Atomic structure of an $\alpha\beta$ T cell receptor (TCR) heterodimer in complex with an anti-TCR Fab fragment derived from a mitogenic antibody

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Each T cell receptor (TCR) recognizes a peptide antigen bound to a major histocompatibility complex (MHC) molecule via a clonotypic $\alpha\beta$ heterodimeric structure (Ti) non-covalently associated with the monomorphic CD3 signaling components. A crystal structure of an $\alpha\beta$ TCR–anti-TCR Fab complex shows an Fab fragment derived from the H57 monoclonal antibody (mAb), interacting with the elongated FG loop of the C β domain, situated beneath the V β domain. This loop, along with the partially exposed ABED β sheet of C β , and glycans attached to both C β and C α domains, forms a cavity of sufficient size to accommodate a single non-glycosylated Ig domain such as the CD3 ϵ ectodomain. That this asymmetrically localized site is embedded within the rigid constant domain module has implications for the mechanism of signal transduction in both TCR and pre-TCR complexes. Furthermore, quaternary structures of TCRs vary significantly even when they bind the same MHC molecule, as manifested by a unique twisting of the V module relative to the C module.

Keywords: crystallography/immune receptors/quaternary structure/signal transduction

Introduction

T cells recognize peptides derived from cell-associated pathogens of viral, bacterial or fungal origin, as well as tumor antigens bound to MHC molecules through a multi-subunit transmembrane surface complex termed the T cell receptor (reviewed in Meuer *et al.*, 1984; Marrack and Kappler, 1986; Clevers *et al.*, 1988; Davis and Bjorkman, 1988; Ashwell and Klausner, 1990). The monomorphic CD3 chains (CD3 γ , δ and ϵ) within this complex were

the first TCR components to be identified serologically and their functional significance was suggested by the ability of CD3 ϵ -specific monoclonal antibodies (mAbs) to inhibit antigen-specific T cell recognition (Reinherz *et al.*, 1980, 1982). Subsequently, through the development of clone-specific (anti-clonotypic) mAbs (Allison *et al.*, 1982; Haskins *et al.*, 1983; Meuer *et al.*, 1983a,b,c), disulfide-linked $\alpha\beta$ heterodimers (Ti α – β) were defined in non-covalent association with the CD3 components (Meuer *et al.*, 1983a,b,c) including CD3 ζ (Samelson *et al.*, 1985; Manolios *et al.*, 1991). In contrast to the CD3 molecules, the Ti α and β subunits are biochemically distinct on each T cell clone analyzed, including multiple clones derived from the same individual (Acuto *et al.*, 1983a,b; Kappler *et al.*, 1983; Reinherz *et al.*, 1983). Peptide mapping of α and β subunits identified conserved as well as variable peptides, implying the existence of constant and variable domains for both Ti α and β , a notion later confirmed by protein microsequencing (Acuto *et al.*, 1984; Fabbi *et al.*, 1984; Sim *et al.*, 1984) and DNA cloning (Chien *et al.*, 1984; Gascoigne *et al.*, 1984; Hedrick *et al.*, 1984a,b; Patten *et al.*, 1984; Saito *et al.*, 1984; Yanagi *et al.*, 1984; Hayday *et al.*, 1985). The anti-clonotypic mAb-mediated crosslinking of TCRs on a given T cell clone selectively activated clonal proliferation and effector function, thereby substituting for the specific combination of MHC and antigen recognized by that clone (Meuer *et al.*, 1983d). Moreover, gene transfer experiments indicated that both the antigen and the MHC specificity of a T cell clone could be conferred to another cell by transfection of an appropriate pair of Ti α and β cDNAs (Dembic *et al.*, 1986; Saito *et al.*, 1987; Kaye and Hedrick, 1988).

Several features of TCRs predicted that they would share with antibodies a common structural basis of ligand recognition (reviewed in Novotny *et al.*, 1986; Chothia *et al.*, 1988; Davis and Bjorkman, 1988). Over the last few years, direct evidence has been provided to support this idea. Crystal structures of $\alpha\beta$ TCR components have now been reported, including the β chain (Bentley *et al.*, 1995; Fields *et al.*, 1996) and a V α –V α homodimer (Fields *et al.*, 1995). Very recently, an intact murine $\alpha\beta$ TCR (Garcia *et al.*, 1996), and a complex between a human TCR, a viral peptide and a human class I MHC molecule (Garboczi *et al.*, 1996), have also been described. As anticipated, the three-dimensional structure of the TCR resembles an antibody Fab fragment in that each of the α and β chains consists of Ig-like variable and constant domains, with the hypervariable loops from the two variable domains (V α and V β) forming the antigen-combining motif. However, there are some clear differences between the TCR and Fab structures. Although each of the four TCR α and β domains has been assigned an Ig fold, deviations are notable in both TCR constant domains (Bentley *et al.*, 1995; Garcia *et al.*, 1996), as

Table I. Crystallographic data and refinement

			Native	Inflection	Peak	Remote				
Crystallographic data										
Wavelength		1.0860		0.9788		0.9790		0.9300		
Resolution limit (Å)		2.8		4.0		4.0		4.0		
R_{merge} (%)		7.8 (26.3) ^a		4.0		4.0		4.3		
R_{anom} (%)				3.2		3.6		3.2		
Completeness (%)		73.5 (51.1) ^a		91.5		91.3		91.0		
Redundancy		1.5 (1.3)		4.8		4.8		4.9		
Mean $[I/\sigma(I)]$		11.6 (3.4)		19.3		19.7		18.1		
Refinement statistics										
			r.m.s. from ideal value			Average B value (Å ²)				
Resolution (Å)	R_{work}	R_{free}	Bond lengths	Bond angles	Impropers		Vα	Vβ	Cα	Cβ
15–2.8	0.243	0.309	0.006 Å	1.2°	0.7°	N15A	19.5	21.1	22.1	14.4
						N15B	17.7	21.2	45.2	23.2

^aThe values shown in parentheses are for the highest resolution bin (2.9–2.8 Å) in native data.

well as the Vα domain (Fields *et al.*, 1995). We have now performed a structural determination of a TCR complexed with an anti-TCR Fab, offering direct analysis of T cell and B cell immune receptors in the same crystal lattice. For this purpose, we employed the murine N15 TCR (Vα8Jα5Cα; Vβ5.2D2Jβ2.6Cβ2) specific for the vesicular stomatitis virus nuclear protein octapeptide (RGY-VYQGL) (VSV8) bound to the murine H-2K^b class I MHC molecule (Shibata *et al.*, 1992; Witte *et al.*, 1997; Chang *et al.*, 1997). We formed a complex between N15 and an Fab fragment derived from the hamster anti-mouse Cβ region-specific mAb H57 (Kubo *et al.*, 1989).

Structural analysis of the N15 TCR was made possible by crystallization of an endoglycosidase H-treated derivative of N15 secreted from Lec3.2.8.1 cells, which synthesize homogeneous GlcNAc₂-Man₅ glycans (Liu *et al.*, 1996). The three-dimensional structure determined at 2.8 Å provides considerable insight into the architecture of the TCR versus Fab. This complex also delineates precisely the H57-binding site on the unusual TCR Cβ FG loop. In addition, because the asymmetric unit of the crystal contains two TCR–Fab complexes in very different crystallographic environments, we can infer characteristics about the intrinsic TCR flexibility versus rigidity within, and between, the individual domains. Our results highlight the overall rigidity of the C domain module and suggest that signaling is unlikely to result from a global conformational change within this part of the αβ heterodimer. Lastly, we have been able to identify a cavity within the C module, bounded by the Cβ FG loop, partially exposed Cβ domain strands, and conserved glycans from both constant domains, which can accommodate a single Ig-like domain, probably representing the CD3ε-binding site.

Results and discussion

Overall structure of the N15 TCR–Fab complexes

The structure of the N15 TCR–Fab complex was determined by the combination of molecular replacement and MAD phasing. The crystallographic data and refinement statistics are reported in Table I. There are two TCR–Fab complexes per asymmetric unit of the crystal. Between the two TCR–Fab complexes, the relative orientation of

the Fab with respect to the TCR differs by ~19° (see the TCR–H57 Fab description below). This is essentially a relative rotation of the Fab molecule around the FG loop of the TCR Cβ domain. In fact, there is no significant conformational difference in the binding region of the TCR loop, or major conformational variation within either Fab between these two complexes. This rotation is a feature of antigen interaction which has not previously been reported and indicates that differences in receptor–ligand orientation may exist even when the antibody and antigen are chemically identical in two complexes. The two TCR molecules are generally very similar. For example, the r.m.s. value for superposition of their Cβ domains is only 0.3 Å. Therefore, unless otherwise noted, in comparisons below we shall be referring to one of the two TCR molecules in the asymmetric unit, termed N15A.

The overall structure of one of the two complexes in the asymmetric unit is shown in Figure 1. The complex consists of a single N15 TCR and a single H57 Fab molecule. A striking feature of the structure is that the unusually long FG loop of the β chain TCR Cβ domain pokes into the Fab's antigen combining pocket in a remarkably complementary way. The TCR and Fab molecules are similar in length (80 versus 78 Å) and are both ~40 Å in depth. However, the width of the TCR is substantially greater than that of the Fab (58 versus 46 Å). In addition, there is an obvious difference between the ligand binding surfaces of the V domain modules of the two receptors, with a relatively flat antigen binding surface in the case of the TCR, versus a concave surface in the case of the Fab. Presumably this TCR surface offers a better interaction with the elongated peptide–MHC ligand, which is itself relatively flat on the face contacting the TCR (Fremont *et al.*, 1992; Zhang *et al.*, 1992). Another difference between immunoreceptors is the symmetry of the Fab molecule compared with the asymmetry of the TCR molecule. This TCR asymmetry results from the unusual arrangement of the Cα and Cβ domains relative to one another, as discussed in detail below.

Assuming the view in Figure 1 represents the disposition of the TCR molecule on T cells, as seen from the side with the TCR lying roughly perpendicular to the cell membrane, then the Fab fragment of the antibody molecule

Fig. 1. Three dimensional structure of the N15 $\alpha\beta$ TCR heterodimer complexed with the H57 anti- $\alpha\beta$ TCR mAb fragment. One of the two complexes in the crystallographic asymmetric unit is represented. The figure was produced using MOLSCRIPT (Kraulis, 1991) with the individual domains of both immunoreceptors labeled. The TCR α chain is in green (residues 1–213), the TCR β chain is in red (residues 1–247), the H57 Fab heavy chain is in gold (residues 1–228) and the H57 Fab light chain is in purple (residues 2–215). All numbering is according to Kabat *et al.* (1991). Note that C_H refers to the C_{H1} domain.

reaches the TCR with its long dimension lying parallel to the membrane. One can envision how TCRs on a T cell surface might be crosslinked by the Fab arms of individual antibody molecules. This is the case for the H57 mAb, which selectively activates all $\alpha\beta$ TCR-bearing T cells but not $\gamma\delta$ T cells (Kubo *et al.*, 1989).

