

# Late Arrival: Recruiting Coreceptors to the T Cell Receptor Complex

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In this issue of *Immunity*, [Jiang et al. \(2011\)](#) provide evidence that the CD8 coreceptor is recruited to the T cell receptor (TCR) complex after initial TCR triggering where it stabilizes the TCR-peptide-major histocompatibility complex interaction.

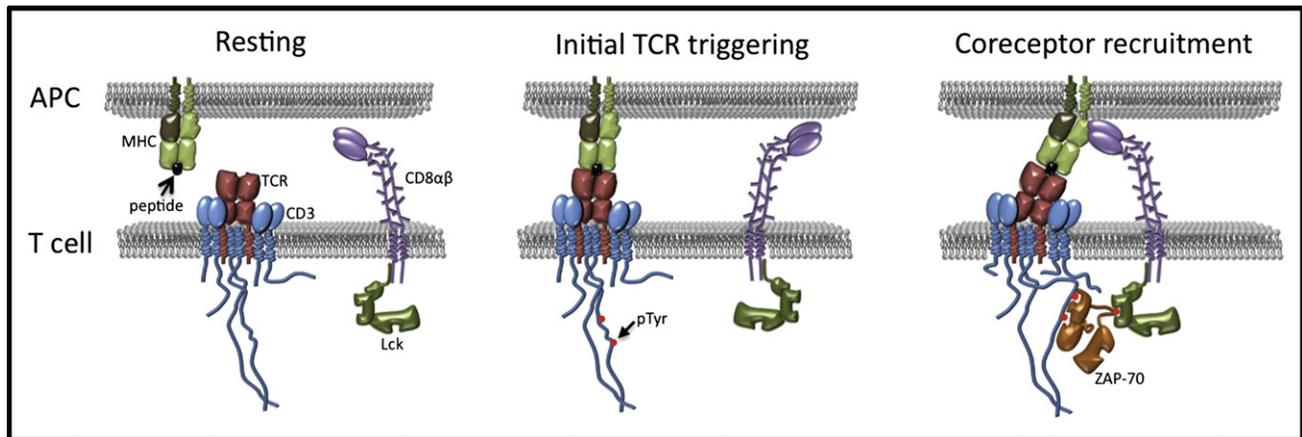
The T cell receptor (TCR) is a multisubunit complex comprising both antigen recognition (TCR) and signaling (CD3) subunits. T cell recognition of antigen requires TCR engagement of a peptide-major histocompatibility complex (pMHC) molecule binary structure on antigen-presenting cells. This interaction is enhanced by the T cell-encoded CD8 or CD4 cell surface receptors (termed coreceptors), which bind MHC class I or II molecules, respectively ([Janeway, 1992](#)). The precise role of coreceptors is not understood: some models postulate that the coreceptors enhance TCR signaling by recruiting the associated tyrosine kinase Lck ([Janeway, 1992](#)); others postulate that coreceptors function mainly to enhance the TCR-pMHC interaction ([Xu and Littman, 1993](#)). This question has been challenging because it is difficult to study the sequence of TCR and coreceptor interactions with pMHC in their native environment at the T cell-target cell interface. In a report in this issue of *Immunity*, [Jiang et al. \(2011\)](#) use a technique developed in their laboratory for this purpose to show that CD8 stabilizes the TCR-pMHC interaction after initial TCR-pMHC binding and triggering. Taken together with data from other groups, this suggests that an initial TCR-pMHC interaction is followed by physical recruitment of CD8 to the TCR-CD3 complex through intermediate signaling molecules, where it stabilizes the TCR-pMHC interaction by binding simultaneously to both the TCR-CD3 complex and the pMHC.

The term “coreceptor” was ascribed to CD8 and CD4 on the assumption that they bound to the same pMHC complexes as the TCR ([Janeway, 1992](#)). The observation that the cytoplasmic tails of coreceptors associate with the tyrosine kinase

Lck, which initiates TCR triggering by phosphorylating the immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic domains of the CD3 subunits, suggested that an important function of coreceptors was to recruit Lck to TCRs that had engaged pMHC, thereby enabling TCR-CD3 ITAM phosphorylation by Lck. An attractive feature of this heterodimerization model was that it provided an elegantly simple mechanism for TCR triggering. However, a number of experimental findings soon cast doubt on this model. First, although Lck association is important for coreceptor function, [Xu and Littman \(1993\)](#) found, by using a CD4-Lck chimera, that the catalytic domain of Lck was entirely dispensable for CD4 function; deletion thereof actually *enhanced* the effect of CD4. Second, it was shown that T cell development and TCR-mediated antigen recognition, although entirely dependent on Lck, can occur in the complete absence of coreceptors (e.g., see [Schilham et al., 1993](#)). These results showed that coreceptors are not required to deliver Lck to the engaged TCR and that Lck contributes to coreceptor function through a noncatalytic mechanism. What could this mechanism be? Lck is also an adaptor protein in that it can interact with multiple proteins simultaneously; it contains SH2 and SH3 domains, which mediate protein:protein interactions by binding phosphorylated tyrosine (pTyr) and proline-rich sequences, respectively. [Xu and Littman \(1993\)](#) showed that a mutation of the Lck SH2 domain that would prevent such binding abrogated CD4-Lck function. These findings, together with numerous other reports that coreceptors physically associate with TCR-CD3 complexes after TCR triggering (reviewed in

