Published in final edited form as:

Curr Opin Hematol. 2013 January ; 20(1): 16–25. doi:10.1097/MOH.0b013e32835a0091.

Lessons from rare maladies: leukocyte adhesion deficiency syndromes

Estelle S. Harris^a, Andrew S. Weyrich^{a,b}, and Guy A. Zimmerman^a

^aDepartment of Internal Medicine, University of Utah School of Medicine, Salt Lake City, Utah, USA

^bProgram in Molecular Medicine, University of Utah School of Medicine, Salt Lake City, Utah, USA

Abstract

Purpose of review—The leukocyte adhesion deficiency (LAD) syndromes are rare genetically determined conditions with challenging clinical features. These immunodeficiencies also provide insights that are broadly relevant to the biology of leukocytes, platelets, intercellular interactions, and intracellular signaling. Recent discoveries merit their review in the context of existing knowledge.

Recent findings—New activities of β_2 integrins, which are deficient or absent in LAD-I, and new β_2 integrin-dependent functions of neutrophils and other leukocytes have recently been identified. Genetic defects and mechanisms accounting for impaired fucosylation of selectin ligands and defective selectin binding and signaling in LAD-II are now apparent. LAD-III, which presents with bleeding similar to that in Glanzmann thrombasthenia and platelet dysfunction in addition to impaired leukocyte adhesion, is now known to be due to absence of KINDLIN-3, a cytoplasmic protein that acts cooperatively with TALIN-1 in activating β_1 , β_2 , and β_3 integrins. Understanding of the leukocyte adhesion cascade and interactions of leukocytes with inflamed endothelium, which are impaired in each of the LAD syndromes, continues to be refined.

Summary—Although LAD syndromes are rare maladies, their investigation is generating new knowledge directly applicable to the diagnosis and care of patients and to fundamental paradigms in immunobiology and hemostasis.

Keywords

immunodeficiency; infection; monocyte; neutrophil; platelet

INTRODUCTION

The leukocyte adhesion deficiency (LAD) syndromes are primary immunodeficiency disorders that are classified as defects in adhesion-dependent functions of myeloid phagocytes, principally polymorphonuclear leukocytes (PMNs; neutrophils) and monocytes [1,2]. Although rare (1 : 1 000 000 births), their investigation has yielded fundamental insights relevant to inflammation, hemostasis, and regulation of cell–cell interactions, in

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Correspondence to Guy A. Zimmerman, MD, 15 North 2030 East, Room 4220, Salt Lake City, UT 84112, USA. Tel: +1 801 585 0950; fax: +1 801 585 0701; guy.zimmerman@u2m2.utah.edu.

Conflicts of interest

Funding sources: The authors' work, including observations mentioned in this article, is funded by awards R37HL44525, R01HL066277, RC1HL100121, R01HL092746, and U54HLH2311 from the National Institutes of Health.

addition to new knowledge applicable to clinical care. The LAD syndromes result from genetic alterations in the number or activation of integrins on leukocytes or in fucosylation of selectin ligands including P-selectin glycoprotein 1 (PSGL-1), a major leukocyte adhesion molecule that binds P-selectin, E-selectin, and L-selectin [3,4,5[■]] (Fig. 1).

Human and murine myeloid leukocytes express integrins – which are critical and widely distributed adhesion molecules [6,7] – of several subclasses in cell-specific patterns. Members of the β_2 subclass of integrins (CD11/CD18; sometimes called the leukocyte, or leukocyte-specific integrins because expression is restricted to these cells) are basally expressed by neutrophils, monocytes, and other circulating myeloid leukocytes (Fig. 1), and are essential for temporally and spatially regulated adhesion of these cells to endothelial cells of postcapillary venules and, under some conditions, other systemic vessels in response to inflammatory signals [3,4,8–11, 12[■],13[■],14–18,19[■],20] (Fig. 2). In addition, β_2 integrins are expressed by macrophages and on lymphocyte subsets, and mediate adhesion and signaling of these immune effector cells [21,22,23[■],24[■]].

P-selectin glycoprotein 1 and other selectin ligands are – like integrins – basally expressed on PMNs, monocytes, and other leukocytes including lymphocyte subclasses. PSGL-1 contributes to P-selectin-dependent interactions of leukocytes with activated platelets [25], in addition to their adhesion to activated endothelium (Fig. 2) and in leukocyte–leukocyte interactions [5[■]].

The paramount clinical feature common to the LAD syndromes is recurrent bacterial infection that is often severe, life-threatening, and difficult to treat [2,26[■]]. Infection by other microorganisms also occurs. Recurrent infection is a consequence of defective leukocyte adhesion to activated endothelium that impairs migration of leukocytes into tissues at sites of microbial entry and injury [2–4,26[■],27] (Fig. 2). This prevents extravascular accumulation of PMNs and monocytes in many patients: a defect in formation of pus [26[■],27]. In addition, many patients with LAD syndromes have basal and persistent neutrophilia in the absence of obvious infection, and a dramatic increase in myeloid leukocyte counts when infected [26[■],27]. Each LAD syndrome also has other manifestations that are not uniformly shared [2,3,26[■],27,28[■],29]: delayed separation of the umbilical cord and dramatically impaired or dysregulated wound healing in LAD-I and LAD-III; mental retardation, short stature, and an abnormal erythrocyte phenotype (deficiency in H antigen; Bombay phenotype) in LAD-II; and ‘Glanzmann-type’ bleeding and, in some patients, hepatosplenomegaly and/or osteopetrosis in LAD-III. There can be substantial variations in phenotype in patients with the same LAD syndrome [27,30,31].

