

# The specificity of TCR/pMHC interaction

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Crystal structures of 11 complexes of TCRs with peptide/MHC (pMHC), that represent 6 independent TCRs, constitute the current structural database for deriving general insights into how  $\alpha\beta$  TCRs recognise peptide-bound MHC class I or class II. The TCRs adopt a roughly diagonal orientation on top of the pMHCs, but the identification of a set of conserved interactions that dictate this orientation is not apparent. Furthermore, the specific interaction of each TCR with its cognate pMHC partner is quite variable and also involves bound water molecules at the TCR/pMHC interface. In two of the systems, the structural basis for binding of altered peptide ligands has illustrated that the only significant conformational changes occur in the TCR/pMHC interface, but their small magnitude is inconsistent with the enormous variation in signalling outcomes. The TCRs adjust to different agonist, partial agonist and antagonist peptides by subtle conformational changes in their complementarity-determining regions, as previously observed in induced-fit mechanisms of antibody/antigen recognition. Alloreactive-complex structures determined or modelled so far indicate increased interactions of the TCR  $\beta$ -chain with the pMHC compared with their syngeneic counterparts.

## Addresses

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**Current Opinion in Immunology** 2002, 14:52–65

0952-7915/02/\$ – see front matter  
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## Abbreviations

<b>APL</b>	altered peptide ligand
<b>BSA</b>	buried surface area
<b>CDR</b>	complementarity-determining region
<b>HV4</b>	hypervariable region 4
<b>pMHC</b>	peptide/MHC
<b>sc</b>	single-chain

## Introduction

Since determination of the first mouse and human TCR and TCR/pMHC (peptide/MHC) structures [1–3,4•] in 1996 and soon afterwards, the expected flurry of new TCR structures and TCR/pMHC complexes has not materialised (Figure 1). Indeed, just four new independent TCR/pMHC complexes, two with MHC class I [5,6•] and two with MHC class II [7,8•], have been published in the past three years (Table 1). This situation attests to the continued difficulty in producing sufficient quantities of TCRs, either intact or as single-chain (sc) TCR Fv-like fragments, for crystallographic studies. In contrast, the number of MHC class I and class II crystal structures has been increasing dramatically (Figure 1), similar to the explosion in antibody structure determinations that began around 1989–1990. Although we have been able to derive

an enormous amount of information already from these six independent TCR/pMHC complexes, history tells us, when considering the antibody/antigen field [9], that not all key structural principles of TCR/pMHC recognition will be gleaned from these first few structures. Nevertheless, given the fact that  $\alpha\beta$  TCRs are restricted to MHC or MHC-like antigens, this situation may be mitigated compared with the almost infinite diversity that must be accounted for in antibody/antigen recognition.

In the past two years, the most significant advances in our understanding of TCR/pMHC interactions have come from the determination of three new TCR/pMHC complexes [6•,7,8•] that now include an MHC class I allogeneic complex and the first complexes with mouse and human MHC class II. In addition, several other complexes have been published with altered peptide ligands (APLs), which include strong agonists, weak agonists and antagonists for two systems — the human A6/HLA-A2 [10] and the mouse 2C/H-2K<sup>b</sup> [4•]. Two allogeneic TCR/pMHC complexes [6•,11•] have illustrated that the general structural features seen in syngeneic complexes apply, although some surprises have come from one of these structures [6•]. Finally, this past year produced the first structure of a  $\gamma\delta$  TCR [12•] that now completes our structural view of primary antigen-recognition receptors — the antibody, the  $\alpha\beta$  TCR and the  $\gamma\delta$  TCR (Figure 2). These new crystal structures from 1999–2001 and the insights they brought are the focus of this review (Table 1). Other notable previous reviews on this subject include [13–20].

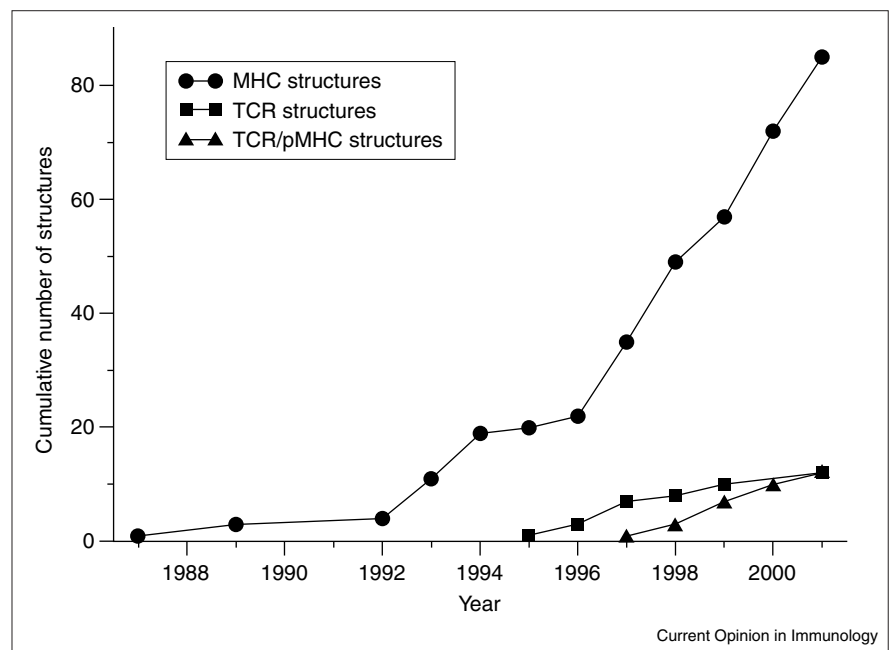
## Production of TCR/pMHC complexes

No general method has yet emerged for rapid production of sufficient quantities of soluble TCRs for crystallographic studies. In a previous review [13], we outlined some of the expression systems that were used to produce TCR protein for structural studies. Here, we will only briefly highlight novel features that facilitated production of TCR/pMHC crystals.

Most of the MHC molecules have been produced in *Escherichia coli* and refolded from inclusion bodies, except for murine H-2K<sup>b</sup> and HLA-DR1, which have also been produced in *Drosophila melanogaster* cells (Table 1). For MHC class II, the peptide is normally covalently attached through a linker to the amino terminus of the  $\beta$ -chain, in an innovative approach pioneered by Kappler and Marrack (see [21]). In another extraordinarily inventive approach, the peptide was covalently connected not to the MHC, but to the amino terminus of the  $\beta$ -chain of the TCR [8•]. This construct was critical in obtaining stable complexes of human TCR HA1.7 with HLA-DR1 and HLA-DR4 that permitted their crystallisation [8•,11•]. This type of

**Figure 1**

Cumulative number of MHC, TCR and TCR/pMHC-complex crystal structures. The number of structures is plotted as a function of their deposition year in the Protein Data Bank (PDB) [72]. The plot does not contain structures that were superseded by re-determination at higher resolution. In order to avoid redundancy, the TCR and MHC structures in TCR/pMHC complexes are not included in the individual numbers. However, MHC and TCR complexes with other molecules, such as superantigens or antibodies, are included. For the TCRs, all fragments and constructs (such as single chains) that were determined by either X-ray diffraction or NMR spectroscopy are included. The first MHC crystal structure was determined in 1987 [36] and, after an approximately 5-year lag, the number of MHC structures increased dramatically, with as many as 15 structures added to the PDB in 2000. Compared with that, the number of new TCR and TCR/pMHC structures is lagging behind considerably.



stabilisation of complexes by reducing the (unfavourable) change in entropy during complex formation through covalent connection of binding partners has been successfully used previously, such as in scFvs [22], scTCRs [6•,7] and the CD3 $\epsilon$  dimer [23•].

