PERSPECTIVES: IMMUNOLOGY

The Immunological Synapse—a Multitasking System

P. Anton van der Merwe and Simon J. Davis

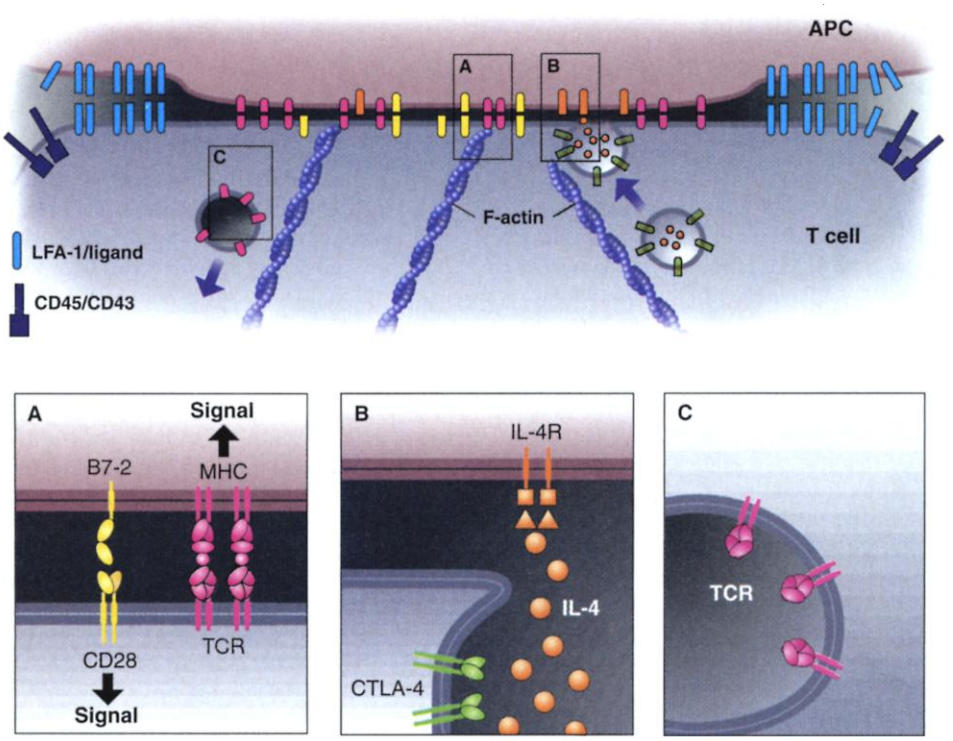
Before T cells of the immune system can recognize pathogens, antigen-presenting cells (APCs) must process pathogen-derived peptides and present them together with major histocompatibility complex (MHC) molecules to T cells. T cells then probe the surface of APCs and become activated when they recognize pathogen-derived peptide bound to MHC (peptide-MHC). This recognition depends on binding of the T cell antigen receptor (TCR) to peptide-MHC, and binding of T cell costimulatory receptors to their ligands also expressed on the APC surface (see the figure). Following initial signaling by the TCR, complexes of TCR bound to peptide-MHC segregate into different areas of the membrane interface between the T cell and APC, a dynamic structure called the immunological synapse (*1*–*3*). As this structure matures, it evolves into a characteristic bull's-eye pattern composed of an outer ring containing adhesion molecules (such as the integrin LFA-1 and its ligand) and a central area where the T cell and APC plasma membranes are in close proximity and the TCR and costimulatory receptors CD2 and CD28 (but not larger molecules) are clustered with their ligands (see the figure). Initially, it was thought that this molecular reorganization enhanced and sustained TCR signaling, and hence subsequent T cell activation. More recently, however, it has been argued that this reorganization is linked to other processes, for example, secondary (non-TCR) signaling and polarized secretion of cytokines by T cells (*4*). On page 1539 of this issue, Lee *et al.* (*5*) provide compelling evidence that formation of the immunological synapse does not initiate or enhance TCR signaling. They show that recognition of peptide-MHC on APCs by naïve CD4 T cells triggers signaling of TCRs well before the mature immunological synapse is formed. This finding implies that TCR signaling does not require formation of the immunological synapse, and that this structure may have other purposes.

