CHAPTER 1

T-Cell Receptor

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Abstract

The T-cell antigen receptor complex (TCR/CD3) is a cell surface structure that defines the T lymphocyte lineage, where it fulfills two basic functions, namely antigen recognition and triggering of signals needed to mount adequate responses to foreign aggression and/or to undergo differentiation. Knowing the precise structure of the complex in terms of its components and their relative arrangement and interactions before and after antigen recognition is essential to understand how ligand binding transforms into functionally relevant T-cell responses. These include not only full responses to foreign peptide antigens by mature T-cells, but also other phenomena like modulation of T-cell activation with altered peptide ligands, positive and negative selection of thymocytes, alloreactivity and autoimmune reactions.

A wealth of new data has accumulated in recent years on the structure of TCR/antigen complexes and CD3 polypeptides and on the stoichiometry of the TCR/CD3 complex and intersubunit interactions. In this review, we discuss how these data fit into a meaningful model of the TCR/CD3 function.

Introduction

In the TCR/CD3 complex, antigen recognition and signal triggering functions are carried out by two distinct molecular modules: the TCR chains are responsible for antigen recognition and the invariant CD3 (CD3ε, CD3γ, CD3δ) and CD247 (ζ) chains are in charge of signal transduction (Fig. 1) (reviewed by refs. 1-6).

The TCR antigen recognition unit exists in three distinct molecular species. In humans and mice, most mature T-lymphocytes express TCRs composed of two class I membrane glycosylated polypeptides termed α and β (αβTCRs). The overall organization of the extracellular region of these TCRs is similar to that of antibody Fab fragments. Each chain contains one variable (V) and one constant (C) Ig domain linked by a disulfide bridge. Some peculiarities of the chain include the flexibility of the external sheet (CFG face) of the small Cα which does not adopt a standard Ig structure, the high interaction surfaces between C domains and intrachain C-V domains and the small C-V angle of the TCR β chain.[7] The Ig-like domains of the TCR α and β chains are followed by a stalk of 19 (α chain) or 15 (β chain) residues, a 22-residue long transme mbrane (TM) domain containing two (α) or one (β) basic residues and a short 4-10 residue long intracellular region (Fig. 1).

On the cell surface, the TCR antigen recognition module is noncovalently associated with the invariant CD3 ε, δ and γ polypeptides and the ζ (CD247) homodimer (Figs. 1,2). These chains are needed to transform ligand binding by the TCR module into signals inside the cell. The CD3 and ζ chains are also involved in regulating the expression of the TCR/CD3 complex on the cell surface (reviewed by refs. 1,2,8).

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Multichain Immune Recognition Receptor Signaling: From Spatiotemporal Organization to Human Disease, edited by Alexander B. Sigalov. ©2008 Landes Bioscience and Springer Science+Business Media.

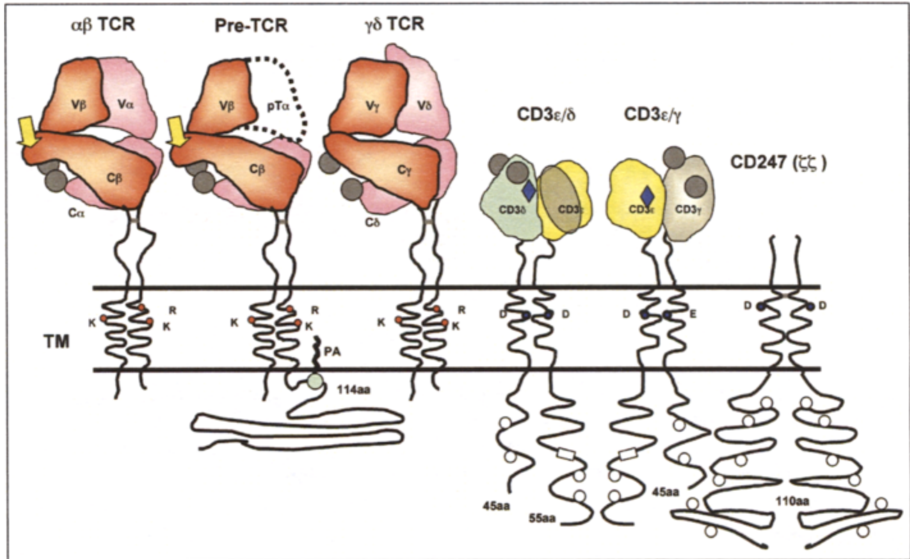

Figure 1. A schematic view of the components of TCR/CD3 complexes. TCR variable (V) and constant (C) domains are as indicated. Grey circles represent N-linked glycans (human Cα $Asn_{,185}$ Cβ $Asn_{,186}$ pTα $Asn_{,51}$ Cγ $Asn_{,184}$ or Cδ $Asn_{,135}$ CD3γ Asn_{30} and $Asn_{,70}$ CD3δ Asn_{16} and Asn_{52}). Arrows indicate the position of the extended F-G loop of TCR β chains. Disulfide links are indicated by grey bars. Acidic and basic residues in the transmembrane (TM) region are indicated by circles. PA: palmitic acid. The binding site for OKT3 and UCHT1 in CD3ε is shadowed. Diamonds indicate the approximate location of N-termini in CD3 chains. The proline-rich region in the cytoplasmic tail of CD3ε is indicated by a white box; open circles indicate ITAM Tyr residues. Where indicated, the number of amino acid residues (aa) refers to the cytoplasmic domain.

