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From Spatiotemporal Organization to Human Disease
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Multichain Immune Recognition Receptor Signaling
From Spatiotemporal Organization to Human Disease

Edited by
Alexander B. Sigalov, PhD

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DEDICATION

This book is dedicated in loving memory to my parents, Galina Ya. Sigalova and Boris L. Sigalov, who are the source of all great things in my life. Without their love, wisdom, understanding, faith, support, and guidance, I would not be the person I am today.
Immunological recognition is a central feature of the adaptive immunity of vertebrates. With the exception of agnathans, which developed an entirely distinct set of immunologically-specific molecules, all vertebrates use a recognition system based on what Achsah Keegan and I suggested in 1992 be termed multichain immune recognition receptors (MIRRs). MIRRs consist of ligand-binding molecules that are immunoglobulin supergene family members associated with signal transducers and enhancers in such a way as both insure precise ligand recognition, discrimination and amplification of the signal.

Two of the prototypic sets of MIRRs, the T-cell and B-cell receptors, are among the most remarkable recognition molecules known. These are extraordinarily diverse molecules in which the range of ligands that can be potentially recognized probably exceeds the actual numbers of lymphocytes in the body. The discovery of the genetic basis of assembling these receptors and understanding how they bind to their cognate antigens are among the most stunning of scientific achievements. Yet these immensely specific binding chains (the heavy/light chain pair for immunoglobulin and the α/β chain pair for most T cells), when expressed as membrane molecules, have no obvious mechanism of signaling. For example, the μH chain cytosolic domain consists of three amino acids (lysine-valine-lysine) and the L chain is not even embedded in the membrane. Furthermore, there is no known direct mechanism to propagate information from the binding domain of the B-cell or T-cell receptors to the membrane-proximal domains of the same chains.

The solution is based on the assemblage of the multichain receptor complex, in which pairing of key chains can occur in the membrane, often based on the presence of oppositely charged residues at critical locations in the transmembrane portion of partner chains. The signaling process then depends on aggregation and/or structural rearrangement induced by binding of multivalent ligands and on the properties of the partner signaling chains, such as Igα and Igβ for the B cell receptor; the CD3 γ, δ and ε and the ζ chains for the T-cell receptor and FcεRI β and γ for the high affinity IgE receptor. These partners contain one or more immunoreceptor tyrosine-based activation motifs (ITAMs). The phosphorylation of tyrosines within the ITAM
motif (YxxL/Ix6-8YxxL/I) is a key early event in signal transduction as a result of engagement and aggregation of the ligand-binding domain.

The multichain system links extraordinarily powerful ligand recognition mechanisms with highly effective signaling pathways capable of both immense amplification and precise discrimination between stimulatory ligands (agonists), inhibitory ligands (antagonists) and even partial agonists.

In *Multichain Immune Recognition Receptor Signaling: From Spatiotemporal Organization to Human Disease*, Alexander Sigalov and his colleagues present a comprehensive examination of the full range of MIRRs, of how they propagate signals, how they discriminate between classes of ligands, and the roles they play in physiologic responses and in various diseases. An outstanding set of scientist scholars have provided their expertise in dealing with virtually every aspect of this fascinating set of receptors and in thus providing a comprehensive treatment of this important and exciting area. The nature of this remarkable set of receptors, how they mediate their functions and the potential for abnormal function due to defects in signal transduction, amplification and discrimination, places this family of molecules at the center of the immune response. Dr. Sigalov is to be congratulated for undertaken this important task. Readers interested in this subject will benefit enormously from this important volume.

