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Not Just Any T Cell Receptor Will Do

Minireview

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Although our structural understanding of T cell recognition has rapidly evolved due to recent crystallographic results, the reality is that detailed answers to many of the most fundamental questions still remain elusive. In this issue, high-resolution insight into the phenomenon of TCR chain bias takes down another brick from the wall.

In perhaps no other branch of biomedical sciences has the potential of structural biology been realized more vividly than in immunology. From the initial illuminating crystal structures of antibody-antigen complexes to the breathtaking first glimpse of an MHC molecule to recent breakthroughs with T cell and other antigen receptor complexes, we have come to expect high drama from the often marathon-like crystallographic endeavors.

In particular regard to T cell recognition, it is still early, but we can glean some "moderate resolution" principles that have emerged from the relatively few unique TCR/pMHC complex structures which comprise the current database (Figure 1A) (two class II complexes, six class I complexes) (reviewed in Hennecke and Wiley, 2001; Rudolph and Wilson, 2002). We use the term moderate resolution not in the crystallographic sense of angstroms but rather in the level of insight these structures have given us into fundamental immunological principles. We can say with some assurance that: (1) all TCRs will recognize pMHC with a roughly diagonal orientation, (2) the CDR3s have a primary role in readout of the bound peptide antigen, while CDRs 1 and 2 tend to recognize conserved features of the MHC helices, (3) conformational change in the TCR CDR loops appears to be a primary mechanism to enhance TCR crossreactivity, and (4) structural complementarity in the TCR/pMHC interface plays an important role in the half-life and functional consequences of TCR/pMHC interactions (Figure 1A).

Given that the technical challenges inherent in crystallization of these complexes remain daunting, an important issue is whether further structures of TCR/pMHC complexes, which incrementally recapitulate these principles, are redundant and of limited value to the field as a whole. Disappointingly, no general strategy has emerged for production and crystallization of the $\alpha\beta$ TCR, as has been the case for class I MHC, for which the Wiley group reported a refolding methodology that has had a profound impact on many areas of immunological research (Garboczi et al., 1992). Hence, the systems chosen for such studies need to be selected very carefully to ensure that the final result will reveal some novel aspects and provide new information key to understanding the immunological experiments.

Future structural challenges in T cell recognition can be broken down into large-scale macromolecular docking questions (Figure 1B) versus those that define the fine atomic details of the TCR/pMHC interaction. These latter details are far less dramatic to the uninformed eye than macromolecular topology questions but are really where the answers lie to many of the most elusive and fundamental immunological questions. The large-scale docking issues are clearly focused on how the various coreceptors all fit together into a functional TCR signaling complex (Figure 1B). While a recent NMR structure of a covalently-linked CD3$\gamma\epsilon$ heterodimer has been reported (Sun et al., 2001), we still do not know how it engages the TCR. Perhaps that odd "protrusion," the FG loop in the Cβ domain of the TCR, has a role in docking to CD3 (Figure 1B)? Studies from the Reinherz and Karjaleinen labs, which have deleted this loop in different TCRs, appear to be at odds with each other, in one case showing a fully functional TCR (Degermann et al., 1999), while in another resulting in aberrant signaling capabilities (Sasada et al., 2002). Alternatively, a structure in this issue of *Immunity* suggests that conformational change in the TCR Cα AB loop could be where the action is (Kjer-Nielsen et al., 2003). Hence, the disposition of the CD3 components (γ, δ, ϵ, ζ), which are an integral fixture of the TCR-CD3 complex, has yet to be determined. We now have complexes of class I and II MHC with CD8 and CD4 (Gao et al., 1997; Wang et al., 2001), respectively, and these have been fantastically revealing in that there appears to be little chance for simultaneous coreceptor binding to both MHC and TCR (Figure 1B) (discussed below). However, in the absence of the entire TCR/CD3/CD4 or CD8 complexes, conclusions about higher order assemblies derived from crystal structures of binary complexes could be deceiving, and remain speculative. The persistent controversy about TCR oligomerization (Baker and Wiley, 2001; Davis et al., 1998) during signaling has not been clarified by structural studies of the incomplete complexes (Figure 1B), which have so far not revealed any dimeric TCR/pMHC complexes but instead have revealed a mixed bag of monomers.

