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TCR mechanobiology: torques and tunable structures linked to early T cell signaling

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Mechanotransduction is a basis for receptor signaling in many biological systems. Recent data based upon optical tweezer experiments suggest that the TCR is an anisotropic mechanosensor, converting mechanical energy into biochemical signals upon specific peptide-MHC complex (pMHC) ligation. Tangential force applied along the pseudo-twofold symmetry axis of the TCR complex post-ligation results in the αβ heterodimer exerting torque on the CD3 heterodimers as a consequence of molecular movement at the T cell–APC interface. Accompanying TCR quaternary change likely fosters signaling via the lipid bilayer predicated on the magnitude and direction of the TCR–pMHC force. TCR glycans may modulate quaternary change, thereby altering signaling outcome as might the redox state of the CxxC motifs located proximal to the TM segments in the heterodimeric CD3 subunits. Predicted alterations in TCR TM segments and surrounding lipid will convert ectodomain ligation into the earliest intracellular signaling events.

Keywords: quaternary change, mechanosensor, T cell signaling, force transduction, antigen recognition

THE TCR STRUCTURE: OVERVIEW

The αβ TCR is a multimeric transmembrane complex composed of a disulfide-linked antigen binding clonotypic heterodimer in non-covalent association with the signal-transducing CD3 subunits (CD3εγ, CD3δε, and CD3ζ) (reviewed in Rudolph et al., 2006; Smith-Garvin et al., 2009). TCR signaling via CD3 dimers evokes T cell lineage commitment and repertoire selection during development, maintains the peripheral T cell pool, and further differentiates naïve T cells into effector or memory cell populations upon immune stimulation. Each CD3ε, γ, and δ subunit contains an extracellular immunoglobulin (Ig)-like domain, a membrane-proximal stalk region, a transmembrane segment, and a cytoplasmic tail. The interaction between an αβ TCR heterodimer on the T cell and a pMHC ligand on an antigen-presenting cell (APC) initiates a cascade of downstream signaling events. These events are transmitted via the immunoreceptor tyrosine-based activation motif (ITAM) elements in the cytoplasmic tails of the associated CD3 subunits, whose lengths are substantial relative to those of the TCR α and β tails (Reth, 1989; Letourneur and Klausner, 1992; Acuto et al., 2008; van der Merwe and Dushek, 2011) The various CD3 chains induce distinct patterns of cellular protein tyrosine phosphorylation upon activation to recruit intracellular adaptors and signaling molecules. Early, intermediate, and late gene activation programs ensue (Crabtree and Cliftstone, 1994). Reviews such as Rudolph et al. (2006) have focused on the structural nature of immune recognition involving Vα and Vβ domains of a given TCR and its pMHC ligand. How recognition of pMHC by a weakly interacting αβ TCR heterodimer on the T cell surface evokes intracellular signaling via the adjacent CD3 components of the TCR complex has remained undefined.

Functional TCR αβ heterodimers were first identified by mAbs on antigen-specific T cell clones and then T cell hybridomas (Acuto et al., 1983; Meuer et al., 1983a,b; White et al., 1983). Subsequent sequence analysis of TCRs predicted that they would share with antibodies a common structural basis of ligand recognition, akin to an antibody Fab fragment (Novotny et al., 1986; Chothia et al., 1988). These results agreed with peptide mapping studies of α and β subunits which identified conserved as well as variable peptides, implying the existence of constant and variable domains in the TCR α and β subunits. The biochemical results were later confirmed and extended by DNA cloning (Chien et al., 1984; Yanagi et al., 1984), and elegantly delineated further by the crystal structure of an intact murine αβ TCR (Garcia et al., 1996) and a complex between a human TCR, viral peptide, and human MHCI molecule that followed (Garboczi et al., 1996). Structures of TCR αβ heterodimers and antibody Fab fragments seem very similar. While each of the four TCR α and β domains, like those of Fab, has been assigned an Ig fold, deviations are notable in both TCR constant domains (Bentley et al., 1995; Garcia et al., 1996) as well as in the Vα domain (Fields et al., 1995). These deviations define fundamental differences between the TCR as a cell surface receptor and antibody as a soluble immune molecule.