These methods clearly have proven useful as a valuable general strategy to obtain homogenous samples for crystallisation. For the TCRs, intact extracellular domains have been produced both in *E. coli* and *D. melanogaster*, whereas scTCRs have been produced in both *E. coli* and myeloma cells. All of the relevant details on these expression constructs can be found in the primary references cited in Table 1.

### The $\gamma\delta$ TCR structure

Antibody structures were first determined in 1971 (see [24–26]), but it took another 25 years to visualise the intact  $\alpha\beta$  TCR structure [1,2]. Five years later (2001), we finally were presented with our first glimpse of a  $\gamma\delta$  TCR [12•] to complement the  $V\delta$  chain structure determined in 1998 [27]. This  $\gamma\delta$  TCR structure confirmed the view that these three sets of key antigen-recognition receptors have similar overall anatomies (Figure 2). The  $\gamma\delta$  TCR has often been called ‘antibody-like’, as compared with the  $\alpha\beta$  TCR, because of its ability to recognise intact proteins ([28]; reviewed in [29]) and in the correspondence of the lengths of their central complementarity-determining region (CDR)3 loops with antibodies rather than with  $\alpha\beta$  TCRs ([30]; reviewed in [31]). The study also described the putative binding site for phospho-antigens, for example pyrophosphates that are derived from mycobacteria [32,33].

### TCR/pMHC orientation

The six independent TCR/pMHC complexes (Table 1) show some fluctuation in the orientation that the TCR adopts on top of the pMHC (Figure 3a–c). This orientation was originally described as diagonal [1,2], but has recently been described for a class II TCR/pMHC complex as orthogonal [7]. However, it appears that the TCR orientation or twist on MHC class I and class II deviates about a relatively restricted mean that is currently spread by about 35° (Figure 3a), but that is still consistent with a generally diagonal orientation and footprint on the pMHC (Figures 3a and 4). However, as noted previously [5,34], the TCR deviates not only in its twist, but also in its roll (range 19°) and tilt (range 30°), which can be represented by the angle of inclination of the pseudo twofold axis between the  $V\alpha$  and  $V\beta$  domains relative to the MHC  $\beta$ -sheet floor (Figures 3b,c). In addition, the TCRs can differ in their  $\alpha\beta$  chain pairings, such that the pseudo  $V\alpha/V\beta$  twofold angle, which currently varies from 166° to 180°, can also contribute to the variation in TCR orientation on the pMHC.

In earlier structures, a relatively constant interaction of  $V\alpha$  with the amino-terminal half of the peptide-binding groove was noted, as compared with the much more variable  $V\beta$  interaction with the carboxy-terminal half (reviewed in [13]). This generalisation is still borne out in the recently described complexes, except for the allogeneic scBM3.3/H-2K<sup>b</sup>/pBM1 complex, where almost no direct contacts are observed between the  $V\alpha$  chain and the pMHC (Figures 3c and 4). In the scBM3.3 structure,  $V\alpha$  hovers just above the surface, creating a voluminous cavity that is occupied by ~30 water molecules [6•]. Otherwise, considerably more variation occurs in  $V\beta$  interactions with

**Table 1****Overview of TCR/pMHC complex structures 1996–2001.**

Complex (TCR/MHC/peptide)	PDB ID	Peptide activity	Constructs and expression systems	References
<b>Class I</b>				
2C/H-2K <sup>b</sup> /dEV8	2ckb	Weak agonist	<i>D. melanogaster</i> , acidic/basic leucine zipper for specific TCR chain-pairing	[1,3]
2C/H-2K <sup>b</sup> /SIYR	1g6r	Superagonist*		[4**]
2C/H-2K <sup>bm3</sup> /dEV8	1jtr	Weak agonist		(a)
scBM3.3/H-2K <sup>b</sup> /pBM1	1fo0	Agonist	Myeloma cells for TCR, <i>E. coli</i> for MHC (refolded from inclusion bodies)	[6**]
B7/HLA-A2/Tax	1bd2	Strong agonist*	<i>E. coli</i> , refolded from inclusion bodies	[5]
A6/HLA-A2/Tax	1ao7	Strong agonist*	<i>E. coli</i> , refolded from inclusion bodies	[2]
A6/HLA-A2/TaxP6A	1qrn	Weak antagonist		[10]
A6/HLA-A2/TaxV7R	1qse	Weak agonist		[10]
A6/HLA-A2/TaxY8A	1qsf	Weak antagonist		[10]
<b>Class II</b>				
scD10/I-A <sup>k</sup> /CA	1d9k	Agonist	<i>E. coli</i> for TCR, refolded from inclusion bodies; CHO cells for MHC. Peptide covalently connected to the MHC	[7]
HA1.7/HLA-DR1/HA	1fyt	Agonist	<i>E. coli</i> for TCR, refolded from inclusion bodies; <i>D. melanogaster</i> for MHC. Peptide covalently connected to the TCR	[8**]
HA1.7/HLA-DR4/HA	1j8h	Agonist		[11*]

\*The designation 'superagonist' or 'strong agonist' is equivalent in these instances. (a) JG Luz, M Huang, KC Garcia, MG Rudolph, L Teyton, IA Wilson, unpublished data. PDB ID, PDB identification number. sc, single-chain Fv fragment of the TCR. Only structures from 2000 onwards are annotated.

pMHC, as illustrated by the clustering of the tips of CDR1 $\alpha$  and CDR2 $\alpha$  loops, compared with the diversity in positioning of the equivalent CDR $\beta$  loops in different complexes (Figures 3a and 5).

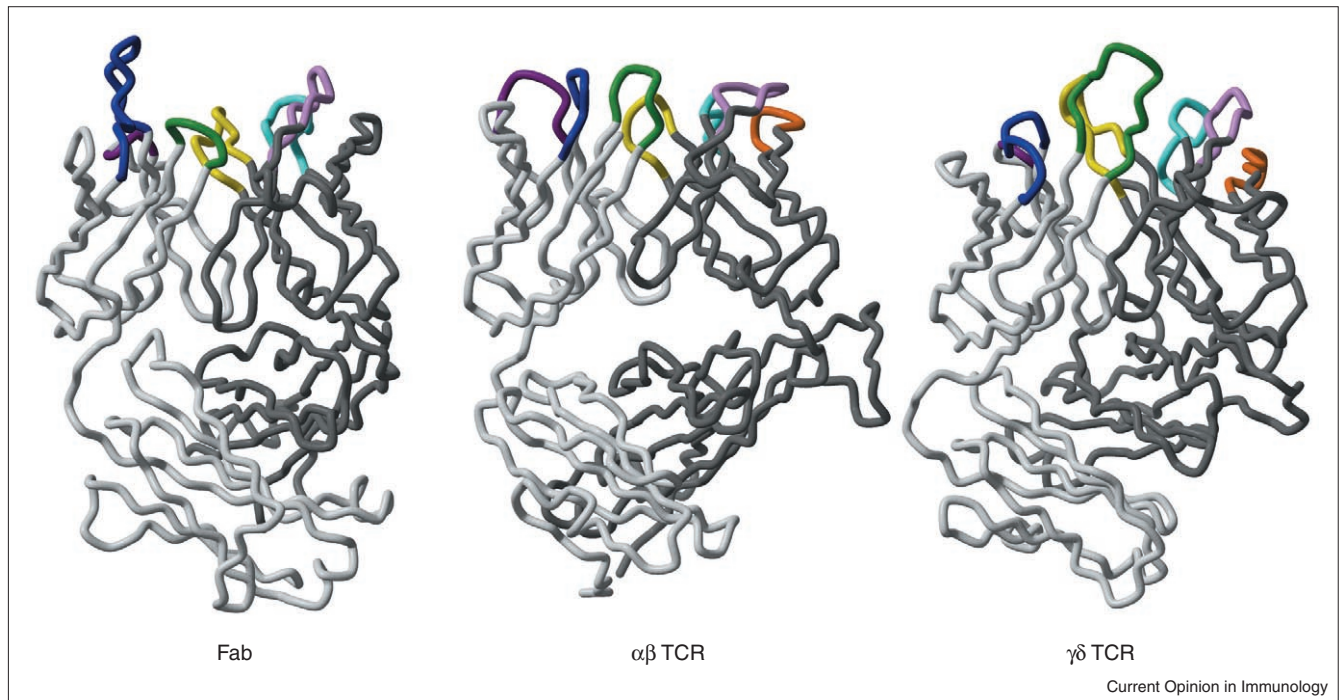
### Analysis of the TCR/pMHC interactions

