water ice, is now known to be about half the volume previously thought. Although the Northern Hemisphere topography is such that water released during the catastrophic floods would have flowed toward the north cap, other processes must have acted as well—the cap is not located at the region of lowest elevation, and it has a "bubble" shape rather than the flat-topped shape that a frozen lake would yield. Together, these require that the last major process acting on the ice was its transport as vapor through the atmosphere and freezing out at the pole; clearly, the polar ice is an indicator of climate-related activities. The "reduction" in the size of the cap exacerbates a water-inventory problem that had been previously recognized. On the basis of the amounts of water thought to have been released to the surface by geological processes and the amounts that currently reside in the polar cap, a substantial amount of water must be somewhere else. Although some may have percolated back into the crust, the majority may have escaped to space along with the early atmosphere.

There is a strong connection between the expectation of finding life on Mars and the presence of liquid water, either at the surface or within the crust. In turn, there is a connection between the presence of liquid water, the geological, geochemical, and geophysical history of Mars, and the history of the atmosphere's interactions with the solar wind. The recent Surveyor results underscore this complicated interweaving of the various processes. At the same time, they point to a history of water and geochemical processes on Mars that would allow the planet to support life (*4, 8, 10*). The question that needs to be addressed, and that will be addressed by sample return missions over the next decade, is whether there ever was life on Mars. Whatever the answer, it will have a large impact on our understanding of the nature and occurrence of life in the universe.

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PERSPECTIVES: IMMUNOLOGY

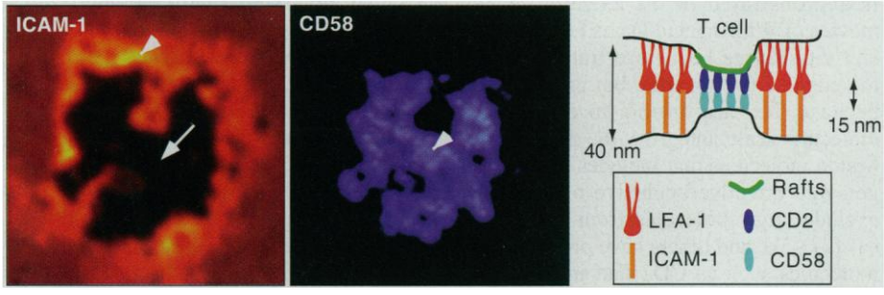
Costimulation: Building an Immunological Synapse

Michael L. Dustin and Andrey S. Shaw

A central event in the development of immunity is the activation of the T cell. At the center of this process is the T cell receptor (TCR), which triggers activation by a specific interaction with antigen [usually a foreign peptide bound to, or "presented by," the organism's own major histocompatibility complex (MHC) molecule on the surface of an antigen-presenting cell]. Because of the small size of the TCR, its low affinity toward antigen, and the limited numbers of antigens on the antigen-presenting cell, an elaborate adhesion complex must be formed to allow the TCR to contact, sample, and then be activated by the rare antigenic ligands (*1*). This specialized contact area has been termed the immunological synapse (*2*, *3*). Reports by Wülfing and Davis in a recent issue (*4*) and Viola *et al.* (*5*) on page 680 of this issue focus attention on how the immune synapse is built and unify what had been thought of as two distinct signals needed for efficient T cell activation.

Efficient T cell activation requires engagement of at least two types of T cell surface receptors. This phenomenon has been interpreted in terms of a "two-signal model," which proposes that T cell activation requires one signal from the TCR and a second signal from a "costimulator" molecule. Although many molecules have been implicated as costimulators, CD28 has become the archetype for costimulatory molecules. Engagement of CD28 either by its ligand on the antigen-presenting cell [B7 (CD80)] or by antibody can strongly enhance TCR signaling responses. Although current models suggest that CD28 functions as a specific activator of the Jun kinase JNK or the nuclear transcription factor NF-κB, CD28 engagement by itself is not sufficient. Activation of either JNK or NF-κB always requires coengagement of the TCR. This has led to a counterproposal that costimulation might function to amplify the signals transduced by the TCR (*1*).

In a recent issue of *Science*, Wülfing and Davis (*4*) demonstrated a novel mechanism for costimulation in formation of the immune synapse. They demonstrate that costimulation initiates active directional transport of protein and lipid domains to the area of cell-cell contact. This transport process requires myosin and correlates with enhanced, as well as sustained, signaling—a hallmark of costimulation. In these experiments, directed transport could be stimulated by either CD28 or LFA-1 engagement, but occurred most efficiently when both were engaged together. Wülfing and Davis propose that costimulation works by activating an actin-myosin–driven transport process that delivers receptors and signaling complexes to the contact area. In this study, however, the transport process appears to be



Topological anatomy of the immune synapse. (Left) The pattern of LFA-1 and CD2 engagement in an activated T cell contact with an artificial membrane containing CD58 (blue) and ICAM-1 (red) (*3*). Arrowhead indicates the area of engagement of respective ligands. Arrow indicates exclusion of 20-nm-diameter ICAM-1 from the 15-nm contact formed by interaction of CD2 and CD58. **(Right)** The position of sequential molecular filters (40 and 15 nm) that are encountered by complexes such as rafts as they are transported toward the center of the immune synapse. The TCR clusters in the central 15-nm region (*13*).