All four domains of the N15 TCR, in both complexes, are well ordered in the crystal. Figure 2 is a stereoview of the N15 TCR molecule. Although each of the four domains is Ig-like, they all differ in a significant way from a canonical Ig domain. The fold of the $C\alpha$ is the most extreme in its deviations. While the A, B, E and D β strands, which form the interface with the $C\beta$ domain, are similar to the corresponding sheet in Ig C regions, the C, F and G strands are highly divergent from the analogous components. In fact there is no outer β sheet *per se*. Rather, there is a loosely tethered connection of structural elements. In particular, after the EF turn, which contains a conserved glycosylation site at Asn185 α , there is an F segment which lacks H-bonds to the neighboring strands. This segment contains a mini α -helix, including Cys191 α which forms the intrachain disulfide bond with Cys141 of the $C\alpha$ domain. This $C\alpha$ structure is consistent with that reported by Garcia *et al.* (1996). The $C\beta$ domain is remarkable for containing a 13 amino acid insertion within the FG loop, resulting in its striking elongation compared with Ig constant domains, as initially reported by Mariuzza and co-workers in the structure of an isolated TCR β subunit (Bentley *et al.*, 1995). Sequence comparisons show that this insertion is not found in other Ig-related

structures, except for TCR $C\gamma$ domains (Kabat *et al.*, 1991). The translocation of the C'' strand of the $V\alpha 8$ domain from the GFCC' face to the BED face, as first recognized in the structure of a $V\alpha$ – $V\alpha$ homodimer (Fields *et al.*, 1995), is also observed in the N15 TCR. In addition, the C'' strand of the N15 $V\beta 5.2$ domain deviates from the canonical Ig V domain position. It shifts away from the BED face and hence in the opposite direction compared with the movement of the corresponding C'' strand in the $V\alpha$ domain.

In the electron density maps there are clear densities for all seven of the potential glycosylation sites, three in the α chain and four in the β chain. Note that of the seven TCR glycans, five are located in the C module, whereas only two are found in the V module. Since the N15 TCR protein was produced in Lec3.2.8.1 cells, which synthesize homogeneous glycans of the type GlcNAc₂–Man₅, and was subjected to endo-H digestion before crystallization, only one *N*-acetylglucosamine residue (GlcNAc) remains attached to the Asn at each of the seven N-linked sites (Liu *et al.*, 1996): Asn21 α , Asn185 α and Asn203 α of the TCR α subunit, and Asn78 β , Asn121 β , Asn186 β and Asn236 β of the TCR β subunit (Figure 2).

Owing to the large relative orientation difference of the Fab with respect to the TCR, and small rotational variation between individual domains, there is no detectable simple non-crystallographic symmetry for the two complexes. Nonetheless, these two complexes are roughly related by a pseudodyad that is slightly tilted away from the crystal 2-fold. The major contacts between the two TCRs are

Fig. 2. Structure of the N15 $\alpha\beta$ TCR heterodimer and associated glycans. Stereo view showing the position of the seven *N*-linked glycans (GlcNAc) as ball and stick figures as well as the position of intra and inter chain disulfide bonds in yellow. The individual strands of the various domains are labeled. Note that the C α F 'strand' is actually a loosely tethered structural element containing a mini helix in which Cys191 lies. The latter pairs with C α Cys141 to form the intrachain disulfide in that constant domain. The other disulfides are between the following cysteines: V α Cys22–V α Cys90, V β Cys23–V β Cys92, C β Cys147–C β Cys212, and C α 213–C β 247. Asparagine residues covalently linking to glycans are V α Asn21, C α Asn185, C α Asn203, V β Asn78, C β Asn121, C β Asn186 and C β Asn236. Strand assignments for V α are as follows: **A**, residues 2–5; **A'**, 8–13; **B**, 18–25; **C**, 31–37; **C'**, 43–49; **C''**, 54–57; **D**, 62–67; **E**, 72–77; **F**, 86–94; **G**, 106–107; **G'**, 110–115. Strand arrangements for V β are as follows: **A**, residues 3–7; **A'**, 9–14; **B**, 20–24; **C**, 31–37; **C'**, 44–50; **C''**, 54–59; **D**, 64–70; **E**, 75–81; **F**, 88–95; **G**, 107–108; **G'**, 112–116. Strand assignments were made using the program DSSP (Kabsch and Sander, 1983).

made by the V β domain, and there is no contact between the two V α domains. This is in contrast to the structure of the V α –V α homodimer, reported previously (Fields *et al.*, 1995). Based on this result, it seems unlikely that TCRs will dimerize physiologically through their V α domains.

Deviations in quaternary structure of TCRs

To mediate their specific antigen-binding functions, antibody molecules assume highly divergent shapes, both locally, as a consequence of unique CDR loop configurations, and more globally, through quaternary structural rearrangement among their domains (Stanfield *et al.*, 1993). To assess the quaternary structure variation among TCRs, we have chosen the N15 TCR molecule A as a reference molecule and compared it with the N15 TCR molecule B within the same asymmetric unit, and with the other crystallographically defined TCR $\alpha\beta$ heterodimers, the mouse 2C TCR (Garcia *et al.*, 1996) and the human A6 TCR (Garboczi *et al.*, 1996). Since the C β domain is highly conserved in all three structures, it was used for the superposition of TCR pairs. The FG loop was excluded in the superposition. The r.m.s. value for the C β domain superposition between N15A and N15B molecules is 0.3 Å and between N15 and 2C molecules is 0.5 Å (107 residues used). The r.m.s. value for the N15–A6 superposition is slightly greater at 0.7 Å (75 residues used). Relative orientation differences of the other domains with respect to the C β domain were then calculated, as listed in

Table II. Quarternary structural variations among TCRs restricted by the same or different MHC molecules

Domain:	Degree rotation		
	<u>Cα</u>	<u>Vβ</u>	<u>Vα</u>
TCR			
N15A ^a	0	0	0
N15B	2.1	3.6	4.4
2C	2.4	7.0	18.0
A6	NA	10.6	10.9

^areference N15A.

The rotation angles were calculated by first superimposing the C β domain of the molecule to be compared with N15A. Subsequently, the transforms of each of the other domains were estimated by further superposition.

Table II. Several points emerge from this analysis. First, it is evident that the C α domain has essentially the same orientation, with respect to the C β domain, in all different TCR molecules ($\leq 2.4^\circ$ rotation), implying a fixed C α –C β domain configuration. We will discuss this relatively rigid C module later. Second, we note a large rotational deviation (18°) between the V α domains of the N15 and 2C TCRs. By contrast, there is only a very small rotation difference (4.4°) between the two N15 V α domains, despite their location in significantly different crystallographic environments. An intermediate deviation (10.9°) is noted between the V α domains of N15 and A6. This

Fig. 3. Superposition of different TCRs defines substantial quaternary structural variations. α -carbon trace comparing the two N15 TCRs in the asymmetric unit (left) as well as the N15A TCR with the 2C TCR (right). N15A (red), N15B (white) and 2C (green) were superimposed using C β framework residues 144–149, 158–161, 172–174, 192–197 and 212–215. The r.m.s. deviation for this C region is 0.19 Å between N15A and N15B, and 0.32 Å between N15A and 2C. The side view (bottom) shows the complete $\alpha\beta$ TCR heterodimers. The top view (top) shows only the overlay of the V modules for simplicity. The variation in quaternary structure between N15 and 2C is quantitated in Table II.

is a striking observation given that the N15 (Shibata *et al.*, 1992; Chang *et al.*, 1997; Witte *et al.*, 1997) and 2C TCRs (Garcia *et al.*, 1996) interact with the same murine class I MHC molecule, namely K^b, but loaded with different peptides (VSV8 versus dEV8), whereas A6 recognizes the human HLA-A2 molecule loaded with a Tax peptide (Garboczi *et al.*, 1996). Third, note also that deviation exists in the relative rotation of the V β domain in the pairwise comparisons (3.6–10.6°), but to a lesser extent than observed with V α . Collectively, these findings indicate that quaternary differences in structures result from variations in at least the disposition of V β and C β domains as well as V β and V α domains.

Figure 3 shows the superpositions of N15A–N15B and N15A–2C TCR pairs. On the left side is the superposition of the two N15 TCRs in the asymmetric unit, whereas on

the right side is the overlay between N15 and 2C. The similarities in the C module are evident in both, while the difference between N15 and 2C quaternary structures involving the V module is most notable in this side view (Figure 3, bottom right). When viewed from the top of the molecules (i.e. V module toward C module), the V module of the 2C TCR has an anticlockwise twist with respect to N15 (Figure 3, top right). The same quaternary variation, although to a lesser extent, is even evident when the two N15 TCRs are compared with each other (Figure 3, top left). This twist is another novel feature of the TCR, reflecting its basic asymmetry. In the case of Fabs, the movement of the V module with respect to the C module is defined by the elbow angle, as the V_H and V_L domains rotate in the same direction. In contrast with the TCR, there is a V α –V β twist rather than a concerted movement

Table III. Comparison of interdomain buried surface areas among TCRs and the H57 Fab

Domain interface	TCR				Domain interface	Fab H57
	N15	2C	A6	14.3.d		
V α -V β	1350	1275	1505	N/A	V _H -V _L	1330
C α -C β	2400	2375	N/A	N/A	C _{H1} -C _L	1800
V α -C α	440	475	N/A	N/A	V _L -C _L	535
V β -C β	810	655	915	755	V _H -C _{H1}	365

N/A - not applicable. Results are given in Å². Buried surface areas were calculated with program MS (Connolly, 1983) with a 1.7 Å probe radius and a density of 4. For calculation of the V α -V β interface, the contribution of residues 116a and 117 were not included.

in the same direction. This orientational variation affects how a given TCR molecule docks onto the peptide-MHC assuming the latter complex is fixed.

