



Review

The TCR/CD3 Complex: Molecular Interactions in a Changing Structure

MARIA JOSÉ FEITO¹, ARTURO JIMÉNEZ-PERIAÑEZ², GLORIA OJEDA², ALEJANDRA SÁNCHEZ¹,
PILAR PORTOLÉS² and JOSÉ M. ROJO^{1*}

¹ Departamento de Inmunología, Centro de Investigaciones Biológicas, CSIC, Madrid, Spain, ² Centro Nacional de Biología Fundamental, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain

Abstract. The T cell receptor-CD3 (TCR/CD3) complex is a multichain structure in charge of antigen recognition in T cells. Despite many genetic, structural, and functional data obtained in recent years, essential questions concerning the TCR/CD3 complex still remain open, including: 1) the precise number of polypeptides in each TCR/CD3 complex, their interactions and spatial arrangement, 2) the role(s) of each polypeptide in antigen recognition and/or in receptor signal transmission, and 3) the relationship between the TCR/CD3 complex and other membrane or cytoplasmic molecules involved in downstream signaling. In this work we shall review data concerning some of these issues, proposing a model of the overall structure of the TCR/CD3 complex to explain its known features.

Key words: T cell receptor; CD3; antigen recognition.

Introduction

The antigen receptor of T lymphocytes (T cell receptor, TCR) is a hallmark molecule whose membrane expression defines the T (thymus-derived) subset of lymphocytes. While antibodies, the antigen-specific effector molecules of the humoral adaptive immune responses, have been known for more than a century, the molecules involved in antigen-specific T lymphocyte responses began to be detected in 1975, with the advent of monoclonal antibody technology. Following the finding that T cells recognize antigen peptides associated to (“restricted by”) major histocompatibility complex (MHC) molecules⁹⁴, monoclonal antibodies were obtained against the CD3 polypeptides, showing its involvement in antigen recognition⁶⁹. Monoclonal anti-

bodies recognizing the TCR which inhibited antigen recognition in an antigen-specific fashion were first described in 1983, as was the physical and functional relationship between the TCR and the CD3 polypeptides, soon followed by the discovery of the genes coding for TCR chains (reviewed in^{16, 60}).

There is agreement that mature TCR/CD3 complexes are formed by 6 different polypeptides (these being the α and β TCR chains, the CD3 γ , δ and ϵ polypeptides, and the ζ chains) necessary and sufficient for the expression of the TCR/CD3 complex, as determined by expression in non-T cells⁴⁵ or in microsomes³⁶.

The TCR/CD3 complex is structured as different modules. One is involved in antigen-specific recognition (TCR), whereas the others, formed by the CD3 γ ,

* Correspondence to: Dr. José M. Rojo, Departamento de Inmunología, Centro de Investigaciones Biológicas, CSIC, Velázquez, 144, E-28006 Madrid, Spain, tel.: +34 91 561 1800, fax: +34 91 562 7518, e-mail: jmrojo@cib.csic.es

δ , ϵ polypeptides and the ζ chains, are organized as dimers (CD3 $\epsilon\gamma$, CD3 $\epsilon\delta$, and ζ - ζ), are non-covalently bound to the TCR moiety, and are involved in signal transduction primarily through the presence of immunoreceptor tyrosine-based activation motifs (ITAM)^{11, 70} in the cytoplasmic tails of the CD3 and ζ chains^{37, 51, 56, 89, 90}. Since it is well established that there are at least two CD3 ϵ chains per TCR/CD3 complex^{7, 20}, a minimal model of the complex includes one TCR heterodimer, one CD3 $\epsilon\gamma$ and one CD3 $\epsilon\delta$ dimer, plus one disulfide-linked ζ chain homodimer (Fig. 1A). In addition, the CD3 and ζ chains fulfill a structural role, being necessary for the correct assembly and expression of the complex in the cell membrane^{1, 48, 59, 77}.