CD3 polypeptides possess an Ig-like ectodomain, a ten-residue long connecting peptide and the TM region that contains one acidic residue. The disulfide-linked homodimeric ζ chains also contain one acid residue in their TM region and have a short nine-residue long extracellular domain. Unlike TCR α and β chains, CD3 and ζ chains possess relatively large 45-110-residue long intracytoplasmic domains that contain immunoreceptor tyrosine-based activation motifs (ITAMs), polyproline motifs, endoplasmic reticulum (ER) retention and endocytosis motifs involved in transmembrane signaling[9,10] and cell surface receptor expression[1,2,8] (Fig. 1). However, it is unlikely that these intracytoplasmic domains have a significant role in the noncovalent interactions between the short-tailed TCR α and β chains and the CD3 and ζ chains, at least in those needed for surface expression of the TCR/CD3 complex.[11-15]

Although most T-lymphocytes express αβ TCRs, a minor subpopulation of functionally distinct mature T-cells expresses TCRs that contain γ and δ polypeptides homologous to the TCR β and α chains, respectively (Fig. 1). Like in immunoglobulins, the observed diversity in the N-terminal V domains of the TCR α, β, γ and δ chains is due to clonotypic rearrangement of V, D and J segments of the relevant genes. T-cells are developmentally selected to express αβ TCRs that specifically recognize short antigenic peptides bound to Major Histocompatibility Complex molecules (peptide-MHCs; pMHCs) on the surface of antigen-presenting cells (APCs). The γδ TCR-expressing cells (γδ T-cells) also bind antigens on the surface of APCs. However, unlike αβT-cells, γδ T-cells do not show restriction by polymorphic MHCs but recognize nonclassical MHC alone or in complexes with small phosphate-containing bacterial antigens.[5,16]

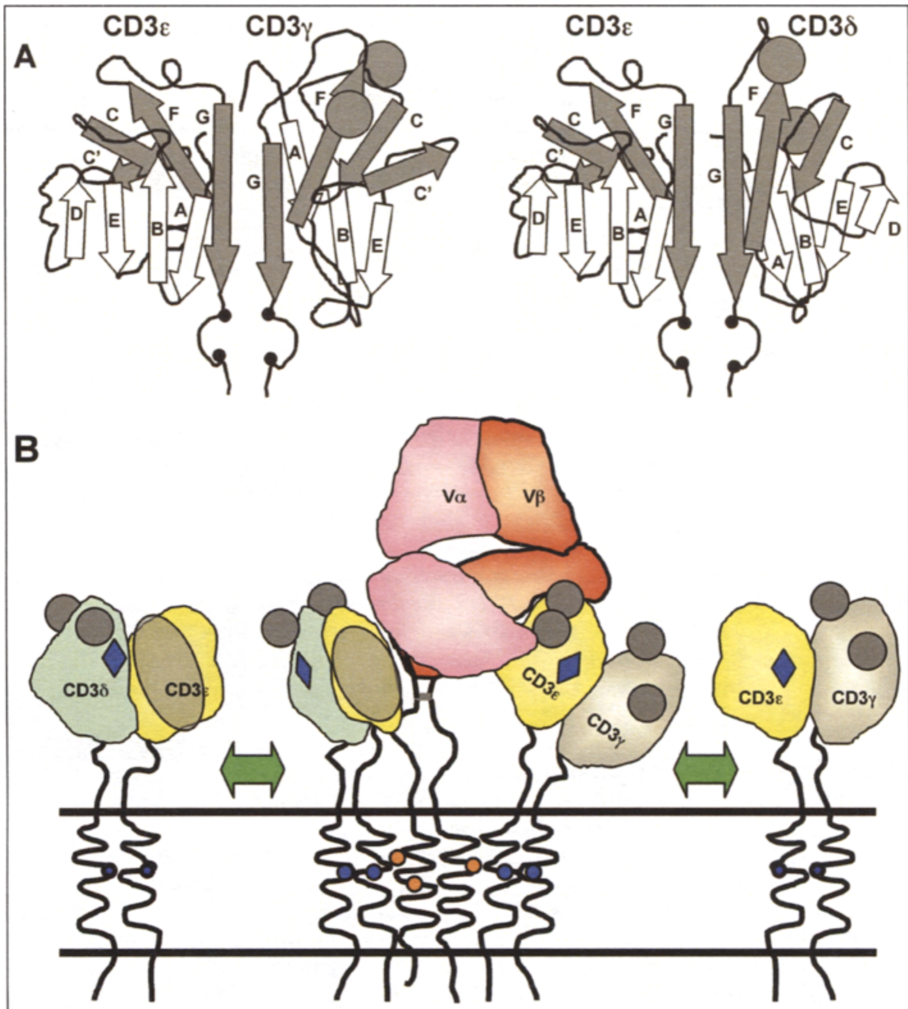

Figure 2. CD3 heterodimer structure and interactions. A) Scheme of the Ig-domain β-sheet structure in human CD3εγ and CD3εδ dimers. Black dots represent Cys residues in RxCxxCxD motifs of CD3 stalks. Circles represent N-linked glycans. B) A model of TCR-CD3 subunit interactions. ζ dimers and the cytoplasmic domains of CD3 are omitted for clarity. Other symbols and indications are as in Figure 1. Modified from references 18, 20, 21.

During differentiation, double negative (DN) thymocytes express TCR β chains complexed to a polypeptide with a long intracellular domain and a single extracellular C domain homologous to Cα (pTα) (Fig. 1). All αβ and γδ TCR/CD3 complexes share the CD3ε and ζ polypeptides and expression of these signaling modules best characterizes the T-cell lineage. This is possibly due to the unique, nonredundant function of CD3ε and ζ in blocking ER retention signals.

Minimal Components and Stoichiometry of the TCR/CD3 Complex

The minimal components of TCR/CD3 complexes, their number and their organization within the complex are essential to understanding the mechanisms of ligand-induced activation.