In the TCR-peptide-MHC complex structures reported by Garboczi *et al.* (1996) and Garcia *et al.* (1996), a common docking feature is that the long dimension of the TCR and MHC binding surface is not parallel but tilted. Garboczi and co-workers argue that this relative orientation is due to the fact that the two highest points on the MHC molecule binding surface impose a restriction on how the TCR can approach the deeply buried peptide. Since the two high points appear to be a topographical feature for both class I and class II MHC molecules, Garboczi *et al.* (1996) speculate further that the 'diagonal binding' mode may be a general one. We have noticed that the fundamental structural basis for having the 'two high points' lies in the inherent twist of the large eight-stranded β sheet that forms the platform of the antigen binding groove. The two helical MHC segments run across the entire sheet diagonally. They must break at the two highest points, defining the relatively flat antigen binding surface in between. Our observation of the V module twist, derived from comparison of 2C with N15 TCRs, raises the possibility that there can be some angular variation in the docking of the TCR during immune recognition events. The result is especially noteworthy in the case of 2C and N15 TCR complexes, as they have identical C modules and recognize the same MHC molecule (K^b), but complexed with different peptides. It should be noted that this statement assumes that no rearrangement in the relative orientation of the variable domains occurs upon antigen binding, which may not be the case.

The TCR V domain module

The hydrophobic core found in V_H-V_L interfaces, and noted in the 2C TCR (Garcia *et al.*, 1996), is largely conserved in N15. It consists of F43 α , L87 α , Y89 α , Y104 α , F106 α , L43 β , F45 β , F91 β , Q106 β and F108 β . As shown in Table III, the V α and V β interface in N15 comprises 1350 Å² of buried surface area (~690 Å² for V α and 660 Å² for V β). This value is close to an average value of 1420 Å² found in Fabs (Stanfield *et al.*, 1993; Wilson and Stanfield, 1993; Padlan, 1994), slightly larger than the value reported for 2C and ~150 Å² less than that calculated for A6. This difference in buried surface area correlates with the length of the CDR3 β residues such that the V α -V β buried surface increases with 2C < N15 < A6 which have CDR3 β loops of 7, 9 and 12 residues, respectively. The contribution of all CDR

residues to the buried surface is ~40% in the case of N15, again near the average observed for an Fab (Padlan, 1994). The finding that the 2C V α -V β domain buried surface area value was at the extreme low end of the range observed for antibodies, suggested the possibility of large V α -V β displacement, which might provide better complementarity for the TCR and peptide-MHC interaction (Garcia *et al.*, 1996). However, based on our observations with N15, this postulated mechanism may be highly dependent on the V module of an individual TCR. Another interesting feature of the interface of the N15 V module is the presence of two interdomain salt bridges which are not conserved in 2C. The first is between K48 α and D100 β . While the K48 α residue is found in both N15 and 2C, the D100 β residue is absent in 2C. This bond serves to connect CDR2 α with CDR3 β regions, stabilizing the loop configuration. The second salt bridge is between K103 α and D56 β and functions in a similar manner, to connect CDR3 α with CDR2 β . In 2C, the lysine at position 103 is replaced by alanine.

Overall, it is clear from Figure 4 that CDR1 α , CDR2 α and CDR1 β are similar in all three known $\alpha\beta$ TCR heterodimeric structures. In fact, many of the interactions that maintain the conformations of CDR1 α and CDR2 α loops are preserved among these TCRs. Although the V domain of N15 belongs to the Kabat β subgroup I, while that of 2C and A6 belong to subgroup II (Kabat *et al.*, 1991), the conformation of CDR1 β of N15 is still very similar to other TCRs. There is one salt bridge, however, between R64 β of the C'D loop and D86 β of the EF loop in the N15 TCR, which is characteristic of the Kabat subgroup I and which is also a hallmark for the variable domains of antibodies. As expected, the CDR3 α and CDR3 β loops of each receptor are idiosyncratic. We will discuss these two CDR3 loops in the next section. While the CDR2 β loops of 2C and A6 are similar to each other, they differ markedly from CDR2 β of N15, largely due to the forward displacement and twist of the V β C' strand noted above. There is a well-defined salt bridge between D56 β of CDR2 β and K103 α of CDR3 α , which appears to play a role in pulling this C' strand away from its canonical position. It is interesting to note that in the N15 A molecule, this C' strand makes a contact with the D strand of the Fab V_H domain, from a symmetry related complex in an anti-parallel way. There is at least one main chain H-bond between R55 β of the TCR molecule and R71 of the V_H domain. However, in the N15 B molecule, the C' strand of V β is free of any crystallographic contact.

Fig. 4. Comparison of V domain modules of N15, 2C and A6. TCRs are superimposed using conserved framework strand residues V α 19–24, 32–37, 63–65, 72–76, 88–91 and V β 20–25, 34–37, 66–68, 74–78 and 90–93. r.m.s. deviation = 1.15 Å for 2C and 0.78 Å for A6. The N15 CDR regions are shown in red and labeled α 1–3 and β 1–3, corresponding to the following residues: CDR1 α , 24–31; CDR2 α , 48–55; CDR3 α , 93–104; CDR1 β , 26–31; CDR2 β , 48–55 and CDR3 β , 95–107. In the case of 2C and A6, the corresponding loops are colored pink (CDR1 α); blue (CDR2 α); green (CDR3 α); dark blue (CDR1 β); orange (CDR2 β) and yellow (CDR3 β).

Therefore, it is safe to conclude that the shift of this C'' strand is an inherent feature of the N15 TCR molecule. Although the C'' strand moves away from its canonical position, it remains well integrated into the β sheet by forming main chain H-bonds between the T54 β –F59 β segment in the C'' strand and the Y50 β –F45 β segment in the C' strand. The net consequence of this movement is that the β sheet has a lesser degree of left-handed twist at the C'' edge. This may be a unique feature of the Kabat V β subgroup I, and will probably impact the antigen recognition process. The shift of the V β C'' strand in an opposite direction, toward the BED face, has recently been reported for another TCR. Therefore, considerable variability in structure may exist among TCRs in this region (Housset *et al.*, 1997).

CDR3 residues critical for MHC-restricted antigen recognition map centrally on the molecular surface of the V module

The importance of CDR3 residues in α and/or β subunits for T cell recognition has been well documented experimentally (Engel and Hedrick, 1988; Danska *et al.*, 1990; Lai *et al.*, 1990; Taylor *et al.*, 1990; Kabat *et al.*, 1991; Casanova *et al.*, 1993; Ehrlich *et al.*, 1993; Kasibhatla *et al.*, 1993; Kelly *et al.*, 1993; Luescher *et al.*, 1995). To examine the role of individual CDR3 α and CDR3 β residues on T cell recognition by the N15 TCR, we

constructed a series of alanine-scanning point mutants which differed by a single amino acid at one or another of the CDR residues. Upon transfection with CD8 $\alpha\beta$ co-receptors into 58 $\alpha\beta^-$ T cells, the mutant TCRs were tested for their ability to alter peptide-triggered IL-2 production (Chang *et al.*, 1997). Critical residues which diminished peptide antigen recognition by 1000–10 000, in molar terms, were identified in both N15 V α (E94A, Y102A and K103A) and V β (R97A, W98A and D100A) CDR3 loops. The V α CDR3 residue, N101A, reduced recognition 100-fold. Figure 5 gives a GRASP surface representation of the N15 $\alpha\beta$ heterodimer, as viewed from the perspective of the peptide–MHC. As is readily apparent, these mutations, which dramatically reduce immune recognition by N15, are centrally placed at the surface interface of the V domain module. Note that not all surface exposed residues alter such recognition function, given that V β residue E105 has no effect on the ability of N15 to recognize VSV8 in the context of K^b. It is tempting to speculate that the depression on the N15 surface around E94 α will accommodate the arginine residue at the p1 position of VSV8 peptide, the hydrophobic W98 β and Y102 α will interact with the p4 valine residue, and the upward pointing R97 β will H-bond to glutamine at p6. The distances of 13–16 Å between the α -carbons of E94 α and R97 β and the α -carbons of Rp1 and Qp6 are consistent with this notion.

Rigidity of the C domain module at the C α -C β interface

As mentioned earlier, the C α domain adopts a peculiar fold, deviating substantially from the conventional Ig-like domain. However, its relative orientation to the C β domain remains the same in all three TCR structures studied to date, two in the N15-Fab crystal and one in the 2C crystal as shown in Table II. This is largely because C α maintains a regular ABED β sheet, facing the C β domain's ABED surface to engage in extensive sheet-sheet packing. Table III lists the calculated buried surface areas for each domain-domain interface in all of the known TCR structures. As is evident from this analysis, the buried surface area of the C α -C β TCR interface is the largest (2 400–

Fig. 5. N15 CDR3 residues involved in recognition of the VSV8/K^b antigen-MHC class I complex. The figure is shown as a GRASP (Nicholls *et al.*, 1991) molecular surface representation, viewing the ligand binding surface of the TCR. The V α domain is shown in blue-grey and the V β domain is shown in pink. Individual CDR3 α and β residues, whose mutation to alanine results in ≥ 1000 -fold reduction in sensitivity of T cell hybridoma transfectants to varying molar concentrations of VSV8 peptide pulsed onto R8 antigen presenting cells, are shown in red. The CDR3 α Asn101 residue which reduces recognition 100 fold is shown in yellow and the CDR3 β Glu105 residue, which has no detectable effect on recognition, is shown in green.

2 375 Å²) when compared with the other domain-domain interfaces.

The qualitative nature of contacts is also distinctive. Except for a few hydrophobic contacts at the one corner of this C module, involving a cluster of aromatic rings (C α F161, C α W183, C β F130 and C β Y179), the C α -C β interface is predominantly composed of hydrophilic interactions, as predicted by Mariuzza and co-workers (Bentley *et al.*, 1995) and noted by Garcia *et al.* (1996), with a skewed distribution of acidic residues in C α and basic residues in C β . We further observe that these interactions cover a very broad surface area. Multiple polar interactions distributed over a large contact area will serve as a stabilizing force to rigidify the C module with respect to the C α -C β interface. This is in striking contrast to the V module where V α -V β dimerization is mediated largely by hydrophobic interaction, as discussed in the preceding section. The non-polar nature of these latter interactions makes the disposition of the V α and V β modules more malleable. From the perspective of antigen recognition, an adaptable V module, which can better undergo V α -V β displacement, may be more able to accommodate ligands of varying characteristics, as has been shown in the case of Fab V_H-V_L interactions upon ligation by antigen (Stanfield *et al.*, 1993; Wilson and Stanfield, 1993). On the other hand, the much more rigid C module may function to maintain the integrity of the heterodimer as well as to aid in physically complexing with the other components of the TCR machinery.