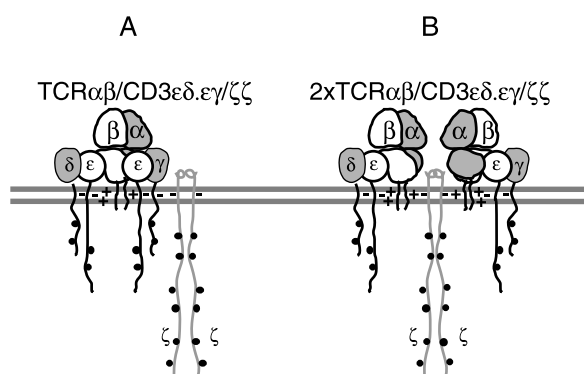


Fig. 1. Alternative models for a minimal stoichiometry of the TCR/CD3 complex. Models containing one (A) or two (B) TCR $\alpha\beta$ heterodimers per complex are depicted. The distribution of negatively and positively charged residues within the transmembrane domains of the polypeptides is shown schematically. Tyrosine residues within cytoplasmic ITAMs are depicted as dots

The Antigen-Recognition Module TCR

In most human and mouse T lymphocytes, the antigen-recognition module is composed of a disulfide-linked heterodimer of two chains, α and β^* . Each one is a type I membrane glycopolypeptide having an extracellular region formed by a V-like and a C-like Ig domain, a stalk of 13 (TCR α) or 19 (TCR β) amino acids connecting these domains to a transmembrane domain, and a short cytoplasmic tail of 4–5 amino acids. Since the discovery of TCR α and β chain sequences, it is clear that, unlike many other membrane receptors, TCR cytoplasmic domains cannot account for signal transmission^{**}. According to crystallographic data, the general structure of the extracytoplasmic TCR α and β heterodimer is roughly that of an immunoglobulin

* The data refers to human chains, unless specified otherwise.

** Nevertheless, the long intracytoplasmic tail of pre-T α chains expressed by immature T lymphocytes is responsible for control mechanisms of pre-TCR expression in the cell surface¹³.

Fab fragment, with some peculiarities^{30, 31}. For instance, the angle formed by the axis of the C and V domains is smaller in TCRs than in Fab fragments. In addition, TCRs have a relatively flat antigen-binding surface and a fourth hypervariable region in the TCR β variable (V) domain, plus a characteristic elbow between the F and G strands of the TCR β constant (C) domain close to the V domain. The position of this elbow shows certain variability depending on neighboring charges⁸⁶. On the other hand, TCRs are a symmetric as a consequence of the arrangement of the TCR C β domains and the small C α domain. Furthermore, the interaction between the C α and C β domains is such that a “pocket” or “cave” is formed below part of the C β domain, located roughly beneath the F to G loop elbow and part of the ABED sheet in the C β domain, with the C to D and E to F loops plus some glycans in the C α domain lining one side⁸⁶. It was proposed that this pocket was the site of interaction between the TCR and CD3 polypeptides^{32, 86}.

It has been shown that the stalks connecting the Ig-like domains and the transmembrane domains are of prime importance for some TCR-CD3 interactions as well as for downstream signaling. This issue has been analyzed in detail by Palmer’s group by means of α and β chain mutation analysis in transfected T cells and transgenic mice. This analysis detected a sequence motif (FETD $\times$ NLN) conserved in different TCR α chains⁵ that is absent from the structurally related TCR δ chains. Changes in this motif produce a weaker interaction with the CD3 δ and ζ chains and a defective response to superantigens restored by calcium ionophores^{5, 6}. Interestingly, in thymocytes this sequence motif is particularly important for interaction with CD3 ϵ . δ dimers⁶ and is linked to selective defects in the ERK MAP pathway activation and the recruitment to membrane rafts of Lck, ZAP-70 and phosphorylated ζ and LAT in thymocytes activated by a positively selecting peptide, a defect which does not extend to other early downstream signaling pathways⁹². This selective defect has been independently observed in thymocytes from mice transgenic for different CD3 δ chain constructs, confirming the importance of distinct CD3 chains and their interaction with the TCR for specific activation pathways²¹.

The TCR β chain connecting peptide has been also analyzed by Palmer’s group, showing that point mutation of one amino acid alters calcium-dependent TCR-mediated signaling⁴.

The transmembrane domain of α (δ) and β (γ) TCR chains are of prime importance for the maintenance of TCR-CD3 interactions. Particularly, these domains

possess one (β and γ) and two (α , δ and pre-T α) conserved, positively charged amino-acid residues. According to mutation analysis, these residues are necessary for interactions with several CD3 polypeptides, whose transmembrane domains possess one negatively charged amino-acid residue each^{2, 43, 58}. Analysis of the transmembrane domain of human TCR β chains has also revealed that the interaction between the TCR and CD3 ζ chains, as well as some TCR signals, depend on other, non-polar amino acids within this domain⁷².