THE Cβ FG LOOP

First noted upon structural analysis was the striking elongation of the FG loop of the Cβ domain connecting its F and G β-strands. Compared to other Ig-like structures, there is a 13 amino acid (aa)
insertion within the FG loop (Kabat et al., 1991). **Figure 1A** shows a side view of the crystallographically resolved murine N15 MHCI-restricted αβ TCR (Wang et al., 1998). The unique protrusion of the Cβ domain’s FG loop is apparent (boxed). Despite its length, the FG loop is internally well-structured, and constitutes an integrated component of a rigid structural entity that connects the Vβ and Cβ domains (Wang et al., 1998). This feature of the FG loop is conserved in αβ TCRs among all mammalian species studied to date, having co-evolved with the development of distinct CD3γ and CD3δ genes (Kim et al., 2010). Consistent with the importance implied by this conservation, removal of the FG loop (AFG) in T cell transfectants as well as TCRs displayed on naïve T cells by transgenesis (Sasada et al., 2002; Touma et al., 2007) affects T cell activation and development. Impaired negative selection resulting from the Cβ FG loop deletion increases the CD4 + CD8+ double positive thymocyte population and causes maturing thymocytes to exit the thymus in larger numbers than their wild-type TCR counterpart.

Second, the overall shape of the TCR C domain module was observed to be remarkably asymmetric. The Cβ domain is about 55 Å in overall length while that of the Ca is only 40 Å. The Cβ domain bends more acutely toward the Vβ domain compared to the angle formed between the Cα and Vα domains. About half of the Cβ domain’s ABED-β-sheet is therefore surface exposed, and does not contact the Ca domain. This asymmetry creates a cave-like structure or cavity underneath the β chain as shown in the right lower corner of **Figure 1A**. This cavity measures ~25 Å in depth, 20 Å in height, and 25 Å in width. The partially exposed ABED-β-sheet of the Cβ domain forms an extensive ceiling. The CD loop and EF loop of the Cα domain (not visualized in this projection) as well as the glycans attached to CaN185, CβN121, and CγN186 form one side-wall while the FG loop of Cβ and the glycans emanating from CβN236 form a canopy and the other side-wall of the cavity. The glycans project outward and hence, will not occlude the cavity. The AB loop of the Ca domain, in contrast, projects into this cave (**Figure 1A**). The floor of the cave is presumably formed by the plasma membrane at the T cell surface. It is noteworthy that the interchain disulfide bond between Ca Cys213 and Cβ Cys247 is positioned below the Ca domain, leaving the cavity unobstructed as the TCR αβ heterodimer projects from the T cell surface membrane. As indicated below, this asymmetric cavity allows for physical and functional linkage of the αβ heterodimer with CD3γε ectodomains. Third, unlike Fab, the αβ TCR heterodimers are heavily N-linked glycosylated (**Figure 1B**). Such adducts can dynamically modify TCR function as noted below. The uniquely kinked conformation of the CD3γ G-strand offers a geometry to accommodate juxtaposition of CD3γ and TCRβ ectodomains and foster quaternary change (see Glossary) (Kim et al., 2010).

**A DENSE N-LINKED GLYCAN ARRAY DECORATES THE TCR**

The NMR and X-ray structures of CD3γε and CD3δε revealed a unique side-to-side hydrophobic interface with conjoined β-sheets between the two C/C2-set Ig-like ectodomains. The parallel pairing of these rigidified dimer modules is a striking structural feature not observed elsewhere (Sun et al., 2001, 2004; Arnett et al., 2004; Kjer-Nielsen et al., 2004). Whereas the CD3ε subunit conformation is virtually identical in CD3εγ and CD3εδ, the CD3δ ectodomain adopts a C-set Ig fold with a narrower GFC front face β-sheet that is more parallel to the ABED back face than those β-sheets in CD3ε and CD3γ. The dimer interface between CD3δ and CD3ε is highly conserved among species and of similar character as in CD3γε. Glycosylation sites in CD3δ are arranged such that the glycans may point away from the membrane, consistent with a model of TCR assembly that allows the CD3δ chain to be in close contact with the TCR α chain (**Figure 1B**). The rigidified CD3 heterodimers are associated with the TCR αβ heterodimer whose own rigid structure is reinforced by the FG loop and peculiar αβ asymmetry in constant domains. Thus, not unexpectedly, comparison of unligated and pMHC ligated TCR αβ structures does not show major conformational changes (reviewed in Rudolph et al., 2006).

