Comment on Kuijpers et al, page 4740

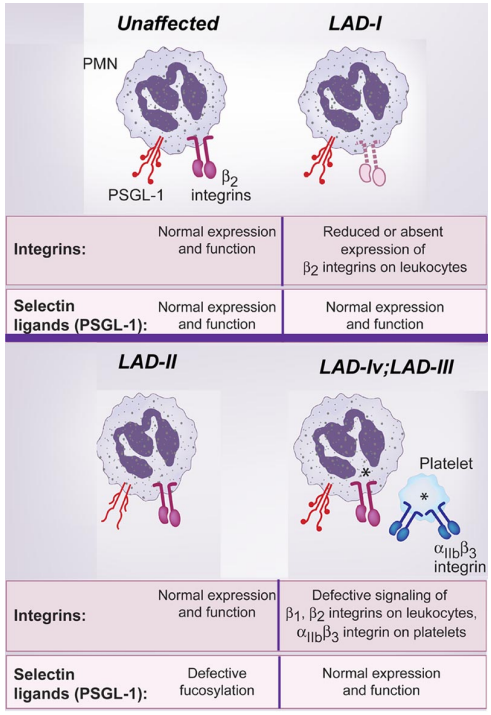
LAD syndromes: *FERMT3* kindles the signal

Guy A. Zimmerman UNIVERSITY OF UTAH

LAD syndromes are uncommon but important genetic disorders of host defense. In this issue of *Blood*, Kuijpers and colleagues now report that one of them, LAD-I variant (LAD-Iv; also called LAD-III), is caused by mutations in *FERMT3*, a gene that encodes an intracellular protein that interacts with cytoplasmic tails of integrin β chains in hematopoetic cells. The observations provide new insights into a morbid and lethal clinical syndrome (which here will be referred to as LAD-Iv/LADIII) that also have basic relevance to the biology of integrins.

Leukocyte adhesion deficiency-1/variant (LAD-Iv/III) is unique among LAD syndromes in that adhesive functions of integrins on platelets—as well as on leukocytes—are disrupted.[1-3] This unusual pattern is thought to be due to one or more defects in activation-dependent alterations of surface integrins that allow them to bind with high avidity to ligands on target cells, a process that is often termed "inside-out signaling."[4] Kuijpers et al, who reported the first case of LAD-Iv/III and subsequently described the natural history of the syndrome in subjects from 7 families of Turkish origin,[3] used homozygosity mapping and haplotype analysis to identify a 13-Mb region on chromosome 11 that contains 3 sequence variants in affected individuals: a premature stop codon in *FERMT3*, an intronic deletion in *NRXN2*, and a putative splice site mutation in *CALDAGGEF1*.[5] Two newly described patients were found to have different stop condon mutations in *FERMT3*. In one, there were no variations in *CALDAGGEF1* or *NRNX2*. These and other findings generated the conclusion that mutations in *FERMT3*, leading to deficiency or absence of its protein product, are sufficient to yield the LAD-Iv/III disease phenotype. *FERMT3* encodes kindlin-3, which directly binds to β chain cytoplasmic tails and triggers integrin activation.[6] Genetic deletion of kindlin-3 in mice resulted in deficient signaling of integrin $\alpha_{IIb}\beta_3$ and a severe bleeding defect with similarities to platelet abnormalities in Glanzmann thrombasthenia.[6] The new observations by Kuijpers et al indicate that genetic deficiency of kindlin-3 disrupts signaling to leukocyte and platelet integrins in humans and is a molecular cause of LAD-Iv/III.

In a recent letter to *Blood*, Mory et al also identified kindlin-3 as a new molecular factor in the pathogenesis of LAD-Iv/III.[7] Earlier, this group reported functional defects in integrins of different classes on leukocytes from an affected subject of Turkish origin and proposed the designation "LAD-III."[2] They further suggested that the LAD-Iv/III disease phenotype may be accounted for by genetic deficiencies that influence key adaptor proteins that mediate rapid signaling of all integrins on hematopoietic cells.[2] In subsequent studies of 2 new Turkish subjects with LAD-Iv/III, they next reported decreased levels of *CALDAGGEF1* mRNA and the protein product in samples from these patients associated with a splice junction mutation in *CALDAGGEF1*.[8] This suggested that the disease phenotype resulted from impaired activity of RAP-1, a small guanosine triphosphatase that is regulated by CALDAGGEF1.[7,8] RAP-1 influences inside-out signaling of platelet and leukocyte integrins.[7,8] Furthermore, mice deficient in Caldaggef1 have impaired RAP-1 signaling and a phenotype suggestive of LAD-Iv/III,[9] a feature consistent with this postulate. Because of the similarities in platelet dysfunction in mice deficient in Caldaggef1 or kindlin-3, Mory et al sequenced *FERMT3* in samples from the 3 patients they previously reported to be deficient in CALDAGGEF1 and found mutations predicted to alter kindlin-3 expression.[7] The same mutation was found in the current report by