CD3 Chains

The CD3 γ , δ and ϵ chains are 20–26 kDa polypeptides which are the products of duplication of a common ancestor gene. In fact, some species of vertebrates have only CD3 ϵ chains plus CD3 γ/δ chains having characteristics and properties of both CD3 γ and CD3 δ chains^{34, 35}.

CD3 ϵ chains form non-covalently bound heterodimers by pairing with CD3 δ and CD3 γ chains shortly after synthesis in the endoplasmic reticulum^{16, 25, 46}. The extracytoplasmic region of CD3 polypeptides is composed of an Ig C2 domain plus a connecting peptide. This peptide stalk includes a highly conserved sequence containing two cysteine residues which is necessary for the pairing of CD3 ϵ with CD3 δ and CD3 γ , possibly through the formation of four-cysteine Zn⁺²-containing bonds between CD3 ϵ and CD3 δ or CD3 ϵ and CD3 γ chains⁹. The interaction between the Ig C2 domains is also needed for ϵ/δ and ϵ/γ heterodimer formation⁷⁹.

CD3 γ , CD3 δ , and CD3 γ/δ chains from different species are glycoproteins with consensus N-glycosylation sites in the extracytoplasmic domain. Glycosylation of CD3 δ chains, but not of CD3 γ , is needed for optimal surface expression of TCR/CD3 complexes, as non-glycosylated CD3 δ chains are bound for degradation²⁶. Potential sites for CD3 γ and CD3 δ glycosylation are located in loops between β strands of the C domain, or in strands not involved in putative C-C domain interactions (Fig. 2, see below). In contrast, CD3 ϵ chains lack glycosylation sites in all species analyzed so far, including birds and toads³⁴. The small size of the extracellular CD3 ϵ C2-like domains, their lack of glycosylation sites, and their mode of interaction with CD3 γ chains⁷⁹ (Fig. 2) suggest that CD3 ϵ chains are responsible for close contacts with the TCR heterodimer, possibly lodged beneath the “cave” formed by the TCR C domains^{32, 86}. Other data favoring this interpretation include the low isoelectric point of the extracellular

domain of CD3 ϵ chains as opposed to the relatively high number of positively charged amino-acid residues present in TCR C domains, favoring an electrostatic interaction between them^{32, 86}. Indeed, it is known that TCR β -CD3 ϵ interactions are dependent on their extracellular domains, whereas TCR α -CD3 ϵ interactions depend on the charged amino acids in the transmembrane domain⁵⁸. Interestingly, the NH₂-terminal region of CD3 ϵ chains contains several negatively charged amino-acid residues. Recent data from our laboratory indicate that the NH₂-terminal region of mouse CD3 ϵ chains can be degraded to variable extents by proteases in different T cells and T cell lines¹⁸ (ROJO, unpublished data). Partial or total loss of these residues by the action of proteases might impair TCR-CD3 ϵ chain interactions. Indeed, we have found that cells having a high proportion of protease-degraded CD3 ϵ chains have looser TCR-CD3 interactions than cells having a low proportion of degraded CD3 ϵ chains¹⁸.

On the other hand, it is well established that the negatively charged amino-acid present in the transmembrane domain of CD3 γ , CD3 δ and CD3 ϵ chains is necessary for the stability of TCR-CD3 interactions^{26, 58}. The cytoplasmic domain of CD3 γ , CD3 δ , or CD3 ϵ polypeptides (and ζ chains⁸⁹, see below) is dispensable for the assembly of the TCR/CD3 complex^{10, 26, 54, 81}. CD3 γ deficiency causes deficient surface TCR/CD3 expression, particularly in CD8⁺ T cells⁴⁴, as well as altered structure of the expressed TCR/CD3 complexes⁹³. The defect in TCR complex expression in cells lacking CD3 γ might be due to its extracellular domain, as shown in experiments of substitution between CD3 γ and CD3 δ extracellular domains⁸⁸. Human CD3 γ and CD3 δ chain constructs lacking the cytoplasmic domain can substitute the mouse counterparts⁵⁴. Taken together, these data suggest that phylogenetically conserved differences between CD3 γ and CD3 δ extracellular domains are responsible for their differential behavior.

