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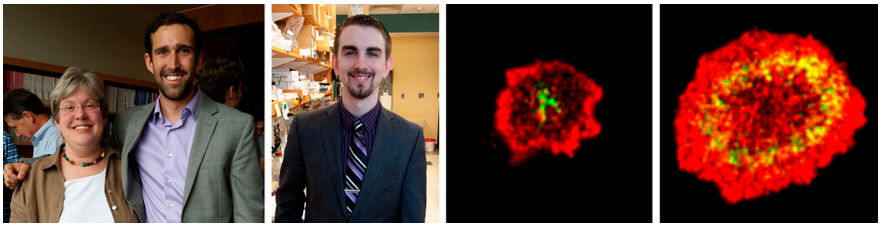
Actin works both sides of the immunological synapse

The cytoskeleton of both T cells and antigen-presenting cells promotes mechanical signaling during T cell activation.

Focal Point



PHOTOS COURTESY OF THE AUTHORS

(Left to right) Jan Burkhardt, Drew Comrie, Alex Babich, and colleagues (not pictured) examine the functions of actin on both sides of the immunological synapses that form between antigen-presenting cells and T cells. The researchers find that actin helps immobilize ICAM-1 on the surface of antigen-presenting dendritic cells. By binding to the T cell integrin LFA-1, immobilized ICAM-1 resists the forces exerted by the T cell's actin cytoskeleton, generating tension that promotes LFA-1's conversion to an active, high-affinity conformation that enhances T cell activation. Immunofluorescence stainings for active (green) and total (red) LFA-1 show that the proportion of the integrin in its active conformation is greater in T cells plated on immobilized ICAM-1 (right) than in cells attached to soluble ICAM-1 (left).

Antigen-presenting cells (APCs) activate T cells by forming a specialized contact site called the immunological synapse (IS). The T cell receptor (TCR) and its downstream signaling molecules cluster in the center of the IS, surrounded by a ring of integrin molecules such as LFA-1, which lower the threshold for T cell priming by both tightly adhering to ligands on the surface of the APC and by activating downstream signaling pathways of their own. Two papers now reveal that the actin cytoskeleton on both sides of the IS promotes the full activation of LFA-1 in order to enhance T cell priming (1, 2).

Stimulation of the TCR induces the T cell's F-actin network to flow toward the center of the IS. Jan Burkhardt and colleagues at the University of Pennsylvania in Philadelphia previously found that this centripetal flow is required to sustain downstream calcium signaling (3). The forces generated by the flow might also support mechanical signaling events at the IS. Whether the TCR itself is capable of mechanotransduction remains controversial but, in other cell types, integrins have been suggested to alter their conformation and ligand affinity in response to mechanical forces. "We decided to ask whether the actin flow causes conformational changes in LFA-1," Burkhardt says.

> "Actin flow... induces a conformational change to the high-affinity form."

Led by graduate students Drew Comrie and Alex Babich, Burkhardt and colleagues initially used conformation-specific antibodies to examine the distribution of the inactive, intermediate, and fully active forms of LFA-1 at the IS (1). "LFA-1's conformational intermediates are not distributed uniformly across the synapse," Burkhardt explains. "The more active forms are located more centrally."

Freezing the centripetal flow of actin disrupted this organization so that the different conformations of LFA-1 became randomly distributed. Moreover, inhibiting actin dynamics lowered the total amount of LFA-1 recruited to the IS and reduced the proportion of molecules in the active conformation. "So the actin flow causes a net accumulation of LFA-1 and induces a conformational change to the high-affinity form," Burkhardt says.

By increasing both the valency and affinity of LFA-1, the actin flow helped ICAM-1, the integrin's ligand on APCs, bind and accumulate at the IS. However, the researchers discovered, ICAM-1 in turn affects the behavior of LFA-1. The integrin was strongly activated when T cells attached to surface-bound ICAM-1 but not when they were stimulated by soluble ICAM-1. This suggests that immobilized ICAM-1 may resist the forces exerted on LFA-1 by the T cell's actin network, enhancing the integrin's shift to the high-affinity conformation. But, the researchers found, resisting these forces in other ways wasn't sufficient to fully activate LFA-1, indicating that the "induced fit" of the integrin's cognate ligand also drives conformational change.

Burkhardt and colleagues wondered whether the APC's actin cytoskeleton helps immobilize ICAM-1 at the cell surface in order to enhance LFA-1 and T cell activation. Little is known about the function of actin on this side of the IS, but actin-depolymerizing drugs inhibit the ability of APCs to prime T cells (4). Comrie et al. found that ICAM-1 was normally immobilized on the surface of antigen-presenting dendritic cells, but depolymerizing the cells' actin network liberated the molecule to roam more freely (2).

ICAM-1's mobility decreased during dendritic cell maturation, a process accompanied by increased expression of two actin-binding proteins, moesin and α-actinin-1, that are known to associate with ICAM-1's cytoplasmic tail. Knocking down these proteins, or deleting ICAM-1's tail domain, increased ICAM-1's mobility at the cell surface. "And if we liberate ICAM-1, we get reduced adhesion to T cells and T cell activation is less efficient," Burkhardt says. LFA-1's conversion to the high-affinity conformation was also less efficient, supporting the idea that ICAM-1's actin-dependent immobilization at the APC surface enhances the integrin's activation by resisting forces generated by the T cell's actin cytoskeleton. The next questions, says Burkhardt, are how the adhesion and signaling activities of active LFA-1 promote T cell priming, and how mechanical forces at the IS affect T cell functions in vivo.

1. Comrie, W.A., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201406121.
2. Comrie, W.A., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201406120.
3. Babich, A., et al. 2012. *J. Cell Biol.* 197:775–787.
4. Al-Alwan, M.M., et al. 2001. *J. Immunol.* 166:1452–1456.