In this regard, the structure of the αβ TCR/CD3 complex is among the best studied examples. In addition to the α and β antigen recognition units, mature αβ TCR complexes contain CD3ε, CD3δ and CD3γ polypeptides as well as ζ homodimers. CD3ε binds noncovalently with CD3δ and CD3γ in a mutually exclusive manner, yielding CD3εδ and CD3εγ heterodimers.[17-21] Results from the coprecipitation experiments performed using human CD3ε-transfected mouse T-cells or human CD3ε-transgenic mice suggest that the TCR/CD3 complexes contain two CD3ε chains.[22,23] Gel-shift analysis of the TCR-CD3 complexes bound by antiCD3 Fab fragments supports this notion.[24]

The number of TCR antigen recognition units in each minimal TCR/CD3 complex has been the matter of long debate. Charged TM residues are known to be important for the stability of the complex and, as expected theoretically, two αβ TCR units should be presented in the complex $(\alpha\beta)_2$:εγ:εδ:ζζ in order to mantain electrostatic equilibrium in the TM region. Estimation of the binding sites for antiTCR or anti CD3 antibodies yielded conflicting TCR:CD3 ratios between 1:1 and 1:2.[25-28] Association of the TCR α and β chains with either CD3εδ or CD3εγ during the TCR/CD3 complex assembly indirectly supported a 1:1 ratio for TCR:CD3 chains.[29] This ratio was also supported by the finding of a unique lodging site for CD3 dimers in a "cave" beneath the F-G loop of the C domain of the TCR β chain (see below).[30] Further experimental evidence was obtained from co-immunoprecipitation and fluorescent resonance energy transfer (FRET) studies performed on the T-cells expressing two different TCRs.[31]

However, gel-shift studies of complete TCR complexes solubilized in digitonin provide convincing data that under these experimental conditions the ratio of TCR αβ chains to CD3 heterodimers is 1:2.[24] Analysis of the TCR/CD3 complex assembly in vitro shows an interaction of TCR β chain with CD3εγ and TCR α chain with CD3εδ and ζζ. These data suggest that in the TM milieu each basic residue in the TCR chains interacts with two acidic charges of the CD3 or ζ dimers.[6,32,33] Thus, current data strongly favor a minimal monovalent αβ:εγ:εδ:ζζ TCR/CD3 complex.

TCR Clusters on the Cell Surface

By blue native electrophoresis of isolated TCR/CD3 complexes, immunostaining and electron microscopy (EM) of fixed cells, TCRs have been shown to exist as monovalent complexes and multivalent clusters.[24,34] On the cell surface, these cholesterol extraction-sensitive clusters form linear structures of closely packed TCR and CD3 units.[24] The mean valency of these complexes varies among different T-cells and this variability is not determined by the nature of the TCRs or their antigen specificity.[24,34] The degree of multivalency is likely to impact the initiation and maintenance of TCR-mediated signals.[35] Consequently, it will be of great interest to determine the factor(s) regulating the multivalency of TCR complexes and the relative orientation of the monovalent units within the multimers. Functionally, it is striking that the presence of these high-order structures of closely packed TCRs on the cell surface does not lead to a permanent state of T-cell activation. Cross-linking of TCRs is essential to signal triggering and it has been proposed as the main, if not the only, factor in TCR activation.[36] The fact that TCR multimers exist in the absence of detectable activation argues in favor of a nonrandom, ordered structure within these linear multimers. This organization may preclude spontaneous TCR activation, suggesting the importance of intermolecular orientation in modulation of cell activation.

Topology of Chain Interactions within TCR/CD3 Complexes

The structural analysis of a large, multichain structure like the TCR/CD3 complex is a formidable task. Recently, invaluable information on the molecular and structural mechanisms of antigen recognition was obtained from structural studies of the ectodomains of CD3 components and about 40 αβ TCR units and more than 20 αβ TCR/pMHC complexes.[5] These structural data, together with biochemical and functional data, shed light on the position and relative orientation of each chain within the complex.

The crystal structure of the TCR, alone or in a complex with the Fab fragment of the H57 antiTCR antibody, localized one possible docking site for CD3 dimers in a "cave" beneath the F-G loop of TCR β chain, sided by the Cα A-B loop that contains an exposed lysine residue and the glycan at the Asn_{185}.[7,30,37] The size of this cave seems to be sufficient to harbor one small, nonglycosylated Ig domain like that of CD3ε. Furthermore, it contains basic residues that could interact with the negatively charged surfaces of CD3ε. H57 antibody has been shown to bind to the F-G loop of TCR Cβ and inhibit the binding of antiCD3 antibodies to at least one of the two CD3 dimers in the TCR/CD3 complex.[37] Partial inhibition of antiCD3 binding by clonotypic antiTCR antibodies has been also observed in other systems.[26] These data confirm the intimate relationship between the TCR and CD3 ectodomains.

The interactions between the CD3 and TCR units can help reconcile other experimental data. For instance, the ectodomains of CD3ε, CD3γ and CD3δ all have an elongated shape; sized about 40x25x25 Å for CD3ε and CD3γ, while the CD3δ molecule is slightly wider.[18-21] Mouse CD3ε and CD3γ ectodomains have a C2-set Ig-fold, whereas human CD3ε and CD3δ have a C1-set Ig-fold (Fig. 2).[18-21] The ectodomains interact mostly through their G strands and the contacts in a continuous β sheet along the dimerization interface result in a rigid "paddle-like," 50-55Å wide structure.[18,21] The short 10-residue stalk region connecting CD3 ecto- and TM domains, contains the RxCxxCxE motif conserved in all CD3 chains. This motif may contribute to the interactions between the CD3 chains and add rigidity to the extracellular CD3 structure, bringing the CD3 TM regions in close proximity to the TCR TM regions to allow interactions between relevant acidic and basic residues.[18,19,38] Together, these data suggest that the CD3 ectodomains are very close to and underneath the TCR α and β C domains. However, despite the close proximity of these ectodomains, no direct interactions between soluble ectodomains of the TCR α and β chains and CD3 heterodimers have been detected.[18,19]