P. A. van der Merwe is in the Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK. E-mail: anton.vandermerwe@path.ox.ac.uk S. J. Davis is at the Nuffield Department of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, UK. E-mail: sdavis@molbiol.ox.ac.uk

If the large-scale segregation of molecules within the mature immunological synapse does not facilitate TCR signaling, then why does it take place? Such reorganization may be required for the polarized secretion by T cells of cytokines directed toward target cells such as B cells. Indeed, the term "immunological synapse" was initially coined by Paul and Seder (*6*) to describe the interface between T cells and B cells, which seemed analogous to the synapse between two neurons across which secreted neurotransmitter travels. Helper CD4 T cells interacting with B cells secrete cytokines that instruct B cells to mature and produce antibody. Likewise, cytotoxic CD8 T cells secrete the contents of their lytic granules in a polarized fashion. Secretion is polarized perhaps to ensure that T cells communicate with or kill only the appropriate target cells: The products that they secrete need to be confined to the T cell: target cell interface.

Recently, Stinchcombe *et al.* showed that molecules at the CD8 cell:target cell interface segregate into a mature immunological synapse in a pattern reminiscent of that for molecules at the T cell:B cell interface. CD8 T cells release the contents of their secretory granules within the central area of the immunological synapse (*7*). It is possible that the close apposition of membranes in this central area may help to retain soluble products at the T cell:target cell or T cell:APC interface, thereby limiting the effects of these products on bystander cells (see the figure).

Typically, activation of T cells by TCR engaging peptide-MHC is dramatically enhanced by interaction of the CD28 costimulatory receptor with its ligands CD80 (B7-1) and CD86 (B7-2) on the APC surface. Using a planar lipid bilayer into which fluorescently labeled lipid-anchored proteins are incorporated, Dustin and colleagues have followed the interactions of CD28 with its ligands during im-



The center of attention. (**Top**) The organization of the immunological synapse formed at the interface between an antigen-presenting cell (APC) and a responding T cell. The central zone of this structure, from which large adhesion molecules such as the integrin LFA-1 (blue) are excluded, contains T cell receptors (TCRs; pink), CD28 costimulatory receptors (yellow), and other smaller molecules (*5*). (**A**) Engagement of the TCR with peptide-MHC on the APC surface results in peptide-MHC signaling within the APC. Interaction of the costimulatory receptor CD28 with its ligands, which results in secondary signaling, is enhanced in the central region of the immunological synapse. (**B**) Key effector molecules, such as the cytokine IL-4 and the inhibitory protein CTLA-4, delivered to the central area by exocytosis, limit effects on bystander cells. (**C**) The immunological synapse may be required for internalization of TCRs, which may be one way that TCR signaling is down-regulated.

munological synapse formation (*8*). On resting T cells, CD28 engaged poorly with lipid-anchored CD80, suggesting that these molecules do not readily interact, perhaps because of the small size, low surface density, and reduced lateral mobility of CD28. However, when formation of the mature immunological synapse was induced, CD28 readily engaged CD80 in the central area where TCRs cluster. Thus, CD28:ligand interactions appear to be enhanced by immunological synapse formation, perhaps through intimate membrane contacts within the central area. Interestingly, the structurally related T cell molecule CTLA-4, which binds to the same ligands as CD28 but blocks T cell activation, is transported from intracellular stores toward the region of the cell surface receiving activation signals (*9*). This suggests that binding of CD28 to its ligand may occur primarily at the center of the mature immunological synapse, and that CTLA-4 may be transported to this site under certain circumstances to block or reverse this effect. More generally, active formation of areas of intimate contact in the immunological synapse may facilitate the engagement of small molecules that enhance (or inhibit) antigen recognition by T cells.