After description of LAD-I (1970s and early 1980s) and LAD-II (1992) [32,33], characterization of a new syndrome in which leukocyte adhesion was defective but expression and sequence of leukocyte integrins was normal and defects in selectin ligands were excluded [3,34–37] (Table 1) generated controversy in the field, including controversial nomenclature [26[■],28[■],42,43]. The new syndrome, now identified as a defect in inside-out signaling of multiple integrin subtypes (Figs 1 and 3), was initially called LAD-I variant (LAD-Iv) [34] – a term that made sense because the defect involves leukocyte integrins, as does LAD-I, and many clinical features are similar [3,27,29]. Nevertheless, it is now commonly termed LAD-III [1,2,26[■],28[■],30] and we will use that designation here. Rare compound mutations and cases in which mutations in the β_2 integrin subunit yields a nonfunctional but expressed $\alpha\beta$ integrin heterodimer are also sometimes called LAD-I/variants [3,26[■]].

LAD-I: ABSENT OR REDUCED EXPRESSION OF β_2 INTEGRINS

Diverse mutations in *ITGB2* at 21q22.3 cause absent or reduced expression of the β_2 integrin subunit and, consequently, reduced expression of β_2 integrin heterodimers [28[■], 31], yielding the characteristic leukocyte phenotype (Fig. 1) and defect in tight adhesion (Fig. 2) in patients with LAD-I [3,26[■]]. Over 80 mutations, many occurring in a 240-residue region encompassed by exons 5–9 in β_2 that is common to all integrin β subunits, have been cataloged [28[■]]. Severe (<2% expression of β_2 integrins) and moderate (2–30% expression of β_2 integrins) LAD-I phenotypes occur [31]. Very rarely, a mutation causes nonfunctional but normally expressed β_2 integrins [28[■],31]. Compound heterozygous and somatic reverse mutations in humans, and spontaneous mutations in large animals, have been reported (reviewed in [26[■],31]).

Patients with moderate LAD-I can survive infancy with antibiotics and careful management, but usually are plagued by severe periodontitis, tooth loss, and impaired or dysplastic remodeling of infected sites and wounds [26[■]]. Dysplastic wound healing may be due to dysregulated macrophage activities and macrophage–neutrophil interactions, with failure to clear apoptotic PMNs and generate molecular signals that modulate inflammation and repair [22,55,56], in addition to defects in tissue surveillance and defense by neutrophils and monocytes (Fig. 2). The efficacy, indications, and biologic strategies for hematopoietic stem cell transplantation in patients with severe and moderate LAD-I are under evaluation (reviewed in [26[■],31, 57]). Mouse models of β_2 integrin deficiency have provided useful insights and new approaches to explore the biology of LAD-I [22,29,55,56,58,59]. For example, murine models [56,60,61[■]] may yield answers to outstanding questions regarding T cell and adaptive immune responses in LAD-I [29,32].

LAD-I causes deficient expression of each of the four members of the β_2 integrin subfamily: $\alpha_L\beta_2$ (CD11a/CD18, LFA-1), $\alpha_M\beta_2$ (CD11b/CD18, MAC-1, CR3), $\alpha_X\beta_2$ (CD11c/CD18, p150/95), and $\alpha_D\beta_2$ (CD11d/CD18) [3,28[■]]. New facets of the biology of β_2 integrins continue to be discovered. Differential activities of $\alpha_L\beta_2$ and $\alpha_M\beta_2$ in PMN adhesion and transmigration (Fig. 2) have recently been identified, based in part on intensive study of $\alpha_L\beta_2$ [62], which is a prototype integrin [24[■]]. The most recently identified member of the β_2 subfamily is $\alpha_D\beta_2$ [63]. Its biology is less well defined than that of the other β_2 integrins. Experiments in mice with targeted deletions of the α_D subunit and, consequently, absent expression of $\alpha_D\beta_2$, indicate that it has intricate activities in host defense, immune regulation, and inflammatory injury [21,64]. Expression of $\alpha_D\beta_2$ is said to be restricted to subsets of macrophages [28[■],62], but this is based on the pattern of basal expression in mice [21,64], which is different from the pattern of expression in humans ([63]; our unpublished studies). Nevertheless, $\alpha_D\beta_2$ on macrophages appears to be important in both experimental animals and humans [21,65].

New activities of PMNs and monocytes involving β_2 integrin-mediated adhesion and signaling also continue to be discovered [22,23[■],66–69]. It is possible that some of these functions, or β_2 integrin activities yet to be identified, are altered in LAD-I. Because integrins are also molecular effectors in inflammatory tissue injury [11], identification of new β_2 -dependent activities of myeloid leukocytes may have relevance beyond LAD syndromes.

LAD-II: A DEFECT IN FUCOSYLATION OF SELECTIN LIGANDS LEADING TO IMPAIRED SELECTIN BINDING AND SIGNALING

Defects in fucosylation of oligosaccharides including sialyl-Lewis X, which is present on PSGL-1 and other key leukocyte glycoconjugates, were previously identified as the

molecular basis of LAD-II (reviewed in [3,31]). A human gene encoding a GDP-fucose-specific transporter present in the Golgi membrane, *SLC35C1*, was subsequently cloned and is mutated in LAD-II (also termed congenital disorder of glycosylation IIc), which is much less common than LAD-I [26[■],28[■],31,70–75]. Mutations resulting in defective function, or dual defects in function and subcellular localization, have been identified [74]. Decreased fucosylation of selectin ligands on leukocytes resulting from mutations in *SLC35C1* (Fig. 1) leads to their impaired tethering and rolling on activated endothelial cells in LAD-II (Fig. 2) [3,26[■],28[■],31]. Impaired signaling through defective PSGL-I and other selectin ligands may also cripple β_2 integrin-mediated slow rolling [5[■],8,9] and, possibly, other functional responses of leukocytes [29] in LAD-II.