Conservation of C α residues in pT α interacting with the C β interface

One extremely interesting feature of the C α -C β domain pairing relates to certain critical C α residues which are preserved in pT α , a 30 kDa glycoprotein whose expression is restricted to early CD4⁺CD8⁻ double negative (DN) T lineage cells (Saint-Ruf *et al.*, 1994; Bruno *et al.*, 1995; Wilson and MacDonald, 1995; Ramiro *et al.*, 1996). pT α is thought to function as a surrogate for the mature TCR α chain. In such progenitor cells, the pre-TCR, a disulfide-linked heterodimer of a functionally rearranged TCR β chain and pT α non-covalently associated with the CD3

A

Mouse pT α	1	LPSGIAGTPF	PSLAPPITLL	VDGRQHMLVV	CLVIDAAPPG	LDNPVWFSAG	50
Human pT α		LPTGVGGTPF	PSLAPPIMLL	VDGKQQMVVV	CLVIDVAPPG	LDSPIWFSAG	
Mouse C α		HI	QNPEPAVYQL	KDPRSQDSTL	CLFTD.....	FDSQINVPKT	
Human C α		NI	QNPDPAVYQL	RDSKSSDKSV	CLFTD.....	FDSQTNVSQS	
			A		B	C	
Mouse pT α	51	NGSALDAFTY	GPSLAEDCTW	TSLAQLSLPS	EELEAWEPLV	CHTRPGAGGQ	100
Human pT α		NGSALDAFTY	GPSLATDCTW	TSLAHLSSLPS	EELASWEPLV	CHTGPGAEGH	
Mouse C α		MESGT.....	...FITDKTV	LDMKAMDSKS	NGAIAWSNQT	SFTCQDIFKE	
Human C α		KSDSV.....	...YITDKTV	LDMRSMDFKS	NSAVAWSNKT	SFACANAFNN	
			D	E	F		
Mouse pT α	101	NRSTHPLQLS	GESSTARSC	119			
Human pT α		SRSTQPMHLS	GEASTARTC				
Mouse C α		T....NATYP	SSDVPC...				
Human C α		SIIPEDTFFP	SPEISC...				
			G				

components, is expressed. During development, the DN to double positive (DP) ($CD4^+CD8^+$) thymocyte transition is induced by an as yet unknown ligand, which binds to the cell surface β -pT α pre-TCR complex. This differentiation then results in formation of mature type $\alpha\beta$ TCR heterodimers on DP thymocytes (Shinkai *et al.*, 1993; Fehling *et al.*, 1995; Levelt *et al.*, 1995; Xu *et al.*, 1996; Koyasu

et al., 1997). The pT α ectodomain in man and mouse is 79% identical, consisting of one Ig-like domain with a short connecting peptide containing the cysteine that forms the interchain disulfide with the TCR β chain (Del Porto *et al.*, 1995).

Although there is only 12% identity between pT α and C α in man and mouse, the C α residues involving important

B

Fig. 6. Alignment of human and mouse pT α and C α domains and conservation of residues involved in the C β interaction. (A) Sequence alignment of human and mouse C α and pT α residues was made using the program PILEUP from GCG (Program Manual for Wisconsin Package, 1994). The structure-based alignment is predicted, based upon N15 and 2C C α domains, with individual strands A–G indicated. Shaded residues are the ones conserved in all four sequences, and those with solid circles make critical polar contacts with C β residues. (B) Partial view of the polar C α –C β interface. The C α main chain is shown in pink and the C β main chain in blue. The C α residues D145, S177, T166, D164 and W183 are shown with their side chains in red, interacting with C β residues R197, R195, R195, Y179 and E181 with side chains shown in white, respectively. H-bonds were calculated with HBPLUS (McDonald and Thornton, 1994) and van der Waal's and salt bridges were calculated with CONTACSYM (Sheriff *et al.*, 1987).

polar interactions with the C β domain are all conserved in the pT α sequences (Figure 6A). These include a complex salt bridge between C α D145 and C β R197, and H-bonds between C α D164 N and C β Y179 OH, C α T166 O and C β R195 NH₂, C α S177 O and C β R197 NH₂, and C α W183 NE1 and C β E181 OE2 (Figure 6B). Given that the conserved structural elements account for close to half of the pT α -C α amino acid sequence identities, it is very obvious that the heterodimer interface of pT α -C β will be extremely similar to that of C α -C β . More importantly, this wide conservation from mouse TCR to human TCR and from pre-TCR to mature TCR, in both species, indicates that the rigid C module is a structural element important for immature, as well as mature, T cell function.

TCR-H57 Fab interaction site involves the well-structured C β FG loop

The binding of the H57 Fab to the TCR surface is largely restricted to an epitope residing within the FG loop of the C β domain. This result is consistent with molecular mutational studies (K.Karjalainen, manuscript in preparation). Collectively, four structure determinations have been made of this loop; two in our N15 TCR-Fab crystal, one in the β chain structure (Bentley *et al.*, 1995) and one in the 2C TCR structure (Garcia *et al.*, 1996). In every case, the sequence of the loop is identical and every conformation of the 12 residue loop is surprisingly well defined, lacking the floppiness which might be anticipated in a loop of this size. A comparison of the 2C and N15 loops is given in Figure 7A. Intricate internal interactions within the loop limit its flexibility. These include a mini hydrophobic patch formed by W225 β at the tip of the loop as well as L219 β and P232 β at the 'root' of the loop (Figure 7B). L219 β and P232 β are the last H-bond-paired residues on the F and G strands, respectively. In this way, the mini hydrophobic patch links to the hydrophobic core of the domain. Moreover, there are multiple H-bonds within the loop, most notable among which are the bifurcated H-bonds between the side chain atom NE1 of W225 β and two carbonyl oxygens, one preceding and the other following the rigid P230 β . Apparently, these polar interactions further anchor the W225 β at the base of the loop. When the unligated 2C TCR, and the H57-bound N15 TCR are superimposed based on the C β domain framework, the FG loop shows several major differences between the ligated and unligated states (Figure 7A). The striking feature is that the residue W225 β maintains the same conformation, while the two 'front' corners of the loop differ substantially. On one side, E227 β flips almost 180°, from being pointed inward to the loop in the unligated state to outward in the ligated conformation. In complex with the Fab, its carboxyl group reaches deeply into the bottom of the Fab-binding pocket, forming a charged H-bond to R50 within CDR2 of the Fab's heavy chain (Figure 7C). This binding mode is somewhat reminiscent of that of a lysozyme-Fab complex (Amit *et al.*, 1986) in the sense that the side chain of one glutamine (Q121) from the antigen penetrates deeply into the antibody combining site. On the other corner, the main chain around E221 β also deviates quite substantially in our ligated structure (Figure 7A). There are two salt bridges between the N15 and Fab here: one involving K224 β in the N15 TCR and D51 in the H57 light chain's

CDR2 loop, and the other involving E221 β in the N15 TCR and K30 in the light chain's CDR1 loop (Figure 7C). Note also the very different conformation of the E221 β side chain in the Fab-ligated N15 TCR (Figure 7B). There is one additional salt bridge outside the FG loop of the C β domain, which involves D14 of the N15 V β domain and K53 in the CDR2 loop of the Fab light chain. Since in the 2C TCR structure only residues 218–220 are involved in crystal packing (Garcia *et al.*, 1996), and no FG loop residues are so affected in the N15 structure, we conclude that the conformational change described here upon N15-Fab interaction may reflect an induced-fit mode of binding. As listed in Table IV, there are also multiple hydrophobic contacts between the TCR epitope and the H57 Fab including a number of aromatic rings such as those of F32 and Y34 from the light chain and W33, Y35 and F98 from the heavy chain. This set of hydrophobic contacts strengthens the aforementioned salt bridges and is consistent with the frequency of aromatics observed by others in antigen combining sites (Mian *et al.*, 1991). The total buried molecular surface area in this complex A TCR-Fab interaction is 1460 Å² (720 Å² for N15 and 740 Å² for H57), consistent with the range of values observed for other intact protein antigen-Fab interactions (Stanfield *et al.*, 1993; Wilson and Stanfield, 1993; Padlan, 1994). One unusual feature of the N15-H57 Fab interaction is the preponderance of contacts made by the light chain (430 Å² buried surface) rather than heavy chain (310 Å² buried surface).

Analysis of the two complexes within a single asymmetric unit shows that each Fab molecule binds to the N15 TCR using essentially the same interacting residues in a given pair, but with significantly different overall orientation, as described above. Figure 7D depicts how the two H57 Fab fragments orient differently relative to N15 when the two TCRs are overlaid on top of each other based on C β domain superposition. As can be seen, there is a 19° rotation around the FG loop of the TCR C β domain. While the major binding surface between the TCR C β FG loop and the Fab remains essentially the same, there are differences in the number of the atomic contacts in complex A versus complex B, as defined in Table IV. This finding implies that the same immune receptor may dock somewhat variably to a single ligand and that slight differences in the binding interaction can result in rather large rotations at the distal C region of the Fab 'lever arm'. Antibodies use several mechanisms to adopt substantially different shapes in order to recognize antigens. These include utilization of diverse CDR loops varying in sequence and size, alteration of the elbow angle between the V module and C module, variation of V_L-V_H pairing, and exploitation of hinge flexibility between Fab and Fc modules (Huber and Bennett, 1987; Stanfield *et al.*, 1993; Wilson and Stanfield, 1993; Padlan, 1994). Compared with the unligated Fab H57 structure, the elbow angle of H57 Fab does change ~20° upon binding to the N15 TCR (data not shown). Nevertheless, both Fab molecules in the two complexes undergo roughly the same elbow angle change, despite their distinctive orientation with respect to the TCR epitope.

A putative CD3 ϵ -binding region

The overall shape of the TCR C domain module is remarkably asymmetric. The C β domain is ~55 Å in

overall length while that of the C α is only 40 Å, as viewed in Figure 2. The C β domain bends more acutely towards the V β domain compared with the angle formed between the C α and V α domains. C β also has an unusually long and well-structured FG loop protruding down from the V β –C β interface. About half of the C β domain's ABED sheet is surface exposed, yet uninvolved in contact with the corresponding C α domain. This asymmetry creates a cave-like structure or cavity underneath the β chain as shown in the right lower corner of Figure 2. This cavity measures ~25 Å in depth, 20 Å in height and 25 Å in width (Figure 8). The partially exposed ABED β sheet of the C β domain forms an extensive ceiling (Figure 2). The CD loop and EF loop of the C α domain, as well as the glycans attached to C α N185, C α N121 and C β N186, form one side wall, while the FG loop of C β and the glycan emanating from C β N236 form the other side wall of the cavity. The glycans appear to project outward and hence, probably will not occlude the cavity. In contrast, the AB loop of the C α domain projects into this cave. The floor of the cave is presumably formed by the plasma membrane on the T cell surface. It is noteworthy how the interchain disulfide bond between C α 213 and C β 247 is positioned below the C α domain (Figure 2), leaving the cavity unobstructed as the TCR $\alpha\beta$ heterodimer projects from the T cell surface membrane. Since the TCR γ subunit is the analogue of the TCR β subunit in $\gamma\delta$ TCRs (Bentley *et al.*, 1995; Krangel *et al.*, 1987), and is predicted to contain an equivalent insertion in the corresponding constant domain loop (Bentley *et al.*, 1995), a similar cavity must exist in the TCR $\gamma\delta$ heterodimer as well.