Eleven TCR/pMHC complex structures have been published (or the results are 'in press') during the past five years (Table 1). A detailed analysis of these complexes, that includes comparison of buried surface area (BSA) at the TCR/pMHC interface, and the contributions to the interface of peptide versus MHC, of V $\alpha$  versus V $\beta$  and of the individual CDR loops, is given in Table 2. The BSA for the TCR/pMHC complex varies extensively — between 1239 Å<sup>2</sup> and 1931 Å<sup>2</sup>. The percentage contribution of the peptide to the pMHC side of that interface is more narrowly confined to a range of 21%–34%, whereas V $\alpha$  has contributed from 37%–74% (average 57%) and, conversely, V $\beta$  from 26%–63% (average 43%) of the TCR buried surface. A similar bias in chain usage has been noted for antibodies, where V<sub>H</sub> usually provides a larger contribution to the antibody/antigen interface [9].

An extraordinary amount of variation also occurs in the contribution of individual CDR loops to their respective

TCR/pMHC interfaces. For V $\alpha$ , the percentage contribution of its CDRs 1–3 to the buried surface varies from an average of 21% for CDR1 $\alpha$  (range 14%–29%), to 12% for CDR2 $\alpha$  (8%–17%), and to 21% for CDR3 $\alpha$  (6%–27%). Hypervariable region 4 (HV4) makes relatively little percentage contribution to either the  $\alpha$  chain (0–11%) or the  $\beta$  chain (0–2%) interface, but could still contribute key orienting contacts or electrostatic interactions (see below). For V $\beta$ , the equivalent values are more extreme, with average contributions for CDR1 $\beta$  being 6% (0–16%), 11% for CDR2 $\beta$  (0–23%) and 24% for CDR3 $\beta$  (10%–39%). In some instances, CDRs 1 $\beta$  and 2 $\beta$  actually contribute little (or nothing) to the TCR/pMHC buried surface, as for complexes of TCRs A6 and B7 with HLA-A2.

In fact, when one analyses the actual number of contacts rather than the buried surface (Table 2, bottom), CDR1 $\beta$  and CDR2 $\beta$  often make minimal contact with the pMHC compared with CDR3 $\beta$ . For V $\alpha$ , this trend mainly applies to CDR2 $\alpha$ , although it is much less pronounced, except for the allogeneic scBM3.3 complex where CDR3 $\alpha$  has almost no contacts. Thus, in most cases, the centrally located CDR3 loops dominate the interactions with pMHC.

**Figure 2**

Overall comparison of the anatomy of antibody Fab,  $\alpha\beta$  TCR and  $\gamma\delta$  TCR structures. Light-grey shading is used for the antibody light (L) chain and TCR  $\alpha$  or  $\delta$  chains; dark-grey shading is used for the antibody heavy (H) chain and TCR  $\beta$  or  $\gamma$  chains. The CDR loops are

colour-coded as follows: for the L,  $\alpha$  and  $\delta$  chains, CDR1 is dark blue, CDR2 is magenta and CDR3 is green; for the H,  $\beta$  and  $\gamma$  chains, CDR1 is cyan, CDR2 is pink, CDR3 is yellow and HV4 is orange (TCRs only).

### The contribution of the bound peptide to TCR interactions

How much does the peptide itself contribute to the pMHC interface? How can the TCR distinguish one peptide from another, when presented by the same MHC molecule? Many immunological studies (e.g. [35]) prior to the determination of a TCR/pMHC structure — combined with the plethora of pMHC structures that first originated from the HLA-A2 structure in 1987 [36] and subsequent single-peptide/pMHC complexes in 1992 [37–39] — suggested that a few up-pointing side-chains of the peptide would be the major determinants that contribute to the specificity of the TCR/pMHC interaction.

#### Physiological ligands

The TCR/pMHC crystal structures have graphically cemented that view, where usually only 2–5 peptide side-chains are involved in direct TCR contact (Table 3). In MHC class I, these interactions are dominated by the peptide residues that bulge most out of the groove and, hence, represent functional hotspots [4\*\*] in the TCR/pMHC interface. For nonamer and octamer peptides, these represent predominantly residues P5, P7 and P8, and P4, P6 and P7, respectively. For MHC class II peptides, the key side-chain contributions are more uniformly dispersed (predominantly P-1, P2, P3, P5 and P8) due to the extensive backbone interactions in the class-II

binding groove that confine their structures to repeating polyproline type-II helix-like conformations [40]. In class II, the peptides also lie slightly deeper in the MHC binding groove (Figure 3d).

Clearly, the peptide is able to dominate the TCR/pMHC interface more in MHC class I due to the differential ability to bulge out of the groove depending on the length of the peptide and the polymorphic residues that line the MHC peptide-binding groove [41\*\*]. Extensive ridges in some MHCs force the peptide to bulge even higher out of the groove and provide more intimate contact with the TCR [42,43]. On the other hand, the contribution of the peptide backbone to TCR interaction is very modest for both MHC class I and class II, where none, or only a handful of, contacts are made (Table 3, bottom). The only exception so far is for the HLA-A2/Tax complex, where the large P4–P5 bulge includes a glycine at P4 that enables the TCR to more easily access the peptide backbone (Figure 3d).

#### Altered peptide ligands

So far, no dramatic structural changes that could account for the magnitude of the different signalling outcomes of various APLs have been noted in the TCR/pMHC structures, when strong agonist, weak agonist and antagonist peptides are presented by the same MHC to the same TCR [4\*\*,10]. Only slight readjustments occur in the TCR/pMHC interface to