A model of the TCR complex of αβ, CD3εγ, and CD3δε ectodomains (Sun et al., 2004) defines a plausible topology and emphasizes its glycan richness (**Figure 1B**). Given that CD3ζ has only a nine amino acid long ectosegment, its extracellular segment is omitted from the Figure as are the connecting peptides (CP) of the TCR α and β chains and the stalk regions of CD3ε, CD3γ, and CD3δ. This rendering incorporates the consequences of several known TCR characteristics: (i) putative transmembrane charge pairs involving TCR subunit chain association with CD3ε–CD3δ–TCRα–CD3γ–CD3ζ as one cluster and CD3ε–CD3γ–TCRβ as a second cluster (Call et al., 2002, 2004), (ii) extracellular domain associations involving other in vitro chain association data (Manolios et al., 1991, 1994), TCR crosslinking results (Brenner, 1985; Koning et al., 1990), and (iii) proximity of one CD3ε subunit to the TCR αβ FG loop revealed by quantitative T cell surface immunofluorescent antibody binding analysis (Ghendler et al., 1998). In addition, structural insights from crystallographic data on the glycosylated N15 TCRαβ heterodimer ectodomain in complex with H57 Fab and the likely position of glycans in both CD3εγ and CD3δε (Wang et al., 1998) are considered. Specifically, CD3εγ is presumed to be near the cavity formed between the TCR Ca CD, EF loops, and the αβ FG loop (Ghendler et al., 1998; Wang et al., 1998). Residues in the TCR Ca AB loop which shows significant conformational change for a LC13 TCR upon pMHC binding (Kjer-Nielsen et al., 2003) were used as target sites for CD3εγ docking in the initial search for possible docking models. CD3δε is docked on the opposite site of the TCR αβ domain where there are less glycans to interfere with the more heavily glycosylated CD3δ subunit (**Figure 1B**), and consistent with known TCRα and CD3δ TM associations from biochemical analysis (Call et al., 2002).

The multiple N-linked glycan adducts of the TCR complex (**Figure 1B**, top panel) help guide pMHC ligands to the TCR recognition surface, reducing entropic penalties by directing binding to the exposed, glycan-free CDR loops. Glycans may also serve a regulatory function, contributing to a galectin–glycoprotein lattice (Demetriou et al., 2001). The more heavily glycosylated CD3δ subunit may influence TCR subunit assembly through steric constraints. The distribution of glycans in the model shown in **Figure 1B** is also consistent with the lack of mAbs elicited against the native CD3δ and CD3γ subunits. Importantly, glycans are large and dynamic. These adducts can affect movement of TCR
FIGURE 1 | Continued.
subunits, thereby impacting signaling. Consistent with this notion, TCR functional avidity was altered by removal of the Ca glycan (Kuball et al., 2009).

Immediately evident in Figure 1B (bottom panel) is the central position of the TCR αβ heterodimer with a vertical dimension of 80 Å projecting from the cell membrane, flanked on either side by the shorter (40 Å) CD3 heterodimers, CD3δεβ and CD3εγα subunits. The width of the CD3εβ and CD3γη components, 50 and 55 Å, respectively, are comparable in size to that of the TCRαβ heterodimer (58 Å), and together (excluding glycans) span ~160 Å. These flanking CD3 ectodomain components will likely impede lateral movement of the TCRαβ heterodimer upon pMHC binding. As previously noted for CD3εγα (Sun et al., 2001), the intradomain disulfide bridge between Cys residues on the B and F strands at the center of each CD3εβ domain reinforces the domain structure. Further rigidity for potential signal transduction comes from the paired G–β-strands in each CD3 heterodimer, coupled with the conserved RxCxxCxE cysteine-coordinated stalks (Sasada et al., 2002) discussed in a separate section.

DYNAMIC QUATERNARY CHANGE UPON TCR LIGATION

The length of the CD3 subunit stalks (5–10 amino acids) is typical for transmembrane proteins observed, for example, for CD2, CD4, and CD58. On the other hand, the CP found in TCRα (25–26 aa) and TCRβ (19 aa) are long. The latter are probably mandated by a requirement for a linker segment of sufficient length to span the 50 Å from the end of the interchain disulfide of the TCR α constant domain to the associated CD3ε and CD3δ transmembrane (TM) segments which are juxtaposed for apparent charge pairing (i.e., between the TCRα lysine and aspartic residues of CD3ε and CD3δ TM, respectively). Similar considerations must be applied to the TCRβ connecting peptide, with charged pairing of the TM TCRβ lysine with an aspartic and a glutamic acid residue of CD3ε and CD3γ TM, respectively. Note that the TCRα TM also includes an arginine residue that is thought to form a charged pair with an arginine residue in each of the CD3ε TM segments (Call et al., 2002).