What might be the utility of this cavity for TCR structure and function? It seems most likely that it serves to physically link the CD3 components with the TCR $\alpha\beta$ heterodimer. The CD3 subunits are known to function as both structural components of the TCR as well as mediators of signal transduction. The importance of their structural role is emphasized by the fact that in the absence of the CD3 ϵ subunit, the TCR cannot be expressed on the cell surface (Sussman *et al.*, 1988). The signaling role of the CD3 subunits was first identified in studies showing that anti-CD3 ϵ mAb could either trigger (van Wauwe *et al.*, 1980) or inhibit activation (Reinherz *et al.*, 1980), depending on the form of the antibody utilized and its ability to cluster receptors. Subsequent studies showed that the various CD3 subunits contain immunoreceptor tyrosine-based activation motifs (ITAMs), which become phosphorylated by TCR triggering and allow recruitment of SH2-containing tyrosine kinases to the TCR (Reth,

1989; Irving and Weiss, 1991; Letourneur and Klausner, 1991; Romeo *et al.*, 1992; Weiss, 1993). The $\alpha\beta$ TCR can be viewed as four associated pairs of dimers: TCR $\alpha\beta$, CD3 $\gamma\epsilon$, CD3 $\delta\epsilon$ and CD3 $\zeta\zeta$ (Koning *et al.*, 1990; Manolios, 1991). The TCR $\alpha\beta$ and CD3 $\zeta\zeta$ dimers are the only ones that are disulfide-linked, and both CD3 $\delta\epsilon$ and CD3 $\gamma\epsilon$ are stable in non-ionic detergents. With regard to interaction between TCR $\alpha\beta$ heterodimers and CD3 components, CD3 ϵ is by far the strongest.

Several general models of the functional TCR have emerged (Blumberg *et al.*, 1990; Koning *et al.*, 1990; de la Hera *et al.*, 1991; Kappes and Tonegawa, 1991). Most of these offer a view in which two CD3 ϵ molecules are present: one associated with TCR β and one associated with TCR α . Our structure shows that if two CD3 ϵ molecules are present per TCR, then only one of these occupies the cavity within the TCR C domain module, leaving the other exposed. While chemical crosslinking studies imply that CD3 γ in man and mouse is in close proximity to the TCR β subunit, the position of CD3 δ relative to the $\alpha\beta$ heterodimer is less certain (Brenner *et al.*, 1985; Koning *et al.*, 1990). We predict that the CD3 $\gamma\epsilon$ dimer is oriented such that its CD3 ϵ molecule is in the cave.

Although, as yet there is no direct structural information on the localization of CD3 ϵ , we propose that this cavity within the C module accommodates the CD3 ϵ subunit for the following reasons. First, binary complex assembly studies demonstrate that the TCR β –CD3 ϵ association is mediated by their ectodomain whereas, in contrast, detectable associations involving TCR β –CD3 δ , TCR α –CD3 ϵ and TCR α –CD3 δ are all mediated through their transmembrane interactions (Manolios *et al.*, 1994). Second, CD3 ϵ is an essential component of both T cell and pre-T cell receptors (Malissen *et al.*, 1995). The nature of the interaction site between CD3 ϵ and TCR $\alpha\beta$ proteins, predicted from our structural analysis, would anticipate this central structural role for CD3 ϵ . As discussed earlier, the pre-TCR retains the same C β domain, and pT α is homologous to C α . Hence, the pT α –C β module is likely to be similar in structure to the C α –C β module, particularly at the dimer interface. In addition, the predicted size of the C α domain's AB loop (five residues), with a single positively charged side chain in all homologues, as well as the conservation of six residues in the neighboring A and B strands (Pro, Leu and Asp; Cys, Leu and Asp, respectively), make it likely that these structural features will be maintained. Consistent with this notion is the observation that the conserved C α glycosylation site at Asn185 is replaced by a glycosylation site at the predicted

Fig. 7. TCR C β FG loop region and its interaction with the H57 Fab. (A) Comparison of N15 (red) and 2C (blue) FG loop region, superimposed by F strand residues 213–216 and G strand residues 233–236 and represented as a ribbon diagram based on α -carbon traces. A clear 4–5 Å displacement of the main chain is evident around E227 β and E221 β . Major side chain reorientation of E221 β and E227 β in H57 β ligated (N15) versus unligated (2C) TCR structures is evident, while the W225 β side chain position in the two structures is similar. (B) Delineation of the hydrophobic core which stabilizes the TCR C β FG loop. Dotted van der Waal's surface is shown around L219 β , W225 β and P232 β . The atom NE1 of W225 β creates bifurcated H-bonds with carbonyl oxygens of P230 β and S229 β . (C) GRASP molecular surface representation of the H57 Fab-binding region. The various CDR regions of H57 L1 (24–34), L2 (50–56), L3 (89–99), H1 (31–35), H2 (50–68) and H3 (95–102) are shown in green, blue, yellow, purple, magenta and brown, respectively. The FG loop of the TCR is represented in white as backbone bonds and the three salt bridges formed by TCR residues E227 β , E221 β and K224 β with Fab residues R50, K30 and D51, respectively, are shown. (D) Variability in H57 Fab docking orientations relative to the N15 TCR. Superposition of the two N15 TCRs in the asymmetric unit uncovers orientational differences in the position of the two Fabs. The Fab of complex B differs by an 18° rotation from the Fab of complex A. The overall view of the complex is as given in Figure 1, with the TCRs on the left and the Fabs on the right. The α -carbon trace of N15–Fab complex A is in red and that of N15–Fab complex B is in green. The C β FG loop is shown in white for both TCRs. (E) MAD electron density map. A stereo view of a portion of the 4 Å MAD map showing the TCR–Fab interface region, with the final refined model fitted is presented. The Fab is in magenta and the TCR in yellow. This map was calculated based only on phases from nine refined Se sites and subjected to the DM procedures (see text).

Table IV. Amino acid residues involved in atomic contacts between the H57 Fab CDRs and the TCR β chain

	H57 light chain	TCR			H57 heavy chain	TCR	
		Complex A	Complex B			Complex A	Complex B
L1	L28	K224	K224 ^a	H1	W33	S229 ^a	S229
	P29		K224 ^a			P230 ^a	P230 ^a
	K30	E221 ^a			Y35	E227 ^a	E227 ^a
		D223 ^a					
	N31	K224	K224	H2	R50	E227 ^a	E227 ^a
	F32	K224 ^a	K224 ^a		N53	P230	P230
			W225 ^a				
		P226	P226	H3	A95	E227 ^a	
L2	Y34	P226	P226			G228	G228
		E227 ^a	E227 ^a		G96	G228 ^a	G228 ^a
					R97	R120	R120 ^a
	M50	R120 ^a	R120 ^a			G228	T123
		P226	P226			S229	N121 ^a
	D51	K224 ^a	K224		F98		D118 ^a
	K53	E14 ^a	E14			R120 ^a	R120 ^a
		D118 ^a				N121	N121
L3	S91		E227 ^a	H100		R120 ^a	R120 ^a
	N95a	D223 ^a	E227			P226 ^a	P226
						E227	G228

^a = distance between 2.5 and 3.4 Å, whereas other residue distances are ≤ 4 Å

Fig. 8. Delineation of a cavity within the TCR C domain module: A putative CD3 ϵ -binding site. GRASP surface representation of the C α (green) and C β (pink) domains are shown with the C β FG loop in red and the C α AB loop in yellow. The two C β glycans represented as single GlcNAc residues (white stick figures), corresponding to attachments at C β Asn121 and 186 and C α 185, form a front wall of the cavity. The C α glycan attached to N203 is outside the site and is not evident in this view. As noted in the text, due to its size, this cavity can only accommodate a single Ig domain.

end of the C strand of human and mouse pT α , which should occupy space adjacent to that occupied by the C α Asn185 glycan. In principle then, the V β -C β -C α TCR module should be very similar to the V β -C β -pT α pre-TCR module in so far as making association with the CD3 ϵ signaling component. Third, the murine CD3 ϵ subunit consists of 87 residues in its extracellular segment and has Ig-like characteristics (Gold *et al.*, 1987), which can be readily folded into a small C2 type Ig domain, a typical example being domain 2 of CD4 (Wang *et al.*, 1990). CD3 ϵ is also the only CD3 subunit with an Ig-like ectodomain in the TCR machinery of man or mouse that is non-glycosylated (Gold *et al.*, 1987). This makes the CD3 ϵ subunit the only subunit that could readily fit into a cavity of this size. Fourth, the ectodomain of the CD3 ϵ

subunit has twice as many acidic residues as basic ones and is therefore negatively charged (pI = 4.5). The cavity, by way of contrast, is filled with more basic residues including the conserved R134 α , which protrudes from the AB loop of the C α domain. Hence, charge complementarity favors this notion. Note that the predicted pIs of CD3 γ and CD3 δ extracellular segments are more typical of ectodomains, being 8.76 and 5.82, respectively.

Implications for signal transduction

The precise functional role of the FG loop in C β is far from clear. However, it is likely to have an important impact on TCR structure and signaling for two reasons. First, this extensive loop will prevent lateral movement of the V β domain relative to the V α domain, and thereby

further rigidify this domain–domain interaction. Hence, perturbation of the V domain CDR loops by peptide–MHC binding can more easily transfer information to the CD3 signaling subunits via this rigid body. Second, although the mini hydrophobic patch of this loop (W225 β , L219 β and P226 β) fixes the overall loop orientation relative to the V β and C β domains, local movement of the main chain atoms around residues E227 β , E221 β and D223 β are permitted. Such perturbation may affect the interaction of C β with CD3 ϵ or other CD3 components. The unique FG loop of the C β domain is widely conserved among sequences of mouse, rat, human and rabbit. Although in some species (fish, chicken and shark), the loop is much shorter (4–7 residues versus 17 residues), a glycosylation site can be found in these foreshortened FG loops which may serve a similar function to that of the peptide in the homologues with longer FG loops (Rast and Litman, 1994).

