ T cells with respect to MHC class II.

In their study on the interaction of MHC class I with the TCR, Sun *et al.* [31•] suggest that different TCRs may have a common orientation on the MHC ligand. Using a panel of H-2K^b mutants, their study proposes an orientation that is parallel to the β pleated strands and diagonal to the α helices. The view point that the orientation of the TCR–MHC interaction is fixed and conserved is also shared by Sim *et al.* [32••] in their study on the role of CDR1 and CDR2 of TCR Vα in the selection of CD4+ and CD8+ T cells. Although all of these studies have provided important information on the orientation of the TCR–MHC interaction, it is obvious that more studies are required before we can make generalizations.

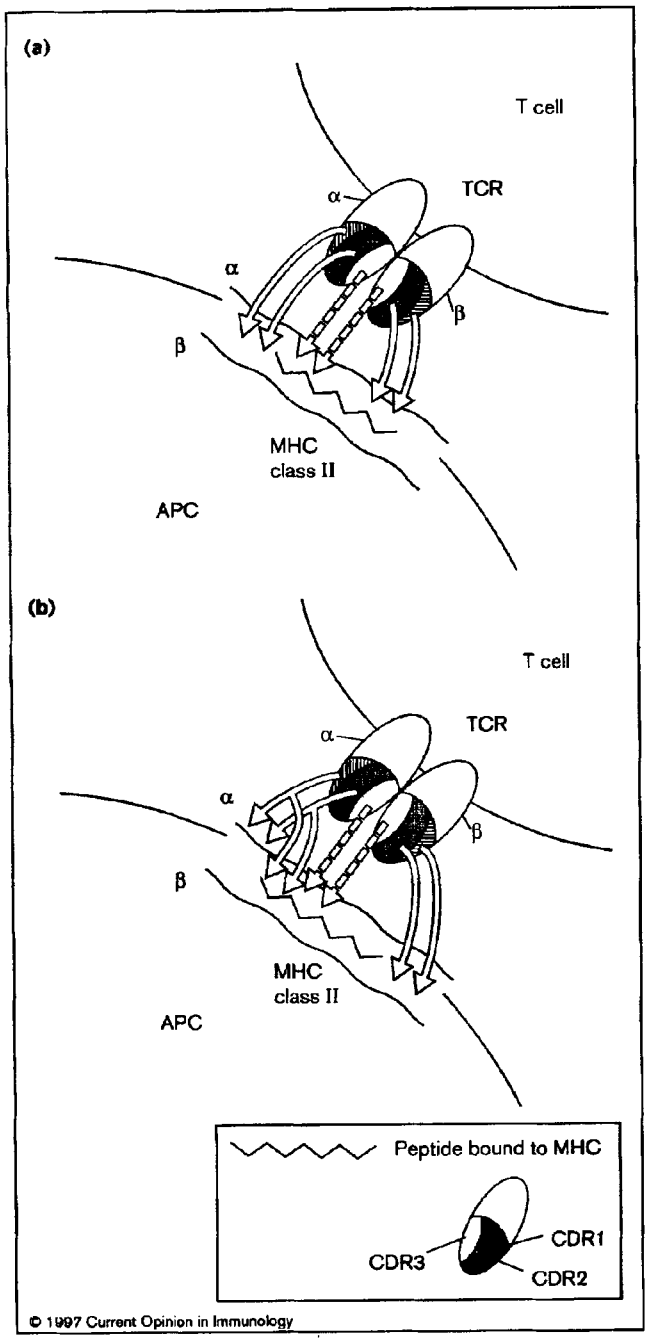
Kinetics and T cell signaling

There have been two models proposed for the activation of a T cell by a MHC–peptide ligand, one favouring affinity of interaction as the critical parameter for T cell activation and the other proposing a conformational change in the TCR post-ligand binding as the requirement for determining responsiveness to a MHC–peptide ligand. Several studies have supported the conformational model [4] but to date there is no direct experimental evidence demonstrating a conformational change in the TCR. Also, the role of aggregation in T cell activation is still not clearly defined as evidence both for and against the need for dimerization/multimerization has been presented [12]. In any case, the two models are not mutually exclusive as there may be a need for a threshold time of TCR occupancy to be achieved to induce a conformational change.