Targeted deletion of *slc35c1* in mice resulted in loss of GDP-fucose import into the Golgi lumen and yielded a complex phenotype with features similar to LAD-II, including neutrophilia, monocytosis, and leukocyte adhesion defects [76]. A second *slc35c1*^{-/-} mouse model also demonstrated phenotypic features consistent with LAD-II, and provided information on differential effects on myeloid leukocyte and T lymphocyte targeting *in vivo* [77]. Treatment of cultured cells from *slc35c1*^{-/-} animals with fucose resulted in partial rescue of fucosylation of glyco-conjugates [76]. Orally administered fucose has corrected features of LAD-II in some, but not all, humans in which this approach has been studied (reviewed in [26[■]]), and resulted in appearance of the fucosylated erythrocyte H antigen and auto-immune neutropenia in one individual [73].

The cellular defects and molecular mechanisms causing growth impairment and psychomotor retardation in LAD-II are yet to be defined [26[■]]. These features may be more severe than immunodeficiency in older patients [26[■],75].

LAD-III: DEFECTIVE INSIDE-OUT SIGNALING OF MULTIPLE INTEGRINS

Patients with LAD-III have clinical features similar to those in LAD-I and a defect in tight adhesion of activated PMNs to endothelial cells and to purified β_2 integrin ligands *in vitro* and/or *in vivo* [3,26[■],27,29] (Figs 1–3). In contrast to LAD-I, β_2 integrins are expressed at expected or near-normal levels (Fig. 1). In addition, they are functional, indicating a defect in inside-out signaling [3,29]. Inside-out signaling of integrins induces transition from an inactive, or low-affinity, state to one that allows avid binding of ligands, and is sometimes termed activation [44]. Furthermore, there is a defect in inside-out signaling of β_1 integrins on activated leukocytes from patients with LAD-III ([3,31]; also see below). In striking contrast to other LAD syndromes [3,26[■],27,29], patients with LAD-III also have a bleeding tendency similar to that in Glanzmann thrombasthenia [78] and defective inside-out signaling of integrin $\alpha_{IIb}\beta_3$, which is the major integrin mediating aggregation and adhesion of platelets [79] (Tables 1 and 2; Fig. 3). There is variability in severity of bleeding and in other phenotypic features of LAD-III [27,30,39,40,48–52,80]. Bone marrow or hematopoietic stem cell transplantation has been effective in some patients [26[■],30,39,48,51].

Clinical presentation and characterization of cells from four index patients originally defined the LAD-III syndrome [27,34–38] (Table 1). Reports of affected siblings and new patients also yielded important clinical, genetic, and molecular insights [27,39,40,48–52,80]. Analysis of defects in function of primary leukocytes and platelets from patients with LAD-III has been a critical feature of these investigations (Table 2).

Independently, studies in genetically altered mice demonstrated that kindlin-3, a cytoplasmic protein, is required for activation of β_1 , β_2 , and β_3 integrins and that its targeted deletion yields leukocyte and platelet adhesion defects that pheno-copy those in LAD-III [83,84] – a breakthrough in the field. Prior to these observations, the molecular defect(s) in inside-out

signaling [44] that causes LAD-III was an unsolved mystery [3,27,29]. After reports of kindlin-3^{-/-} mice [83], genotyping of patients with LAD-III rapidly identified mutations in *FERMT3* and deficiency of its protein product, KINDLIN-3, in cells from LAD-III patients [30,40, 48,50]. *FERMT3* mutations have now been found in all patients with LAD-III in whom genotyping has been reported [30,39,40,48–53,80] (Fig. 3c).

On the basis of studies of genetically altered mice [85,86] and patients with LAD-III [87], it was proposed that mutations in *CALDAGGEF1* (*RASGRP2*) leading to impaired RAP-1 signaling is a molecular cause of LAD-III (reviewed in [4,44,88]). Genotyping of patients with LAD-III from seven Turkish families, the most common background of patients with this syndrome [31], yielded three sequence variations, including alterations in *CALDAGGEF1* and *FERMT3*, but unequivocal evidence that mutations in *FERMT3* and deficiency of KINDLIN-3 are sufficient to cause LAD-III [30]. Furthermore, patients with LAD-III and mutations in *FERMT3* but without variations in *CALDAGGEF1* have been identified [30,38–40,48], and engineered expression of KINDLIN-3, but not *CALDAGGEF1*, has rescued adhesion deficiency in cells derived from LAD-III patients [39,40,48,88]. The impact of variations in *CALDAGGEF1* on functions of leukocytes and platelets in patients who also have mutations in *FERMT3* remains controversial [26[■],28[■], 41,49,54,87], although it is possible that isolated mutations in the *CALDAG-GEF1*-regulated pathway – or in other inside-out signaling intermediates yet to be identified – could cause LAD-III [4,88]. The functional impact of mutations in *FERMT3* and deficiency of KINDLIN-3 is, however, clear.

KINDLIN-3 deficiency also likely contributes to impaired outside-in signaling by leukocyte and platelet integrins [46,83], a key feature of their biology [6,79], in LAD-III. Further, deficiency of KINDLIN-3 likely contributes to features of LAD-III apart from impaired leukocyte interaction with endothelium and platelet functional defects. In *kindlin-3*^{-/-} mice, defective inside-out signaling of β_1 , β_2 , and β_3 integrins on monocyte-lineage osteoclasts impairs podosome formation and bone resorption, culminating osteopetrosis [89[■]], as occurs in some patients with LAD-III [45,48,50, 51,80,87]. Recently, KINDLIN-3 was reported to be present in human erythrocytes, as the ortholog is in mice [90], and its deficiency proposed to be a cause of poikilocytosis and anemia in LAD-III [49]. Anemia was reported in three of the four index patients (Table 1), although the contributions of bleeding and other causes were not determined.