One additional observation argues that V domain ligation may alter the quaternary structure of CD3 ϵ within the C domain module or, at least, the strength of association between CD3 ϵ and the Ti $\alpha\beta$ heterodimer. Antibodies to CD3 ϵ coprecipitate the CD3 components γ , δ and ϵ as well as the Ti $\alpha\beta$ heterodimer in mature T cells (Meuer *et al.*, 1983c; Reinherz *et al.*, 1983). In contrast, under the same experimental conditions as used to immunoprecipitate with anti-CD3 ϵ mAb, all anti- $\alpha\beta$ clonotypic antibodies fail to detect the CD3 components (Meuer *et al.*, 1983b,c). Assuming that physiological ligation of TCRs by peptide–MHC, like the anti-clonotypic mAbs, alters the interaction of CD3 ϵ with the TCR cavity, this change may be a basis for initiation of signal transduction. Clearly, future mutational studies guided by the atomic detail provided herein will test the validity of this speculation.

Materials and methods

Data collection and processing

Protein production and crystallization were as previously described except that a mono Q column was used to further purify the H57 Fab (Liu *et al.*, 1996). The crystals belong to the space group P2₁ (unit cell $a = 74.7$ Å, $b = 122.3$ Å, $c = 115.8$ Å and $\beta = 108.0^\circ$) containing two complexes in one asymmetric unit with 50% solvent. Each of the data sets was collected using an MAR research imaging plate system at the Brookhaven National Synchrotron Light Source on beamline X12C, using one single frozen crystal under -165°C . The mosaicity is only 0.2° – 0.3° for native crystals. Raw data were integrated and merged using DENZO and SCALEPACK (Otwinowski and Minor, 1997). Further processing was performed with the CCP4 suite (Science and Engineering Research Council collaborative computer project 4; Daresbury Laboratory, Daresbury, UK) (CCP4, 1994).

Molecular replacement

The program packages AMoRe (Navaza, 1994) and X-PLOR (Brunger, 1992) were used to carry out molecular replacement calculations. The search models were the 2C TCR (Garcia *et al.*, 1996) and the Fv module of the partially refined H57 Fab structure (Lim *et al.*, manuscript in preparation). Surface side chains were truncated where the sequence differed between 2C and N15 TCR. A two-type (TCR and Fv)–four-body search protocol approach (Navaza, 1994) was taken and the normalized structure factors were used as experimental data. The solution was quite clear at 4 Å resolution. The correlation coefficient for the correct solution after fitting was 22.8, with the next highest peak value 16.3. Multiple domain rigid body refinement using X-PLOR (Brunger, 1992) reduced the R -factor from 54 to 50.1%. The result became immediately convincing when the models were displayed on graphics where the crystal packing was observed to be reasonable and the Fab–

TCR binding mode made biological sense. The constant module of the Fab was found at this stage by systematically changing the elbow angle in rigid body refinement. When a 20° elbow angle change was applied to the original unligated Fab model, after rigid body refinement the R -factor dropped from 0.442 to 0.412 at 4 Å and the map began to show reasonable density.

MAD data

MAD data were collected using a crystal in which all six TCR methionine residues were replaced by selenomethionine. Cell growth and crystallization conditions were as previously described (Liu *et al.*, 1996) except that cells were preincubated for 10 h in GMEM medium containing 50 $\mu\text{g}/\text{ml}$ seleno-L-methionine (Sigma #3132), followed by fresh medium replacement containing 2 mM Na butyrate and supernatant harvested after four days for protein processing. One single frozen crystal was used to collect three data sets at the inflection point, peak point and remote point respectively, with 'Friedel flip' geometry. The integrated data sets were local-scaled together carefully with the program DSCALEAD (Rould, 1997) to obtain accurate dispersive differences. Difference-Fourier maps were calculated using the initial phases from the preliminary refined model derived with molecular replacement. Nine of 12 Se sites showed up in both anomalous and dispersive difference maps, and the sites agree with the methionine positions in the model. The MAD data were treated as a special case of multiple isomorphous replacement (Ramakrishnan *et al.*, 1993) and the nine Se sites were refined and used for phasing with MLPHARE in the CCP4 package (CCP4, 1994).

Model building and refinement

Initially the experimental electron density maps phased by the MAD data at 4 Å were used to rebuild the model to avoid any bias from the molecular replacement. The maps were first subjected to the density-modification procedure incorporated into the program DM (Cowtan and Main, 1993). Multiple domain averaging, solvent flattening, and histogram matching were applied. Care was taken to gradually improve domain by domain transforms relating the two complexes so that the DM map was of quite high quality. Model building and refinement were alternated using the programs O (Jones *et al.*, 1991) and X-PLOR (Brunger, 1992). An alternative position and individual temperature factor refinement protocol was used. There were 10 rounds of model building and refinement. At the fourth round, the model phases started to be incorporated and at the sixth round, only model phases were used. During the refinement, a bulk solvent correction and the overall B factor were applied on the Fo. The resolution was extended gradually to 2.8 Å, and $2 F_o - F_c$ as well as $F_o - F_c$ difference maps were used for further model building. The simulated annealing omit maps were used for checking and rebuilding. The seven glycans were seen clearly on the maps and could be built easily. NCS restraint for each individual pair of domains was applied in the refinement until the last several cycles. In the final model, four residues of the appended linker sequence following the interchain disulfide of the TCR α and β chains at the C-termini are absent due to disorder. This final model contains 13 892 atoms and has R_{free} 0.309 and R_{work} 0.243 at 15–2.8 Å, with good geometry (r.m.s. values for bond length and angle are 0.006 Å and 1.2° , respectively, and there is only 0.6% of the structure in the disallowed region on the Ramachandran plot generated from PROCHECK) (Laskowski *et al.*, 1983). The statistical data are listed in the Table I. The coordinates have been deposited in the Protein Data Bank (access code INFD).

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References

- Acuto, O., Meuer, S.C., Hodgdon, J.C., Schlossman, S.F. and Reinherz, E.L. (1983a) Peptide variability exists within α and β subunits of the T cell receptor for antigen. *J. Exp. Med.*, **158**, 1368–1373.