We hypothesize that, based on the structures of CD3εβ and CD3εγα, the highly selective TCR signaling may require dynamic interaction rather than static on-and-off switching, such that the interfaces between the extracellular domains of the TCR αβ heterodimer and CD3 dimers may be quite small. With this current model, no detailed information on the interfaces is warranted, being one of a range of acceptable structures. Nonetheless, we envisage the ectodomains of TCR αβ chains being supported by the CD3 heterodimers, while components of the TCR αβ dimer, such as the Cβ FG loop (Wang et al., 1998) and the α-CP (Backström et al., 1998; Werlen et al., 2000) may serve as levers and/or tension elements to help control vertical movements of CD3 subunits for signal transduction through the critical TM segments. Given apparently weak ectodomain association between CD3 and TCR αβ heterodimers (see Arnett et al., 2004; Touma et al., 2007 and references therein), it is likely that this assembly undergoes dynamic quaternary change upon TCR ligation and triggering, thereby affecting cytoplasmic CD3 signaling regions. According to the model, the five helices of the CD3ε–CD3γ–TRCα–CD3ε–CD3γ component lie closer to the TCR α subunit and the three helices of the CD3ε–CD3γ–TRCβ component lie closer to the TRCβ subunit (Call et al., 2002).

THE TCR AS AN ANISOTROPIC MECHANOSENSOR

The squat and rigid CD3 connecting segments (Touma et al., 2007) contrast sharply with the long and flexible TCR α and β CP linking their respective constant domains to the transmembrane segments. Structural insight into a basis for this contrasting arrangement first came from analysis of interactions of activating and non-activating anti-CD3ε monoclonal antibodies, which bind to the CD3εγα ectodomains with virtually identical affinity on T cells. Activating antibodies footprint to the membrane distal CD3ε lobe which they approach diagonally, adjacent to the lever-like Cβ FG loop noted above to facilitate pMHC-triggered activation. In contrast, a non-activating mAb (17A2) was found to bind to the cleft between CD3ε and γ in a perpendicular mode (Kim et al., 2009; Figure 1D). Thus, polystyrene bead-bound 17A2 antibody became stimulatory only upon application of ~50 pN of external tangential force to the bead. Specific bead-bound pMHC (but not irrelevant peptide bound to the same MHC) activates a T cell upon application of a similar force via optical tweezers to initiate intracellular calcium flux (Figure 2). These findings imply that the TCR is a mechanosensor, converting mechanical energy into a biochemical signal upon specific pMHC ligation that occurs as a T cell moves over APCs during the course of immune surveillance. As shown in Figure 1C (left panel), the pMHC on the APC is first ligated by a specific TCR. However, as the T cell continues to move prior to a stop movement signal mediated through inside-out integrin affinity up-regulation, pMHC functions as a force transducing handle to pull on the TCR αβ heterodimer (Figure 1C,
The CxxC motif in CD3ε, CD3γ, and CD3ζ: A potential role in structural stabilization and redox-sensitive signaling attenuation

Studies on murine CD3γ (Touma et al., 2007) and human CD3γ (Thomassen et al., 2006; Xu et al., 2006) attest to the importance of the cysteines in the CxxC motif for TCR function and/or assembly. Similar findings have been shown for CD3ζ (Martínez-Martín et al., 2009; Wang et al., 2009). Given that the two cysteines in each CD3 CxxC motif are adjacent to the TM helix (Figure 3) and in view of a recent study showing that a CxxC motif is found at the N-termini of α-helices, stabilizing α-helical structures, this juxtaposition is noteworthy (Iqbalsyah et al., 2006). Assuming an intrachain disulfide is formed in each stalk region (vide infra), one possibility is that the CD3 TM helix is stabilized and perhaps even extended as an elongated helix above the plane of the cell membrane. Alternatively, this CxxC motif may support a tight β turn (Hsu et al., 2006). In either case, the disposition of the CD3 ectodomain relative to the cell membrane may be affected if a disulfide bond is removed, attenuating signaling, and altering TCR quaternary structure. The disulfide bonds would ensure that lever action on the TM helices of the various CD3 domains would be simultaneous, parallel, and in phase. Whether physiologic modification of the redox state of the CD3 heterodimer is regulated during development or T cell activation can be determined. However, given that TCR crosslinking on murine and human T lymphocytes generates hydrogen peroxide and superoxide ions (Paní et al., 2000; Devadas et al., 2002) and that oxidative stress from macrophages alters the native CD3ζ association with the TCR (Otsuji et al., 1996), it is possible that redox reactivity of CD3 stalk cysteines is critical for modulating TCR quaternary structure, subunit conformation, and functional responsiveness. Rapid conversion between oxidized and reduced forms under
physiologic circumstances may be important for TCR triggering and downregulation, respectively. Assuming a direct link between redox state, TCR function, and TM structure is demonstrated, future efforts can be directed toward design of deliverable redox regulators using mAb or other materials to modify T cell responses.