Molecular defects in LAD syndromes. In each syndrome, leukocytes have defects in adhesion and targeting to sites of microbial invasion and tissue injury.[1-3,8] Molecular defects in LAD syndromes result in recurrent infections with bacteria and fungal pathogens, inability to form pus at extravascular sites, and other clinical features. LAD-I is caused by mutations in the gene for the β2 subunit of the β2 family of integrins (also termed leukocyte integrins and CD11/CD18 integrins), resulting in decreased or absent levels of these heterodimers on circulating leukocytes. This impairs their ability to rapidly adhere tightly to activated, "inflamed" endothelial cells in response to signaling molecules (PAF, chemokines) displayed on the endothelial surface and to emigrate from vessels at sites of infection or injury. Leukocytes from subjects with LAD-II have intact β2 integrin expression and function but impaired adhesive functions of selectin ligands, chiefly P-selectin glycoprotein ligand 1 (PSGL-1). This defect is caused by mutations in a fucose transporter and consequent defective fucosylation of PSGL-1 and other glycoproteins, and results in impaired tethering and rolling of leukocytes on activated endothelial cells. These are initial events in the multistep process of leukocyte adhesion and emigration.[1] In LAD-Iv/III, expression of integrin heterodimers and selectin ligands is normal, but there is defective activation-dependent signaling (inside-out signaling; starbursts in the figure) of β2 integrins resulting in adhesion defects similar to those in LAD-1. There is also defective signaling of $\alpha_{IIb}\beta_3$ integrin on platelets, resulting in impaired aggregation and a Glanzmann-like bleeding disorder. In addition, there is evidence for impaired β1 integrin activation on hematopoietic cells in LAD-Iv/III. Adapted from Bunting et al.[1]

Kuijpers et al. Mory et al interpreted their findings as together indicating that the disease phenotype may be due to combined deficiencies in kindlin-3 and CALDAGGEF1.[7] In contrast, Kuijpers et al found no evidence for CALDAGGEF1 or RAP-1 deficiency in subjects that they studied.[3] In their article in this issue of *Blood*, they interpret their results as indicating that the *CALDAGGEF1* splice site mutation in 7 of the subjects is in linkage disequilibrium with *FERMT3* but is silent and not itself disease-causing.[5] These differences await reconciliation. Nevertheless, it is likely that deficiency of kindlin-3 or CALDEGGEF1 could individually cause LAD-Iv/III, and that as yet unidentified mutations in other signaling pathways or adaptor proteins may also cause this syndrome. This is important because other index cases of LAD-Iv/III (referenced in Bunting et al,[1] Alon and Etzioni,[2] and Kuijpers et al[3]) were not of Turkish origin and may have harbored unique syndrome-causing mutations. Nevertheless, deficiency of kindlin-3 appears to be a consistent molecular defect in LAD-Iv/III. Recently, 3 additional reports of mutations in *FERMT3* in patients with the syndrome and/or evidence that loss of kindlin-3 function causes an LAD-Iv/III phenotype in human or murine blood cells appeared online (*Nature Medicine,* 22 February 2009).

Kindlin-3, like talin, appears to be a general regulator of integrin activation.[6] It may also kindle signals in the "outside-in" process, contributing to the bidirectional functionality of integrins.[4-6]

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Bunting M, Harris ES, McIntyre TM, et al. Leukocyte adhesion deficiency syndromes: adhesion and tethering defects involving β2 integrins and selectin ligands. Curr Opin Hematol. 2002;9:30-35.
2. Alon R, Etzioni A. LAD-III, a novel group of leukocyte integrin activation deficiencies. Trends Immunol. 2003;24:561-566.
3. Kuijpers TW, van Bruggen R, Kamerbeek N, et al. Natural history and early diagnosis of LAD-1/variant syndrome. Blood. 2007;109:3529-3537.
4. Luo B-H, Carman CV, Springer TA. Structural basis of integrin regulation and signaling. Annu Rev Immunol. 2007;25:619-647.
5. Kuijpers TW, van de Vijver E, Weterman MAJ, et al. LAD-I/variant syndrome is caused by mutations in *FERMT3*. Blood. 2009;113:4740-4746.
6. Moser M, Nieswandt B, Ussar S, et al. Kindlin-3 is essential for integrin activation and platelet aggregation. Nat Med. 2008;14:325-330.
7. Mory A, Feigelson SW, Yarali N, et al. Kindlin-3: a new gene involved in the pathogenesis of LAD-III. Blood. 2008;112:2591.
8. Pasvolsky R, Feigelson SW, Kilic SS, et al. A LAD-III syndrome is associated with defective expression of the Rap-1 activator CalDAG-GEFI in lymphocytes, neutrophils, and platelets. J Exp Med. 2007;204:1571-1582.
9. Bergmeier W, Goerge T, Wang H, et al. Mice lacking the signaling molecule CalDAG-GEFI represent a model for leukocyte adhesion deficiency type III. J Clin Invest. 2007;117:1699-1707.