Three- and four-chain assembly studies using an in vitro translation system show that the TCR α chain interacts with one CD3εδ heterodimer, whereas the TCR β chain binds to one CD3εγ heterodimer.[32] A unique role of the extracellular domain of CD3γ chain in the TCR/CD3 complex assembly has been also suggested.[39] In addition, association of the CD3εδ heterodimer with the TCRαβ unit is lost in cells expressing the TCR α chain in which an original stalk region with the FETDxNLN motif is substituted by the shorter TEKVN sequence presenting in a TCR δ chain.[40,41] The favored association of CD3εδ with TCR α and the proximity of CD3εγ to TCR β, as shown by chemical cross-linking,[42] and mutational analysis of TCR Cβ F-G loop,[43] indicate that the CD3εγ heterodimer usually occupies the site close to the TCR β chain. According to docking models, the probable location of CD3εγ in one specific site suggests that another CD3 heterodimer, CDεδ, might be located on the opposite, free of interfering glycan side of the TCR/CD3 complex. This potential site of CD3εγ location could include part of the exposed faces of the TCR α and β chains[18,19] and conserved regions of the TCR α chain facing the membrane or close to it.[21]

Mouse γδ TCR/CD3 complexes have been reported to contain only CD3εγ, but not CD3εδ heterodimer.[44,45] This is in agreement with the normal development of γδ T-cells in $CD3\delta^{-/-}$ mice.[46] Interestingly, γδ TCR/CD3 complexes still maintain the stoichiometry of two CD3ε-containing CD3 heterodimers per complex.[46] Perhaps because of the common evolutionary origin of CD3γ and CD3δ,[4] there is a certain degree of functional and structural redundancy of these chains varying among species. For instance, $CD3\delta^{-/-}$ mice develop γδ T-cells, whereas humans with CD3δ deficiency do not.[47] Quite the contrary, CD3γ-deficient humans, but not mice, produce some γδ T-cells (Regueiro, J.R. personal communication). The eventual incorporation of ζ dimers into the partial TCR/CD3 complex allows export of the mature complexes to the cell surface (reviewed by ref. 8). Charged residues in the ζ TM domain interact with the TM region of the TCR α chain.[6] Additionally, the short extracellular region of ζ and a conserved TM Tyr residue of the TCR β chain also have been suggested to contribute to the TCR/CD3 complex assembly and function.[48-50]

The structures of human CD3εγ and CD3εδ complexed to the anti-CD3 antibodies OKT3 and UCHT1 have been determined.[20,21] Both antibodies bind exposed overlapping regions mainly

located in the CD3ε C'-CFG sheet. In human CD3γ and CD3δ, N-glycosylation sites are located in the CD3γ B-C loop, on top of CD3γ G strands and in the CD3δ F-G loop (Fig. 2).[18-21] This suggests that the conserved CD3ε ABE strands are most likely to interact with the TCR unit under Cβ FG loop. This arrangement brings the acidic residues conserved in the N-terminal sequence and D-E loop of human CD3ε (or in the C'-D loop in mouse CD3ε) close to basic residues in the TCR unit (Fig. 2). Additionally, in this arrangement much of the CD3γ or CD3δ extracellular domains face the cell membrane and thus remain inaccessible to antibody binding.

Binding of monovalent Fab fragments of anti-CD3 antibodies OKT3 or UCHT1, but not anti-TCR β chain JOVI.1 Fab fragment, to the TCR/CD3 complex induces association of the adaptor protein Nck to the cytoplasmic domain of the CD3ε chain.[9] This suggests that the binding of the monovalent anti-CD3 antibodies may change TCR-CD3 interactions in a way resembling physiological TCR ligands.[9] Thus, although it is assumed that OKT3 or UCHT1 antibodies bind to an exposed face of CD3ε, it is possible that full exposition of the relevant epitopes is achieved upon conformational change during the binding process as suggested by Kjer-Nielsen et al.[20]

Interactions between the TCR and Antigen—Role of CD4 and CD8 Coreceptors

Antigen peptides recognized by αβ TCR are located in a "groove" formed by two alpha helices and a beta-sheet floor in domains α1 and α2 of class I MHCs or in the homologous domains α1 and β1 of class II MHCs. The position of αβ TCRs is approximately perpendicular to the plane defined by the peptide and the alpha helices on the top of α1 and α2 domains or α1 and β1 domains of MHCs class I and II, respectively.[5] T-cells that recognize peptides bound to class I MHCs express the CD8αβ coreceptor, whereas those recognizing peptides bound to class II MHCs express the CD4 coreceptor (Fig. 3). Coreceptors are strongly associated with lck tyrosine kinases and provide these enzymes to the TCR-mediated signaling pathways, thus setting a biochemical basis of the linkage between CD8 and CD4 expression and MHC class I or class II restriction.[51]