A substantial body of evidence indicates that integrin cytoplasmic domains – particularly the β subunit cytoplasmic tail – regulate conformational changes that result in ligand binding, and are key ‘trigger’ regions in inside-out signaling (reviewed in [44]). KINDLIN-3, which is one of three kindlin orthologs in mammals, binds to cytoplasmic domains of β_1 , β_2 , and β_3 integrins [46,83,84] and works in cooperation with TALIN 1, a cytoplasmic protein that links integrin β tails to the actin cytoskeleton (reviewed in [44,47[■]]). The actin cytoskeleton is critical for stabilization of integrin bonds and platelet and leukocyte adhesiveness [91], but early experiments indicated that impaired actin polymerization does not underlie defective adhesion of neutrophils in LAD-III [34–36]. TALIN 1 has an N-terminal head domain (THD) with a FERM (4.1, ezrin, radixin, moesin) motif and four subdomains in its structure; overexpression of the THD is sufficient to activate β_1 , β_2 , and β_3 integrins [44]. This and other observations demonstrate that TALIN 1 is critical in inside-out signaling of integrins on leukocytes and platelets (reviewed in [44,47[■]]). Nevertheless, TALIN 1 is widely expressed, and its mutation or deletion is unlikely to account for the unique cellular defects in LAD-III; global deletion of talin 1 in mice is embryonic lethal [47[■]]. KINDLIN-3, on the contrary, is largely restricted to blood cells [46,54], although it is also found in endothelial cells [92]. KINDLIN-3 and other kindlins contain a FERM domain similar in sequence to that in TALIN but in which the F₂ subdomain is split by an embedded

pleckstrin homology domain [46,54]. The interrupted kindlin FERM domain and, particularly, the F₃ subdomain mediate binding to integrin β tails at a motif that is distinct, and distal to, the THD-binding region (reviewed in [44,46,47[■]]). The mechanisms by which KINDLIN-3 and TALIN cooperatively modify integrin conformation, affinity, and avidity, and interact in inside-out signaling and activation of β_1 , β_2 , and β_3 integrins on platelets and leukocytes are not yet precisely defined [44,46,47[■]] and are being aggressively explored [93–99] – an explosion of work triggered, in part, by recognition that KINDLIN-3 deficiency is the molecular basis for LAD-III.

CONCLUSION

Rare maladies of humans teach observant physicians and translational biologists much, as was recognized by Harvey and Garrod [3]. There have been many such lessons from the LAD syndromes. Ongoing investigations of the intricate nature of integrins, selectin binding, and signaling, and inside-out and outside-in signal transduction mechanisms suggest that other such lessons are in the offing, and that the molecular mechanisms that underlie LAD-I, LAD-II, and LAD-III will be further illuminated.

Acknowledgments

The authors thank Alex Greer for skillful preparation of the manuscript and Diana Lim for her creative contributions and drafting of the figures. They also thank Tammy Smith for help in evaluating information on gene sequence and variations and contributions to reference [39], and other members of their research group and collaborators for contributions to work cited.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- ■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Al-Herz W, Bousfiha A, Casanova JL, et al. Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol.* 2011; 2:54. [PubMed: 22566844]
2. Holland, SM.; Gallin, JI. Disorders of granulocytes and monocytes. In: Longo, DM.; Kasper, DL.; Jameson, JL., et al., editors. *Harrison's principles of internal medicine*. 18. New York, NY: McGraw Hill Professional; 2011. p. 472-485.
3. Bunting M, Harris ES, McIntyre TM, et al. Leukocyte adhesion deficiency syndromes: adhesion and tethering defects involving beta 2 integrins and selectin ligands. *Curr Opin Hematol.* 2002; 9:30–35. [PubMed: 11753075]
4. Zimmerman GA. LAD syndromes: FERMT3 kindles the signal. *Blood.* 2009; 113:4485–4486. [PubMed: 19423742]
5. ■ Zarbock A, Ley K, McEver RP, Hidalgo A. Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. *Blood.* 2011; 118:6743–6751. An excellent review of selectin ligands and their structure and function. Also see reference [13[■]]. [PubMed: 22021370]
6. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell.* 2002; 110:673–687. [PubMed: 12297042]