One feature of the mature immunological synapse that is not explained by these events is the central clustering of TCRs. The actin cytoskeleton seems to be involved, possibly by interacting directly with the TCR-CD3 complex (*2*, *3*). Furthermore, clustered TCRs appear to be immobile (*2*), suggesting that they remain tethered to the cytoskeleton, perhaps acting as an organizing center for cytoskeletal remodeling processes that ensure close membrane contacts and contribute to secretion. Another possible reason for TCR clustering is that some peptide–MHC class II complexes on B cells are associated with a signal-transducing heterodimeric protein Igα/β, which requires engagement with TCR for intracellular signaling (*10*). We propose that TCR clustering in the immunological synapse enhances the accumulation of complexes of peptide–MHC II–Igα/β at the interface, thereby enhancing engagement with TCR and Igα/β signaling. In this way, activated CD4 T cells could selectively signal to B cells that present sufficient amounts of peptide–MHC II–Igα/β. Lee *et al.* propose an alternative purpose for immunological synapse formation. They argue that TCR clustering in the immunological synapse precedes endocytosis of TCRs, which may be one way that TCR signaling is down-regulated.

The Lee *et al.* results suggest that the dramatic molecular rearrangements that characterize the formation of the mature immunological synapse do not primarily enhance or sustain TCR signaling (*5*). We would like to propose that the immunological synapse is a dynamic multitasking system that integrates multiple cellular processes required for T cell activation and the execution of T cell effector activities.

References and Notes

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PERSPECTIVES: ENZYMOLOGY

A Moving Story

Joseph J. Falke

More and more structures of proteins and nucleic acids, including enzymes that catalyze chemical reactions, are known with high accuracy. Much less is known about the dynamics of these macromolecules and their role in biological function. For example, motions within an enzyme molecule may be necessary to lower the transition state barrier. On page 1520 of this issue, Eisenmesser *et al.* (*1*) use an elegant nuclear magnetic resonance (NMR) method to investigate the motions linked to substrate turnover in the enzyme cyclophilin A. Their approach should facilitate the search for motions linked to catalytic events in other enzymes.

A complete analysis of the dynamics of a typical macromolecule requires knowledge of thousands of atomic trajectories as a function of time. This remains challenging, but NMR and other methods have revealed some basic features of thermally driven macromolecular dynamics, particularly in proteins.

The author is in the Molecular Biophysics Program and the Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, USA. E-mail: falke@colorado.edu

Early protein NMR experiments detected ring-flipping motions of aromatic side chains, providing some of the first evidence that proteins are dynamic structures (*2*). Modern spectroscopic, time-resolved crystallographic, and computational studies have detected complex side chain and backbone thermal motions over time scales ranging from picoseconds to seconds (*3–8*). Chemical methods such as hydrogen exchange and disulfide trapping have probed thermal motions on longer time scales of microseconds to hours, revealing motional amplitudes as large as 1.5 nm on the millisecond time scale (*9–11*).

A protein in solution thus undergoes constant random thermal motions within a stable equilibrium structure. These motions involve displacements of individual atoms, bonds, functional groups, side chains, local regions of the backbone, secondary structure elements, and entire folded domains. Many proteins also undergo thermally driven transitions, called conformational changes, between two or more equilibrium structures.

Both types of motions can play important functional roles (*12–16*). Random thermal motions act as a molecular lubricant during conformational changes, allowing the protein to sample conformational space. Random thermal motions and the average conformation can both change substantially when a protein is modified by substrate or ligand binding, docking to another macromolecule, or covalent modification (such as phosphorylation). Such changes often have important functional consequences for the tuning of binding affinities and the switching of regulatory proteins. Yet, in a given macromolecule, only a subset of motions is important for biological function. The challenge is to identify these functionally relevant motions.

Dynamics play a role in certain aspects of enzyme function, but the links between dynamics and catalysis remain unclear. In the first step of an enzyme-catalyzed reaction, substrate binding typically induces a conformational change within the enzyme, thereby enclosing the substrate in a cavity protected from solvent or places the catalytic residues near the substrate (*14–16*).

An even more fascinating structural rearrangement may occur during the catalytic step of the reaction (*17*), when the complex moves from the ground state to the transition state. Such a transient rearrangement could simply serve to accommodate the structural changes in the substrate as the transition state is reached or could actively contribute to catalysis by preferentially stabilizing the transition state. Yet, because the transition state exists only fleetingly, enzyme dynamics during transition state formation and decay have never