Turning to the details, perhaps the holy grail of TCR/pMHC structural studies is to describe the structural basis of MHC restriction. It may come as a surprise to many readers of *Immunity*, but we still have no idea how or why, at a structural level, the TCR repertoire is biased toward recognition of MHC. How is it that all $\alpha\beta$ TCRs have some degree of reactivity with all MHC molecules (Germain, 1990), as necessitated for thymic education (Nikolic-Zugic and Bevan, 1990) and TCR scanning of MHC in the periphery (Wu et al., 2002)? Despite roughly similar diagonal docking orientations over MHC ($\pm$35° from diagonal for all complexes to date), the TCR/pMHC

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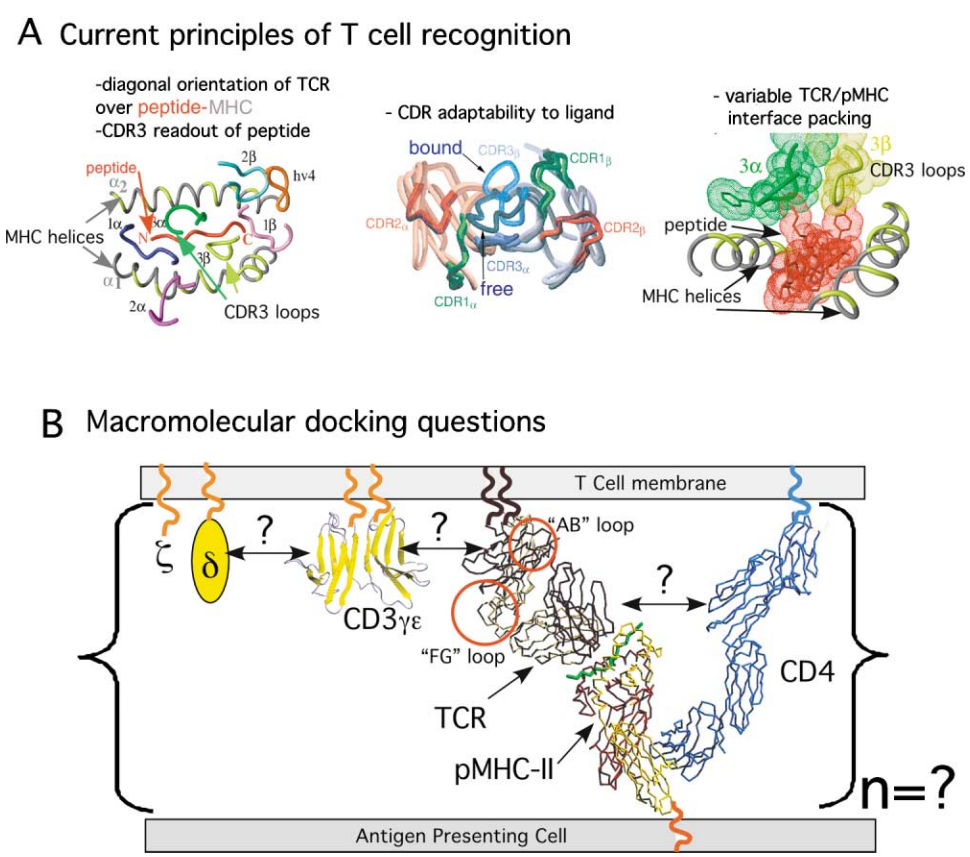

Figure 1. Past, Present, and Future Structural Issues in T Cell Recognition

(A) Recurrent structural features of TCR/pMHC complexes to date.

(B) Model of TCR-CD3-CD4 components within a T cell/APC interface (adapted from Wang et al., 2001).

