A New Angle on TCR Activation

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Functional cognate T cell recognition is mediated via the interaction of a T cell receptor complex with its pMHC ligand. Adams et al. (2011) in this issue of *Immunity* provide evidence that docking geometry may impact 2D binding and T cell activation.

The αβ T cell receptor (TCR) heterodimer is similar to an immunoglobulin Fab fragment in overall quaternary structure and domain conformation, with each subunit consisting of one variable (V) and one constant (C) immunoglobulin (Ig) domain in the extracellular segment (Garcia et al., 1996). Nevertheless, close inspection reveals several important differences: the αβ TCR heterodimer is wider, with a rather flat binding surface reaching out from the membrane to match its pMHC ligand interaction site; it carries a unique 12 amino acid residue insertion in the Cβ domain, termed the Cβ FG loop, reinforcing the rigid connectivity between Vβ and Cβ; and it manifests a peculiar asymmetric disposition of Cβ relative to Cα to serve as a dynamic CD3εγ docking site (Wang et al., 1998). The arrangement of the squat CD3 heterodimers, CD3εγ and CD3εδ, lateral to the centrally placed αβ heterodimer in a loose confederation of heavily glycosylated ectodomains fixed by interacting transmembrane segments is noteworthy (Kim et al., 2009). The CD3ζ homodimer, which is virtually without an ectodomain, also forms part of the transmembrane bundle. The CD3 components each have cytoplasmic tails containing immunoreceptor tyrosine-based activation motifs (ITAMs) involved in signaling upon pMHC ligation, contrasting with the short ITAM-less α and β cytoplasmic stumps. These elements collectively comprise the TCR complex.

How pMHC ligation of the αβ heterodimer initiates signaling via the CD3 components in conjunction with Lck kinase-linked CD4 or CD8 coreceptors is a matter of intense investigation. That thermodynamic or kinetic parameters of pMHC binding only loosely correlate with T cell activation outcome and that there are no discernible αβ TCR heterodimer-pMHC structural changes to distinguish agonist from nonagonist pMHC ligands (Ding et al., 1999) has further added to the mystery of this pivotal immune receptor. The TCR holds the secret of self- versus nonself-discrimination essential for protective host immunity in mammals. When TCR function goes awry, autoimmunity or immunodeficiency may follow. Thus, we need to understand all features of this extraordinary receptor of adaptive T cell immunity.

In this issue, Adams et al. (2011) compare a crystal structure of the alloreactive 42F3 TCR αβ heterodimer in complex with the QL9 nonamer peptide of 2-oxogluterate dehydrogenase bound to H2-L^d (L^d) with that of the 2C TCR αβ heterodimer bound to the same pMHC. By using yeast-displayed H2-L^d peptide libraries whose peptide sequences were randomized in three different ways in conjunction with 42F3 tetramers and flow cytometry sorting, they recovered peptides presented by L^d with TCR binding sequences divergent from QL9 to varying degrees (e.g., 3A1 and QL9 are entirely different peptides, with no single position identical). In contrast, among the nine peptide residues, 4B10 diverged from QL9 at three TCR residues, and 5E8 diverged at three MHC residues. None of these peptides exists in known proteins. 42F3 complexes with each of these pMHC ligands were crystallized and structurally studied. In addition, solution-binding affinities of recombinant 42F3 with the various pMHCs were determined by surface plasmon resonance (SPR) (3D), as well as 2D binding affinity of 42F3 cellular transfectants and their respective capacities, to produce interleukin-2 (IL-2).