The availability of soluble MHC and TCR molecules has made it possible to carry out kinetic measurements of MHC–TCR interactions *in vitro*. Earlier studies by Matsui *et al.* [33] and Weber *et al.* [34] suggested an affinity of the order of 10^{-5}M. Although these two studies employed very different strategies, with the former analyzing inhibition by soluble peptide-complexed MHC in the binding of an anti-TCR antibody to the TCR on the T cell surface and the latter looking at inhibition of T cell responses by soluble TCR–Ig chimeras, the fact that they both obtained similar values indicated that the tripartite interaction was of low-affinity. On the other hand Sykulev *et al.* [35] reported higher affinity interactions between TCRs and class I restricted allogeneic and syngeneic ligands.

The first kinetic parameters for TCR–MHC binding using soluble MHC–peptide complexes, soluble TCRs and surface plasmon resonance were reported by Corr *et al.* [36] and Matsui *et al.* [37]. Although the dissociation rates in the two systems were in the same range, the two interactions differed significantly in their association rates. Considering that Corr *et al.* reported a class I restricted alloreactive system whereas Matsui *et al.* dealt with an I-E^k restricted TCR, the differences were thought to either represent a

Figure 3



Diagrammatic representation of the proposed orientations of the MHC–peptide–TCR interaction. **(a)** Orientation proposed by Jorgenson *et al.* [27]. The TCR is oriented perpendicular to the MHC–peptide complex with CDRs 1 and 2 interacting primarily with MHC (TCR α chain with MHC β chain and TCR β chain with MHC α chain) and CDR3 making contacts with the peptide. **(b)** Orientation proposed by Hong *et al.* [29] and later confirmed by Sant'Angelo *et al.* [30]. The TCR and MHC–peptide complex in this model are parallel to each other and therefore all the CDRs can interact with both the MHC and the peptide.

range of association rates in the TCR–MHC system or differences in the requirements of CD8+ versus CD4+ T cells. Whether the latter generalization can be made awaits the determination of the affinities of TCR–MHC–peptide interactions in other systems. There was a good correlation between affinity and T cell activation in the study reported by Matsui *et al.* Results of Al-Ramadi *et al.* [38•] with the alloreactive system analyzed earlier by Corr *et al.*, however, suggest that kinetic measurements based on surface plasmon resonance may not always correlate with T cell activation. They showed that an MHC–peptide complex differing from the parent complex by one amino acid in the peptide could still activate the T cell to a level equivalent to the parental peptide. This MHC–peptide complex did not bind to the soluble TCR, however, and this was suggestive of a 10-fold or greater decrease in the affinity in this particular system. This study also suggested a role for coreceptors, especially in situations in which MHC–peptide complex *per se* may not be a very potent T cell activator. The role of coreceptors (CD4, CD8) in the MHC–TCR interaction during T cell activation is further strengthened by recent data showing interaction sites for these molecules on the TCR [39,40•].

More recent studies [37,41••,42•] have demonstrated that the affinity of a TCR for a MHC–peptide complex is a critical factor in determining T cell activation both in the periphery and in the thymus. T cells in the thymus go through a process of complex development which eventually leads to either positive selection resulting in the generation of the mature peripheral T cell pool or in negative selection promoting programmed cell death of thymocytes. The process of selection is mediated by the interaction of clonally distinct TCRs with the MHC molecules loaded with self-peptides on the surface of thymic APCs [3]. The selection of T cells in the thymus is also believed to be an outcome of differential signaling as a result of either variations in conformational changes in the TCR complex post MHC–peptide ligand binding or differences in the affinity of the MHC–peptide complex for the TCR [3,4]. Again, as for peripheral T cell activation, these two models may not be mutually exclusive. The affinity/avidity model suggests that a MHC–peptide complex with an optimal affinity for the TCR would promote positive selection whereas one with a higher affinity would favour programmed cell death (negative selection; see Fig. 4). Peptides with low dissociation rates from MHC, resulting in high avidity ligands, were shown to promote negative selection of thymocytes [43•]. The strongest evidence, however, in support of a direct correlation between the affinity of TCR for MHC–peptide complex and the outcome of thymic selection was recently provided by Alam *et al.* [41••]. Using a set of peptides which had been previously shown to promote either positive or negative selection in fetal thymic organ cultures of TCR transgenic mice, this study once again demonstrated rapid dissociation and slow association in the interaction between MHC–peptide complex and the TCR. More importantly, the data show that MHC–peptide complexes favouring positive selection