complex crystal structures do *not* share sets of common contacts (Hennecke and Wiley, 2001; Rudolph and Wilson, 2002). One argument is that our V gene repertoires have evolved to only retain segments that will assume diagonal docking on the MHC. However, one can also argue that there may be many alternative orientations possible by random αβ pairs, but the thymus serves as a filtering step through which only the diagonal orientations can pass. The early notion that the thymic filter was the monomorphic CD4 and CD8 coreceptors bridging TCR and MHC through conserved binding epitopes on both, and thus enforcing a convergent topology on all TCR/MHC complexes, no longer appears plausible. Recent structures of both CD8 and CD4 complex with MHC I and II, respectively, show that it is unlikely that these coreceptors will make contact with the TCR (Figure 1B) (Gao et al., 1997; Wang et al., 2001). Hence, the TCR is free to assume any rotational docking topology on the MHC, yet somehow a common solution is enforced. The answer to this enigma likely lies within the interatomic contacts of the TCR/MHC interface, but the database of structures is not yet large enough for the conserved or cryptic epitope(s) to reveal itself in an obvious fashion. Perhaps, similar to the notion of anchor motifs defining rules for peptide binding to MHC, there will exist sets of anchor points between certain αβ pairs and MHC haplotypes (Madden, 1995) that define an overall geometry, while the remaining contacts impart specificity. It will require a large number of structures, in concert with focused immunogenetic experiments, to glean the rules—if they exist.

A related question, whose answer lies within the structural details of TCR/MHC complexes, is the basis for the phenomenon of chain bias. For MHC recognition, in principle, any αβ TCR pair should serve as an acceptable framework to endow with specificity for any pMHC through CDR3 diversification. However, experimental evidence has shown that certain antigens induce a highly restricted TCR repertoire from the almost limitless number of possible αβ pairs, while other antigens select a roughly random set of TCR heterodimers. One of the most striking examples is in the CTL response that arises naturally to Epstein Barr Virus. In this issue of *Immunity*, Kjer-Nielsen et al. describe the first structure of a TCR/pMHC complex composed of a "public" TCR (LC13) that is almost exclusively selected by HLA-B8 individuals for reactivity toward the latent antigen EBNA 3A (Kjer-Nielsen et al., 2003). As virtually all CTL against this antigen use the LC13 TCR, it is the most extreme example of chain bias known and, therefore, serves as a perfect target for structural studies to explain this phenomenon. Prior to this result, one could fathom a number of possible explanations to account for TCR chain bias (Figure 2) (Wallace et al., 2000). Some explanations have nothing to do with the structural aspects of the antigen recognition. For instance, perhaps regulatory T cells are

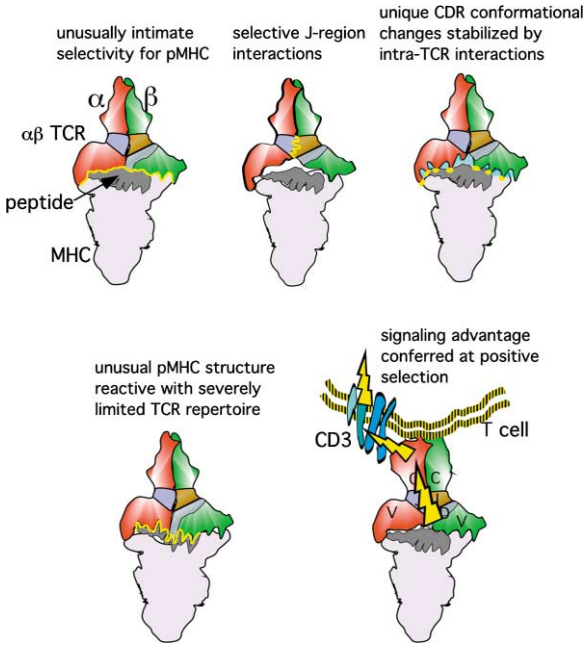

Figure 2. Possible Selective Pressures that Could Lead to Chain Bias in a TCR Repertoire