**Table 2****Crystal structures and analysis of TCR/pMHC-class-I complexes.**

TCR	2C	2C	scBM3.3	B7	A6	A6	A6	A6	scD10	HA1.7	HA1.7	Average
MHC	H-2K <sup>b</sup>	H-2K <sup>b</sup>	H-2K <sup>b</sup>	HLA-A2	HLA-A2	HLA-A2	HLA-A2	HLA-A2	I-A <sup>k</sup>	HLA-DR1	HLA-DR4	values
Peptide	dEV8	SIYR	pBM1	Tax	Tax	TaxP6A	TaxV7R	TaxY8A	CA	HA	HA	
Resolution (Å)	3.0	2.8	2.5	2.5	2.6	2.8	2.8	2.8	3.2	2.6	2.4	–
BSA (Å <sup>2</sup> )	1891	1795	1239	1651	1801	1767	1753	1666	1733	1931	1915	1740
<b>% Contribution to buried surface</b>												
Peptide	24	24	21	32	34	33	34	27	23	33	32	29
V <sub>α</sub>	54	52	37	67	64	66	64	74	61	46	47	57
CDR1	23	18	14	28	25	23	23	29	22	15	15	21
CDR2	13	16	17	13	10	13	10	12	15	8	9	12
CDR3	16	16	6	23	25	25	26	27	22	22	23	21
HV4	2	0	0	0	4	11	6	7	1	0	0	3
V <sub>β</sub>	46	48	63	33	36	34	36	26	39	54	53	43
CDR1	16	15	10	0	2	2	2	0	3	8	8	6
CDR2	17	21	14	11	0	1	0	0	13	23	16	11
CDR3	10	11	39	22	33	31	34	26	16	22	23	24
HV4	1	2	1	0	0	0	0	0	0	1	1	1
<b># Contacts</b>												
MHC	63	39	60	46	76	76	90	82	98	84	82	72
Peptide	23	35	34	60	47	60	58	30	34	37	34	41
Total	86	74	94	106	123	136	148	112	132	121	116	113
V <sub>α</sub>	69	40	29	72	74	98	96	88	70	46	51	67
CDR1	23	19	11	25	24	23	21	23	28	13	16	21
CDR2	17	1	16	17	3	4	8	9	16	2	5	9
CDR3	26	20	2	30	41	60	56	49	23	30	29	33
HV4	3	0	0	0	6	9	5	7	2	0	0	3
V <sub>β</sub>	17	34	65	34	49	38	57	24	62	75	66	47
CDR1	7	16	1	0	3	4	4	0	0	15	13	6
CDR2	6	2	9	3	0	0	0	0	31	28	17	9
CDR3	4	15	55	31	46	34	53	24	24	22	25	30
HV4	0	0	0	0	0	0	0	0	0	0	0	0

BSA was calculated with MS [73] using a 1.7 Å probe radius. Contacts were calculated with HBPLUS [74] and CONTACSYM [75].

accommodate different up-pointing peptide side-chains. In the A6 system, the number of peptide/TCR contacts does not correlate with the degree of agonism and antagonism (Table 3), although there is a slight, but probably insignificant, increase in the overall BSA for the strong agonist, Tax (Table 2). Similarly, in the 2C system [4\*\*], the BSA does not change much, but the complementarity and the number of TCR/pMHC contacts increases despite the relatively minor substitution of an arginine (in the strong agonist, SIYR) for a lysine (in the weak agonist, dEV8) at P4 (Table 3). Again, no gross conformational changes in the TCR or pMHC are observed, but slight rearrangements in the CDR loops accommodate the different peptides (Figure 5b), as particularly exemplified by the conformational adjustments seen in the CDR3β loop in the A6 TCR on binding different pMHCs (Figure 5c).

The correlation between the half-life of a complex [44] and the degree of agonism or antagonism is also not clear-cut. In both

2C and A6, the strong agonists (SIYR and Tax) have a longer half-life (9.2 s and 7.5 s) than weak agonists (3.7 s for H-2K<sup>b</sup>/dEV8 and 1.5 s for HLA-A2/TaxV7R). However, by using surface plasmon resonance (SPR), agonists have been found in the A6 system that have shorter half-lives than antagonists [45\*]. In this study, an antagonist was converted to an agonist by stepwise filling of a cavity in the TCR/pMHC interface and the biological activity paralleled the TCR/pMHC affinity, not the half-life of the complex [45\*]. So questions arise as to whether these SPR measurements using soluble receptors really represent what is happening on the cell surface. Longer half-lives of TCR/pMHC complexes on the cell surface could lead to additional stabilisation by the co-receptors CD4 and CD8 [46]. Although solution studies yielded contradictory results for TCR/pMHC oligomerisation [47,48], lateral interactions among the TCR/pMHC signalling complexes or interactions with other co-stimulatory or inhibitory receptors, as in the immunological synapse, may only form above a certain threshold of TCR/pMHC-complex half-life [49].

### Positive selection and TCR bias

A question that we have been specifically asked to address with respect to the current set of TCR/pMHC crystal structures is how positive selection on self-pMHC molecules produces a repertoire highly biased to effective recognition of unknown foreign peptides bound to the same allelic form of an MHC molecule.

This question could be restated to ask how it is that positively selected T cells retain sensitivity to peptides they encounter in the periphery. Clearly, foreign peptides are not really any different from self-peptides and, indeed, some may be antagonists as well as agonists. Which ones are which depends on the complementarity of their interaction with the TCR in the context of the pMHC. The conserved docking mode of the TCR on the pMHC offers some explanation for how a collection of different peptides — the peptide repertoire — is recognized in the context of the same MHC. The same interactions that steer the TCR towards its generally diagonal orientation place the CDR1 and CDR2 loops over the long  $\alpha 1$  and  $\alpha 2$  ( $\beta 1$ ) helices of the MHC class I (II) molecule. Indeed, given the more conserved interactions for the V $\alpha$  CDR1 and CDR2 loops with pMHC that have been shown so far, the V $\alpha$  domain would appear to be more critical in determining the orientation and hence in setting up the readout of the peptide sequence. These relatively conserved germline CDR1 and CDR2 interactions [13] provide the basic affinity of the TCR for the generic MHC allele, where the CDR3 loops are positioned to primarily contact the peptide.

Various mutagenesis experiments have shown that no single contact or set of contacts dominates the TCR/pMHC interaction [50], contrary to what is often observed in antibody/antigen interactions, where somatic mutation can allow a small number of high-affinity interactions to dominate the energy landscape [51]. For TCRs, if the affinity (or half-life) of the complex in the thymus becomes too high (or long) due to excessive interactions with the peptide, negative selection will occur.

Thus, interaction of the CDR1 and CDR2 loops with the MHC-helices, or even the peptide backbone, is likely to be responsible for positive selection, whereas the CDR3 loops play a more important role in negative selection. The latter leads directly to priming of the TCRs for sensitivity against any peptide encountered in the periphery: CDR1 and CDR2 loops provide for basal affinity, and CDR3 loops for specificity. Again, lack of any somatic mutation in the TCRs that would drive high-affinity engagement allows the TCR to be relatively permissive for substitutions of peptide side-chains in the interface. Certain key positions in the peptide, usually those that protrude highest from the groove, provide the basis for discrimination of peptide and for altering the affinity or half-life of the TCR/pMHC interaction. As described for the 2C system [4<sup>••</sup>], the central P4 and P6 up-pointing residues form a

functional ‘hotspot’ around which the TCR is highly sensitive to changes in the particular peptide residues.

Furthermore, in some linked examples of alloreactive complexes, substantial bulging of the peptide out of the groove may dominate the interaction so as to allow cross-reactivity with ‘foreign’ MHC that may even disrupt the standard V $\alpha$  interactions [6<sup>••</sup>]. In normal situations, the generally diagonal orientation places the CDR3 loops in the center of the groove where, for class I peptides, substantial bulging of the peptide backbone or protrusion of the central peptide side-chains can arise. The curvature of two interacting globular surfaces of the TCR and pMHC leads to greater distances between the peptide at the amino terminus, and to some extent at the carboxyl terminus, than in the middle, again explaining the sensitivity of the central CDR3 loops to reading-out the central peptide sequence. For class II peptides, the central P1–P9 residues are of more constant depth and do not rise towards the TCR except at their ends.