The interaction between the relatively flat and oblong—with a size of about 40x20 Å—CDR surface of αβ TCR V domains and the pMHC complex takes place in a precisely oriented fashion. With some angle variation among different TCR/pMHC pairs, the long axis of this surface is centered diagonally to the groove formed by the two alpha helices of the N-terminal domains of MHC molecules[7] (reviewed by ref. 5). The highly variable CDR3 loops are the main, but not exclusive, zone contact with solvent-exposed side chains of the antigenic peptide, whereas CDR1 and CDR2 tend to interact with the less variable α helices of MHC molecules. The reasons for the diagonal orientation of the TCR to the pMHC in the complex are not known. The mode of interaction of a given CDR loop with MHC or peptide residue varies among known TCR/pMHC complexes, making unlikely an intrinsic affinity of CDR1 and CDR3 of each V domain for MHC molecules. CD8 and CD4 interactions with MHC molecules can restrict the orientation of the TCR recognition unit toward the antigen peptide complexed to the same MHC molecule, thus setting permissive limits for optimal activation. In class I MHCs, the major binding site for CD8 V-like domains of CD8αα dimer is located in the C-D loop of the α3 domain, close to the APC membrane.[52] The about 40-residue long disulfide-linked connecting peptide of CD8 is thought to have relative flexibility and might be located close to the TCR/CD3 complex. In fact, there is biochemical evidence for an interaction between CD8αβ dimers and the CD3δ chain.[53,54]

CD4 has four IgSF ectodomains and interacts with MHC molecules through its N-terminal D1 V-like domain. The CD4 binding site on class II MHC molecules is located at the junction of the α2 and β2 domains and is oriented similarly to that for binding with CD8.[55] The intact soluble CD4 crystal structure reveals that D1-D2 and D3-D4 form rigid rods and two CD4 molecules dimerize through the D4 membrane-proximal domains.[56] Based on the crystal structure of a complex containing the human CD4 N-terminal two-domain fragment and the MHC class II molecule, ternary complexes of TCR and CD4 bound to one single peptide-MHC molecule were modeled, suggesting that both TCR and CD4 are tilted to the T-cell surface rather than oriented vertically.[55] The relative orientation of TCR and CD4 and the position of the membrane-proximal

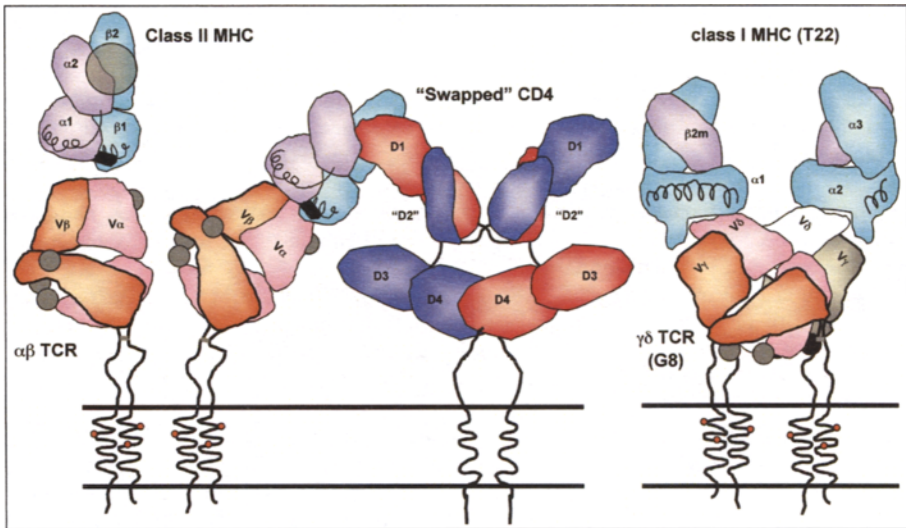

Figure 3. Models for the intercomponent interactions in the αβ TCR-peptide-MHC class II-dimeric CD4 complex (left panel) and in the γδ TCR(G8) dimer-peptide-MHC class I (T22) complex (right panel). The region of MHC-CD4 interaction interface is shown as a shadowed circle. The αβ TCR and γδ TCR units are as in Figures 1 and 2. CD4 dimers rearrange and exchange part of their D2 domains, forming "swapped" disulfide-linked domains. The γδ-γδ domain interactions are mediated by Vδ domains facing each other. The TCR γ and δ chains in the back are shown in grey and white, respectively, with glycans shown as black circles. Adapted from refs. 16,58.

D3-D4 CD4 domains have been thought to prevent TCR-CD4 interactions in the same complex. However, D4- and D2-mediated CD4 dimerization has been recently reported to be very relevant to the CD4 coligand and coreceptor functions.[57,58] In the D2-mediated dimerization, a large conformational change takes place whereby, in a CD4 dimer, D2 domains swap their parts (the so-called D2 swapping) and form two interchain disulfide bonds involving Cys_{130} and Cys_{159} of each CD4 monomer (Fig. 3).[58] This rearrangement does not interfere with the D1 binding to MHC or with the CD4 dimerization through D4 domains. Under these conditions, in ternary TCR-pMHC-CD4 complex, the CD4 D3-D4 domains are oriented towards the pMHC-TCR so that Lys_{279} in CD4 D3 is in close range with Glu_{59} located in the MHC β1 alpha helix (Fig. 3).[58] Thus, CD4 might orientate close to the δ chain of the CD3δε heterodimer[54] and distal to the cave beneath the TCRβ chain FG loop.[58] This might explain the functional data suggesting the proximity of CD3 and CD4 during TCR-mediated cell activation.[26,59] Formation of this trimolecular TCR/CD3/CD4 complex with the TCR unit tilted to the T-cell membrane upon pMHC recognition suggests that CD4 dimers may function as active cross-linkers between nearby TCR/pMHC complexes (Fig. 3). As mentioned above, the assumption that TCR clusters exist on the cell surface as ordered structures could impose a restriction on the orientation of αβ TCR cross-linking upon antigen recognition and this restriction would be independent, but not mutually exclusive, on the restrictions posed by coreceptors.