induced by EBV that suppress a broad TCR repertoire against antigens necessary for the persistence of this virus in its latent form. Or possibly LC13 may be endowed with nearly perfect affinity/avidity characteristics, giving it a significant signaling advantage during positive selection, while other αβ pairs may be deleted due to crossreactivity with self-antigens (Figure 2). These scenarios necessarily imply that, in principle, many possible TCR αβ pairs in the germline repertoire can effectively recognize and lyse cells that present the HLA-B8 peptide complex (HLA-B8/FLRGRAYGL). However, there may be a "hole in the repertoire," such that the LC13 TCR comprises the only pair of αβ sequences in our entire V gene set, which, working in concert as a heterodimer, is capable of recognizing this antigen (Figure 2). This latter scenario is unlikely, given that heterozygotic HLA-B8/B44 individuals, who cannot select the LC13 TCR due to self-tolerance, generate a robust and diverse repertoire of TCRs with specificity for HLA-B8/FLRGRAYGL. Finally, work from the Carbone group in the Herpes Simplex Virus (HSV) system has shown that TCR immunodominance can arise from preferential J region usage, which is neighboring and can be part of the binding interface with pMHC (Figure 2) (Wallace et al., 2000). Since Jα and Jβ form part of the VαVβ domain interface in the αβ heterodimer, it may be that the set of interactions between the particular J regions of LC13 are exclusive to one another and force the chain bias, rather than a unique antigen structure. This fact, though, would not explain the convergence on identical CDR3 amino acid sequences, which are not entirely encoded in the J region, that interact with the bound EBNA peptide antigen.

The crystal structure clearly tells us that the invariant nature of LC13 is due to the fine-tuned and refined structural chemistry required for efficient recognition of HLA-B8/FLRGRAYGL. In the complex, the principal finding is that an interconnected network of interactions from both V and J germline segments, as well as recombined N-, P-, and D-encoded segments, form specific contacts with both peptide and MHC. Many of these contacts are only possible for the TRAV26-2 Vα and TRBV7-8 Vβ used by LC13. The indirect nature of some of the highly specific contacts is exemplified, for instance, by a water-mediated hydrogen bond between the most critical peptide residue P7-Tyr and Vα framework residue His48 and CDR1α residue Tyr31. But the origin of the exquisite specificity is far more complicated than is revealed by simply adding up TCR/pMHC contacts. Upon binding the pMHC, a number of the CDR loops have undergone extensive conformational changes, disrupting their canonical unbound structures, to new conformations dictated by the recognition of HLA-B8/FLRGRAYGL. Astonishingly, one residue in CDR3α, Pro93, appears to play a key role as a "crumple point" to enable this CDR to adopt to a conformation optimal for peptide readout. The necessity of Pro93α, then, is not for direct antigen recognition but rather as an enabler of an ancillary structural rearrangement. And yet, such enabling residues throughout the LC13 binding site are no less critical to the final recognition solution as the residues participating directly in interatomic contacts. While more dramatic examples of TCR conformational change have been previously documented (Reiser et al., 2002), the concept of conserved noncontact enabling residues is very enlightening and suggests new levels of complexity in understanding TCR/pMHC interactions. In total, both germline and nongermline encoded sequences conspire to orchestrate a cooperative relay of inter- and intramolecular interactions by, and within, the LC13 binding site to achieve the final recognition mode. Underscoring the selective pressure on the LC13 TCR is the observation that many of the individual clones settle on the same CDR3α and β amino acid sequences using different recombination strategies, revealed at the nucleotide sequence level (Kjer-Nielsen et al., 2002). Hence, the restriction to LC13 is not based on some advantage conferred during recombination by the LC13 germline DNA segments. Given multiple possible CDR3 sequence solutions for recognition of HLA-B8/FLRGRAYGL, there is an exclusive convergence on LC13. This unequivocally tells us that the answer to why LC13 lies at the heart of the TCR/pMHC interaction chemistry.

What, then, is the advantage of this recognition solution over other possible modes of recognition by other TCR αβ pairs? The structure clearly tells us why LC13 is a very good choice, but it does not tell us why it is the *only* TCR which CTL will use in these individuals. Certainly, LC13 has not achieved some notably higher affinity for pMHC than other TCRs, as the rough estimate of K_d is only ~50 μM. One clue may be inferred from an interesting structural feature in this complex, a conformational change in the Cα domain AB loop (Kjer-Nielsen, 2003). As these authors had the opportunity to compare bound versus free TCR structures (Kjer-Nielsen et al., 2002), they observed a significant difference in a loop on the surface of the Cα domain that would appear poised for contact with CD3 subunits. From the initial TCR structures, the unusual noncanonical Ig-fold structure of the TCR Cα domain has been a puzzling deviation