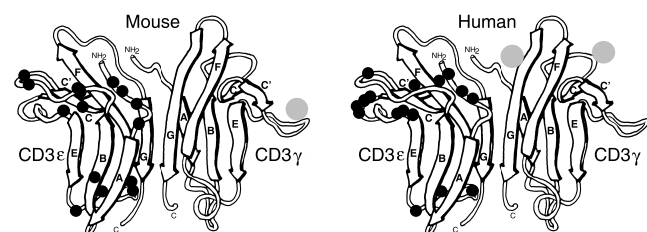


Fig. 2. Schematic location of CD3 ϵ acidic residues (black dots) and CD3 γ glycosylation sites (grey circles) in the external domains of mouse and human CD3 $\epsilon\gamma$ heterodimers. Ribbon diagrams of CD3 $\epsilon\gamma$ dimers as described by SUN et al.⁷⁹, with some modifications. The position of CD3 ϵ amino-terminal residues before strand A is hypothetical

The cytoplasmic domain of CD3 ϵ chains is part of one of the two "signal transduction modules" detected in the TCR/CD3 complex^{51, 89}, the other being the cytoplasmic domain of ζ chains³⁷ (see^{83, 90} for reviews). CD3 ϵ chain cytoplasmic domain sequences are very conserved among species and contain one typical ITAM motif involved in signal transduction. *In vitro*, tyrosine-phosphorylated ITAMs from CD3 γ , δ , ϵ and ζ bind different SH2 domain-containing proteins involved in the early steps of TCR signaling, including the ZAP-70 and Lck tyrosine kinases, the adapter proteins Grb-2 and Shc, or the p85 regulatory subunit of the phosphatidylinositol 3-kinase^{19, 65}. Recently, it has been determined that a conserved proline-rich region within the CD3 ϵ cytoplasmic tail binds Nck³³. Nck is an adapter protein known to have an important role in TCR-mediated T cell activation⁷⁵. Indeed, blocking Nck-CD3 ϵ interactions inhibits TCR activation, including maturation of T cell-APC "immunological synapses"³³. Interestingly, although Nck associates through its SH3-1 domain to isolated CD3 ϵ cytoplasmic chains, it does not bind to CD3 ϵ within TCR/CD3 complexes unless there is a TCR/CD3 activation signal. Furthermore, Nck association to CD3 ϵ upon activation is not dependent on tyrosine phosphorylation and temperature, but on some kind of conformational change or re-organization within the TCR/CD3 complex, which can be induced by monovalent ligands (i.e. Ig Fab fragments) of the TCR³³.

The cytoplasmic domains of CD3 γ and CD3 δ chains also possess one conserved ITAM motif each. CD3 γ chains also have a di-leucine internalization motif (SxxSxxx LL) which is hidden when these chains are within the TCR/CD3 complex. This motif is exposed upon protein kinase C (PKC)-mediated phosphorylation, producing PKC-mediated internalization of TCR/CD3 complexes^{23, 24}. TCR/CD3 complexes also possess other ligand-dependent and -independent internalization mechanisms^{8, 53, 76}.

Zeta Chains

Unlike the other elements of the TCR/CD3 complex, ζ chains do not belong to the Ig superfamily, but to a family of polypeptides involved in membrane signaling which includes the Fc ϵ RI γ chains⁴⁹. The ζ gene undergoes alternative splicing, producing quantitatively minor products (η , θ , ι) which can also be found in TCR/CD3 complexes^{15, 41, 64, 91}, as can also happen with Fc ϵ RI γ chains in particular instances⁵². Zeta chains comprise a short extracytoplasmic domain of 9 amino

acids, a transmembrane domain of 21 amino acids containing a cysteine involved in homodimerization as well as a negatively charged amino-acid residue, and a cytoplasmic domain of 112 amino acids containing three tandem ITAM motifs. In TCR/CD3 complexes, ζ chains are found as homodimers linked by a disulphide bridge in the extracellular domain, with minor amounts of heterodimers of ζ chains and η or other splicing products.

