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Disabling multiple integrins from the inside out

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In the days before it became routine to knock out genes in mice, the identification of the genetic basis for rare immunodeficiencies in humans was often critical to establishing the physiological significance of a gene product. A prime example of the scientific utility of these immunodeficiences has been in the analysis of integrins, a family of αβ heterodimeric cell surface receptors that mediate cell adhesion to other cells and to extracellular matrix components (1). First reported in 1979, leukocyte adhesion deficiency–1 (LAD-1) is a rare autosomal-recessive immunodeficiency characterized by recurring, life-threatening bacterial infections and persistent leukocytosis (2, 3). The genetic basis for LAD-1 lies in germline mutations in the gene encoding the β2 integrin subunit (CD18), which impair cell-surface expression of all three members of the β2 integrin subfamily: lymphocyte function associated antigen–1 (LFA-1), Mac-1, and p150,95. Consequently, neutrophil function is dramatically impaired in LAD-1 patients, as neutrophils utilize β2 integrins to adhere to endothelium, migrate, and ingest C3bi-coated microorganisms. Lymphocytes and NK cells also utilize β2 integrins, and in vitro studies of T cells and NK cells from LAD-1 patients revealed impaired functional responses (4). However, LAD-1 patients generally do not suffer from severe viral infections, and delayed-type hypersensitivity responses in vivo are not significantly altered. This suggests that other integrin subfamilies, such as the β1 integrin subfamily, can compensate for the loss of lymphocytic β2 integrin expression in LAD-1 patients. Similar studies of an equally rare bleeding disorder known as Glanzmann thrombasthenia (GT) revealed various germline mutations in the gene encoding the β3 integrin subunit, which is expressed at high levels on platelets (2). As with LAD-1, these mutations typically result in loss of expression of the αIIbβ3 integrin on the platelet surface. Thus, platelets from GT patients fail to aggregate during the wound response, since this adhesive response requires binding of soluble fibrinogen by the αIIbβ3 integrin.

While both LAD-1 and GT indicate critical roles for β2 and β3 integrins in the function of hematopoietic cells, in vitro studies show that impairing cell-surface expression is not the only way to disable an integrin. The functional activity of integrins expressed on circulating hematopoietic cells is fairly dormant. Upon exposure to an appropriate external stimulus, such as components of a damaged vessel wall (for platelets), chemokines expressed on an endothelial surface (neutrophils and lymphocytes), or antigen-specific signals from target cells (T lymphocytes), the functional activity of integrins is rapidly enhanced, resulting in increased adhesion that does not require changes in levels of integrin expression on the cell surface. Such "inside-out" signaling to integrins likely involves both cytoskeletal-dependent reorganization of integrins on the cell surface and activation-dependent increases in integrin affinity caused by conformational changes (5–9).

In this issue of the *JCI*, McDowall et al. describe a patient with characteristics of both LAD-1 and GT (10). This patient presented in the clinic with an abnormal bleeding response, typical of GT, as well as recurrent bacterial infections, typical of LAD-1. Neutrophil as well as lymphocyte counts in the circulation were elevated. In vitro studies of platelets, neutrophils, and lymphocytes isolated from this patient indicated defective functional activity of β2 and β3, as well as β1, integrins. Various inside-out signals failed to enhance platelet aggregation or binding of soluble fibrinogen, neutrophil adhesion to fibrinogen, or T cell adhesion to various β2 and β1 integrin ligands. However, unlike in classical LAD-1 or GT, integrin expression on hematopoietic cells was normal. Furthermore, agonists that directly stimulate integrin activity were able to induce increased adhesion, suggesting that hematopoietic cells from this patient express structurally intact integrins. A constitutively enhanced clustering of the LFA-1 integrin on T cells from this patient was also noted and is particularly interesting in light of in vitro studies suggesting a critical role for LFA-1 clustering in T cell receptor–mediated activation of LFA-1 (6, 7, 11, 12).

Two other patients have also been reported with a clinical presentation strikingly similar to that of the patient analyzed by McDowall et al. (10). Harris et al. analyzed a patient suffering from severe and frequent bacterial, as well as viral, fungal, and protozoan, infections (13). Neutrophils and Epstein-Barr virus–transformed B cell lines derived from this patient also had

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Nonstandard abbreviations used: leukocyte adhesion deficiency–1 (LAD-1); lymphocyte function associated antigen–1 (LFA-1); Glanzmann thrombasthenia (GT); adhesion and degranulation-promoting adapter protein (ADAP).

normal levels of β2 and β1 integrin expression, but defective aggregation and adhesion responses upon stimulation. In addition, defective platelet aggregation was noted, although β3 integrin expression on platelets was not reported. An earlier report by Kuijpers et al. also identified a patient suffering from bacterial infections and leukocytosis (14). Similarly to what occurred in the patient analyzed by McDowall et al. (10), β2 integrins were expressed on neutrophils and lymphocytes isolated from this patient, but inside-out signaling responses were impaired. A defect in platelet aggregation was also noted, although this was not detected until a later follow-up examination. However, unlike in the patient in the study by McDowall et al. (10), inside-out signaling to β1 integrins was intact.

The analysis of these patients has several major implications for our understanding of integrin function. First, the clinical severity manifested by these patients provides compelling evidence that inside-out signaling to integrins is an essential effector response in vivo for platelets, neutrophils, and lymphocytes. Second, the recurrent viral infections suffered by the patient analyzed by Harris et al. (13) illustrate that β1 integrins are also critical in mediating lymphocyte-dependent responses. Third, these studies suggest common features in the pathway by which various activation signals regulate multiple integrins expressed on different types of hematopoietic cells. Since loss of β1 integrin function in mice results in embryonic lethality (15), it is likely that the intracellular factor responsible for the loss of integrin-activation responses in some or all of these patients is restricted in expression to hematopoietic cells. What might this factor be? Unfortunately, our current understanding of inside-out signaling to integrins does not lead to a clear suspect. The dysregulated clustering of LFA-1 in the patient analyzed by McDowall et al. (10) suggests that it may be important to focus on cytoskeletal proteins or proteins that regulate the cytoskeleton. However, T cells from this patient expressed normal levels of several cytoskeletal proteins, and 5 GTPases (Rac-1, Rac-2, Rap-1, RhoA and Cdc42) that have been implicated in regulating integrin function (16–19). Studies with knockout mice have recently highlighted functions for the Vav-1 guanine nucleotide exchange factor and the adhesion and degranulation-promoting adapter protein (ADAP) in TCR-mediated regulation of β2 and β1 integrin-mediated adhesion and clustering (11, 12, 20). However, expression of both Vav-1 and ADAP was normal in T cells from the patient presented in the McDowall study. Furthermore, integrins expressed on ADAP-deficient platelets and neutrophils appear to function normally, and both ADAP-deficient and Vav-deficient T cells exhibit enhanced adhesion in response to phorbol ester stimulation. Since phorbol esters induce cytoskeletal-dependent changes in the membrane distribution of the LFA-1 integrin (6, 21), the inability of cells from this patient to enhance integrin activity in response to phorbol esters may be a critical clue to the ultimate identification of the factor that is defective in these patients. Although McDowell et al. do report a 2.5-fold increase in PKC-α expression in this patient's T cells when compared to controls, the inability to activate integrins in response to PKC-independent calcium mobilizers suggests that this change in PKC-α expression is not responsible for the defect in integrin activation in this patient. Although the precise genetic lesion in these patients remains a mystery for now, what we know already suggests that novel, clinically relevant mechanisms of regulating integrin activation in hematopoietic cells remain to be uncovered.

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