- Acuto, O., Hussey, R.E., Fitzgerald, K.A., Protentis, J.P., Meuer, S.C., Schlossman, S.F. and Reinherz, E.L. (1983b) The human T cell receptor: Appearance in ontogeny and biochemical relationship of α and β subunits on IL-2 dependent clones and T cell tumors. *Cell*, **34**, 717–726.
- Acuto, O., Fabbri, M., Smart, J., Poole, C.B., Protentis, J.P., Royer, H.D., Schlossman, S.F. and Reinherz, E.L. (1984) Purification and NH₂-terminal amino acid sequencing of the β subunit of a human T cell antigen receptor. *Proc. Natl Acad. Sci. USA*, **81**, 3851–3855.
- Allison, J.P., McIntyre, B.W. and Black, D. (1982) Tumor specific antigen and murine T lymphoma defined with monoclonal antibody. *J. Immunol.*, **129**, 2293–2300.
- Amit, R.A., Mariuzza, R.A., Phillips, S.E.V. and Poljak, R.J. (1986) Three-dimensional structure of an antigen-antibody complex at 2.8 Å resolution. *Science*, **233**, 747–753.
- Ashwell, J.D. and Klausner, R.D. (1990) Genetic and mutational analysis of the T-cell antigen receptor. *Annu. Rev. Immunol.*, **8**, 139–167.
- Bentley, G.A., Boulton, G., Karjalainen, K. and Mariuzza, R.A. (1995) Crystal structure of the β chain of a T cell antigen receptor. *Science*, **267**, 1984–1987.
- Blumberg, R.S., Ley, S., Sancho, J., Lonberg, N., Lacy, E., McDermott, F., Schad, V., Greenstein, J.L. and Terhorst, C. (1990) Structure of the T cell antigen receptor: evidence for two CD3 ϵ subunits in the T cell receptor-CD3 complex. *Proc. Natl Acad. Sci. USA*, **87**, 7220–7224.
- Brenner, M.B., Trowbridge, I.S. and Strominger, J.L. (1985) Cross-linking of human T cell receptor proteins: association between the T cell idiotype β subunit and the T3 glycoprotein heavy subunit. *Cell*, **40**, 183–190.
- Brunker, A.T. (1992) X-PLOR Version 3.1: A system for X-ray crystallography and NMR. Yale University Press, New Haven, CT.
- Bruno, L., Rocha, B., Rolink, A., von Boehmer, H. and Rodewald, H.-R. (1995) Intra- and extra-thymic expression of the pre-T cell receptor α gene. *Eur. J. Immunol.*, **25**, 1877–1882.
- Casanova, J.-L., Martinon, F., Gournier, H., Barra, C., Pannetier, C., Regnault, A., Kourilsky, P., Cerottini, J.-C. and Maryanski, J.L. (1993) T cell receptor selection by and recognition of two major class I major histocompatibility complex-restricted antigenic peptides that differ at a single position. *J. Exp. Med.*, **177**, 811–820.
- Chang, H.-C., Smolyar, A., Spoerl, R., Witte, T., Yao, Y., Goyarts, E.C., Nathenson, S.G. and Reinherz, E.L. (1997) Topology of T cell receptor/peptide class I MHC interaction defined by charge reversal complementation and functional analysis. *J. Mol. Biol.*, **271**, 278–293.
- Chien, Y., Becker, D.M., Lindsten, T., Okamura, M., Cohen, D.I. and Davis, M.M. (1984) A third type of murine T cell receptor gene. *Nature*, **312**, 31–35.
- Chothia, C., Boswell, D.R. and Lesk, A.M. (1988) The outline structure of the T cell $\alpha\beta$ receptor. *EMBO J.*, **7**, 3745–3755.
- Clevers, H., Alarcon, B., Wileman, T. and Terhorst, C. (1988) The T cell receptor/CD3 complex: A dynamic protein ensemble. *Annu. Rev. Immunol.*, **6**, 629–662.
- Collaborative Computational Project No. 4. (1994) Daresbury UK. *Acta Crystallogr.*, **D50**, 760–763.
- Connolly, M.L. (1983) Analytical molecular surface calculation. *J. Appl. Crystallogr.*, **16**, 548–558.
- Cowan, K. and Main, P. (1993) Improvement of macromolecular electron-density maps by the simultaneous application of real and reciprocal space constraints. *Acta Crystallogr.*, **D49**, 148–157.
- Danska, J.S., Livingstone, A.M., Paragas, V., Ishihara, T. and Fathman, C.G. (1990) The presumptive CDR3 regions of both T cell receptor α and β chains determine T cell specificity for myoglobin peptides. *J. Exp. Med.*, **172**, 27–33.
- Davis, M.M. and Bjorkman, P.J. (1988) T-cell antigen receptor genes and T-cell recognition. *Nature*, **334**, 395–402.
- de la Hera, A., Muller, U., Olsson, C., Isaaz, S. and Tunncliffe, A. (1991) Structure of the T cell antigen receptor (TR): Two CD3 ϵ subunits in a functional TCR/CD3 complex. *J. Exp. Med.*, **173**, 7–17.
- Del Porto, P., Bruno, L., Mattei, M.G., von Boehmer, H. and Saint-Ruf, C. (1995) Cloning and comparative analysis of the human pre-T cell receptor α chain gene. *Proc. Natl Acad. Sci. USA*, **92**, 12105–12109.
- Dembic, Z., Haas, W., Weiss, S., McCubrey, J., Kiefer, H., von Boehmer, H. and Steinmetz, M. (1986) Transfer of specificity by murine α and β T cell receptor genes. *Nature*, **320**, 232–238.
- Ehrlich, E.W., Devaux, B., Rock, E.P., Jorgensen, J.L., Davis, M.M. and Chien, Y.-H. (1993) T cell receptor interaction with peptide/major histocompatibility complex (MHC) and superantigen/MHC ligands is dominated by antigen. *J. Exp. Med.*, **178**, 713–722.
- Engel, I. and Hedrick, S.M. (1988) Site-directed mutations in the VDJ junctional region of the T cell receptor β chain cause changes in antigenic peptide recognition. *Cell*, **54**, 473–484.
- Fabbri, M., Acuto, O., Smart, J.E. and Reinherz, E.L. (1984) Homology of Ti α subunit of a T cell antigen/MHC receptor with immunoglobulin. *Nature*, **312**, 269–271.
- Fehling, H.J., Krotkova, A., Saint-Ruf, C. and von Boehmer, H. (1995) Crucial role of the pre-T cell receptor α gene in development of $\alpha\beta$ but not $\gamma\delta$ T cells. *Nature*, **375**, 795–798.
- Fields, B.A., Ober, B., Malchiodi, E.L., Lebedeva, M.I., Braden, B.C., Ysern, X., Kim, J.K., Shao, X., Ward, E.S. and Mariuzza, R.A. (1995) Crystal structure of the V α domain of a T cell antigen receptor. *Science*, **270**, 1821–1824.
- Fields, B.A., Malchiodi, E.L., Li, H., Ysern, X., Stauffer, C.V., Schlievert, P.M., Karjalainen, K. and Mariuzza, R.A. (1996) Crystal structure of a T cell receptor β chain complexed with a superantigen. *Nature*, **384**, 188–192.
- Fremont, D.H., Matsumura, M., Stura, E.A., Peterson, P.A. and Wilson, I.A. (1992) Crystal structures of two viral peptides in complex with murine MHC class I H-2K^b. *Science*, **257**, 919–927.
- Garboczi, D.N., Ghosh, P., Utz, U., Fan, Q.R., Biddison, W.E. and Wiley, D.C. (1996) Structure of the complex between human T cell receptor, viral peptide and HLA-A2. *Nature*, **384**, 134–141.
- Garcia, K.C., Degano, M., Stanfield, R.L., Brunmark, A., Jackson, M.R., Peterson, P.A., Tayton, L. and Wilson, I.A. (1996) An $\alpha\beta$ T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. *Science*, **274**, 209–219.
- Gascoigne, N.R., Chien, Y., Becker, D.M., Kavalier, J. and Davis, M.M. (1984) Genomic organization and sequence of T cell receptor β chain constant and joining region genes. *Nature*, **310**, 387–391.
- Gold, D.P., Clevers, H., Alarcon, B., Dunlap, S., Novotny, J., Williams, A.F. and Terhorst, C. (1987) Evolutionary relationship between the T3 chains of the T cell receptor complex and the immunoglobulin supergene family. *Proc. Natl Acad. Sci. USA*, **84**, 7649–7653.
- Haskins, K., Kubo, R., White, J., Pigeon, M., Kappler, J. and Marrack, P. (1983) The major histocompatibility complex-restricted antigen receptor on T cells. *J. Exp. Med.*, **157**, 1149–1169.
- Hayday, A.C., Diamond, D.J., Tanigawa, G., Heilig, J.S., Folsom, V., Saito, H. and Tonegawa, S. (1985) Unusual organization and diversity of T cell receptor α chain genes. *Nature*, **316**, 828–832.
- Hedrick, S.M., Nielsen, E.A., Kavalier, J., Cohen, D.I. and Davis, M.M. (1984a) Sequence relationships between putative T cell receptor polypeptides and immunoglobulins. *Nature*, **308**, 153–158.
- Hedrick, S.M., Cohen, D.I., Nielsen, E.A. and Davis, M.M. (1984b) Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. *Nature*, **308**, 149–153.
- Housset, D., Mazza, G., Gregoire, C., Piras, C., Malissen, B. and Fontecilla-Camps, J.C. (1997) The three-dimensional structure of a T cell antigen receptor V α V β heterodimer reveals a novel arrangement of the V β domain. *EMBO J.*, **16**, 4205–4216.
- Huber, R. and Bennett, W.S. (1987) Antibody-antigen flexibility. *Nature*, **326**, 334–335.
- Irving, B.A. and Weiss, A. (1991) The cytoplasmic domain of the T cell receptor ζ chain is sufficient to couple to receptor-associated signal transduction pathways. *Cell*, **64**, 891–901.
- Jones, T.A., Zou, J.-Y., Cowan, S.W. and Kjeldgaard, M. (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr.*, **A47**, 110–119.
- Kabat, E.A., Wu, T.T., Perry, H.M., Gottesman, K.S. and Faeller, C. (1991) Sequences of proteins of immunological interest. US Dept of Health and Human Services, DHS, NIH, Bethesda, MD.
- Kabsch, W. and Sander, C. (1983) Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*, **22**, 2577–2637.
- Kappes, D.J. and Tonegawa, S. (1991) Surface expression of alternative forms of the TCR/CD3 complex. *Proc. Natl Acad. Sci. USA*, **88**, 10619–10623.
- Kappler, J., Kubo, R., Haskins, K., Hannum, C., Marrack, P., Pigeon, M., McIntyre, B., Allison, J. and Trowbridge, J. (1983) The major histocompatibility complex-restricted antigen receptor on T cells in mouse and man: identification of constant and variable peptides. *Cell*, **35**, 295–302.
- Kasibhatla, S., Nalefski, E.A. and Rao, A. (1993) Simultaneous involvement of all six predicted antigen binding loops of the T cell receptor in recognition of the MHC/antigenic peptide complex. *J. Immunol.*, **151**, 3140–3151.