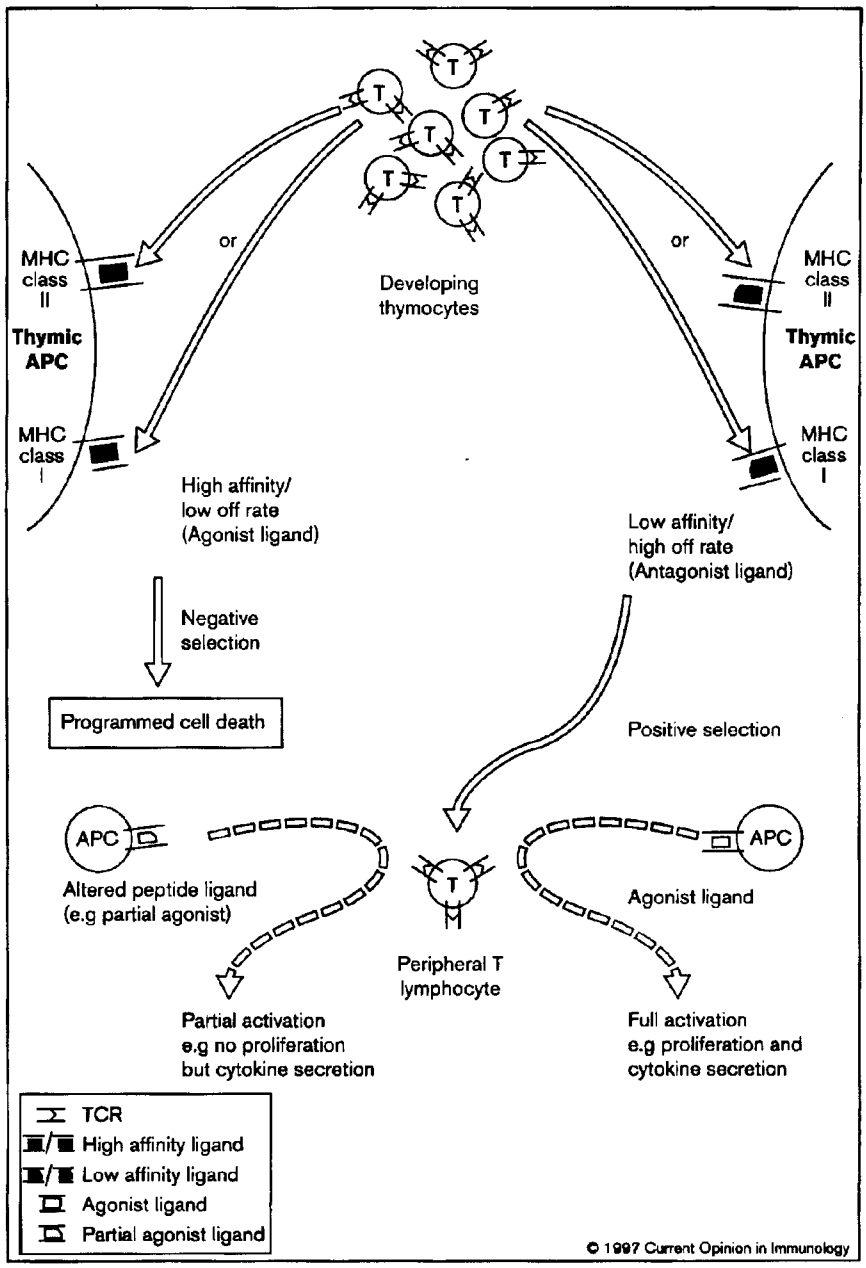
differed in their affinities for the TCR from those which promoted negative selection. A Kd of approximately 10 μM (and lower) resulted in negative selection of a TCR recognizing an ovalbumin peptide bound to H-2K^b and a threefold lower affinity resulted in positive selection. A correlation between TCR binding affinity and the number of cell surface molecules required for T cell activation has also been reported recently [44•]. The stage has been set and we will most likely see more of kinetic experimentation, which in turn might lead to general rules concerning the affinity requirements for positive versus negative selection in the thymus and T cell signaling in the periphery.