The interaction of ζ chain dimers with the rest of the TCR/CD3 complex is only observed after the incorporation of TCR α or β chains with preformed ϵ δ or ϵ γ dimers^{7, 14, 46, 77}. Interestingly, the short extracytoplasmic ζ domain, and particularly the acidic amino-acid residue within it, is needed for ζ chain association to the TCR/CD3 complex, and efficient surface expression and TCR-mediated activation⁴². An interaction between ζ homodimers and TCR $\alpha\beta$ chains is also suggested by analysis of immunoprecipitates using TCR- or CD3-specific monoclonal antibodies, whereby ζ chain co-precipitation with the TCR is much stronger than with CD3 polypeptides under conditions favoring TCR-CD3 dissociation (Rojo, unpublished). These data, plus the importance of the TCR α and TCR β connecting peptide in ζ chain association, all indicate that, within the complete complex, ζ chains interact with the TCR heterodimer rather than with CD3 dimers. Association of ζ chains to the complex might hide endoplasmic reticulum retention sequences of CD3 ϵ chains⁵⁷, allowing efficient transport of the complete TCR/CD3 complex to the Golgi compartment for maturation and eventual expression in the cell membrane. Interestingly, it has been observed that ζ chains lacking most of the cytoplasmic domain can also fulfill this role in TCR/CD3 transport and maturation, suggesting that this masking is due to a re-organization of the complex, rather than a direct effect of the ζ chain cytoplasmic tails^{3, 89}.

The functional role of the three tandem ζ chain ITAMs has been the subject of much speculation, particularly concerning the question of whether they are redundant or have distinct, specific functions. ITAM are essential to ζ chain-mediated signaling, and all of them can separately or together induce activating signals in transformed cells, such as Jurkat, when expressed as isolated membrane fusion proteins (reviewed in^{47, 90}). Upon activation of T lymphocytes, ζ chain ITAM are phosphorylated by Src tyrosine kinases, such as Lck and Fyn, allowing the binding of SH2-domain-containing proteins. All the phosphorylated ζ chain ITAM bind the ZAP-70 tyrosine kinase that is essential to TCR-mediated signaling. However, it has been re-

cently shown that ITAM phosphorylation proceeds in a precise and controlled sequence which depends on the strength of TCR stimuli⁶³. In addition, tyrosine-phosphorylated ITAM differ in their affinity towards SH2 domains from different proteins, suggesting a partially redundant, yet distinct role of each ITAM in TCR signaling^{19, 65}. Conflicting results exist as to the importance of ζ chain ITAMs in normal mature T cells. MALISSEN and SCHMITT-VERHULST⁵⁶ have recently shown normal TCR/CD3 assembly and T cell development and function in mice expressing ζ chains lacking cytoplasmic tails. However, ζ chain ITAMs might be essential to the development of T cells with different TCR specificities and affinities⁷⁸.

The TCR/CD3 Complex: a Model for Subunit Interactions

Despite enormous progress in recent years, including the determination of TCR and CD3 heterodimer structures, the precise structure of the TCR/CD3 complex is not known. Relevant questions include: 1) how many polypeptide chains there are per complex, i.e., the precise stoichiometry of the complex; 2) how these polypeptides interact among themselves and their spatial relationships in the cell membrane; and 3) whether or not the TCR/CD3 complex undergoes changes in its structure and interactions upon binding to MHC-antigen or other activating ligands.

Before proposing our model, we have carefully evaluated the available data on the TCR/CD3 complex, including 1) known interactions between its polypeptides in the cell membrane and during complex assembly; 2) the biochemical and immunochemical data on the size of the TCR/CD3 complex, and the minimal number of polypeptides per complex; 3) the known structural data, particularly crystallographic data, of the different elements of the TCR/CD3 complex and related molecules, including MHC-antigen complexes and co-receptors, plus the physical constraints posed on their interactions by other factors such as size or glycosylation; and 4) functional analysis of TCR/CD3-mediated signaling, including the effect of distinct antibodies or the selective loss of functions in TCR/CD3 mutants.