- Kaye, J. and Hedrick, S.M. (1988) Analysis of specificity for antigen, MIs, and allogenic MHC by transfer of T cell receptor α and β chain genes. *Nature*, **336**, 580–583.
- Kelly, J.M., Sterry, S.J., Cose, S., Turner, S.J., Fecondo, J., Rodda, S., Fink, P.J. and Carbone, F.R. (1993) Identification of conserved T cell receptor CDR3 residues contacting known exposed peptide side chains from a major histocompatibility complex class I-bound determinant. *Eur. J. Immunol.*, **23**, 3318–3326.
- Koning, F., Maloy, W.L. and Coligan, J.E. (1990) The implications of subunit interactions for the structure of the T cell receptor–CD3 complex. *Eur. J. Immunol.*, **20**, 299–305.
- Koyasu, S., Clayton, L.K., Lerner, A., Heiken, H., Parkes, A. and Reinherz, E.L. (1997) Pre-TCR signaling components trigger transcriptional activation of a rearranged TCR α gene locus and silencing of the pT α locus: Implications for intrathymic differentiation. *Int. Immunol.*, **9**, 1475–1480.
- Krangel, M.S., Bierer, B.E., Devlin, P., Clabby, M., Strominger, J.L., McLean, J. and Brenner, M.B. (1987) T3 glycoprotein is functional although structurally distinct on human T cell receptor γ T lymphocytes. *Proc. Natl Acad. Sci. USA*, **84**, 3817–3821.
- Kraulis, P.J. (1991) MOLSCRIPT: a program to produce both detailed and schematic plots of protein structure. *J. Appl. Crystallogr.*, **24**, 946–950.
- Kubo, R.T., Born, W., Kappler, J.W., Marrack, P. and Pigeon, M. (1989) Characterization of a monoclonal antibody which detects all murine $\alpha\beta$ T cell receptors. *J. Immunol.*, **142**, 2736–2742.
- Lai, M.-Z., Jang, Y.-J., Chen, L.-K. and Gelfand, M.L. (1990) Restricted V-(D)-J junctional regions in the T cell response to λ -repressor. *J. Immunol.*, **144**, 4851–4856.
- Laskowski, R.A., MacArthur, M.W., Moss, S.D. and Thornton, J.M. (1983) PROCHECK: a program to check the stereochemical quality of protein structure. *J. Appl. Crystallogr.*, **26**, 283–287.
- Levelt, C.N., Wang, B., Ehrfeld, A., Terhorst, C. and Eichmann, K. (1995) Regulation of T cell receptor (TCR)- β locus allelic exclusion and initiation of TCR- α locus rearrangement in immature thymocytes by signaling through the CD3 complex. *Eur. J. Immunol.*, **25**, 1257–1261.
- Liu, J., Tse, A.G.D., Chang, H.-C., Liu, J.-H., Wang, J., Hussey, R.E., Chisti, Y., Reinhold, B., Spoerl, R., Nathenson, S.G., Sacchetti, J.C. and Reinherz, E.L. (1996) Crystallization of a deglycosylated T cell receptor (TCR) complexed with an anti-TCR Fab fragment. *J. Biol. Chem.*, **271**, 33639–33646.
- Luescher, I.F., Anjuere, F., Peitsch, M.C., Jongeneel, C.V., Cerottini, J.-C. and Romero, P. (1995) Structural analysis of TCR–ligand interactions studied on H-2K^d restricted cloned CTL specific for photoreactive peptide derivative. *Immunity*, **3**, 51–63.
- Letourneur, F. and Klausner, R.D. (1991) T-cell and basophil activation through the cytoplasmic tail of T-cell-receptor ζ family proteins. *Proc. Natl Acad. Sci. USA*, **88**, 8905–8909.
- McDonald, I.K. and Thornton, J.M. (1994) Satisfying H-bonding potential in proteins. *J. Mol. Biol.*, **238**, 777–793.
- Malissen, M., Gillet, A., Ardouin, L., Bouvier, G., Trucy, J., Ferrier, P., Vivier, E. and Malissen, B. (1995) Altered T cell development in mice with a targeted mutation of the CD3 ϵ gene. *EMBO J.*, **14**, 4641–4653.
- Manolios, N., Letourneur, F., Bonifacio, J.S. and Klausner, R.D. (1991) Pairwise, cooperative and inhibitory interactions describe the assembly and probable structure of the T cell antigen receptor. *EMBO J.*, **10**, 1643–1651.
- Manolios, N., Kemp, O. and Li, Z.G. (1994) The T cell antigen receptor α and β chains interact via distinct regions with CD3 chains. *Eur. J. Immunol.*, **24**, 84–92.
- Marrack, P. and Kappler, J. (1986) The antigen-specific, major histocompatibility complex-restricted receptor on T cells. *Adv. Immunol.*, **38**, 1–30.
- Meuer, S.C., Fitzgerald, K.A., Hussey, R.E., Hodgdon, J.C., Schlossman, S.F. and Reinherz, E.L. (1983a) Clonotypic structures involved in antigen-specific human T cell function: Relationship to the T3 molecular complex. *J. Exp. Med.*, **157**, 705–719.
- Meuer, S.C., Acuto, O., Hussey, R.E., Hodgdon, J.C., Fitzgerald, K.A., Schlossman, S.F. and Reinherz, E.L. (1983b) Evidence for the T3-associated 90K heterodimer as the T cell antigen receptor. *Nature*, **303**, 808–810.
- Meuer, S.C., Cooper, D.A., Hodgdon, J.C., Hussey, R.E., Fitzgerald, K.A., Schlossman, S.F. and Reinherz, E.L. (1983c) Identification of the antigen/MHC receptor on human inducer T lymphocytes. *Science*, **222**, 1239–1242.
- Meuer, S.C., Hodgdon, J.C., Hussey, R.E., Protentis, J.P., Schlossman, S.F. and Reinherz, E.L. (1983d) Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.*, **158**, 988–993.
- Meuer, S.C., Acuto, O., Hercend, T., Schlossman, S.F. and Reinherz, E.L. (1984) The human T cell receptor. *Annu. Rev. Immunol.*, **2**, 23–50.
- Mian, I.S., Bradwell, A.R. and Olson, A.J. (1991) Structure, function and properties of antibody binding sites. *J. Mol. Biol.*, **217**, 133–151.
- Navaza, J. (1994) AMoRe: an automated package for molecular replacement. *Acta Crystallogr.*, **A50**, 157–163.
- Nicholls, A., Sharp, K.A. and Honig, B. (1991) Protein folding and association: Insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins: Struct. Funct. Genet.*, **11**, 281–296.
- Novotny, J., Tonegawa, S., Saito, H., Kranz, D.M. and Eisen, H.N. (1986) Secondary, tertiary, and quaternary structure of T-cell-specific immunoglobulin-like polypeptide chains. *Proc. Natl Acad. Sci. USA*, **83**, 742–746.
- Otwinowski, Z. and Minor, W. (1997) Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.*, **276**, 307–326.
- Padlan, E.A. (1994) Anatomy of the antibody molecule. *Mol. Immunol.*, **31**, 169–217.
- Patten, P., Yokota, T., Rothbard, J., Chien, Y., Arai, K. and Davis, M.M. (1984) Structure, expression and divergence of T cell receptor β chain variable regions. *Nature*, **312**, 40–46.
- Program Manual for the Wisconsin Package. (1994) Version 8, Genetics Computer Group, Inc., September.
- Ramakrishnan, V., Finch, J.T., Graziano, V., Lee, P.L. and Sweet, R.M. (1993) Crystal structure of globular domain of histone H5 and its implications for nucleosome binding. *Nature*, **362**, 219–223.
- Ramiro, A.R., Trigueros, C., Marquez, C., Millan, J.L.S. and Toribio, M.L. (1996) Regulation of pre-T cell receptor (pT α -TCR β) gene expression during human thymic development. *J. Exp. Med.*, **184**, 519–530.
- Rast, J.P. and Litman, G.W. (1994) T cell receptor gene homologs are present in the most primitive jawed vertebrates. *Proc. Natl Acad. Sci. USA*, **91**, 9248–9252.
- Reinherz, E.L., Hussey, R.E. and Schlossman, S.F. (1980) A monoclonal antibody blocking human T cell function. *Eur. J. Immunol.*, **10**, 758–762.
- Reinherz, E.L., Meuer, S.C., Fitzgerald, K.A., Hussey, R.E., Levine, H. and Schlossman, S.F. (1982) Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. *Cell*, **30**, 735–743.
- Reinherz, E.L., Meuer, S.C., Fitzgerald, K.A., Hussey, R.E., Hodgdon, J.C., Acuto, O. and Schlossman, S.F. (1983) Comparison of T3-associated 49 and 43 kilodalton cell surface molecules on individual human T cell clones: Evidence for peptide variability in T cell receptor structures. *Proc. Natl Acad. Sci. USA*, **80**, 4104–4108.
- Reth, M. (1989) Antigen receptor tail clue. *Nature*, **338**, 383–384.
- Romeo, C., Amiot, M. and Seed, B. (1992) Sequence requirements for induction of cytolysis by the T cell antigen/Fc receptor ζ chain. *Cell*, **68**, 889–897.
- Rould, M.A. (1997) Screening for heavy-atom derivatives and obtaining accurate isomorphous differences. *Methods Enzymol.*, **276**, 461–472.
- Saint-Ruf, C., Ungewiss, K., Groettrup, M., Bruno, L., Fehling, H.G. and von Boehmer, H. (1994) Analysis and expression of a cloned pre-T cell receptor gene. *Science*, **266**, 1208–1212.
- Saito, H., Kranz, D.M., Takagaki, Y., Hayday, A.C., Eisen, H.N. and Tonegawa, S. (1984) A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. *Nature*, **312**, 36–40.
- Saito, T., Weiss, A., Miller, J., Norcross, M.A. and Germain, R.H. (1987) Specific antigen-Ia activation of transfected human T cells expressing murine T $\alpha\beta$ -human T3 receptor complexes. *Nature*, **325**, 125–130.
- Samelson, L.E., Harford, J.B. and Klausner, R.D. (1985) Identification of the components of the murine T cell antigen receptor complex. *Cell*, **43**, 223–231.
- Sheriff, S., Hendrickson, W.A. and Smith, J.L. (1987) Structure of myohemerythrin in the azidomet state at 1.7/1.3 Å resolution. *J. Mol. Biol.*, **197**, 273–296.
- Shibata, K., Imai, M., van Bleek, G.M., Joyce, S. and Nathenson, S.G. (1992) Vesicular stomatitis virus antigenic octapeptide N52–59 is anchored into the groove of the H-2K^b molecule by the side chains of three amino acids and the main-chain atoms of the amino terminus. *Proc. Natl Acad. Sci. USA*, **89**, 3135–3139.
- Shinkai, Y., Koyasu, S., Nakayama, K.-I., Murphy, K.M., Loh, D.Y., Reinherz, E.L. and Alt, F.W. (1993) Restoration of T cell development in RAG-2 deficient mice by functionally rearranged TCR transgenes. *Science*, **259**, 822–825.

- Sim,G.K., Yague,J., Nelson,J., Marrack,P., Palmer,E., Augustin,A. and Kappler,J. (1984) Primary structure of human T cell receptor α chain. *Nature*, **312**, 771–775.
- Stanfield,R.L., Takimoto-Kamimura,M., Rini,J.M., Profy,A.T. and Wilson,I.A. (1993) Major antigen-induced domain rearrangements in an antibody. *Structure*, **1**, 83–93.
- Sussman,J.J., Bonifacino,J.S., Lippincott-Schwartz,J., Weissman,A.M., Saito,T., Klausner,R.D. and Ashwell,J.D. (1988) Failure to synthesize the T cell CD3 ζ chain: Structure and function of a partial T cell receptor complex. *Cell*, **52**, 85–95.
- Taylor,A.H., Haberman,A.M., Gerhard,W. and Caton,A.J. (1990) Structure-function relationships among highly diverse T cells that recognize a determinant from influenza virus hemagglutinin. *J. Exp. Med.*, **172**, 1643–1651.
- van Wauwe,J.P., de Mey,J.R. and Goosens,J.G. (1980) OKT3: a monoclonal anti-human T lymphocyte antibody with potent mitogenic properties. *J. Immunol.*, **124**, 2708–2713.
- Wang,J., Yan,Y., Garrett,T.P.J., Liu,J., Rodgers,D.W., Garlick,R.L., Tarr,G.E., Husain,Y., Reinherz,E.L. and Harrison,S.C. (1990) Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. *Nature*, **348**, 411–418.
- Weiss,A. (1993) T cell antigen receptor signal transduction: a tale of tails and cytoplasmic protein-tyrosine kinases. *Cell*, **73**, 209–212.
- Wilson,I.A. and Stanfield,R.L. (1993) Antibody–antigen interactions. *Curr. Opin. Struct. Biol.*, **3**, 113–118.
- Witte,T., Smolyar,A., Spoerl,R., Goyarts,E.C., Nathenson,S.G., Reinherz,E.L. and Chang,H.-C. (1997) MHC recognition by immune receptors: differences among TCR versus antibody interactions with the VSV8/H-2^b complex. *Eur. J. Immunol.*, **27**, 227–233.
- Wilson,A. and MacDonald,H.R. (1995) Expression of genes encoding the pre-TCR and CD3 complex during thymus development. *Int. Immunol.* **7**, 1659–1664.
- Xu,Y., Davidson,L., Alt,F.W. and Baltimore,D. (1996) Function of the pre-T cell receptor α chain in T cell development and allelic exclusion at the T cell receptor β locus. *Proc. Natl Acad. Sci. USA*, **93**, 2169–2173.
- Yanagi,Y., Yoshikai,Y., Leggett,K., Clark,S.P., Aleksander,I. and Mak,T.W. (1984) A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature*, **308**, 145–149.
- Zhang,Z., Young,A.C.M., Imarai,M., Nathenson,S.G. and Sacchettini,J. (1992) Crystal structure of the major histocompatibility complex class I H-2K^b molecule containing a single viral peptide: implications for peptide binding and T-cell receptor recognition. *Proc. Natl Acad. Sci. USA*, **89**, 8403–8407.

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