How stringent is the TCR in its ligand recognition?

The exquisite specificity of a TCR for its ligand has been thought to indicate that a small change in the TCR-recognizable part of the ligand would result in lack of responsiveness of the T cell. Recent studies have demonstrated, however, that the recognition by the TCR is rather flexible in that more than one type of peptide

Figure 4

Avidity model of T cell selection and activation. Interaction of a TCR on a developing thymocyte with a high-affinity MHC-peptide ligand on thymic APC results in negative selection via programmed cell death. The low-affinity ligand mediates positive selection. Activation of a mature T cell in the periphery can result in full or partial activation depending on the avidity of the MHC–peptide–TCR interaction (agonist/partial agonist/antagonist ligands are shown on separate APCs only for simplicity).



can induce a response [45]. The quality of the T cell response evoked by such so called 'altered peptide ligands' (APLs) can, however, be dramatically different with some inducing stimulatory functions and others totally switching off T cell activation. The first experimental evidence demonstrating flexibility in the recognition was provided by Evavold and Allen [46] who showed that a T cell which normally responded to the recognition of a MHC–peptide complex by proliferating and secreting IL-4 would now respond by only secreting IL-4 and not proliferation if the peptide was modified at one residue. This partial T cell activation mediated by a variant peptide was the prelude to many studies which have demonstrated partial agonism and antagonism in T cell activation. Madrenas *et al.* [47••] and Sloan-Lancaster *et al.* [48] have analyzed intracellular signaling events in T cells in response to APLs and reported a distinct pattern of ζ chain phosphorylation accompanied by a failure to activate ZAP-70 kinase. The response was not just a reflection of a weaker signal with the partial agonists as compared to the agonists but a strict difference in the quality of the response; the pattern did not change even when high concentrations of the variant peptide were used. The results suggest that recognition of a variant peptide by the TCR results in the activation of selective intracellular signaling events or possibly partial activation of certain events and no activation of others. For example, T cell stimulation by an APL may lead to efficient activation of Ras and costimulation pathways whilst simultaneously showing a major decrease in the ζ/ZAP-70/NFAT pathway with a result of partial T cell activation [45]. Trigerring of a subset of early T cell signals by APLs is also supported by a recent study of Rabinowitz *et al.* [49•].

Recent studies [41••,42•] provide data directly correlating the affinity of the interaction between a TCR and MHC–peptide ligands with the agonistic and antagonistic properties of the peptides bound to MHC molecules (Fig. 4). An agonist MHC–peptide ligand comprising an ovalbumin peptide bound to MHC class I had high-affinity for cognate TCR with a K_{off} ~0.02^{s-1} whereas antagonist MHC–peptide ligands demonstrated lower affinities with K_{off}s in the range of $0.039–0.146^{s-1}$ [41••]. Direct evidence in support of the affinity model has also been provided in the class II system by Lyons *et al.* [42•]. The antagonistic mouse cytochrome c peptides complexed with I-E^k had affinities 10–50 times lower than the wild-type MHC–peptide complex and this decrease was primarily due to a higher off rate. Interestingly, the study with the MHC class I system did not find a strict correlation between off rate and responsiveness. Taking into account the differences in signaling patterns reported by Sloan-Lancaster *et al.* [48] and Madrenas *et al.* [47••], the results obtained from these kinetic measurements suggest that short-lived occupancy (rapid dissociation/low-affinity) would result in only certain signaling reactions through the TCR–CD3 complex whereas slow dissociation/longer occupancy would result in the completion of the entire sequence of signaling events necessary for full activation of the T cell. This would fit well with the kinetic proofreading model or kinetic discrimination model proposed by McKeithan [50] and Rabinowitz *et al.* [51] respectively.

The biological relevance of APLs is thus obvious from their ability to mediate positive or negative selection and activation of T cells *in vitro*. In the studies reported by Sloan-Lancaster *et al.* [48,52] the stimulation of a T cell by an APL induced anergy, a phenomenon that is important for the development of self tolerance. It is also possible that such ligands might be critical for the maintenance of T cell memory in the periphery. The ability of APLs to modulate T cell responses has been already demonstrated in experimental autoimmune encephalomyelitis [53•,54••]. APLs, therefore, have obvious potential in selective immunotherapy, particularly in T-cell-mediated autoimmunity.