The key new findings are 4-fold. First, 3A1-L^d has the highest solution 3D affinity for 42F3 by SPR equilibrium analysis (3.9 μM). Second, if the membrane-bound 42F3 is used for 3A1-L^d interaction analysis in which membrane confinement properties are in play, this so-called "2D" interaction shows a less-favorable association. This 2D measurement correlates with lack of IL-2 production of 42F3 T cell transfectants stimulated by peptide-pulsed antigen-presenting cells or pMHC oligomers. Third, the TCR-pMHC docking geometry of 42F3 to 3A1-L^d, the only nonagonist described here, is divergent from the agonist L^d complexes (QL9, 4B10, and 5E8) with 42F3, as well as that between 2C and QL9. TCR 42F3 binds diagonally with respect to the peptide in the agonist pMHC complexes, as commonly observed in many other agonist pMHC-TCR complexes, whereas 42F3 aligns more parallel to the peptide in the nonagonist 3A1 complex. It seems that the "nondiagonal" docking observed in the crystal structure may not be compatible with the biologically more relevant 2D binding. Fourth, even when there is a similar docking mode among the same TCR bound to the same MHC but loaded with different peptides, the chemistry of interaction is highly diverse. Different TCR-pMHC complexes can be more or less Vα or Vβ centric in conserved germline contacts. These findings underscore how plasticity of a single TCR in binding ligand can diversify peptide recognition. At the same time, these data make it abundantly clear that there are no simple recognition "rules" allowing ab initio prediction of cognate interaction for the highly evolved mammalian T cell immune system.

Figure 1 shows the docking of 42F3 bound to the agonist QL9-L^{d} in comparison to that of the nonstimulatory 3A1-L^{d} complex. Only the 3A1 peptide is shown in the MHCI groove for clarity. The view is from the side, down the long axis of the peptide and MHC helices. It is obvious that 42F3 straddles the groove in the QL9-L^{d} complex interacting with the L^{d} α1 and α2 helices. In contrast, when bound to 3A1-L^{d}, 42F3 docks much more parallel to the α1 helix, making few contacts with α2. The 3A1 peptide points its two Leu and two Trp side chains up to the TCR V module, providing hydrophobic contacts for robust TCR interaction and readily explaining the high affinity in solution of the 42F3-3A1-L^{d} interaction and its highest crystal structure resolution among the pMHC-TCR complexes. As stated by the authors, the register of the two 42F3 Vβ domains, when binding to QL9-L^{d} versus 3A1-L^{d}, is much more similar than that of the corresponding Vα domains.

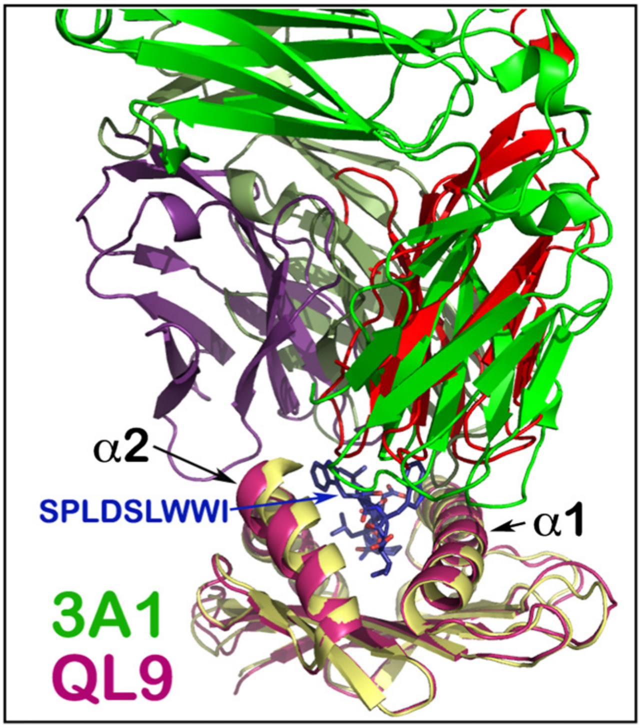

Figure 1. Superposition of Crystal Structures of 3A1-L^{d}-42F3 with QL9-L^{d}-42F3

Shown here are the α1 and α2 domains of MHC molecule L^{d} in complex with the 42F3 TCR αβ heterodimer. Only 3A1 peptide (SPLDSLWWI) is shown for clarity in the peptide-binding groove. TCR 42F3 heterodimeric subunits in the 3A1-L^{d}-42F3 complex are in dark green (β subunit) and light green (α subunit), respectively, and in red (β subunit) and purple (α subunit) in the QL9-L^{d}-42F3 complex. The superposition shown is based on the MHC structure.