7. Barczyk M, Carracedo S, Gullberg D. Integrins. *Cell Tissue Res.* 2010; 339:269–280. [PubMed: 19693543]
8. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol.* 2007; 7:678–689. [PubMed: 17717539]
9. McEver RP, Zhu C. Rolling cell adhesion. *Annu Rev Cell Dev Biol.* 2010; 26:363–396. [PubMed: 19575676]
10. McIntyre TM, Prescott SM, Weyrich AS, Zimmerman GA. Cell-cell interactions: leukocyte-endothelial interactions. *Curr Opin Hematol.* 2003; 10:150–158. [PubMed: 12579042]
11. Phillipson M, Kubes P. The neutrophil in vascular inflammation. *Nat Med.* 2011; 17:1381–1390. [PubMed: 22064428]
- 12■. Vestweber D. Novel insights into leukocyte extravasation. *Curr Opin Hematol.* 2012; 19:212–217. An informative recent review. Also see reference [19■]. [PubMed: 22395664]
- 13■. Stadtmann A, Brinkhaus L, Mueller H, et al. Rap1a activation by CalDAG-GEFI and p38 MAPK is involved in E-selectin-dependent slow leukocyte rolling. *Eur J Immunol.* 2011; 41:2074–2085. This study identifies Caldag-gef1 and Rap-1 as key components of a signaling pathway from selectin ligands to $\alpha_1\beta_2$ -mediated slow rolling and leukocyte recruitment in murine models. [PubMed: 21480213]
14. Lorant DE, Patel KD, McIntyre TM, et al. Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. *J Cell Biol.* 1991; 115:223–234. [PubMed: 1717478]
15. Wang HB, Wang JT, Zhang L, et al. P-selectin primes leukocyte integrin activation during inflammation. *Nat Immunol.* 2007; 8:882–892. [PubMed: 17632516]
16. Kuwano Y, Spelten O, Zhang H, et al. Rolling on E- or P-selectin induces the extended but not high-affinity conformation of LFA-1 in neutrophils. *Blood.* 2010; 116:617–624. [PubMed: 20445017]
17. Shao B, Yago T, Coghill PA, et al. Signal-dependent slow leukocyte rolling does not require cytoskeletal anchorage of P-selectin glycoprotein ligand-1 (PSGL-1) or integrin $\alpha\text{L}\beta\text{2}$. *J Biol Chem.* 2012; 287:19585–19598. [PubMed: 22511754]
18. Spertini C, Baisse B, Spertini O. Ezrin-radixin-moesin-binding sequence of PSGL-1 glycoprotein regulates leukocyte rolling on selectins and activation of extracellular signal-regulated kinases. *J Biol Chem.* 2012; 287:10693–10702. [PubMed: 22311979]
- 19■. Hyun YM, Sumagin R, Sarangi PP, et al. Uropod elongation is a common final step in leukocyte extravasation through inflamed vessels. *J Exp Med.* 2012; 209:1349–1362. These studies indicate that adhesion and migration of leukocytes mediated by β_1 and β_2 integrins are critical uropod elongation as a late step in the leukocyte adhesion cascade. The conclusions are based on genetically modified mouse models. See also [12■] for an excellent review. [PubMed: 22711877]
20. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. *Nat Rev Immunol.* 2004; 4:432–444. [PubMed: 15173832]
21. Miyazaki Y, Bunting M, Stafforini DM, et al. Integrin $\alpha\text{D}\beta\text{2}$ is dynamically expressed by inflamed macrophages and alters the natural history of lethal systemic infections. *J Immunol.* 2008; 180:590–600. [PubMed: 18097061]
22. Sindrilaru A, Peters T, Schymeinsky J, et al. Wound healing defect of Vav3^{-/-} mice due to impaired β_2 -integrin-dependent macrophage phagocytosis of apoptotic neutrophils. *Blood.* 2009; 113:5266–5276. [PubMed: 19147786]
- 23■. Costantini C, Calzetti F, Perbellini O, et al. Human neutrophils interact with both 6-sulfo LacNAc + DC and NK cells to amplify NK-derived IFN γ : role of CD18, ICAM-1, and ICAM-3. *Blood.* 2011; 117:1677–1686. This study identifies interactions between neutrophils and adaptive immune effector cells dependent in part on β_2 integrins. See references [66] and [68] for useful reviews. [PubMed: 21098395]
- 24■. Hogg N, Patzak I, Willenbrock F. The insider's guide to leukocyte integrin signalling and function. *Nat Rev Immunol.* 2011; 11:416–426. An informative and topical review with information relevant to LAD syndromes. [PubMed: 21597477]

25. Vieira-de-Abreu A, Campbell RA, Weyrich AS, Zimmerman GA. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. *Semin Immunopathol.* 2012; 34:5–30. [PubMed: 21818701]
- 26■. Hanna S, Etzioni A. Leukocyte adhesion deficiencies. *Ann N Y Acad Sci.* 2012; 1250:50–55. A recent comprehensive review from a group that has made important contributions to the field. [PubMed: 22276660]
27. Kuijpers TW, van Bruggen R, Kamerbeek N, et al. Natural history and early diagnosis of LAD-1/variant syndrome. *Blood.* 2007; 109:3529–3537. [PubMed: 17185466]
- 28■. van de Vijver E, Maddalena A, Sanal O, et al. Hematologically important mutations: leukocyte adhesion deficiency (first update). *Blood Cells Mol Dis.* 2012; 48:53–61. A recent comprehensive catalog of mutations in LAD-I, LAD-II, and LAD-III from investigators who have made important clinical and basic observations characterizing LAD syndromes. [PubMed: 22134107]
29. Etzioni, A.; Harlan, JM. Cell adhesion and leukocyte adhesion defects. In: Ochs, HD.; Smith, CIE.; Puck, JM., editors. *Primary immunodeficiency disease a molecular and genetic approach.* 2. New York, NY: Oxford University Press; 2007. p. 550-564.
30. Kuijpers TW, van de Vijver E, Weterman MA, et al. LAD-1/variant syndrome is caused by mutations in FERMT3. *Blood.* 2009; 113:4740–4746. [PubMed: 19064721]
31. Etzioni A. Genetic etiologies of leukocyte adhesion defects. *Curr Opin Immunol.* 2009; 21:481–486. [PubMed: 19647987]
32. Anderson DC, Springer TA. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Annu Rev Med.* 1987; 38:175–194. [PubMed: 3555290]
33. Etzioni A, Frydman M, Pollack S, et al. Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency. *N Engl J Med.* 1992; 327:1789–1792. [PubMed: 1279426]
34. Kuijpers TW, Van Lier RA, Hamann D, et al. Leukocyte adhesion deficiency type 1 (LAD-1)/variant. A novel immunodeficiency syndrome characterized by dysfunctional beta2 integrins. *J Clin Invest.* 1997; 100:1725–1733. [PubMed: 9312170]
35. Harris ES, Shigeoka AO, Li W, et al. A novel syndrome of variant leukocyte adhesion deficiency involving defects in adhesion mediated by beta1 and beta2 integrins. *Blood.* 2001; 97:767–776. [PubMed: 11157496]
36. McDowall A, Inwald D, Leitinger B, et al. A novel form of integrin dysfunction involving beta1, beta2, and beta3 integrins. *J Clin Invest.* 2003; 111:51–60. [PubMed: 12511588]
37. Alon R, Aker M, Feigelson S, et al. A novel genetic leukocyte adhesion deficiency in subsecond triggering of integrin avidity by endothelial chemokines results in impaired leukocyte arrest on vascular endothelium under shear flow. *Blood.* 2003; 101:4437–4445. [PubMed: 12595312]
38. Alon R, Etzioni A. LAD-III: a novel group of leukocyte integrin activation deficiencies. *Trends Immunol.* 2003; 24:561–566. [PubMed: 14552841]
39. Harris ES, Smith TL, Springett GM, et al. Leukocyte adhesion deficiency-I variant syndrome (LAD-Iv, LAD-III): molecular characterization of the defect in an index family. *Am J Hematol.* 2012; 87:311–313. [PubMed: 22139635]
40. Svensson L, Howarth K, McDowall A, et al. Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nat Med.* 2009; 15:306–312. [PubMed: 19234463]
41. Manevich-Mendelson E, Feigelson SW, Pasvolsky R, et al. Loss of Kindlin-3 in LAD-III eliminates LFA-1 but not VLA-4 adhesiveness developed under shear flow conditions. *Blood.* 2009; 114:2344–2353. [PubMed: 19617577]
42. Etzioni A, Alon R. LAD III versus LAD I variant. *Blood.* 2007; 110:4129. author reply 4129–4130. [PubMed: 18024798]
43. Kuijpers T, Baas F, Weterman MA, et al. Response: adherence to the LAD variant form. *Blood.* 2007; 110:4129–4130. [PubMed: 18024798]
44. Shattil SJ, Kim C, Ginsberg MH. The final steps of integrin activation: the end game. *Nat Rev Mol Cell Biol.* 2010; 11:288–300. [PubMed: 20308986]