Conclusions

The recent X-ray crystallographic structures of α and β TCR chains have finally provided long awaited experimental proof in favour of structural similarity between Igs and TCRs. The structure of a complete TCR modeled on the basis of the two crystal structures suggests that the TCR on the surface of a T cell might undergo dimerization after binding to cognate ligand which may in turn be involved in T cell signaling. This dimerization model is not necessarily supported by all presently available experimental data, but is certainly provocative and testable. The kinetic measurements of interactions between soluble TCRs and MHC–peptide molecules strongly favour a model in which the affinities between the TCRs and their ligands play a critical role in the activation of T cells in the periphery as well as during positive and negative selection in the thymus. Affinity and conformational models may not be mutually exclusive, however. Although we are still far from making generalizations about the orientation of the TCR with respect to the MHC–peptide ligands and the role of coreceptors in T cell signaling, these recent studies with soluble TCR molecules and their MHC–peptide ligands have no doubt set the stage for understanding precisely the molecular events of T cell development and activation at the level of the tripartite interaction.

Note added in proof: TCR finally gets to meet its ligand (in private) in 3 dimensions

Many uncertainties about how the α and β chains of the TCR interact with each other and how the TCR interacts with its ligand, the MHC–peptide complex, discussed earlier in this review, were resolved soon after

this review was written. In the 11 October issue of Science, Garcia *et al.* [55••] reported an αβ TCR structure at 2.5 Å and a low-resolution structure of its complex with the MHC class I molecule H-2K^b. The group made use of *Drosophila melanogaster* cells to express functional TCR and MHC molecules. The TCR shows a quaternary structure very much like that of the antigen-binding region of an antibody and structures of the Vβ–Cβ and Vα domains are similar to the structures reported earlier [1••,2••]. Cα, however, seems to deviate from the canonical immunoglobulin fold and shows an unusual top-strand topology. The structure also demonstrates that the mode of Cα–Cβ association is more similar to antibody C_H3–C_H3 than it is to the C_H1–V_L. A lower-resolution complex of this TCR complexed with H-2K^b-bound self peptide dEV8 reveals the footprint of TCR–MHC–peptide interaction. The interaction of the trimolecular complex seems to be diagonally orientated and is more consistent with the model proposed by Sant'Angelo *et al.* [30•] and Sun *et al.* [31•] than with other models involving an orientation in which TCR is perpendicular to the MHC molecule [27].

Only one month later, the precise identification of contacts between MHC, peptide and TCR was reported by Garboczi *et al.* [56••] who described the high-resolution crystal structure (2.5 Å) of a human TCR–MHC–peptide complex. The TCR in this case was HLA-A2-restricted and recognized a Tax peptide of human T cell lymphotropic virus, HTLV1 (for this study, the TCR and MHC were both produced in bacteria). The basic topology of the TCR–MHC–peptide interaction is similar to that proposed by Garcia *et al.* [55••]. The CDR1 and CDR3 of both α and β chains show interaction with the peptide. In this structure, although all three CDRs of the α chain contact MHC helices, unexpectedly only CDR3 of the β chain shows such interactions. There is more extensive interaction with the α2 helix of MHC class I than with the α1 helix and the TCR Vβ chain seems to be shadowing the carboxy-terminal half of the α1 helix without making any contacts.

The 3D structure of the interaction of a TCR Vβ–Cβ domain with the superantigens SEC2 and SEC3 was also recently reported [57••]. Unlike MHC–peptide recognition, TCR recognition of superantigen takes place primarily through interaction via the Vβ chain. Fields *et al.* [57••] show that the principal superantigen contact site is CDR2 of the β chain of the TCR, which, incidentally, does not make any contact with MHC in the structure of Garboczi *et al.* [56••]. The model proposed in this study suggests that in the TCR–superantigen interaction the TCR α chain binds to MHC class II whilst the β chain and MHC are contacted through the superantigen. This is consistent with data indicating that α chain usage can affect superantigen recognition [58,59].

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