How can one explain the lack of functional activity of the 3A1-L^{d} complex? This is the key question posed by the structural and functional data. The authors explore two ideas to address this question. On the one hand, if a TCR or pMHC were to form dimers or higher-order oligomers, then the 3A1 docking topology may be outside that allowed for a productive orientation. The notion that the TCR forms dimers and/or that pMHC forms dimers or pseudodimers has not been confirmed structurally. Nonetheless, its existence has been suggested based on the notion that TCRs must dimerize in order for signaling to occur in vitro (see below). Alternatively, even if the TCR, including its coreceptor, functions as a unitary signaling complex, 3A1 docking orientation may not be allowed, the authors suggest. These possibilities are predicated on concepts of static binding geometries and cannot readily resolve the enigma. As pointed out by Adams et al., for example, 2D biophysical parameters manifested by 3A1-L^{d}-42F3 interaction that fail to activate 42F3 are equivalent to those parameters found to be stimulatory for pMHC-TCR binding associated with the OT1 TCR. Thus, there is not an absolute 2D threshold for T cell triggering that has been uncovered. Likewise, the CD1d-lipid antigen (αGalCer) recognition by NKT15, a functional iNKT cell αβ TCR, is even more parallel to the α1 helix of CD1d than 42F3 to p3A1-L^{d} (Borg et al., 2007). iNKT cells may or may not express coreceptors, but, as Adams suggests, interface specificity is engrafted in the TCR V domains in the absence of coreceptor position. Coreceptors themselves do not bind to the αβ heterodimer. In the case of CD4, for example, in which the ectodomain structure is known in its entirety, TCR αβ and CD4 bind to the same pMHC in a bidentite manner with their membrane-proximal regions splayed apart by ≥130 Å (Wang et al., 2001).

A dynamic rather than a static model of TCR ligation and activation may help to explain the exciting current findings. In this regard, several groups have recently provided evidence that physical force applied to TCR components activates T cells (Husson et al., 2011; Kim et al., 2009; Li et al., 2010). This activation requires that force 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). These findings were revealed through structural analysis of the binding of CD3ε monoclonal antibodies in conjunction with optical tweezer experiments. More importantly, when specific pMHC-bound beads were approximated to the T cell membrane, triggering of T cell activation occurred only after tangential force application (Kim et al., 2009). Such forces can be exerted by cognate pMHC on APCs through binding the TCR on an interacting T cell as the T cell moves when performing immune surveillance prior to a stop movement signal. The greater the functional affinity between pMHC signals and TCR αβ heterodimer, the greater the pull, potentially. The pull from pMHC most probably causes the Cβ FG loop to push on the upper outer lobe of CD3ε. How ectodomain quaternary changes alter the TCR complex transmembrane segments, surrounding membrane lipid and cytoplasmic tail structures to transduce signals from outside to inside the cell, is a future great scientific challenge.

Parenthetically, bivalent or multimeric crosslinking can torque the TCR in a manner similar to a monomeric interaction with physical load applied. Thus, the importance of bivalent interaction in mediating TCR activation may not be due to the requirement for TCR dimerization per se but rather to torque application. In this regard, the angle of interaction between TCR αβ heterodimer and pMHC will affect the torque and, hence, physical force placed on the CD3 subunits. Catch bonds may form under load, and these could also change the nature of TCR-pMHC interaction.

Lastly, unlike antibody Fabs, the TCR αβ and CD3γ and CD3δ elements are highly N-linked glycosylated. Glycans are dynamic, large, and tunable. Those adducts will affect movement of the TCR subunits and subsequent signaling. A study consistent with this notion shows that TCR functional avidity is altered by removal of a Cα glycan, for example (Kuball et al., 2009). Given the detailed information available from Adams et al., 42F3 may be an ideal system to explore the effect of these variables on T cell signaling. It now appears that MHC-restricted TCR recognition is "restricted" by additional parameters. The structural and functional studies in the current paper serve well to underscore the need for further investigation.

REFERENCES

Adams, J.J., Narayanan, S., Liu, B., Birnbaum, M.E., Kruse, A., Bowerman, N.A., Chen, W., Levin, A.M., Connolly, J.M., Zhu, C., et al. (2011). Immunity *35*, this issue, 681–693.