Concerning TCR/CD3 stoichiometry, available data support a minimal stoichiometry of two TCR heterodimers per complex plus two CD3 ϵ chains forming heterodimers with CD3 γ chains and CD3 δ chains, and one ζ - ζ chain homodimer ($(\alpha\beta)_2$, $\epsilon\gamma$, $\epsilon\delta$, ζ_2 , Fig. 1B). The experimental data supporting this model can be sum-

marized as follows: the existence of two different CD3 ϵ chains^{7, 20} and two different TCR $\alpha\beta$ heterodimers per complex²⁹; the ability of both TCR α and TCR β chains to associate to CD3 $\epsilon\gamma$ and CD3 $\epsilon\delta$ dimers during assembly⁷⁷; a 1:1 stoichiometry for anti-CD3 and anti-TCR monoclonal antibody binding sites^{18, 61, 67}, the size of isolated TCR/CD3 complexes determined by ultracentrifugation²⁸; and the fact that $(\alpha\beta)_2$, $\epsilon\gamma$, $\epsilon\delta$, ζ_2 complexes achieve electrostatic equilibrium of the 6 positive charges and 6 negative charges within their transmembrane domains that are involved in interchain interactions. Furthermore, having two TCR, this structure has two "caves"⁸⁶ to harbor the two CD3 ϵ heterodimers present in the complex.

Data against this minimal complex comes from experiments showing a 1:1.5 or a 1:2 ratio of anti-TCR to anti-CD3 binding sites^{68, 80}, as well as the lack of detectable co-precipitation of TCRs in cells from tetra-transgenic mice⁶⁸. These results might be due to inaccurate determination of the FITC-to-protein ratios in the first case, and to difficulties to detecting and discriminating the co-precipitating TCR in the second (see²⁹ for a discussion). Further evidence suggesting the association of two CD3 dimers to each TCR heterodimer comes from experiments showing that anti-CD3 antibodies can block the binding of certain anti-TCR antibodies such as H57³², whereas the same anti-TCR antibodies inhibit at best by 50% the binding of anti-CD3 antibodies. These data, plus the detection of two putative CD3 docking sites in the TCR constant domains, provides grounds for the idea of two CD3 dimers associated to each TCR $\alpha\beta$ heterodimer^{32, 79}. However, the binding sites for the competing anti-TCR and anti-CD3 antibodies are located in different, non-covalently linked polypeptides, and it is difficult to draw definitive conclusions from interactions whose complexity we are beginning to envisage. For instance, we have observed complete blocking by anti-CD3 antibodies of the binding of one anti-TCR antibody (C193.5A) and partial blocking of anti-CD3 binding by anti-TCR⁶⁷, though complete blocking could be achieved under appropriate conditions²². Consequently, we shall propose an alternative role for the two CD3 docking sites in the TCR (see below).

Nuclear magnetic resonance data on the CD3 $\epsilon\gamma$ heterodimer of the Ig-like domains have been recently published showing that they form a rigid structure with interactions between A and F strands and their G β strands, indicating that the cysteine-rich sequences of the connecting peptides also involved in interchain interactions^{9, 79} are placed close together⁷⁹ (Fig. 2). Interestingly, potential glycosylation sites in CD3 γ (and

CD3 δ) chains from different species are in or close to the B to C, C' to C'', or F to G loops^{26, 79}, roughly distal to the membrane surface and suggesting that they are part of the external, exposed surface of the CD3 ϵ . γ (or CD3 ϵ . δ) heterodimer, rather than the site of interaction with the TCR (Fig. 2). In contrast, CD3 ϵ chains are devoid of sugar moieties in all species analyzed, and possess a series of negatively charged amino-acid residues in their Ig-like domain, conferring them a low pI of 4.6 in the mouse. These charged residues have been proposed to interact with positively charged residues lining the accessible surface of the "cave" formed by the TCR α and TCR β constant domains^{32, 86}. An analysis of the location of negatively charged residues of mouse CD3 ϵ within CD3 ϵ . γ chain dimers shows that they are not randomly distributed, but clustered in the B to C loop, in a row with conserved acidic residues of the NH₂-terminal region before strand A (Fig. 2). A second cluster of membrane-proximal acidic residues is located in the E to F loop (Fig. 2). These negatively charged residues are close to the phylogenetically conserved ABE surface of the CD3 ϵ domain, and are not involved in interactions with the CD3 γ domain, strongly suggesting an interaction of this surface with the constant domains of the TCR heterodimer. The location of CD3 ϵ . γ and CD3 ϵ . δ heterodimers close to the TCR C β domain F to G loop is further supported by the strong inhibition by anti-CD3 antibodies of the binding of one anti-TCR monoclonal antibody (H57) that binds to the F to G loop of mouse TCR C β domains^{22, 32}.