Other TCRs

The recently reported structure of the G8 γδ TCR in complex with its ligand, the nonclassical MHC molecule T22[16] (reviewed by ref. 5), suggests that antigen- mediated ordered cross-linking of TCRs and their following reorientation (tilting) toward the T-cell surface may both play a role in productive T-cell activation. Compared to αβ TCRs, the γδ TCRs have some distinctive

features, including a canonical Ig C-fold of δ chain C domains and a normal F-G loop in γ chains which have a very acute interdomain angle of 41°. The interaction of G8 with T22 is dominated by the prominent CDR3 δ-chain and is neither centered nor vertical on top of T22. Furthermore, there is no diagonal orientation of the CDRs toward the MHC molecule. Still, T22-interacting G8 TCRs are likely to dimerize so that the productive antigen recognition may be a result of a precisely ordered structure formation with bridging noncontiguous MHC molecules and re-orientation of the γδ TCRs relative to the T-cell surface (Fig. 3), as for αβ TCRs in the presence of coreceptors. Interestingly, many γδ T-cells do not express coreceptors which reinforces the idea that coreceptors contribute to constraints on the orientation of TCR/MHC interactions permissive for productive activation.

No ligand has been defined for preTCR complexes and the structure of the pTα ectodomain complexed with the TCR β chain has not been determined, although it is assumed that this structure should be similar to that of αβ TCRs. It is known that pTα is susceptible to palmitoylation (Fig. 1) and preTCRs are resident in rafts with constitutive signaling.[60]

Are All TCRs Equal, or Are Some TCRs More Equal Than Others?

Aside from the differences in the TCR V domains, some qualitative differences have been noted in the TCR/CD3 complexes expressed by T-cells of different lineages or in distinct differentiation steps. For instance, the relative abundance of CD3δ and CD3γ within the cells might have an impact on the proportion of these chains in TCR/CD3 complexes.[17,61] As mentioned before, in mouse γδ T-cells the TCR complexes contain CD3εγ but not CD3εδ heterodimers.[44,45] Also, the CD3γ chain in activated mouse γδ T-cells is so heavily glycosylated that it was mistaken for the CD3δ chain. In addition, these γδ T-cells have been shown to incorporate FcεRIγ chains instead of ζ chains.[44,45] Ectodomain glycosylation can have an important impact on the shape and size of the TCR unit and CD3γ and CD3δ chains, setting physical limits to their interactions with other molecules.[62] Thus, while anti-CD3 WT31 antibody binds to αβ T-cells, its binding to γδ T-cells is only possible upon deglycosylation. Epitope scanning of anti-CD3 binding sites in normal $CD4^+$ or $CD8^+$ human cells have also detected strong dependence of binding on glycosylation.[63] The UCHT1 antibodies bind to $CD4^+$ cells better than to $CD8^+$ cells whereas the RW2-8C8 antibodies bind better to $CD8^+$ than to $CD4^+$ cells. These differences are linked to differential glycosylation of the TCR/CD3 chains in each subset.[63]

Another source of variability among different TCR/CD3 complexes comes from the results on stepwise proteolytic degradation of the acidic residue-rich, N-terminal sequence of mouse or human CD3ε (ref. 64 and Bello R et al, unpublished data). As analyzed by isoelectric focusing, different T-cells present a distinct profile of CD3ε isoforms (Bello R et al, unpublished results). Loss of the negative N-terminal charges weakens interactions between the TCR and CD3 units,[64] facilitates the recognition by certain anti-CD3 antibodies,[64] and might lower the threshold for TCR activation (Rojo J.M et al, unpublished results). Because of the weak association between the ectodomains of the CD3 and TCR units, all these variations might affect the quaternary changes upon TCR ligation and thus contribute to fine-tuning of the TCR-mediated responses.

Future Directions

Although future developments cannot be anticipated, it is safe to say that current interests include establishing the precise topology of the TCR/CD3 complex ectodomains and particularly the exact sites and modes of interaction between the TCR antigen recognition unit and the CD3 signaling heterodimers. Other important topics include the nature and mechanisms of the structural changes/re-arrangements in the TCR/CD3 complex organization upon ligand stimulation with qualitatively different TCR ligands. The past history of structure-function studies of antigen recognition by T-cells has been a surprise box to immunologists and surely there are still many surprises to come in the future.

Acknowledgements

Many important publications have been cited indirectly due to space limitations and we apologize to the authors. Supported by grants ISCIII-05/054 (Ministerio de Sanidad y Consumo, Spain) and SAF2004-06852 (Ministerio de Ciencia y Tecnología, Spain). R.B. is recipient of a Predoctoral Fellowship of Ministerio de Ciencia y Tecnología, Spain.

