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Blood orchestrates a leukocyte integrin trio

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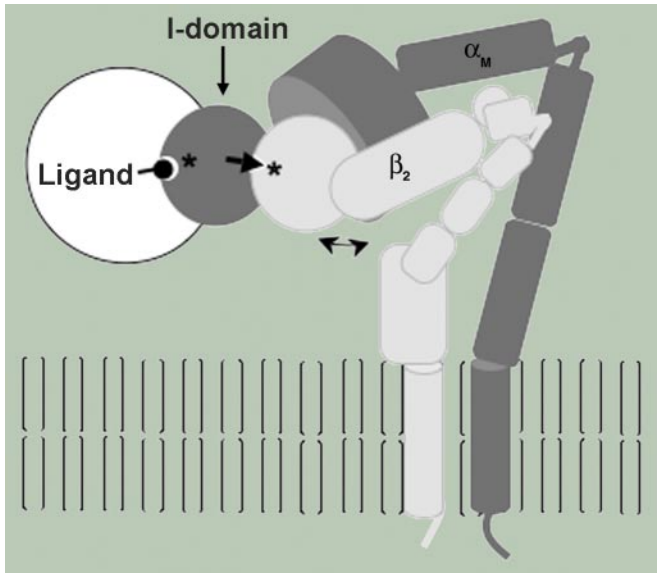
The regulated activation of leukocyte integrins is essential for normal host defense because it restricts their activity to sites of bacterial invasion and inflammation and spares normal tissue from toxicity. In this issue of *Blood*, 3 papers focused on the regulation of β_2 integrins in leukocytes shed further light on the structure and function of this essential family of proteins.



The bent conformation of the leukocyte integrin $\alpha_M\beta_2$ can bind ligands. See the complete figure in the article beginning on page 3513.

This is an exciting time to be an "integrinologist." The recent publication of crystal structures for the extracellular domains of the integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, the discovery of specific interactions between integrin subunit transmembrane domains, and solution structures for integrin cytoplasmic domains, either alone or bound to cytoplasmic ligands, have provided details about integrins at the atomic level.

The crystal structure of the extracellular headpiece of inactive $\alpha_v\beta_3$ revealed that it was bent in half such that its membrane-distal and membrane-proximal domains were in proximity and its ligand-binding site was pointed toward, rather than away, from the cell membrane.[1] There are 2 schools of thought as to how this structure can be converted to an active integrin. One suggests that the bent conformation can be active after contact between proximal and distal segments of the β subunit is disrupted[2]; the other suggests that active integrins are extended, not bent, and constraints on activation are located in the transmembrane and cytoplasmic domains.[3] Gupta and colleagues addressed this controversy, using the leukocyte integrin $\alpha_M\beta_2$ (aka, Mac-1 or CD11b/CD18), by mutating a 4-residue sequence in a membrane-proximal loop of β_2 that according to the $\alpha_v\beta_3$ crystal structure should be in contact with the membrane-distal domain. The authors found these mutations produced constitutive $\alpha_M\beta_2$ activation. Moreover, the mutants were unable to interact with a monoclonal antibody that binds to the extended conformation of β_2. Thus, these results suggest that integrins can be both bent and active and that contact between membrane-distal and membrane-proximal β subunit segments modulates the activation process (see figure). Nonetheless, this is not the last word on the conformation of active integrins because at the very least, the geometry of a bent integrin and the accessibility of its ligand-binding site still remain difficult to reconcile.

Accessibility of the ligand-binding site on $\alpha_M\beta_2$ is the topic of the paper by Eisenhardt and colleagues. $\alpha_M\beta_2$ belongs to an integrin subgroup whose α subunits contain an I-domain (or inserted domain) that is responsible for ligand binding. Although one might predict that the I-domain–binding site would be an apt target for generating integrin inhibitors, this has proven not to be the case, and previous attempts to target I-domains have produced only allosteric activation inhibitors.[4] Using an elegant phage display technique, Eisenhardt et al were able to generate 3 single-chain antibodies that specifically bound to the active conformation of the α_M I-domain. This work is notable for 3 reasons. First, the antibodies bound at the ligand-binding site, indicating that this site is readily accessible to exogenous molecules. Second, cyclic peptides whose sequences were derived from the antibody heavy chain CDR3 regions were able to inhibit the $\alpha_M\beta_2$-mediated adhesion of cells to a fibrinogen matrix, suggesting that the antibodies, or their derivatives, may be useful anti-inflammatory agents. Lastly, antibody binding to monocytes from patients with sepsis was increased, implying that these antibodies could be used to diagnose the septic state.

The identity of signaling pathways that cause integrin activation in cells is not clear. In this context, Kuijpers and colleagues report studies of the fascinating leukocyte adhesion deficiency type 1 (LAD-1)/variant syndrome in which patients manifest symptoms of both LAD-1 and Glanzmann thrombasthenia. While integrins are expressed normally on the leukocytes and platelets of these patients, they cannot be activated, implying that common signaling pathways regulate integrins in leukocytes and platelets. Defective Rap1 activation was reported previously in an Arab patient with the syndrome,[5] but Kuijpers et al found that activation of this small GTPase was normal in their patients, all of whom were descendents of Turkish ancestors from Anatolia. Thus, the defects responsible for the LAD-1/variant syndrome appear to be heterogeneous. Nonetheless, deciphering these defects should provide important clues as to how cellular signaling pathways regulate integrin function.

Taken together, these reports show that despite atomic-level understanding of integrin structure, there is still much to learn about integrin function. It is still good to be an integrinologist!

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