45. McDowall A, Svensson L, Stanley P, et al. Two mutations in the KINDLIN3 gene of a new leukocyte adhesion deficiency III patient reveal distinct effects on leukocyte function in vitro. *Blood*. 2010; 115:4834–4842. [PubMed: 20357244]
46. Moser M, Legate KR, Zent R, Fassler R. The tail of integrins, talin, and kindlins. *Science*. 2009; 324:895–899. [PubMed: 19443776]
- 47■. Ye F, Petrich BG. Kindlin: helper, co-activator, or booster of talin in integrin activation? *Curr Opin Hematol*. 2011; 18:356–360. A recent review outlining key questions regarding the mechanistic activities of kindlins and talin in inside-out signaling of integrins. References [91–99] are examples of recent primary observations aimed at some of these questions and related issues. Also see references [44,46,54]. [PubMed: 21730832]
48. Malinin NL, Zhang L, Choi J, et al. A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans. *Nat Med*. 2009; 15:313–318. [PubMed: 19234460]
49. Meller J, Malinin NL, Panigrahi S, et al. Novel aspects of Kindlin-3 function in humans based on a new case of leukocyte adhesion deficiency III. *J Thromb Haemost*. 2012; 10:1397–1408. [PubMed: 22564402]
50. Mory A, Feigelson SW, Yarali N, et al. Kindlin-3: a new gene involved in the pathogenesis of LAD-III. *Blood*. 2008; 112:2591. [PubMed: 18779414]
51. Jurk K, Schulz AS, Kehrel BE, et al. Novel integrin-dependent platelet malfunction in siblings with leukocyte adhesion deficiency-III (LAD-III) caused by a point mutation in FERMT3. *Thromb Haemost*. 2010; 103:1053–1064. [PubMed: 20216991]
52. Robert P, Canault M, Farnarier C, et al. A novel leukocyte adhesion deficiency III variant: kindlin-3 deficiency results in integrin- and nonintegrin-related defects in different steps of leukocyte adhesion. *J Immunol*. 2011; 186:5273–5283. [PubMed: 21441448]
53. Taaning EBM, Shah BF, Schejbel LD, et al. Leukocyte adhesion deficiency type III due to a novel mutation in the FERMT3 gene. *Vox Sanguinis*. 2012; 103:270.
54. Malinin NL, Plow EF, Byzova TV. Kindlins in FERM adhesion. *Blood*. 2010; 115:4011–4017. [PubMed: 20228270]
55. Peters T, Sindrilaru A, Hinz B, et al. Wound-healing defect of CD18(–/–) mice due to a decrease in TGF-beta1 and myofibroblast differentiation. *EMBO J*. 2005; 24:3400–3410. [PubMed: 16148944]
56. Peters T, Sindrilaru A, Wang H, et al. CD18 in monogenic and polygenic inflammatory processes of the skin. *J Invest Dermatol Symp Proc*. 2006; 11:7–15.
57. Malech HL, Hickstein DD. Genetics, biology and clinical management of myeloid cell primary immune deficiencies: chronic granulomatous disease and leukocyte adhesion deficiency. *Curr Opin Hematol*. 2007; 14:29–36. [PubMed: 17133097]
58. Wang H, Lim D, Rudd CE. Immunopathologies linked to integrin signalling. *Semin Immunopathol*. 2010; 32:173–182. [PubMed: 20217414]
59. Cullere X, Lauterbach M, Tsuboi N, Mayadas TN. Neutrophil-selective CD18 silencing using RNA interference in vivo. *Blood*. 2008; 111:3591–3598. [PubMed: 18216298]
60. Grabbe S, Varga G, Beissert S, et al. Beta2 integrins are required for skin homing of primed T cells but not for priming naive T cells. *J Clin Invest*. 2002; 109:183–192. [PubMed: 11805130]
- 61■. Peters T, Bloch W, Pabst O, et al. Adaptive immune response to model antigens is impaired in murine leukocyte-adhesion deficiency-1 revealing elevated activation thresholds in vivo. *Clin Dev Immunol*. 2012; 2012:450738. Interesting observations in a mouse model of LAD-I that address unresolved issues in adaptive immune function in this syndrome. Also see references [60] and [64] and clinical correlates in references [29] and [32]. [PubMed: 22474478]
62. Lefort CT, Ley K. Neutrophil arrest by LFA-1 activation. *Front Immunol*. 2012; 3:157. [PubMed: 22701459]
63. Van der Vieren M, Le Trong H, Wood CL, et al. A novel leukointegrin, alpha d beta 2, binds preferentially to ICAM-3. *Immunity*. 1995; 3:683–690. [PubMed: 8777714]
64. Wu H, Rodgers JR, Perrard XY, et al. Deficiency of CD11b or CD11d results in reduced staphylococcal enterotoxin-induced T cell response and T cell phenotypic changes. *J Immunol*. 2004; 173:297–306. [PubMed: 15210787]