William Paul, PhD

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Multichain immune recognition receptors (MIRRs) represent a family of surface receptors expressed on different cells of the hematopoietic system and function to transduce signals leading to a variety of biologic responses. These receptors share common structural features including extracellular ligand-binding domains and intracellular signaling domains intriguingly carried on separate subunits. Another important feature that links members of the MIRR family is the presence of one or more copies of a cytoplasmic structural module termed the immunoreceptor tyrosine-based activation motif (ITAM). ITAMs consist of conserved sequences of amino acids that contain two appropriately spaced tyrosines (YxxL/Ix6-8YxxL/I, where x denotes non-conserved residues). Following receptor engagement, phosphorylation of ITAM tyrosine residues represents one of the earliest events in the signaling cascade. Although the MIRR-mediated ligand recognition and the MIRR-triggered downstream signaling cascades are believed to be among the best studied in biology in recent years, at present the spatial organization of the MIRRs, its reorganization in response to ligand binding as well as the molecular mechanisms underlying the initiation of MIRR signaling remain to be elucidated.

MIRR-mediated signal transduction plays an important role in both health and disease, making these receptors attractive targets for rational intervention in a variety of immune disorders. Thus, future therapeutic strategies depend on our detailed understanding of the molecular mechanisms underlying MIRR triggering and subsequent transmembrane signal transduction. In addition, knowing these mechanisms would provide a new handle in dissecting the basic structural and functional aspects of the immune response.

The central idea of this book is to show that the structural similarity of the MIRRs determines the general principles underlying MIRR-mediated transmembrane signaling mechanisms and also provides the basis for existing and future therapeutic strategies targeting MIRRs. The reviews assembled in this book detail the progress in defining and controlling the spatiotemporal organization of key events in immune cell activation. An improved understanding of MIRR-mediated signaling has numerous potential practical applications, from the rational design of drugs and vaccines to the engineering of cells for biotechnological purposes. Section I reviews
the spatial organization and physiological function of MIRR family members such as T-cell receptor, B-cell receptor, Fc receptors, natural killer cell receptors and the platelet collagen receptor glycoprotein VI. Section II focuses on current models of MIRR triggering and highlights modern technologies available to visualize cell-cell interaction contacts such as immunological synapse and also to measure protein-protein interactions in space in real time. Potential therapeutic strategies targeting MIRR-mediated signaling are briefly reviewed in Section III.

This book summarizes current knowledge in this field and illustrates how control of MIRR-triggered signaling could become a potential target for medical intervention, thus bridging basic and clinical immunology. Describing the molecular basis of MIRR-mediated transmembrane signaling, this volume addresses a broad audience ranging from biochemists and molecular and structural biologists to basic and clinical immunologists and pharmacologists.

Alexander B. Sigalov, PhD
ABOUT THE EDITOR...

ALEXANDER SIGALOV, PhD, is a Research Assistant Professor in the Department of Pathology at the University of Massachusetts Medical School in Worcester, Massachusetts, USA. His main research interests include protein intrinsic disorder and oligomericity in the context of transmembrane signal transduction, the molecular mechanisms underlying immune receptor-mediated signaling and ways to control these processes and thus to modulate the immune response, as well as the development and applications of novel targets and strategies for innovative immune therapy. He discovered and investigated a very unusual and unique biophysical phenomenon, the homooligomerization of intrinsically disordered proteins, thus providing the first evidence for the existence of specific interactions between unfolded protein molecules. In the field of immunology, he unraveled a long-standing mystery of transmembrane signaling and immune cell activation triggered by multichain immune recognition receptors. Later, he developed a novel concept of platelet inhibition and invented a novel class of platelet inhibitors. In the field of immune therapy, he proposed new therapeutic strategies for a variety of malignancies and immune disorders, including immunodeficiencies, inflammatory and autoimmune diseases, allergy and HIV. He is a member of the American Association for the Advancement of Science and the Biophysical Society USA. Alexander Sigalov received his academic degrees (MSc in Chemistry and a PhD in Organic Chemistry) from Moscow State University, Russia.
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CHAPTER 1