References

1. Clevers H, Alarcón B, Wileman T et al. The T-cell receptor/CD3 complex: A dynamic protein ensemble. Ann Rev Immunol 1988; 6:629-662.
2. Klausner RD, Lippincott-Schwartz J, Bonifacino JS. The T-cell antigen receptor: Insight into organelle biology. Annu Rev Cell Biol 1990; 6:403-431.
3. Kuhns MS, Davis MM, Garcia KC et al. Deconstructing the form and function of the TCR/CD3 Complex. Immunity 2006; 24:133-9.
4. Göbel TWF, Bolliger L. Evolution of the T-cell receptor signal transduction units. Curr Top Microbiol Immunol 2000; 248:303-320.
5. Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides and coreceptors. Annu Rev Immunol 2006; 24:419-466.
6. Call ME, Wucherpfennig KW. The T-cell receptor: Critical role of the membrane environment in receptor assembly and function. Annu Rev Immunol 2005; 23:101-125.
7. García KC, Degano M, Stanfield RL et al. An αβ T-cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. Science 1996; 274:209-219.
8. Alarcón B, Gil D, Delgado P et al. Initiation of TCR signaling: Regulation within CD3 dimers. Immunol Rev 2003; 191:38-46.
9. Gil D, Schamel WWA, Montoya M et al. Recruitment of Nck by CD3ε reveals a ligand-induced conformational change essential for T-cell receptor signaling and synapse formation. Cell 2002; 109:901-912.
10. Cambier JC. New nomenclature for the Reth motif (or ARH1/TAM/ARAM/YxxL). Immunol Today 1995; 16:110.
11. Buferne M, Luton F, Letorneur F et al. Role of CD3δ in surface expression of the TCR/CD3 complex and in activation for killing analyzed with a CD3δ-negative cytotoxic T-lymphocyte variant. J Immunol 1992; 148:657-664.
12. Dietrich J, Neising A, Hou X et al. Role of CD3γ in T-cell receptor assembly. J Cell Biol 1996; 132:299-310.
13. Luton F, Buferne M, Legendre V et al. Role of CD3γ and CD3δ cytoplasmic domains in cytolytic T-lymphocyte functions and TCR/CD3 down-modulation. J Immunol 1997; 158:4162-4170.
14. Transy C, Moingeon P, Stebbins C et al. Deletion of the cytoplasmic region of the CD3 epsilon subunit does not prevent assembly of a functional T-cell receptor. Proc Natl Acad Sci USA 1989; 86:7108-7112.
15. Wegener AMK, Letourneur F, Hoeveler A et al. The T-cell receptor/CD3 complex is composed of at least two autonomous transduction modules. Cell 1992; 68:83-95.
16. Adams EJ, Chien Y-H, Garcia KC. Structure of a γδ T-cell receptor in complex with the nonclassical MHC T22. Science 2005; 308:227-231.
17. Alarcón B, Ley SC, Sánchez-Madrid F et al. The CD3-γ and CD3-δ subunits of the T-cell antigen receptor can be expressed within distinct functional TCR/CD3 complexes. EMBO J 1991; 10:903-912.
18. Sun Z-YJ, Kim ST, Kim IC et al. Solution structure of the CD3εδ ectodomain and comparison with CD3εγ as a basis for modeling T-cell receptor topology and signaling. Proc Natl Acad Sci USA 2004; 101:16867-16872.
19. Sun ZJ, Kim KS, Wagner G et al. Mechanisms contributing to T-cell receptor signaling and assembly revealed by the solution structure of an ectodomain fragment of the CD3εγ heterodimer. Cell 2001; 105:913-923.
20. Kjer-Nielsen L, Dunstone MA, Kostenko L et al. Crystal structure of the human T-cell receptor CD3εγ heterodimer complexed to the therapeutic mAb OKT3. Proc Natl Acad Sci USA 2004; 101:7675-7680.
21. Arnett KL, Harrison SC, Wiley DC. Crystal structure of a human CD3-ε/δ dimer in complex with a UCHT1 single-chain antibody fragment. Proc Natl Acad Sci USA 2004; 101:16268-16273.
22. Blumberg RS, Ley S, Sancho J et al. Structure of the T-cell antigen receptor: Evidence for two CD3 ε subunits in the T-cell receptor complex. Proc Natl Acad Sci USA 1990; 87:7220-7224.
23. de la Hera A, Müller U, Olsson C et al. Structure of the T-cell antigen receptor (TCR): Two CD3ε subunits in a functional TCR/CD3 complex. J Exp Med 1991; 173:7-17.