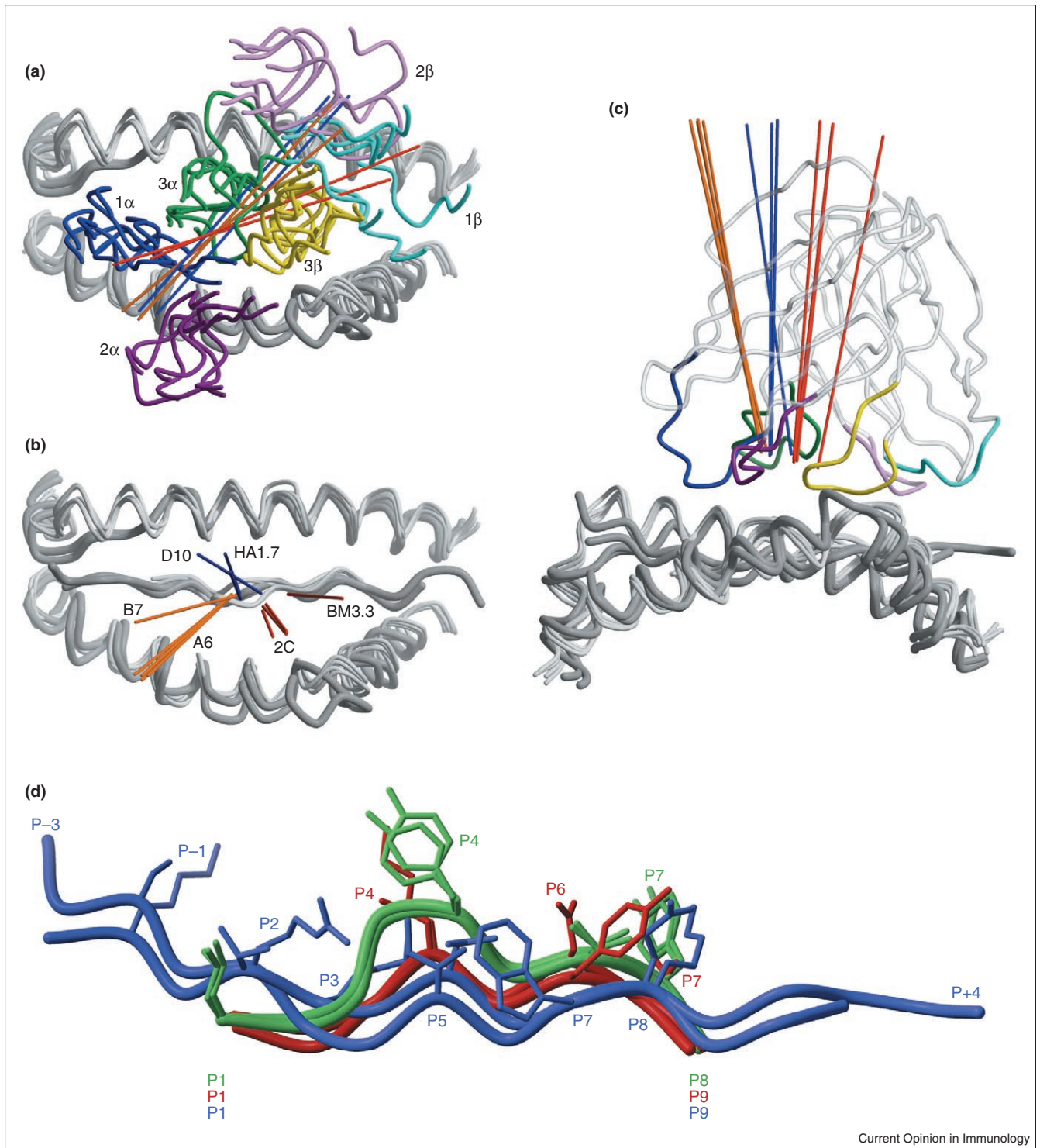
Such effects are tabulated in Table 3 where, for class I, the main peptide contribution to TCR recognition are at P5, P7 and P8 for nonamers, and P4, P6 and P7 for octamers. For class II, the corresponding residues are P5 and P8, but P2 also becomes a player, because its backbone interaction with the class II MHC raises the amino end of the class II peptide out of the groove in relation to the more deeply embedded amino end of the class I peptides. The greater conservation of the TCR  $\alpha$  chain interaction and location in the syngeneic complexes determined so far suggests that the  $\alpha$  chain may play a more important role in setting up the initial pMHC interaction, whereas the  $\beta$  chain then is able to adapt to fit whatever is encountered in the form of peptide at the carboxy-terminal half of the groove.

### Alloreactivity

Alloreactivity may occur in response to the polymorphic variation in the MHC or in selection of a different peptide repertoire as a result of these ‘mutated’ residues in the MHC. So far, three complexes have been used to address this issue ([6<sup>••</sup>, 11<sup>•</sup>]; JG Luz, M Huang, KC Garcia, MG Rudolph, L Teyton, IA Wilson, unpublished data).

The complex of the scBM3.3 TCR with the allogeneic MHC H-2K<sup>b</sup> is perhaps the most structurally distinct so far, but the corresponding syngeneic complex is currently not known. The scBM3.3 TCR tilts substantially towards the  $\beta$ -chain side (Figures 3c and 4), with the  $\alpha$ -chain making few direct contacts with the MHC (Table 2). In fact, the long central CDR3 $\alpha$  is flared back such that it makes no contacts with the peptide and only two with the MHC (Table 2). The majority of the interactions are with the  $\beta$ -chain, consistent with that proposed for the interaction of H-2L<sup>d</sup> with TCR 2C, where an extreme bulge in the carboxy-terminal half of the peptide is likely to increase its interaction with the TCR  $\beta$ -chain [43]. Two recent structural studies ([11<sup>•</sup>]; JG Luz, M Huang, KC Garcia,

Figure 3



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MG Rudolph, L Teyton, IA Wilson, unpublished data) confirm that subtle changes in allogeneic MHCs may alter the peptide conformation and location such that the same peptide is presented differently to the TCR. Thus, these structural studies conclude that TCR interaction with the bound peptide strongly affects the alloresponse.

### TCR conformational variation and changes

Sufficient numbers of TCR structures are now available to assess the extent of conformational variation that arises in their antigen-combining sites (Figure 5a). As expected, the four TCR outer CDRs 1 and 2 adopt canonical conformations [52•], as first described for antibodies [53,54]. A small

**Figure 3 legend**

Relative orientation of the TCR on top of the MHC and comparison of peptide conformations in TCR/pMHC (class I versus class II) complexes. The MHC helices are shown as light and dark grey tubes for class I and class II, respectively. The CDR loops are coloured as in Figure 2. Lines and axes are coloured blue for class II TCRs and orange and red for human and mouse class I TCRs, respectively. **(a)** Variation in the diagonal (twist) orientation of the six independent TCR/pMHC complexes. The projection of a linear least-squares fit through the centres of gravity of the CDR loops is shown for the six different TCRs. **(b,c)** Variation in the tilt and roll of TCR/pMHC complexes. The pseudo two-fold axes (colour code as above) that relate the V $\alpha$  and V $\beta$  domains of the TCRs to each other are shown for twelve TCR/pMHC structures of six different TCR molecules (scD10, HA1.7, B7, A6, 2C and

scBM3.3). This gives a good estimate of the inclination (roll, tilt) of the TCR on top of the MHC, which is a function of the TCR, not the pMHC ligand. One extreme case is the allogeneic scBM3.3 TCR, which is shown as a transparent C $\alpha$  trace in the side view of (c). **(d)** The C $\alpha$  traces of the bound peptides (removed from their respective MHCs) are drawn as tubes with the TCR-contacting side-chains (see Table 3) as stick representations. Only a representative peptide for each of the six independent TCR/pMHC complexes is shown (Table 1). Class-I-bound peptides from mouse and human are coloured red and green, respectively. Peptides from class II complexes are coloured blue. The peptides are oriented with their TCR-contacting residues pointing upward. The  $\beta$ -sheet floors of the peptide-binding sites of the MHC molecules were superimposed to align the peptides.

number of discrete canonical conformations may be able to describe most of the known sequences of the  $\alpha$ 1,2 and  $\beta$ 1,2 loops. At present, 3–4 canonical structures have been

defined for each of these loops [52<sup>\*</sup>]. What makes the TCR different from antibodies is the enormous variation seen in both of the central CDR3s (Figure 5a). In antibodies,