MECHANOSensing AT THE IMMUNOLOGICAL SYNAPSE

Our data involve a model wherein tangential forces applied along the pseudo-twofold symmetry axis of the TCR αβ heterodimer exerts a highly selective signaling torque on the CD3 components. This directionally specified vector precludes non-specific activation and fosters antigen-specific events. In turn, activation leads to stop movement and formation of the immunological synapse. Mechanoreceptor function is most likely additionally tunable by inducible actin cytoskeletal interactions with TCR and pMHC on T cells and APCs, respectively, since torque will be greatest in their presence. Within the synapse, force could be generated on the TCR via microcluster formation and actin-based trafficking (Yokosuka et al., 2008). Mechanosensing can be further amplified at the synapse where intermembrane distances (~150 Å) are optimal for TCR/pMHC ligation in conjunction with other signaling molecules to sustain activation from both p-SMAC and c-SMAC components. The facilitating roles of adhesion molecules and CD4 and CD8 co-receptors have been discussed in detail previously (Kim et al., 2009 and references therein) and will not be reviewed here. Rapid transport of TCRs to the immunological synapse and TCR signaling after disengagement of pMHC likely be reviewed here. Rapid transport of TCRs to the immunological synapse where intermembrane distances (CD3γ C82 and C85 cysteines only shown for clarity) and the transmembrane domains of unknown structures (cylinders) drawn to scale (fourma et al., 2007).

FUTURE DIRECTIONS

Details of the mechanobiology of TCR function remain to be fully elucidated. Force threshold requirements and effects on signaling of loading rate and directionality of pMHC ligand movement relative to the TCR need to be established. Function/structure studies of alterations attenuating TCR rigidity with respect to T cell activation are needed.

The precise mechanotransduction of TCR signaling upon modification of the redox state of the CD3 heterodimer, for example, can be measured using a combined fluorescence and optical trap microscope for simultaneous trapping and fluorescence imaging (Törsta et al., 2007). This newly established methodology offers nanometer level position, piconewton level force, and low light single molecule fluorescence sensitivity. Furthermore, quaternary motion and the mechanical properties of the CD3 heterodimer via single molecule studies on intact T cells can be compared. Finally, the physical force relayed from the ectodomain onto the TM during mechanosensor function may be transmitted to the cytoplasmic tail directly and/or indirectly by modification of the membrane lipid organization (Zech et al., 2009; Nika et al., 2010). The CD3ε cytoplasmic tail maintains close interaction with the plasma membrane via basic CD3ε residues and acidic phospholipids enriched in the inner leaflet of the plasma membrane (Xu et al., 2008; Deford-Watts et al., 2009). Two key tyrosine residues in the CD3ε ITAM are deeply inserted in the hydrophobic core of the lipid bilayer. Release of sequestered tyrosines must occur for phosphorylation by Lck to follow. CD3ζ phosphorylation is also lipid-dependent (Aivazian and Stern, 2000) and the importance of the six ITAMs in the ζζ homodimer for signaling is not to be underestimated (Acuto et al., 2008 and references therein).

T cells are exposed to stresses from fluid and cell motions during immune surveillance. pMHC ligands are tethered on APCs via their own TM segments. pMHC mobility may be tunable via actin cytoskeletal connections during dendritic cell maturation, thereby altering T cell activation. T cells may exploit stress and geometrical cues from this greater micro-environment to discern proper signaling from noise, identifying appropriate TCR–pMHC interactions. The dynamic quaternary structure of the TCR may not only be controlled through glycosylation and redox modification but through non-linear response to forces through mechanisms like catch bonds, where pushing and pulling on the TCR facilitates mechanisms such as conformational change, allostery and stabilization that the TCR may exploit for robust and proper signaling. Aside from offering basic scientific insight into multisubunit receptor mobility and function, that understanding of early TCR signal initiation will be advantageous for drug development aimed at modifying T cell activation.

GLOSSARY

Shear force  Force which acts tangentially to a surface. Here, we use “tangential force” interchangeably.
Tensile force  Force which acts perpendicular to a surface, like the force on a rope.

Catch bond  Type of intermolecular bonds which strengthens upon application of force. Typically, force accelerates bond breakage as in “slip bonds.”

Torque  The tendency of force to generate rotation of a body about an axis.

Lever  Force amplifying device which consists of a fulcrum, fixed pivot, and a rigid beam.

Chemical force  Force derived from intermolecular bonding.

Mechanotransduction  Cell signaling through means of a mechanical input.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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