As human CD3 ϵ can structurally and functionally replace mouse CD3 ϵ in the mouse TCR/CD3 complex^{7, 20, 85}, comparing the characteristics of mouse and human CD3 ϵ chains might help in understanding TCR-CD3 interactions. Fig. 2 shows that human and mouse CD3 ϵ have a broadly similar distribution of acidic residues, despite the low homology in the amino-terminal region, the B to C and the C' to E loops. Thus, it has three amino-terminal acidic residues, plus two in the B to C loop, although the highest concentration of acidic residues is in the C' to E loop, close to the B to C loop (Fig. 2). In addition, human CD3 ϵ (and the CD3 ϵ from other mammalian species) conserves three of the four negatively charged amino-acids present in the mouse E to F loop (Fig. 2). Thus, acidic amino acids within CD3 ϵ chains are mainly distributed in membrane-distal and membrane-proximal "hot spots". Initial analysis of TCR heterodimer crystals suggested that the 2 nm high "cave" formed beneath the TCR C β F to G loop might be enough to harbour a small Ig domain like that predicted for CD3 ϵ chains^{32, 86}. Instead, the extracellular

domains of CD3 ϵ . γ heterodimers form a rigid structure 4 nm high, making unlikely the interaction between membrane-distal CD3 ϵ negative charges and basic residues in the TCR C α membrane proximal A to B and F to G loops, as suggested by SUN et al.⁷⁹. It is also unlikely that these basic residues can interact with the conserved, membrane-proximal acidic residues in the E to F loop of CD3 ϵ , as these are concealed within the TCR cave. Consequently, we believe that the most likely place of association of CD3 ϵ . γ or CD3 ϵ . γ dimers to the resting TCR is in the "second" binding site formed by CC' and FG strands in the external surface of TCR C β ⁷⁹ plus, perhaps, the interaction of CD3 ϵ amino-terminal acidic residues with basic residues in the prominent TCR C β F to G loop (Fig. 3).

Recent data indicate that the CD4 co-receptor interacts with TCR/MHC complexes forming a "V", where the T cell membrane would be located on top of the V and the APC surface in the V angle, with the interacting TCR and MHC forming one side and CD4 the other⁸⁷. Considerations about the size of the molecules, their mobility constraints and the geometry of CD4-MHC interactions suggest that, for the TCR to recognize MHC-antigen, it has to tilt its axis by about 45°

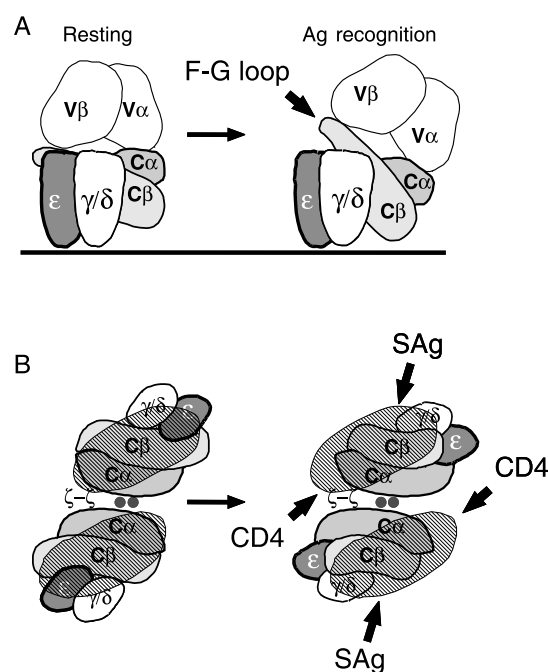


Fig. 3. A model of the interactions between the extracellular domains of the TCR/CD3 complex and the structural changes produced by antigen recognition. The extracellular components of the TCR/CD3 complex are viewed from one side (A) or from the top (B). In A, the TCR/CD3 half in the back has been omitted for clarity and the approximate position of the TCR C β F to G loop is shown; in B, the position of the TCR V domains is shadowed, and the approximate sites for interactions between the TCR-MHC-antigen and staphylococcal superantigens (SAG) and CD4 are indicated