**Table 3****Interactions of the peptide component of the pMHC with the TCR.**

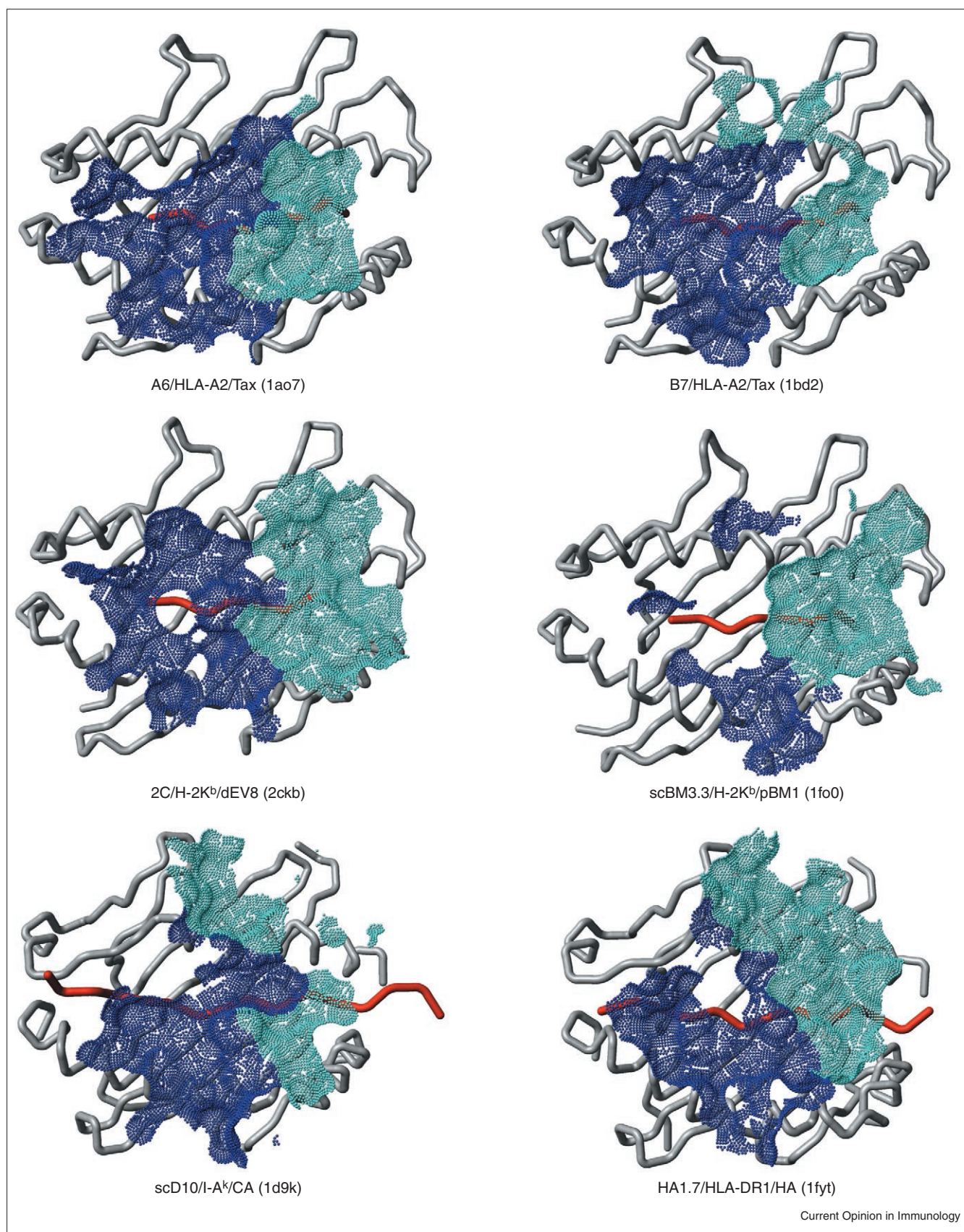
TCR/MHC/peptide	#Peptide residues	Peptide residue (P) and # contacts per residue												Total # contacts
Peptide side-chain contacts with TCR														
Human class I				P1	P2	P3	P4	P5	P6	P7	P8	P9		
A6/HLA-A2/Tax	9			1	–	–	–	15	1	5	11	–		33
A6/HLA-A2/TaxP6A	9			–	–	–	–	27	–	5	12	–		44
A6/HLA-A2/TaxV7R	9			1	–	–	–	19	–	11	14	–		45
A6/HLA-A2/TaxY8A	9			4	–	–	–	15	–	1	–	–		20
B7/HLA-A2/Tax	9			4	–	–	–	29	–	4	10	–		47
Mouse class I				P1	P2	P3		P4		P5	P6	P7	P8	
scBM3.3/H-2K <sup>b</sup> /pBM1	8			–	–	–		1		–	20	8	–	29
2C/H-2K <sup>b</sup> /dEV8	8			–	–	–		18		–	3	2	–	23
2C/H-2K <sup>b</sup> /SIYR	8			–	–	–		14		–	21	–	–	35
Human/mouse class II		P–2	P–1	P1	P2	P3	P4	P5	P6	P7	P8	P9	P+1	
scD10/I-A <sup>k</sup> /CA	16	–	1	–	11	–	–	8	–	4	7	–	–	31
HA1.7/HLA-DR1/HA	13	–	6	–	2	4	1	6	–	–	8	–	–	27
HA1.7/HLA-DR4/HA	13	–	4	–	4	5	–	3	–	–	8	–	–	24
Peptide main-chain contacts with TCR														
Human class I				P1	P2	P3	P4	P5	P6	P7	P8	P9		
A6/HLA-A2/Tax	9			–	1	–	10	–	1	1	1	–		14
A6/HLA-A2/TaxP6A	9			–	1	–	11	–	2	1	1	–		16
A6/HLA-A2/TaxV7R	9			–	1	–	10	1	–	–	1	–		13
A6/HLA-A2/TaxY8A	9			–	1	–	9	–	–	–	–	–		10
B7/HLA-A2/Tax	9			–	–	–	5	–	1	4	3	–		13
Mouse class I				P1	P2	P3		P4		P5	P6	P7	P8	
scBM3.3/H-2K <sup>b</sup> /pBM1	8			–	–	–		–		–	2	3	–	5
2C/H-2K <sup>b</sup> /dEV8	8			–	–	–		–		–	–	–	–	–
2C/H-2K <sup>b</sup> /SIYR	8			–	–	–		–		–	–	–	–	–
Human/mouse class II		P–2	P–1	P1	P2	P3	P4	P5	P6	P7	P8	P9	P+1	
scD10/I-A <sup>k</sup> /CA	16	–	–	–	–	–	–	–	1	1	1	–	–	3
HA1.7/HLA-DR1/HA	13	–	–	–	–	–	–	1	2	1	3	–	–	7
HA1.7/HLA-DR4/HA	13	–	–	–	–	–	–	–	1	2	4	–	–	7

There are no TCR contacts with peptide residues P3 or P9 in any class I complex, whereas no TCR contacts are observed for peptide residues P1, P9 (P8 for octamers), or P+1 in any class II complex. All interactions were

calculated with HBPLUS [74] and CONTACSYM [75] using standard van der Waals radii and a probe radius of 1.7 Å. The Tax peptide residue P4 is a glycine, which may explain its accessibility for main-chain contacts.