65. Thomas AP, Dunn TN, Oort PJ, et al. Inflammatory phenotyping identifies CD11d as a gene markedly induced in white adipose tissue in obese rodents and women. *J Nutr.* 2011; 141:1172–1180. [PubMed: 21508205]
66. Nathan C. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol.* 2006; 6:173–182. [PubMed: 16498448]
67. McDonald B, Pittman K, Menezes GB, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. *Science.* 2010; 330:362–366. [PubMed: 20947763]
68. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol.* 2011; 11:519–531. [PubMed: 21785456]
69. van Gisbergen KP, Sanchez-Hernandez M, Geijtenbeek TB, van Kooyk Y. Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J Exp Med.* 2005; 201:1281–1292. [PubMed: 15837813]
70. Luhn K, Wild MK, Eckhardt M, et al. The gene defective in leukocyte adhesion deficiency II encodes a putative GDP-fucose transporter. *Nat Genet.* 2001; 28:69–72. [PubMed: 11326279]
71. Lubke T, Marquardt T, Etzioni A, et al. Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. *Nat Genet.* 2001; 28:73–76. [PubMed: 11326280]
72. Etzioni A, Sturla L, Antonellis A, et al. Leukocyte adhesion deficiency (LAD) type II/carbohydrate deficient glycoprotein (CDG) IIc founder effect and genotype/phenotype correlation. *Am J Med Genet.* 2002; 110:131–135. [PubMed: 12116250]
73. Hidalgo A, Ma S, Peired AJ, et al. Insights into leukocyte adhesion deficiency type 2 from a novel mutation in the GDP-fucose transporter gene. *Blood.* 2003; 101:1705–1712. [PubMed: 12406889]
74. Helmus Y, Denecke J, Yakubenia S, et al. Leukocyte adhesion deficiency II patients with a dual defect of the GDP-fucose transporter. *Blood.* 2006; 107:3959–3966. [PubMed: 16455955]
75. Gazit Y, Mory A, Etzioni A, et al. Leukocyte adhesion deficiency type II: long-term follow-up and review of the literature. *J Clin Immunol.* 2010; 30:308–313. [PubMed: 20099014]
76. Hellbusch CC, Sperandio M, Frommhold D, et al. Golgi GDP-fucose transporter-deficient mice mimic congenital disorder of glycosylation IIc/leukocyte adhesion deficiency II. *J Biol Chem.* 2007; 282:10762–10772. [PubMed: 17276979]
77. Yakubenia S, Frommhold D, Scholch D, et al. Leukocyte trafficking in a mouse model for leukocyte adhesion deficiency II/congenital disorder of glycosylation IIc. *Blood.* 2008; 112:1472–1481. [PubMed: 18541720]
78. Nurden A, Nurden P. Advances in our understanding of the molecular basis of disorders of platelet function. *J Thromb Haemost.* 2011; 9(Suppl 1):76–91. [PubMed: 21781244]
79. Collier BS, Shattil SJ. The GPIIb/IIIa (integrin α IIb β 3) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend. *Blood.* 2008; 112:3011–3025. [PubMed: 18840725]
80. Sabnis H, Kirpalani A, Horan J, et al. Leukocyte adhesion deficiency-III in an African-American patient. *Pediatr Blood Cancer.* 2010; 55:180–182. [PubMed: 20213844]
81. Feigelson SW, Grabovsky V, Manevich-Mendelson E, et al. Kindlin-3 is required for the stabilization of TCR-stimulated LFA-1:ICAM-1 bonds critical for lymphocyte arrest and spreading on dendritic cells. *Blood.* 2011; 117:7042–7052. [PubMed: 21536861]
82. van de Vijver E, De Cuyper IM, Gerrits AJ, et al. Defects in Glanzmann thrombasthenia and LAD-III (LAD-1/v) syndrome: the role of integrin β 1 and β 3 in platelet adhesion to collagen. *Blood.* 2012; 119:583–586. This study suggests a molecular mechanism that accounts for bleeding that is clinically more severe in LAD-III than in Glanzmann thrombasthenia. Deficiency of KINDLIN-3 in LAD-III likely impairs function of β 1 and β 3 integrins on platelets, including α 2 β 1, whereas integrin α IIb β 3-dependent hemostatic functions alone are impaired in Glanzmann thrombasthenia. Deficiency of KINDLIN-3 and defects in function of multiple integrins may also impair antimicrobial activities of platelets in LAD-III [25]. [PubMed: 22065596]
83. Moser M, Nieswandt B, Ussar S, et al. Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat Med.* 2008; 14:325–330. [PubMed: 18278053]
84. Moser M, Bauer M, Schmid S, et al. Kindlin-3 is required for β 2 integrin-mediated leukocyte adhesion to endothelial cells. *Nat Med.* 2009; 15:300–305. [PubMed: 19234461]