[Janeway, 1992](#)), led them to propose a recruitment model of coreceptor function ([Figure 1](#)). This model postulates that TCR engagement of pMHC leads to Lck-mediated tyrosine phosphorylation of the TCR-CD3 complex by a mechanism independent of coreceptors, and that this is followed by recruitment of coreceptors to the triggered TCR-pMHC complex by interactions of the SH2 domain of coreceptor-associated Lck with pTyr in the TCR-CD3 complex.

However, Xu and Littman’s coreceptor recruitment model has been largely overlooked by subsequent studies. One reason for this is the lack of direct evidence for a two-stage binding mechanism. Now [Jiang et al. \(2011\)](#) have provided such evidence by using a mechanical adhesion frequency assay previously developed in their laboratory. This method involved using micropipettes to repeatedly bring into contact and retract ligand-coated red blood cells (RBCs) and cells bearing a candidate receptor. Receptor-ligand binding is detected by deformation of the RBC upon retraction. By varying the duration of contact and repeating the contact-retraction cycle many times, it is possible to obtain a plot of binding probability versus contact time from which the affinity and rate constants of the receptor-ligand interaction can be determined. Because the concentrations of cell surface molecules are two-dimensional (molecules per  $\mu\text{m}^2$  surface area), these are referred to as 2D binding constants. By using pMHC-coated RBCs and T cells expressing CD8 and the appropriate TCR, Cheng Zhu and colleagues have previously studied the CD8-MHC ([Huang et al., 2007](#)) and the TCR-pMHC ([Huang et al., 2010](#)) interactions in isolation. In both cases binding



**Figure 1. Coreceptor Recruitment Model**

Initially proposed by Xu and Littman (1993) based on studies of the CD4 coreceptor, this model postulates that TCR-CD3 and coreceptors are normally not associated in the resting state (left), and that TCR recognition of pMHC leads to tyrosine phosphorylation of the TCR-CD3 ITAMs (middle) by Lck not associated with coreceptor, which leads in turn to recruitment of coreceptor (CD8 in this figure) via the SH2 domain of Lck associated with its cytoplasmic tail (right). Subsequent work suggested the one mechanism of coreceptor association with TCR-CD3 is binding of the Lck SH2 domain to a pTyr residue on ZAP-70 (Thome et al., 1995). APC, antigen-presenting cell.

is simple (monophasic) and it was possible to estimate 2D binding constants, revealing that CD8-MHC class I interaction has much (100-fold) lower affinity than the TCR-pMHC interaction, consistent with measurements of the solution or 3D affinity (van der Merwe and Davis, 2003). This indicates that CD8 binding to MHC class I is minimal at the usual surface densities of CD8 and MHC.

In the present study, Jiang et al. (2011) showed that, when all three binding partners are present, TCR-pMHC binding was more complex, being divided into two discrete phases. Binding rapidly reached an initial plateau (within 0.3 s), but after about 1 s it increased again to reach a second, significantly higher plateau. By using pMHC mutants and blocking antibodies, they were able to show that the initial phase represented TCR binding pMHC whereas the second phase represented CD8 binding the same pMHC complex as the TCR. Importantly, the second phase was eliminated when Lck was inhibited. Thus CD8 was able to stabilize the TCR-pMHC interaction but only after initial TCR engagement and CD3 phosphorylation by Lck. The simplest explanation for these results is that TCR engagement of pMHC leads to physical recruitment of CD8 to the TCR-CD3 complex where it binds to the same pMHC complex, as predicted by Xu and Littman (1993)'s coreceptor recruitment model (Figure 1).

The work by Jiang et al. (2011) provides strong support for the coreceptor recruitment model and paves the way for future questions. One important question is the precise mechanism of coreceptor recruitment to the TCR-CD3 complex. Thome et al. (1995) have provided evidence that the SH2 domain of coreceptor-associated Lck binds to a pTyr residue on tyrosine kinase ZAP-70, but it is unclear whether this is the only mechanism of coreceptor recruitment to the TCR-CD3 complex. Given that there are multiple pTyr residues in the TCR-CD3 complex and up to ten ITAMs that can bind ZAP-70, this raises the question as to whether all these ITAM-ZAP-70 complexes can recruit coreceptor in an orientation suitable for pMHC binding, or whether particular ITAMs are favored. Palmer and colleagues have argued that coreceptor recruitment involves a conserved motif in the TCR $\alpha$  stalk region that is required for incorporation of CD3 $\delta$  into the TCR-CD3 complex, raising the possibility of a special role for the CD3 $\delta$  ITAM in coreceptor recruitment (Palmer and Naeher, 2009). Interestingly,  $\gamma\delta$ TCRs, which don't require coreceptors for ligand recognition, lack CD3 $\delta$  in their TCR-CD3 complexes. A second outstanding question is whether, given that there are multiple pTyr residues in a triggered TCR-CD3 complex, multiple copies of coreceptors are recruited to a triggered TCR-CD3 complex? If so, these corecep-

tors could help aggregate TCR-CD3 complexes by binding other pMHC molecules, as proposed in the pseudodimer model (Irvine et al., 2002).

