Integrin-associated protein (CD47): an unusual activator of G protein signaling

Commentary

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Integrin-associated protein (IAP), also known as CD47, was first discovered as a protein physically and functionally associated with the integrin αvβ3 but now is known to associate with additional integrins, such as α2β1 and αIIbβ3, and also to have ligands of its own. This very unusual member of the immunoglobulin (Ig) superfamily includes a single Ig domain, followed by a very hydrophobic region that spans the membrane five times, and a short cytoplasmic tail that demonstrates tissue-specific expression of four alternatively spliced isoforms. IAP binds both the large plasma and ECM glycoprotein thrombospondin (TSP) and a cell-based ligand, SIRPα, independent of its association with integrins. Ligation of the integrin/IAP complex on a variety of cells can induce adhesion, chemotaxis, spreading, secretion, and other sequelae of cell activation.

The work by Brittain et al., reported in this issue of the *JCI* (1), suggests that IAP-mediated activation may extend to circulating reticulocytes, cells not normally thought to respond to environmental cues by changing adhesive behavior. IAP may therefore play an important role in sickle-cell disease, in which large numbers of reticulocytes circulate and can adhere within small blood vessels. This work not only has implications for the pathophysiology and treatment of sickle cell anemia, but also raises important questions about the mechanisms of IAP signal transduction.

The IAP/integrin complex initiates signaling by an unusual mechanism. Ligand engagement of the integrin/IAP complex can activate heterotrimeric G protein signal transduction (2). To date, the only Gα subunit identified that is associated with this membrane complex is $G\alpha_i$, and indeed, most of the identified functions of the complex are sensitive to pertussis toxin, which targets this subunit.

Activation of heterotrimeric G protein signaling by the integrin/IAP complex

Integrin, IAP, and heterotrimeric G proteins can be coprecipitated as a complex whose integrity is absolutely dependent on cholesterol. Since most other receptors that activate heterotrimeric G proteins are members of the heptaspanin family, one appealing model for activation by IAP/integrin holds that the five membrane-spanning segments of IAP and the two membrane-spanning domains of the heterodimeric integrin form an ad hoc seven-transmembrane receptor. Ligation of this complex with an adhesive ligand or TSP could activate GTPase activity in a manner analogous to that of conventional heptaspanins.

The pertussis sensitivity of TSP signaling on SS reticulocytes, demonstrated in the present study (1), might suggest that IAP ligation, even in the absence of integrin association, is sufficient to activate heterotrimeric G protein signaling. However, erythroid precursors certainly do express integrins, and work from 15 years ago suggested that loss of integrin-mediated adhesion was necessary for release of mature erythrocytes from the bone marrow (3). Thus, it remains possible that there are IAP-associating integrins on reticulocytes that might be necessary for the signaling demonstrated in the work from Brittain et al. An interesting alternative possibility is suggested by the prominent requirement for shear stress in IAP-induced reticulocyte adhesion to TSP, since shear has not been previously shown to have a role in IAP signaling events. The requirement for shear might imply that integrin and IAP do not need physical association to activate G protein signaling, but that some necessary signal common to integrin ligation and shear stress is required for an effective IAP–G protein link. A further possibility is that an IAP-containing complex exists on the surface of reticulocytes that does not contain integrins, but does contain other receptor molecules, perhaps heptaspanins, that provide a link to G_i. Elucidation of whether IAP associates with other molecules in a signaling complex on reticulocytes — and, if so, the nature and response to shear of the associated molecules — will certainly shed new light on the molecular mechanisms of IAP signal transduction.

G protein–dependent and –independent functions of IAP

While neutrophil activation, platelet activation, and now reticulocyte activation appear to occur downstream of G_i, a number of other functions of IAP in other cell types may not. Ligation of IAP can synergize with T cell receptor or CD28 signaling to induce IL-2 production, an effect that is not inhibited by pertussis toxin (4–6). Whether other IAP-dependent signals in lymphocytes and monocytes/macrophages are pertussis-sensitive has not been determined. For instance, ligation of IAP on T cells and B cells can induce apoptosis and failure of Th1 development (7, 8). Similarly, ligation of macrophage IAP inhibits IL-12 synthesis in response to IFN-γ (9), and ligation of the αvβ3/IAP complex by sCD23 induces monocyte production of IL-6 (10). Thus, it remains possible that IAP also may initiate G protein–independent signal transduction. While pertussis-insensitive IAP signaling can cause actin polymerization and translocation of the θ isoform of protein kinase C in T cells (11), nothing is known of the molecular basis for initiation of this pathway. It has recently been suggested that IAP can aid self-nonself discrimination, since the absence of IAP on erythrocytes leads to their rapid clearance from the circulation (12). This role for IAP seems to be intimately tied to its recognition by SIRPα on splenic macrophages; it is not clear that IAP signal transduction is required in this circumstance at all.