24. Schamel WWA, Arechaga I, Risueno RM et al. Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. J Exp Med 2005; 202:493-503.
25. Meuer S, Acuto O, Hussey RE et al. Evidence for the T3-associated 90K heterodimer as the T-cell antigen receptor. Nature 1983; 303:808-810.
26. Portolés P, Rojo J, Golby A et al. Monoclonal antibodies to murine CD3ε define distinct epitopes, one of which may interact with CD4 during T-cell activation. J Immunol 1989; 142:4169-4175.
27. Punt JA, Roberts JL, Kearse KP et al. Stoichiometry of the T-cell antigen receptor (TCR) complex: Each TCR/CD3 complex contains one TCR α, one TCR β and two CD3ε chains. J Exp Med 1994; 180:587-593.
28. Thibault G, Bardos P. Compared TCR and CD3ε expression on αβ and γδ T-cells. Evidence for the association of two TCR heterodimers with three CD3ε chains in the TCR/CD3 complex. J Immunol 1995; 154:3814-3820.
29. San José E, Sahuquillo AG, Bragado R et al. Assembly of the TCR/CD3 complex: CD3ε/δ and CD3ε/γ dimers associate indistinctly with both TCRα and TCRβ chains. Evidence for a double TCR heterodimer model. Eur J Immunol 1998; 28:12-21.
30. Wang J, Lim K, Smoylar A et al. Atomic structure of an αβ T-cell receptor (TCR) heterodimer in complex with an anti-TCR Fab fragment derived from a mitogenic antibody. EMBO J 1998; 17:10-26.
31. Fernández-Miguel G, Alarcón B, Iglesias A et al. Multivalent structure of an αβ T-cell receptor. Proc Natl Acad Sci USA 1999; 96:1547-1552.
32. Call ME, Pyrdol J, Wiedmann M et al. The organizing principle in the formation of the T-cell receptor-CD3 complex. Cell 2002; 111:967-979.
33. Call M, Pyrdol J, Wucherpfennig K. Stoichiometry of the T-cell receptor-CD3 complex and key intermediates assembled in the endoplasmic reticulum. EMBO J 2004; 23:2348-2357.
34. Hellwig S, Schamel WWA, Pflugfelder U et al. Differences in pairing and cluster formation of T-cell receptor α- and β-chains in T-cell clones and fusion hybridomas. Immunobiology 2005; 210:685-694.
35. Schamel WWA, Risueno RM, Minguet S et al. A conformation- and avidity-based proofreading mechanism for the TCR-CD3 complex. Trends Immunol 2006; 27:176-182.
36. Cochran JR. Cochran JR, Cameron TO et al. Receptor proximity, not intermolecular orientation, is critical for triggering T-cell activation. J Biol Chem 2001; 276:28068-28074.
37. Ghendler Y, Smolyar A, Chang H-C et al. One of the CD3ε subunits within a T-cell receptor complex lies in close proximity to the Cβ FG loop. J Exp Med 1998; 187:1529-1536.
38. Borroto A, Mallabiabarrena A, Albar JP et al. Characterization of the region involved in CD3 pairwise interactions within the T-cell receptor complex. J Biol Chem 1998; 273:12807-12816.
39. Wegener AMK, Hou X, Dietrich J et al. Distinct domains of the CD3-γ chain are involved in the surface expression and function of the T-cell antigen receptor. J Biol Chem 1995; 270:4675-4680.
40. Bäckström BT, Milia E, Peter A et al. A motif within the T-cell receptor α chain constant region connecting peptide domain controls antigen responsiveness. Immunity 1996; 5:437-447.
41. Werlen G, Hausmann B, Palmer E. A motif in the αβ T-cell receptor controls positive selection by modulating ERK activity. Nature 2000; 406:422-426.
42. Brenner MB, Trowbridge IS, Strominger JL. Cross-linking of human T-cell receptor proteins: Association between the T-cell idiotype β subunit and the T3 glycoprotein heavy subunit. Cell 1985; 40:183-190.
43. Touma M, Chang H-C, Sasada T et al. The TCR Cβ FG loop regulates αβ T-cell development. J Immunol 2006; 176:6812-6823.
44. Hayes SM, Laky K, El-Khoury D et al. Activation-induced modification in the CD3 complex of the γδ T-cell receptor. J Exp Med 2002; 196:1355-1361. Erratum in: J. Exp. Med. 196: 1653.
45. Hayes SM, Love PE. Distinct structure and signaling potential of the γδ TCR complex. Immunity 2002; 16:827-838.
46. Hayes SM, Love PE. Stoichiometry of the murine γδ T-cell receptor. J Exp Med 2006; 203:47-52.
47. Dadi HK, Simon AJ, Roifman CM. Effect of CD3δ deficiency on maturation of α/β and γ/δ T-cell lineages in severe combined immunodeficiency. New Eng J Med 2003; 349:1821-1828.
48. Johansson B, Palmer E, Bolliger L. The extracellular domain of the ζ-chain is essential for TCR function. J Immunol 1999; 162:878-885.
49. Rodríguez-Tarduchy G, Sahuquillo AG, Alarcón B et al. Apoptosis but not other activation events is inhibited by a mutation in the transmembrane domain of T-cell receptor β that impairs CD3ζ association. J Biol Chem 1996; 271:30417-30425.
50. Teixeiro E, Daniels MA, Hausmann B et al. T-cell division and death are segregated by mutation of TCRβ chain constant domains. Immunity 2004; 21:515-526.
51. Janeway Jr. CA. The T-cell receptor as a multicomponent signalling machine: CD4/CD8 Coreceptors and CD45 in T-cell activation. Ann Rev Immunol 1992; 10:645-674.

52. Gao FG, Tormo J, Gerth UC et al. Crystal structure of the complex between human CD8αα and HLA-A2. Nature 1997; 387:630-634.
53. Doucey M-A, Goffin L, Naeher D et al. CD3δ establishes a functional link between the T-cell receptor and CD8. J Biol Chem 2003; 278:3257-3264.
54. Suzuki S, Kupsch J, Eichmann K et al. Biochemical evidence of the physical association of majority of the CD3δ chains with the accessory/co receptor molecules CD4 and CD8 on nonactivated T-lymphocytes. Eur J Immunol 1992; 22:2475-2479.
55. Wang J, Meijers R, Xiong Y et al. Crystal structure of the human CD4 N-terminal two-domain fragment complexed to a class II MHC molecule. Proc Natl Acad Sci USA 2001; 98:10799-10804.
56. Wu H, Kwong PD, Hendrickson WA. Dimeric association and segmental variability in the structure of human CD4. Nature 1997; 387:527-530.
57. Moldovan M-C, Yachou A, Levesque K et al. CD4 dimers constitute the functional component required for T-cell activation. J Immunol 2002; 169:6261-6268.
58. Maekawa A, Schmidt B, Fazekas de St. Groth B et al. Evidence for a domain-swapped CD4 dimer as the coreceptor for binding to Class II MHC. J Immunol 2006; 176:6873-6878.
59. Dianzani U, Shaw A, Al-Ramadi BK et al. Physical association of CD4 with the T-cell receptor. J Immunol 1992; 148:678-688.
60. Saint-Ruf C, Panigada M, Azogi O et al. Different initiation of preTCR and γδTCR signalling. Nature 2000; 406:524-527.
61. Wilson A, MacDonald HR. Expression of genes encoding the preTCR and CD3 complex during thymus development. Int Immunol 1995; 7:1659-1664.
62. Rudd PM, Wormald MR, Stanfield RL et al. Roles for glycosylation of cell surface receptors involved in cellular immune recognition. J Mol Biol 1999; 293:351-366.
63. Zapata DA, Schamel WWA, Torres PS et al. Biochemical differences in the alpha beta TCR-CD3 surface complex between $CD8^+$ and $CD4^+$ human mature T-lymphocytes. J Biol Chem 2004; 279:24485-24492.
64. Criado G, Feito MJ, Ojeda G et al. Variability of invariant mouse CD3ε chains detected by anti-CD3 antibodies. Eur J Immunol 2000; 30:1469-1479.