from the more standard domain structures of the rest of the TCR (Garcia et al., 1996). The loose packing of the top strands has been proposed to be an ideal location for an induced fit-type interaction with CD3. One of the most critical issues in T cell recognition remains how TCR engagement leads to activation. Somehow, the CD3 subunits, which are docked alongside the TCR constant domains, must sense ligand binding. The TCR/pMHC recognition event is not simply a means to bring two molecules together, but the TCR is a conduit for sensing subtle structural differences and translating these differences into graded biological responses. The structural rearrangement seen in the LC13 Cα loop is by far the most thought-provoking evidence, so far, that a type of conformational transduction may accompany ligand binding, as has been recently shown for CD3 itself (Gil et al., 2002). The weakness of this supposition, though, is that we have not seen such a change in numerous other TCR/pMHC complexes and also that the conformational change could simply reflect an inherent flexibility in that region of the structure. Further, the key missing link is a structural explanation of how ligation at the TCR binding site is communicated to this small loop that is a great distance away from the TCR/pMHC interface. As always, this structure can now inform and focus further functional experiments aimed at addressing these important questions.

Our goal in this minireview is to give a sense that, although our structural understanding of T cell recognition is being furthered by studies of the kind described by Kjer-Nielsen et al. in this issue, it is far from complete. The picture of a TCR/pMHC complex may say a thousand words, but the dictionary for the language of T cell recognition will contain millions of words. Many, if not most, of the core questions in T cell immunology remain future challenges for structural biology. Unlike antibody-antigen and most receptor-ligand interactions, TCRs appear to possess the ability to respond differentially to MHC-bound peptides of very different chemical and structural composition. So far, we have seen structures of single TCRs with highly related peptide variants, but we have yet to see how a TCR can recognize vastly different structural entities, as has been clearly demonstrated in functional studies. The concept of molecular mimicry in autoimmunity, whereby a microbial pathogen induces autoreactivity to a self-antigen remains only a hypothesis (Oldstone, 1987). We do not have structures of an autoimmune TCR complexed with both autoantigen and crossreactive, perhaps microbial, peptide-MHC complexes. Is there really "structural mimicry" or in fact are the different pMHC ligands being recognized in entirely unique fashions? The extension of the crossreactivity question is how both structurally similar and dissimilar ligands can activate the TCR to varying extents. The simple relation between TCR/MHC complex half-life and bioactivity now has numerous exceptions, so what are the physical principles by which the recognition at the TCR combining site is translated into a signaling outcome?

As a last point, structural studies in T cell recognition increasingly need to dovetail with the emerging concept of the immunological synapse (Grakoui et al., 1999). Some of the results from fluorescent imaging experiments of TCR/APC interactions run counter to many of the models that structural biologists have proposed using the "lego building block" strategy to piece together larger assemblies from smaller pieces. For instance, in the mature synapse of the 2B4 TCR complexed with I-E^k, CD4 appears to be excluded from the central core to a ring surrounding the TCR/MHC clusters (Grakoui et al., 1999). Obviously, this puts the idea of a stable trimolecular TCR/MHC/CD4 signaling complex into question. A recent experiment from the Davis lab proposes the rather novel idea that the bridging ability of CD4 may, in fact, rely on the CD4 ectodomain grasping MHC-II at the N-terminal end, while the C-terminal region of the same CD4 molecule grasps another TCR/pMHC complex to form a "pseudo-dimer" (Irvine et al., 2002). In any case, the common knowledge that soluble CD4, CD8, and CD3 ectodomain constructs do not have measurable solution affinities for soluble TCR suggests we are all in for a surprise when we finally see how they all fit together. It may be that lipid and membrane components indigenous to rafts are necessary to reconstitute these multipartite signaling complexes.

No structure of a TCR/pMHC complex is completely redundant at this early stage of the game; each significantly adds to our knowledge base. Over 100 crystal structures of antibody-antigen complexes have been deposited in the PDB database, yet we continue to learn new principles about antibody-antigen and, more generally, protein-protein recognition with new structures from carefully chosen and revealing systems. Will the delineation of principles for T cell recognition require the same plethora of structural information as antibody-antigen complexes? Most certainly not, given that we are much more computationally advanced at modeling and analyzing protein structures than at any point in the past. However, the message for the future is clearly to identify systems whose structural study will lift us from our current plateau to the next levels of understanding, either in the large-scale complex docking issues or in the fine details of peptide-MHC recognition and restriction.

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