It is possible that TSP and SIRPα, the two different ligands for IAP, have very different consequences for IAP signal transduction. TSP1 binds to IAP via a peptide sequence in its carboxyterminal domain — RFYVVMWK — that is conserved among the products of all five TSP genes (13). Thus, although it has never been tested, it is likely that all TSP isoforms bind to IAP. In contrast to SIRPα, which clearly binds to the IAP Ig domain, the interaction site on IAP for TSP has not been mapped. Peptides, chemokines, catecholamines, and other ligands bind to the extracellular loops or hydrophobic pockets within the multiply multiple-membrane-spanning domains of conventional G protein–coupled receptors (14). Thus, it is possible that TSP binds to the multiply membrane-spanning domain of IAP, rather than, or in conjunction with, its binding to the Ig domain. This could be a critical difference in the effects of IAP ligation by TSP and by SIRPα: whereas TSP and the RFYVVMWK peptide can activate G_i signaling, this has never been shown for SIRPα.

Whether SIRPα and TSP1 can bind to IAP simultaneously is unknown; synergistic or antagonistic effects of the two ligands on IAP signaling are equally possible. This unanswered question is of particular relevance to the role of IAP in sickle-cell disease, since endothelial cells can express SIRPα. Thus, both IAP ligands may be present at sites of vascular thrombosis; the net effect of their simultaneous presence on IAP signaling is unknown. Although IAP has been shown to be involved in a variety of inflammatory and immune responses, the study by Brittain et al. is the best demonstration to date of how IAP ligation could contribute to pathophysiology of a specific disease. Further understanding of the biochemistry and function of this interesting, ubiquitous, and apparently unique molecule may give clues to where — and whether — IAP-directed therapy will have a role in treatment of sickle-cell or other diseases.

1. Brittain, J.E., Mlinar, K.J., Anderson, C.S., Orringer, E.P., and Parise, L.V. 2001. Activation of sickle red blood cell adhesion via integrin-associated protein/CD47–induced signal transduction. *J. Clin. Invest.* **107**:1555–1562.
2. Brown, E.J., and Frazier, W.A. 2001. Integrin-associated protein (CD47) and its ligands. *Trends Cell Biol.* **11**:130–135.
3. Patel, V.P., Ciechanover, A., Platt, O., and Lodish, H.F. 1985. Mammalian reticulocytes lose adhesion to fibronectin during maturation to erythrocytes. *Proc. Natl. Acad. Sci. USA.* **82**:440–444.
4. Ticchioni, M., et al. 1997. Integrin-associated protein (CD47) is a comitogenic molecule on CD3-activated human T cells. *J. Immunol.* **158**:677–684.
5. Waclavicek, M., et al. 1997. T cell stimulation via CD47: agonistic and antagonistic effects of CD47 monoclonal antibody 1/1A4. *J. Immunol.* **159**:5345–5354.
6. Reinhold, M.I., Lindberg, F.P., Kersh, G.J., Allen, P.M., and Brown, E.J. 1997. Costimulation of T cell activation by integrin-associated protein (CD47) is an adhesion-dependent, CD28-independent signaling pathway. *J. Exp. Med.* **185**:1–11.
7. Mateo, V., et al. 1999. CD47 ligation induces caspase-independent cell death in chronic lymphocytic leukemia. *Nat. Med.* **5**:1277–1284.
8. Avice, M.N., Rubio, M., Sergerie, M., Delespesse, G., and Sarfati, M. 2000. CD47 ligation selectively inhibits the development of human naive T cells into Th1 effectors. *J. Immunol.* **165**:4624–4631.
9. Armant, M., et al. 1999. CD47 ligation selectively downregulates human interleukin 12 production. *J. Exp. Med.* **190**:1175–1182.
10. Hermann, P., et al. 1999. The vitronectin receptor and its associated CD47 molecule mediates proinflammatory cytokine synthesis in human monocytes by interaction with soluble CD23. *J. Cell Biol.* **144**:767–775.
11. Rebres, R.A., Green, J.M., Reinhold, M.I., Tichioni, M., and Brown, E.J. 2001. Membrane raft association of CD47 is necessary for actin polymerization and protein kinase C-theta translocation in its synergistic activation of T cells. *J. Biol. Chem.* **276**:7672–7680.
12. Oldenborg, P.A., et al. 2000. Role of CD47 as a marker of self on red blood cells. *Science.* **288**:2051–2054.
13. Bornstein, P., and Sage, E.H. 1994. Thrombospondins. *Methods Enzymol.* **245**:62–85.
14. Coughlin, S.R. 1994. Expanding horizons for receptors coupled to G proteins: diversity and disease. *Curr. Opin. Cell Biol.* **6**:191–197.