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indiscriminate, and the key cargo was not clearly identified.

In this issue of *Science*, Viola *et al.* (*5*) show that the cargo for the actin-based transport mechanism is the 70-nm-diameter lipid rafts also referred to as caveolae or detergent-insoluble glycolipid domains (*6*). The rafts are initially distributed evenly on the T cell surface and remain so after engagement of TCRs by beads coated with antibody to the TCR. Remarkably, engagement of CD28 together with the TCR recruits essentially all the rafts to the contact area. This correlates with an increased lifetime for tyrosine phosphate, which may occur through phosphatase exclusion, and increased consumption of the Lck kinase, indicative of greater tyrosine kinase activation. It has been suggested recently that engaged TCRs migrate into rafts (*7*). Viola *et al.* now demonstrate that it is the rafts that migrate to engaged TCRs and CD28.

These studies suggest that costimulation modulates the signaling environment around the engaged TCRs. Rafts are rich in kinases and adapter molecules that are required for T cell activation (*8*). In addition, the rafts' topological features are also compatible with their promoting sustained TCR engagement. Because glycolipids and small glycophosphatidylinositol-anchored molecules such as CD59, DAF, alkaline phosphatase, and Thy-1 are concentrated in rafts, these domains may represent regions of reduced steric hindrance where interaction of the short TCR and MHC would be favored. In addition, cholesterol in lipid rafts may increase membrane rigidity and enhance the affinity of membrane protein interactions.

The costimulation-initiated transport mechanism appears capable of transporting anything linked to actin. Because both positive and negative regulators of T cell signaling may be associated with the actin cytoskeleton (*9*), how does the process achieve selectivity? One type of selectivity is demonstrated in the extreme by the movies of Wülfing and Davis (*10*): size selectivity. Large beads are transported to the edge of the synapse, but are excluded because they are too big to enter. On a molecular scale, integrins, the group of adhesion molecules that includes LFA-1, can generate effective occlusive barriers that exclude large molecules from contact areas (*11*). We and others have proposed that molecules such as CD2 that interact with ligands to generate very small gaps (<15 nm) between apposed membrane are also involved in large-molecule exclusion (*1*, *12*). If the actin-based transport process can convey molecules to the center of the immunological synapse, then these barriers could be conceived of as molecular filters allowing only small molecules to enter the contact area, while excluding molecules with larger ectodomains (see the figure).

The conventional view of T cell signaling was that each type of receptor generates its own distinct signal or has its own "voice." This collection of independent voices from the surface was then harmonized (integrated) in the nucleus to regulate transcription. The new concept that is emerging suggests that the immune synapse functions to tune, adjust, and amplify a single voice, the signal transduced by the TCR. TCR signaling is intimately associated with contact formation because extended cell contact is required to maintain TCR engagement. This new concept is supported by recent studies of molecular organization of components in the immunological synapse and by the demonstration that specific transport mechanisms organize the contact area. Although we do not yet know whether CD28 and LFA-1 produce specific biochemical signals to initiate this transport process, a new paradigm for immunological costimulation is emerging that is built around the central role of contact formation in T cell activation.

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PERSPECTIVES: SIGNAL TRANSDUCTION

The Path to Specificity

Charles S. Zuker and Rama Ranganathan

Signal transduction systems in a typical eukaryotic cell consist of a network of proteins that transform multiple external stimuli into appropriate cellular responses. Molecules that form this network can be placed into ordered biochemical pathways in which signal propagation occurs through the sequential establishment of protein-protein and small molecule–protein interactions. A major challenge in the study of intracellular signaling has been the elucidation of the physical and biological principles by which the network of signaling molecules is assembled to execute temporally and spatially ordered signaling programs.

How does specificity arise in connecting a given input signal with the appropriate cellular response? How is "crosstalk" between pathways avoided when detrimental but promoted when necessary? In addressing these questions, recent work has begun to focus on the organization signaling components into macromolecular assemblies. These assemblies are mediated by multifunctional adapter proteins that are critical for both efficiency and specificity of signaling. By recruiting the appropriate assortment of signaling proteins together, adapters organize signaling pathways into distinct functional entities (*1*, *2*). Adapter molecules range from very simple to complex multidomain proteins that contain different numbers, varieties, and combinations of modular protein-protein interaction motifs.

Some of the best studied intracellular cascades are the tyrosine kinase and G protein–coupled receptor (GPCR) pathways. In the case of receptor tyrosine kinases, recruitment of specific adapter proteins (Grb2 and Shc, for example) creates a tyrosine phosphoprotein scaffold that is anchored at the plasma membrane and serves as an organizing center for components of the mitogen-activated protein (MAP) kinase pathway (*1*). Proteins assembling into this complex vary in different receptor systems, thus allowing functional diversity through modular reorganization of the signaling complex. Recently, multi–PDZ domain proteins have been shown to act as scaffolds for organizing neuronal G protein–coupled signaling proteins. In *Drosophila* photoreceptors, a five–PDZ domain protein known as InaD assembles components of the visual signaling pathway into a macromolecular complex (*3*, *4*). Flies homozygous for a null allele of InaD show mislocalization of all target proteins in photoreceptor cells and dramatic loss of signaling (*4*). Thus, in the world of intracellular real estate, location, location, and location are key determinants of in vivo function.

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