Borg, N.A., Wun, K.S., Kjer-Nielsen, L., Wilce, M.C., Pellicci, D.G., Koh, R., Besra, G.S., Bharadwaj, M., Godfrey, D.I., McCluskey, J., and Rossjohn, J. (2007). Nature *448*, 44–49.

Ding, Y.H., Baker, B.M., Garboczi, D.N., Biddison, W.E., and Wiley, D.C. (1999). Immunity *11*, 45–56.

Garcia, K.C., Degano, M., Stanfield, R.L., Brunmark, A., Jackson, M.R., Peterson, P.A., Teyton, L., and Wilson, I.A. (1996). Science *274*, 209–219.

Husson, J., Chemin, K., Bohineust, A., Hivroz, C., and Henry, N. (2011). PLoS ONE *6*, e19680.

Kim, S.T., Takeuchi, K., Sun, Z.Y., Touma, M., Castro, C.E., Fahmy, A., Lang, M.J., Wagner, G., and Reinherz, E.L. (2009). J. Biol. Chem. *284*, 31028–31037.

Kuball, J., Hauptrock, B., Malina, V., Antunes, E., Voss, R.H., Wolfl, M., Strong, R., Theobald, M., and Greenberg, P.D. (2009). J. Exp. Med. *206*, 463–475.

Li, Y.C., Chen, B.M., Wu, P.C., Cheng, T.L., Kao, L.S., Tao, M.H., Lieber, A., and Roffler, S.R. (2010). J. Immunol. *184*, 5959–5963.

Wang, J.-H., Lim, K., Smolyar, A., Teng, M.-K., Liu, J.-H., Tse, A.G.D., Liu, J., Hussey, R.E., Chishti, Y., Thomson, C.T., et al. (1998). EMBO J. *17*, 10–26.

Wang, J.-H., Meijers, R., Xiong, Y., Liu, J.-H., Sakihama, T., Zhang, R., Joachimiak, A., and Reinherz, E.L. (2001). Proc. Natl. Acad. Sci. USA *98*, 10799–10804.

αβ T Cell Receptors Come Out Swinging

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It is unclear how an effective T cell repertoire is built from a limited array of T cell receptor (TCR) genes. In this issue of *Immunity*, Stadinski et al. (2011) demonstrate that TCR variable (V) α chains can indirectly affect Vβ-mediated recognition of the major histocompatibility complex (MHC) molecule.

T cell immunity relies on expression of a single αβ T cell receptor (TCR) within individual T cells. TCRs are antigen receptors that recognize peptide fragments complexed to either the major histocompatibility complex (MHC) class I or class II glycoproteins (pMHC). TCRs are derived from random rearrangement of a limited set of variable (V), diversity (D), and junctional (J) or of the V and J gene segments found in the TCRβ and TCRα gene loci, respectively. Regions of hypervariability, called complementary determining regions (CDR) regions, are encoded within the Vα and Vβ chain and form the antigen-binding site. Moreover, the same set of TCR gene segments are used to recognize MHC-like molecules such as CD1 family members. During T cell development, only those T cell precursors expressing a TCR capable of interacting with self-MHC molecules develop into mature T cells, a process called positive selection. This process ensures that T cells become specific for a particular MHC class with mature CD8$^+$ and CD4$^+$ T cells typically restricted to MHC class I (MHC I) and class II (MHC II), respectively.

A long-standing paradox has been just how does the limited set of available TCR genes within the genome combine to generate a T cell repertoire diverse enough to enable recognition of the seemingly infinite array of highly polymorphic MHC alleles and bound peptide within an outbred population? This question has dogged the field for decades and has been compounded by the structural data, whereby TCRs can bind the pMHC in a variety of ways (Burrows et al., 2010). Recent studies have revisited this central question and determined that some closely related MHC II-restricted TCRs, all of which express the same TCR Vβ8.2 gene, make similar contacts with their cognate pMHC (reviewed by Marrack et al., 2008). These studies indicate that MHC restriction might be predetermined within the germline TCR V-region repertoire via conserved interactions between CDR2β germline-encoded residues that direct MHC binding.

As the adage goes, rules are meant to be broken, and the boundaries