Jiang et al. (2011)'s results help resolve a debate that emerged when the structure of coreceptor-pMHC complexes were determined (reviewed in van der Merwe and Davis, 2003). These studies found that both CD8 and CD4 engaged the membrane-proximal portion of MHC class I and II, respectively, at an angle that was approximately perpendicular to the long axis of the TCR-pMHC complex. This appeared to preclude direct physical association of the coreceptor and TCR ectodomains, because they would be too far apart. This led to the proposal that when a TCR physically associates with a coreceptor, the TCR and coreceptor bind *different* pMHC molecules (Irvine et al., 2002). In fact, Jiang et al. (2011)'s results suggest that this is not necessarily the case; when TCR and CD8 associate through their cytoplasmic domains and intermediate molecules such as ZAP-70 and Lck, they are able to bind the same pMHC molecule (Figure 1).

Finally, Jiang et al. (2011)'s results provide some clues into coreceptor function. Coreceptors evidently play a role directing development of T cell precursors into functionally distinct CD8 or CD4 subsets, contingent on whether their TCR recognizes peptides presented on MHC class I or II, respectively. But what

is their role in mature peripheral T cells? The finding that coreceptors are recruited *after* TCR signaling is initiated by Lck phosphorylation explains why coreceptors are dispensable for T cell antigen recognition. They may, however, play an important modulatory role. For example, coreceptors amplify TCR triggering, thereby enabling recognition of low-affinity or rare pMHC complexes. Because affinity and abundance are both important limiting factors in pMHC recognition, this would broaden and increase the sensitivity of the TCR repertoire. A second possible role arises from the positive-feedback effect of coreceptor recruitment, whereby initial pMHC binding sufficient to induce TCR-CD3

phosphorylation is stabilized by coreceptor recruitment, which stabilizes the interaction and possibly enhances TCR-CD3 signaling. One effect of such a positive-feedback mechanism would be to improve the ability of T cells to discriminate between pMHC ligands with small differences in affinity.

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## CNS Autoimmune Inflammation: RICK Must NOD!

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In this issue of *Immunity*, Shaw et al. (2011) report that the NOD-RICK signaling axis is required for the activation of dendritic cells infiltrating the central nervous system, leading to reactivation of antigen-specific T cells and autoimmune inflammation.

The onset of autoimmunity is triggered by both environmental and genetic elements, contributing to the disruption of central and peripheral tolerance, resulting in the escape of autoreactive T cells from normal selection. In this issue of *Immunity*, work from the Kanneganti laboratory (Shaw et al., 2011) has shed some light on the mechanistic understanding how infections may trigger central nervous system (CNS) autoimmune inflammation. Infectious agents have long been suspected to play a role in the activation of autoreactive T cells, and recent studies have begun to unravel a relationship between infections and certain chronic autoimmune inflammatory diseases of the central nervous system (CNS), including multiple sclerosis (MS) (Getts and Miller, 2010). Multiple sclerosis is an autoimmune disease in which autoaggressive T lymphocytes specifically reactive to myelin

antigens are stimulated to initiate an inflammatory response in the CNS, leading to demyelination and subsequent axonal injury. Importantly, peptidoglycan (PGN), a major bacterial cell wall component, has been detected in APCs located in the brains of MS patients (Schrijver et al., 2001). One salient feature of MS is the relapsing and remitting nature of the disease. Interestingly, bacterial infections have been associated with the increased risk of MS exacerbations. Therefore, the mechanistic understanding of how infections might trigger CNS autoimmune inflammatory response will probably offer novel therapeutic strategies for the treatment of MS patients.

Because PGN-containing APCs have been detected in the brains of MS patients, Shaw et al. (2011) set out to determine the role of PGN-mediated signaling in the CNS autoimmune inflam-

matory response, which might yield important mechanistic understanding about the link between infections and MS exacerbations. Nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) NOD1 and NOD2 are the known cytosolic sensors of fragments of bacterial peptidoglycan, whereas Toll-like receptor 2 (TLR2) detects peptidoglycan at the cell surface. A dual-specificity protein kinase RICK (receptor-interacting protein-like interacting caspase-like apoptosis regulatory protein kinase, also called RIP2 or CARDIAK) functions downstream of NOD1 and NOD2 to mediate NF- $\kappa$ B and mitogen-activated protein kinase (MAPK) activation in a TLR-independent manner (Park et al., 2007). By using an animal model for CNS inflammation, experimental autoimmune encephalomyelitis (EAE), Shaw et al. (2011) attempted to investigate the relative