Figure 4



**Figure 4 legend**

Relative contributions of the TCR V $\alpha$  and V $\beta$  domains to the BSA of TCR/pMHC complexes. The view is from the TCR onto the peptide-binding site of the MHC. Peptide and MHC C $\alpha$  traces are shown as red and grey tubes, respectively. The surface buried by

the TCR V $\alpha$  (dark blue) and V $\beta$  (cyan) domains on the pMHC is represented as dots. Note the substantially reduced direct contacts of V $\alpha$  with the pMHC in the allogeneic scBM3.3/H-2K<sup>b</sup>/pBM1 complex.

CDR L3 adopts a well-defined set of canonical structures, but the equivalent CDR3 $\alpha$  loop is, in fact, the most variable in the current set of TCR structures. Thus, the prediction [55] that these central CDRs would be most variable and adapt to the pMHC primarily (but not exclusively [6•]) through contact with the peptide has been borne out.

Induced fit has also been addressed for TCRs as it was for antibodies [9]. Only one published example of free and bound TCRs is available for the 2C TCR [1,3], where substantially different conformations are seen for CDR1 $\alpha$  and CDR3 $\alpha$ . What has yet to be resolved is whether any of the CDRs can change their canonical structures upon pMHC binding. In antibodies, these CDRs, including CDR L3, usually only go through segmental shifts in structure (~1 Å–3 Å) that change their location, but not their overall shape [9,54,56]. The prediction is that TCRs will also conform to that notion [52•] and, in fact, the tip of the TCR 2C CDR1 $\alpha$  loop moves in a hinge motion by 17°, without changing its overall shape (Figure 5b). For the central CDRs, it is more likely that these loops will also rearrange on ligand binding, as seen especially for the CDR H3 loops of antibodies [9].

Two examples are available to assess the extent of conformational variation in the CDR loops in the presence of an APL. For TCR 2C, only small variations are seen in CDR3 $\beta$  (Figure 5b) but, for TCR A6, these conformational rearrangements are much larger (Figure 5c). Evidence for flexibility in the TCR has also been derived from kinetic and thermodynamic studies [57–59]. Whether these data support a model in which flexible CDRs stabilise or rearrange upon pMHC binding remains an unanswered question. What is certainly consistent so far in both the structural and kinetic/thermodynamic experiments is that conformational rearrangements of the CDRs can provide better complementarity of the TCR to both the MHC [3] and the peptide [4•,10].

**The role of bound water**

Several TCR/pMHC complexes contain bound water molecules in their TCR/pMHC interfaces. The ability of water molecules to provide additional complementarity by the filling of cavities in the interface is well documented for antibodies [60]. The highest resolution TCR/pMHC complexes (2.4 Å–2.5 Å; Table 2) contain 17 (2C/H-2K<sup>bm3</sup>/dEV8 [JG Luz, M Huang, KC Garcia, MG Rudolph, L Teyton, IA Wilson, unpublished data]), 39 (scBM3.3/H-2K<sup>b</sup>/pBM1 [6•]) and 15 (HA1.7/HLA-DR4/HA [11•]) waters in their

interface with 6, 12 and 6, respectively, mediating contact between the TCR and pMHC. No specific waters are conserved among these structures, indicating that their presence is dependent on the individual sequences of both the TCR and pMHC. In the allogeneic scBM3.3 complex, ~30 interfacial waters are sequestered in a cavity between the V $\alpha$  and the pMHC, as a result of the TCR V $\alpha$  domain lifting up (Figure 4) from the pMHC surface [6•].

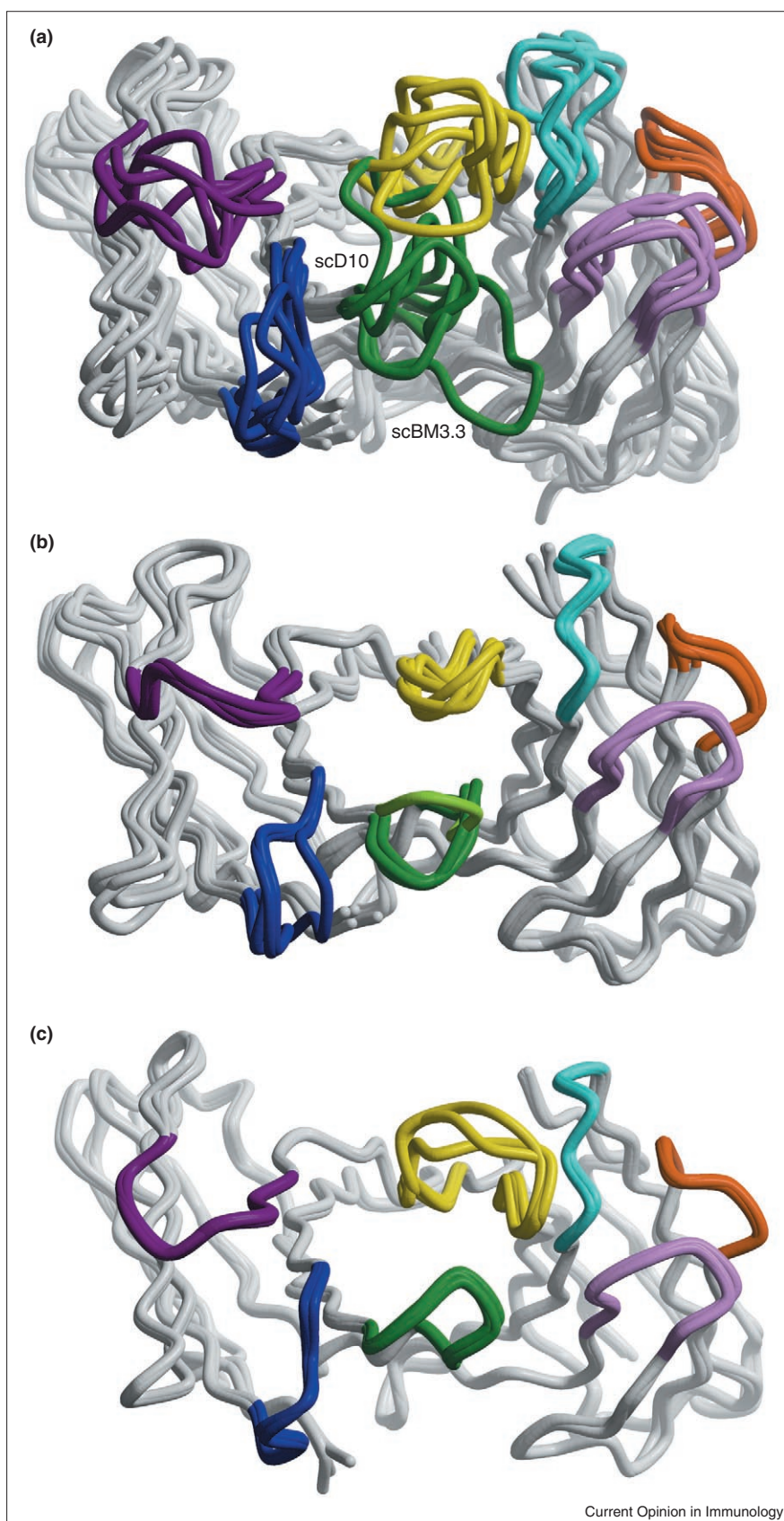
Thus, these recent, higher resolution TCR/pMHC structures indicate a strong involvement of bound water to provide complementarity and specificity to the recognition process. Indeed, small sequence and structure changes in either the peptide (APLs) or the MHC (as in alloreactive complexes) can be amplified on the pMHC surface by redistribution or acquisition of bound waters in the TCR/pMHC interface. A particularly good example of the role of waters in adding complementarity to pMHC interactions is found for the allogeneic H-2K<sup>bm8</sup> complex, where water can partially substitute for loss in buried side-chain functional groups [61•]. In addition, such buried MHC substitutions, which occur frequently in allogeneic MHC, can transmit their effects by altering the water structure and the electrostatic properties on the surface, even though their mutated residues are not directly ‘seen’ by the TCR ([11•]; JG Luz, M Huang, KC Garcia, MG Rudolph, L Teyton, IA Wilson, unpublished data).