T-Cell Receptor

Jose M. Rojo,* Raquel Bello and Pilar Portolés

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 (CD3e, 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 (αβ TCRs). 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. 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 transmembrane (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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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 Asn₁₈₅, Cβ Asn₁₈₆, pα Asn₁₅₁, Cy Asn₁₈₄ or Cδ Asn₁₃₅, CD3γ Asn₁₉₀ and Asn₁₇₀, CD3δ Asn₁₆₀ and Asn₁₄₀). 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 ε chains also contain one acidic residue in their TM region and have a short nine-residue long extracellular domain. Unlike TCR α and β chains, CD3 and ε chains contain 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 and cell surface receptor expression (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 ε chains, at least in those needed for surface expression of the TCR/CD3 complex.

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 αβ T-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.
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, ε dimers. 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 \([(αβ)_2:ε:δ:ζ]\) in order to maintain 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 ζ. 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:ε:δ:ζ 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.\(^{24,35}\) 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 anti-TCR antibody, localized one possible docking site for CD3 dimers in a "cave" beneath the F-G loop of TCR β chain, sided by the Ca A-B loop that contains an exposed lysine residue and the glycan at the Asn185. 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 β and inhibit the binding of anti-CD3 antibodies to at least one of the two CD3 dimers in the TCR/CD3 complex. Partial inhibition of anti-CD3 binding by clonotypic anti-TCR antibodies has been also observed in other systems. 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. Mouse CD3ε and CD3γ ectodomains have a C2-set Ig-fold, whereas human CD3ε and CD3δ have a C1-set Ig-fold. 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," 50-55 Å wide structure. The short 10-residue stalk region connecting CD3 ε- 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. 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.

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 CD3γδ heterodimer. A unique role of the extracellular domain of CD3γ chain in the TCR/CD3 complex assembly has been also suggested. 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. The favored association of CD3εδ with TCR α and the proximity of CD3εγ to TCR β, as shown by chemical cross-linking, and mutational analysis of TCR CB F-G loop, 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, CD8ε, 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 and conserved regions of the TCR α chain facing the membrane or close to it.

Mouse γδ TCR/CD3 complexes have been reported to contain only CD3εγ, but not CD3εδ heterodimer. This is in agreement with the normal development of γδ T-cells in CD3δ−/− mice. Interestingly, γδ TCR/CD3 complexes still maintain the stoichiometry of two CD3ε-containing CD3 heterodimers per complex. Perhaps because of the common evolutionary origin of CD3γ and CD3δ, there is a certain degree of functional and structural redundancy of these chains varying among species. For instance, CD3δ−/− mice develop γδ T-cells, whereas humans with CD3δ deficiency do not. Quite the contrary, CD3γ-deficient humans, but not mice, produce some γδ T-cells (Regueiro, J.R. personal communication). The eventual incorporation of γ 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 ε TM domain interact with the TM region of the TCR α chain. Additionally, the short extracellular region of ε 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.

The structures of human CD3εγ and CD3εδ complexed to the anti-CD3 antibodies OKT3 and UCHT1 have been determined. Both antibodies bind exposed overlapping regions mainly
located in the CD3ε C'-CFG sheet. In human CD3γ and CD3β, N-glycosylation sites are located in the CD3γ B-C loop, on top of CD3γ G strands and in the CD3β F-G loop (Fig. 2). 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 CD3β 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. This suggests that the binding of the monovalent anti-CD3 antibodies may change TCR-CD3 interactions in a way resembling physiological TCR ligands. 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.

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. 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 Ick 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.

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 (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. 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 CD3ε chain.

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. 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. 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. 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. 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 Cys340 and Cys359 of each CD4 monomer (Fig. 3). 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 Lys279 in D3 is in close range with Glu59 located in the MHC β1 alpha helix (Fig. 3). Thus, CD4 might orientate close to the δ chain of the CD3δε heterodimer and distal to the cave beneath the TCRβ chain FG loop. This might explain the functional data suggesting the proximity of CD3 and CD4 during TCR-mediated cell activation. 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 (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