from the T cell surface, rather than be vertical. Interestingly, we recently proposed ligand-induced TCR tilting to explain certain differences in CD3 recognition by monoclonal antibodies¹⁸. Reinherz's group also indicated that the nature of CD4-MHC interactions make unlikely the formation of MHC dimers or CD4 homodimerization during antigen recognition, as well as the proximity and the direct interaction between CD4 and the TCR heterodimer directly involved in antigen recognition. However, in our model the membrane-proximal domains of the CD4 bound to the MHC might lie close to CD3 associated to the opposite heterodimer in the complex (Fig. 3B). This would agree with many experimental data supporting the idea that CD4, particularly its membrane-proximal domains, interacts with the TCR/CD3 complex, possibly in the proximity of the CD3 subunits^{22, 27, 67, 84} (see^{38, 39, 50} for reviews).

Our model for the TCR/CD3 complex takes all these considerations into account, plus other factors, such as the geometry of interactions between TCR, MHC antigens and superantigens^{31, 40}, to propose a symmetric model of the TCR/CD3 complex formed by two heterodimers placed close together, associated by the ζ chain homodimer in a central position, modified from our previously proposed models^{18, 74} (Fig. 3). In resting T cells, the axis of the TCR heterodimer would be roughly vertical to the cell surface (Fig. 3A). The CD3 $\epsilon\gamma$ and CD3 $\epsilon\delta$ dimers would be placed each by one of the two TCR units of the complex, where interactions through the acidic residues of the CD3 ϵ external domain and the positively charged residues in the TCR C β domain would be of prime importance. Upon TCR ligation by antigen/MHC (or anti-TCR or anti-CD3 antibodies), the tilting of the TCR might favor the access of CD3 ϵ to other positive charges in the TCR C α and C β , causing a structural change in the position of CD3 $\epsilon\gamma$ /CD3 ϵ dimers and a more stable complex (Fig. 3). Enhanced stability of the TCR/CD3 interaction upon anti-CD3 ligation has been observed by different groups^{18, 61, 62}. Such a change might be the basis for a number of known effects of TCR ligands that seem to depend on conformational TCR/CD3 changes, including ligation-dependent association of the Nck adapter to proline-rich sites in the cytoplasmic domain of CD3 ϵ chains³³, the differential activating effect of certain anti-TCR antibodies⁷³, or the changes detected in TCR/CD3 radioactive labeling upon ligand binding⁶⁶. It should be noted again that these changes are compatible with studies of TCR structures bound to antigen-MHC complexes showing a rigid TCR heterodimer where structural changes induced to maximize

complementarity of antigen recognition surfaces in V domains do not extend to C domains³¹. The existence and relevance of structural/conformational changes versus receptor cross-linking in TCR/CD3 complex activation has been the subject of much debate since it was first proposed by JANEWAY et al.³⁸ to explain certain activation phenomena in T lymphocytes (see, e.g.^{12, 55, 71, 82} for recent discussions of this topic). Recent data obtained with class II MHC-antigen dimers generated by chemical cross-linking of the N-terminal amino acids at the end of $\alpha 2$ or $\beta 2$ domains has been taken as definitive evidence that it is the degree of cross-linking, and not the orientation of TCR-MHC interactions, that is relevant to efficient activation¹⁷. However, this interpretation is arguable, as the situation of the amino-acid residues involved might produce similar orientations in MHC dimers linked through the $\alpha 2$ or $\beta 2$ domains, and a minimal flexibility of the MHC dimer subunits would be enough to allow crosslinking interactions with the TCR complex proposed in Fig. 3.

A ligand-induced displacement of the TCR with respect to the CD3 dimers has been recently proposed by GIL et al.³³ as a mechanism to explain certain cross-linking-independent activation events. We propose a model whereby TCR-CD3 rotation is triggered by changes in the TCR due to ligand binding. This would also be in agreement with our recent data showing that cells having CD3 ϵ chains that undergo protease degradation in the negatively charged amino-terminal regions have a looser interaction with the TCR, but are more easily activated by antigen than cells expressing CD3 ϵ chains with "complete" amino-terminal sequences, as loss of negative charges in CD3 ϵ would favor the changes in CD3 ϵ -TCR interactions¹⁸.

There is no doubt that, in the near future, approaches from different points of view and different experimental systems will continue to shed new light on the structural and functional intricacies of the TCR/CD3 complex.

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