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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 (CD3γ, CD3δ, 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 CD3γ, 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 Clipstone, 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 the TCR constant domains (Bentley et al., 1995; Yanagi et al., 1996), and nicely 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). The Cα 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)
ABED β allows for physical and functional linkage of the cell surface membrane. As indicated below, this asymmetric cavity region (Kim et al., 2010). The uniquely kinked conformation of the CD3 adducts can dynamically modify TCR function as noted below.

The NMR and X-ray structures of CD3 εδ ectodomains (Wang et al., 2004) defines a plausible topology and emphasizes its glycan richness (Figure 1B). Given that CD3εδ 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 CD3εδ, 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–CD3ε–CD3δ 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, 1983; Koning et al., 1990), and (iii) proximity of one CD3ε subunit to the TCR β 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 CD3εδ (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 β 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. CD3εδ is docked on the opposite site of the TCR εδ domain where there are less glycans to interfere with the more heavily glycosylated CD3δ ectodomain (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 ø heterodimer with a vertical dimension of 80 Å projecting from the cell membrane, flanked on either side by the shorter (40 Å) CD3 heterodimers, CD3 øε and CD3 øγ subunits left and right, respectively. CD3 ø is in light blue, CD3 ε is in yellow, and CD3 γ is in green. CPK representation (convention for distinguishing atoms of different chemical elements in molecular models) of all glycans is indicated by the rust color spheres. The view is from the side with the T cell membrane at the bottom. In the top panel, a model of TCR complex glycans surrounding the pMHC binding site from the pMHC perspective is shown. Individual glycans in CPK representation are numbered and subunit origin color-coded. The ø FG loop is denoted with an asterisk. (C) Mechanosensor function of the TCR complex. The left panel shows the pMHC (orange) bound to a TCR complex. As the T cell continues to move prior to stopping, a pull by pMHC is converted to a push onto CD3 øγ amplified by the ø FG loop (magenta loop) above CD3 øβ (blue) and accompanying signaling events follow (right panel). (D) Anti-CD3 mAb binding. The right panel shows that the activating 2C11 anti-CD3 mAb binds to the exposed outer lobe of CD3 øβ (highlighted in red) while the non-activating 17A2 anti-CD3 mAb binds perpendicular to the membrane between CD3 øε and CD3 øγ ectodomain subunits (highlighted in red). Anti-CD3 Fab arms are in light blue and salmon colors.

**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 TCRø (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 ø constant domain to the associated CD3 ε and CD3 γ transmembrane (TM) segments which are juxtaposed for apparent charge pairing (i.e., between the TCRø lysine and aspartic residues of CD3 ø and CD3 øγ 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 CD3 ø and CD3 øγ TM, respectively. Note that the TCRø TM also includes an arginine residue that is thought to form a charged pair with an aspartic residue in each of the CD3 øε TM segments (Call et al., 2002).

We hypothesize that, based on the structures of CD3 øε and CD3 øγ, 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 ø 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 øβ chains being supported by the CD3 heterodimers, while components of the TCR øβ dimer, such as the ø FG loop (Wang et al., 1998) and the ø-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 øβ 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 CD3 øε–TPR ø–CD3 øγ–CD3 øβ component lie closer to the TCR ø subunit and the three helices of the CD3 øε–CD3 øγ–TPR øβ component lie closer to the TCRβ 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 ø 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-CD3 ø monoclonal antibodies, which bind to the CD3 øγ ectodomains with virtually identical affinity on T cells. Activating antibodies footprint to the membrane distal CD3 øε lobe which they approach diagonally, adjacent to the lever-like ø FG loop noted above to facilitate pMHC-triggered activation. In contrast, a non-activating mAb (17A2) was found to bind to the cleft between CD3 øε 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 øβ heterodimer (Figure 1C,
right panel). This force is amplified and exerted on CD3εγ by the lever arm where the TCRβ TM acts as a fulcrum. For activation, force must be applied to the TCR complex tangentially and not perpendicular to the plane of the T cell membrane, showing that the TCR is an anisotropic mechanosensor (i.e., direction matters; Kim et al., 2009). The rupture force and bond lifetime under load between pMHC and TCRαβ heterodimer are potentially important parameters which can determine the potency of pMHC stimulation. The pull from pMHC most probably causes the Cβ FG loop to push on the upper outer lobe of CD3εγ. During this force driven quaternary change, TCR-decorating glycans can serve as steric and spring-like barriers that require force to overcome in order to deliver signaling to CD3 subunits. Several groups have recently provided evidence that physical force applied to TCR components activates T cells (Kim et al., 2009; Li et al., 2010; Ma and Finkel, 2010; Husson et al., 2011; Judokusumo et al., 2012).

That the TCR is a mechanosensor activated by direction-specific physical force has several immediate implications. First, since the total force applied to the T cell surface is essentially defined during movement of the T cell relative to that of the APC, ligation of a small number of TCRs by several cognate pMHCs on the opposing APC will exert a greater physical force on each individual TCR than ligations of multiple TCRs on the same T cell by a large number of pMHCs on the APC. Hence, specificity and sensitivity are built into TCR mechanosensor function. Second, in principle, shear forces generated by T cell movement during immune surveillance can form catch bonds at the TCR–pMHC interface to enhance binding and/or confer additional ligand specificity. These bonds, which are strengthened by tensile force, have been described for cell adhesion molecules (Marshall et al., 2003). Catch bond characteristics may allow for non-linear response in the lifetime of TCR–pMHC bonding where it is maximal at certain force levels enabling specific pMHC ligand to drive quaternary change, yet allow for quick release for other pMHC ligand binding. Because the torque exerted by the TCR–pMHC interaction around the Cβ FG loop/CD3εγ “flywheel” is dependent on the force applied, the length of the αβ–pMHC lever arm and the angle between the force vector and lever arm, TCR docking topology is important.

Consistent with this view, a recent paper demonstrated that docking orientation rather than affinity of 3D binding correlated with the ligand’s T cell activating potential (Adams et al., 2011). This finding is also in agreement with a docking orientation difference between the T cell activating 2C11 versus non-activating 17A2 antibodies (Kim et al., 2009; Figure 1D). Bacterial superantigens stimulate up to 20% of the entire T cell population by simultaneously interacting with class II MHC and TCRβ molecules on APC and T cells, respectively (Sundberg et al., 2002). Not surprisingly, therefore, these interactions foster TCR docking vectors similar to those of activating TCR–pMHC interactions (Reinherz et al., 1999). Precedent for mechanoreceptors in the hematopoietic system is the von Willebrand factor (VWF) receptor on platelets where tensile stress on bonds between the GPIbα subunit and the VWF-A1 domain under fluid dynamic conditions triggers integrin αIIbβIIIa activation to support platelet adhesion (Doggett et al., 2002; Ruggeri, 2007).

THE CxxC MOTIF IN CD3ε, CD3γ, AND CD3β: 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 CD3δ (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 (Pani et al., 2000; Devadas et al., 2002) and that oxidative stress from macrophages alters the native CD3ξ 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. Force which acts tangentially to a surface. Here, we use “tangential force” interchangeably.

**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 (Tärsa 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 CD3ζ cytoplasmic tail maintains close interaction with the plasma membrane via basic CD3ζ 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 CD3ζ 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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