The release of bound waters upon TCR/pMHC complex formation increases the entropy and thus may also contribute favourably to the binding energy. In the 2C system, it is estimated that the TCR must displace at least 15 ordered water molecules from the pMHC, mainly by the outer CDR1 and 2 loops of both V $\alpha$  and V $\beta$  (JG Luz, M Huang, KC Garcia, MG Rudolph, L Teyton, IA Wilson, unpublished data) that primarily contact the MHC. However, due to the paucity of (high resolution) structural examples and the lack of biochemical data, it is difficult to assess the generality or extent of the contribution of water to the TCR/pMHC binding energetics.

**Conclusions and future perspectives**

The completion of a  $\gamma\delta$  TCR structure has finally enabled visualisation of the complete arsenal of primary antigen-recognition receptors. These receptors are remarkably similar in the overall structure of their antigen-binding domains (Figure 1) but, in the case of the  $\alpha\beta$  TCR, have become specialised to interact primarily with MHC or MHC-like molecules that can present peptides, lipids or glycolipid fragments [62] for immune surveillance.



**Figure 5**

Structural comparison of  $\alpha\beta$  TCRs.

**(a)** Overlay of the V $\alpha$ /V $\beta$  domains of six different  $\alpha\beta$  TCRs from TCR/pMHC structures. The CDR and HV4 loops are coloured as in Figure 2. The central CDR3 loops are the most structurally diverse and recognise mainly the peptide whereas the CDR1 and CDR2 loops recognise the mostly conserved structural features on the MHC. The two most divergent CDR3 $\alpha$  loops are labelled and belong to MHC class I (scBM3.3)- and class II (scD10)-restricted TCRs. **(b)** Overlay of the unliganded 2C TCR with the three liganded structures. The unliganded 2C TCR structure shows significant conformational differences of both its CDR3 $\alpha$  loop (light green) and CDR1 $\alpha$  loop (furthest right of the dark blue loops). **(c)** Overlay of the four liganded A6 TCR structures. The A6 CDR3 $\beta$  loop (yellow) shows some conformational variability in response to the different Tax-peptide mutants in the HLA-A2 complexes.

What is most remarkable is the evolution of a common docking mode that enables the  $\alpha\beta$  TCR to survey the contents of the MHC binding-groove. It is not the common binding orientation that is in itself remarkable, but that the six independent complex-structures determined so far have not yet revealed the basis for this conserved orientation. No absolutely conserved pairs of interactions are apparent in these different TCR/pMHC-complex interfaces that would account for their relatively fixed docking orientations, especially when one considers the extreme variability in the V $\beta$  interactions with different pMHCs.

The variability in the tilt, twist and roll of the TCR indicates that individual solutions to the docking problem differ in the details in order to provide sufficient complementarity for binding (i.e. a  $K_d$  in the  $\mu$ M range) and, thus, for signalling. In most cases, the TCR V $\alpha$  interactions with the MHC seem to predominate and, hence, provide some basis for a conserved orientation. But the alloreactive scBM3.3 TCR is inconsistent with that generality, as most of its interactions with pMHC are due to the  $\beta$ -chain. However, some fortuitous interactions not present in the syngeneic complex may have altered the relative distribution of its interactions. Additionally, glycosylation may play a role in facilitating docking, as both the TCR and MHC are highly glycosylated which, hence, could sterically restrict the range of possible orientations [16,19].

Electrostatic interactions could also help pre-orient the TCR and pMHC. In TCRs, Lys68 in HV4 $\alpha$  is close to a negatively charged residue (Asp76 $\beta$  in MHC class II or Glu166 $\alpha$  in MHC class I) and may provide some orientation effects [63]. Although salt bridges and hydrogen bonds between these residues have not been conserved in all TCR/pMHC class I complexes (with variable distances of 2.9 Å–10 Å between the side-chain functional groups), electrostatic effects, especially for orienting purposes, can work at a distance [64,65]. A further potential key electrostatic interaction [8 $\bullet\bullet$ ] has now been observed in the two MHC class II complexes [7,8 $\bullet\bullet$ ,11 $\bullet$ ], between Lys39 $\alpha$  (in a loop that projects out from the floor of the  $\beta$ -sheet) and Glu56 $\beta$  of the TCR CDR2 $\beta$ .

Another major unresolved issue is how the exceedingly small changes in the TCR/pMHC interface in response to APLs can transmit such different signals via the TCR signalling complex. The slight (or no) increase in complementarity, in BSA or in the number of contacts in agonist versus antagonist complexes are difficult to reconcile with the substantial differences in signalling outcomes that can be generated. Although the trend of increased half-life for agonist versus antagonist TCR/pMHC complexes is so far maintained, exceptions have been found that belie this as a general rule. A telling question that was posed to one of us at a recent international meeting was: if you were given the crystal structures of several agonist and antagonist complexes, could you predict their biological behaviour — can you structurally differentiate the strong from the weak agonist, or the agonist

from the antagonist? The answer is most likely no, since visual inspection of such similar structures cannot resolve the subtle differences in kinetic and thermodynamic properties that lead to such different signalling outcomes.

The TCR itself seems to adapt to small changes in the pMHC ligand by small conformational changes or rearrangements of its central CDR loops. Water molecules themselves may enhance the differences in the interface between agonists and antagonists. The juxtaposition of two membranes, such as in the immunological synapse, many substantially enhance receptor/receptor interactions and exaggerate the extremely small differences in kinetic and thermodynamic parameters that we observe in solution between stable constructs of agonist and antagonist pMHCs.

The future direction of research demands further TCR/pMHC complex structures in order to address these issues and to allow all of the general principles that govern TCR/pMHC recognition to be fully extracted. Perhaps the most important breakthrough of all would be determination of a complete  $\alpha\beta$  TCR signalling complex, including CD4/CD8 and the CD3 $\gamma$ ,  $\delta$ ,  $\epsilon$  and  $\zeta$  chains. Meanwhile, models of the TCR/pMHC/co-receptor (CD4 or CD8) complex can be assembled from the component pieces [16], which include the distal globular domains of CD8/pMHC class I complexes [66,67], the recent low resolution CD4/pMHC class II complex [68] and the CD3 $\epsilon\gamma$  NMR structure [23 $\bullet$ ]. Any global changes that could influence TCR signalling events might become apparent from these more complex assemblies. However, the lack of the membrane-anchoring domains in constructs normally used for structure determination will still be a problem until we can routinely crystallise intact membrane proteins. Notwithstanding, substantial advances have certainly been made in the past two years in our understanding of the recognition of MHC class I and now class II by TCRs, as well as structural insights into alloreactivity and graft rejection, and response to APLs. Future studies should also deal with the extent to which bulky ligands — such as substantially bulged peptides [41 $\bullet\bullet$ ], glycopeptides [69,70] or glycolipids in the case of CD1 [71] — can be accommodated in the TCR/pMHC interface.

## Acknowledgements

We thank Robyn Stanfield for substantial help with figures and advice on calculations, Jens Hennecke and Don Wiley for coordinates, Samantha Greasley and Don Wiley for discussions, the Section Editors for comments and Dagmar Klostermeier for critical comments on the manuscript. Our TCR/pMHC research is supported by National Institutes of Health (NIH) grants AI-42266 (to IAW) and CA-58896 (to IAW), a postdoctoral fellowship of the German Academic Exchange Service (to MGR) and the Skaggs Institute for Chemical Biology.

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