85. Chrzanowska-Wodnicka M, Smyth SS, Schoenwaelder SM, et al. Rap1b is required for normal platelet function and hemostasis in mice. *J Clin Invest.* 2005; 115:680–687. [PubMed: 15696195]
86. Bergmeier W, Goerge T, Wang HW, 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. [PubMed: 17492052]
87. Kilic SS, Etzioni A. The clinical spectrum of leukocyte adhesion deficiency (LAD) III due to defective CalDAG-GEF1. *J Clin Immunol.* 2009; 29:117–122. [PubMed: 18709451]
88. Hidalgo A, Frenette PS. When integrins fail to integrate. *Nat Med.* 2009; 15:249–250. [PubMed: 19265824]
89. Schmidt S, Nakchbandi I, Ruppert R, et al. Kindlin-3-mediated signaling from multiple integrin classes is required for osteoclast-mediated bone resorption. *J Cell Biol.* 2011; 192:883–897. Important observations demonstrating that kindlin-3-deficient mice have major defects in adhesion and spreading of osteoclasts and severe osteopetrosis, which is also seen in some patients with LAD-III. [PubMed: 21357746]
90. Kruger M, Moser M, Ussar S, et al. SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell.* 2008; 134:353–364. [PubMed: 18662549]
91. Rullo J, Becker H, Hyduk SJ, et al. Actin polymerization stabilizes alpha4beta1 integrin anchors that mediate monocyte adhesion. *J Cell Biol.* 2012; 197:115–129. [PubMed: 22472442]
92. Bialkowska K, Ma YQ, Bledzka K, et al. The integrin co-activator Kindlin-3 is expressed and functional in a nonhematopoietic cell, the endothelial cell. *J Biol Chem.* 2010; 285:18640–18649. [PubMed: 20378539]
93. Bouaouina M, Goult BT, Huet-Calderwood C, et al. A conserved lipid-binding loop in the kindlin FERM F1 domain is required for kindlin-mediated alphaIIb beta3 integrin coactivation. *J Biol Chem.* 2012; 287:6979–6990. [PubMed: 22235127]
94. Kahner BN, Kato H, Banno A, et al. Kindlins, integrin activation and the regulation of talin recruitment to alphaIIb beta3. *PLoS One.* 2012; 7:e34056. [PubMed: 22457811]
95. Kim C, Ye F, Hu X, Ginsberg MH. Talin activates integrins by altering the topology of the beta transmembrane domain. *J Cell Biol.* 2012; 197:605–611. [PubMed: 22641344]
96. Feng C, Li YF, Yau YH, et al. Kindlin-3 mediates integrin alphaL beta2 outside-in signaling, and it interacts with scaffold protein receptor for activated-C kinase 1 (RACK1). *J Biol Chem.* 2012; 287:10714–10726. [PubMed: 22334666]
97. Lefort CT, Rossaint J, Moser M, et al. Distinct roles for talin-1 and kindlin-3 in LFA-1 extension and affinity regulation. *Blood.* 2012; 119:4275–4282. [PubMed: 22431571]
98. Hyduk SJ, Rullo J, Cano AP, et al. Talin-1 and kindlin-3 regulate alpha4beta1 integrin-mediated adhesion stabilization, but not G protein-coupled receptor-induced affinity upregulation. *J Immunol.* 2011; 187:4360–4368. [PubMed: 21911599]
99. Kliche S, Worbs T, Wang X, et al. CCR7-mediated LFA-1 functions in T cells are regulated by 2 independent ADAP/SKAP55 modules. *Blood.* 2012; 119:777–785. [PubMed: 22117043]

KEY POINTS

- Leukocyte adhesion deficiency (LAD) syndromes are rare genetic immunodeficiency disorders that are caused by defects in adhesion and signaling of leukocytes and platelets.
- Each LAD is dominated by impaired interactions of neutrophils and monocytes with endothelial cells, but also involves other cells and other functional responses.
- LAD-I results from absent or reduced expression of β_2 integrins, a critical subset of integrin heterodimers that is restricted to leukocytes.
- LAD-II is caused by mutations in a Golgi-specific GDP-fucose transporter, leading to impaired fucosylation of ligands that are recognized by selectins and mediate leukocyte adhesion and signaling.
- LAD-III results from impaired inside-out signaling of β_1 , β_2 , and β_3 integrins, yielding defective leukocyte adhesion and signaling and a Glanzmann-like bleeding tendency, and is caused by mutations in *FERMT3* and consequent deficiency of KINDLIN-3.

	Normal	LAD-I	LAD-II	LAD-III
	Integrins	Present	Absent or decreased β_2 integrins	Normal	Defective inside-out signaling of β_1 , β_2 , β_3 integrins
Selectin ligands	Present	Normal	Defective fucosylation	Normal
Functional defects	None	Tight adhesion, emigration	Rolling	Integrin activation, adhesion, emigration, others
Mutations	None	<i>ITGB2</i>	<i>SLC35C1</i>	<i>FERMT3</i>

FIGURE 1.

The LAD syndromes are caused by molecular defects in integrin expression, fucosylation of selectin ligands, or inside-out signaling ('activation') of integrins on leukocytes and platelets. The adhesive phenotypes of PMNs from healthy individuals and patients with LAD syndromes [3,4], the corresponding functional defects in leukocyte interactions with endothelium, and known mutated genes are shown. In LAD-III, platelet integrin signaling is also defective. In addition to PMNs, monocytes, other myeloid leukocytes, and lymphocytes also basally express integrins and selectin ligands; the patterns of adhesion molecules on human leukocyte subsets are different but overlapping. Similarly, mouse leukocytes display integrins and selectin ligands, and mouse platelets express integrin $\alpha_{IIb}\beta_3$ and other integrins. There are murine models of each LAD syndrome. See text for details. Diagnosis of the LAD syndromes is commonly made based on the clinical presentation, PMN phenotype [surface expression of β_2 integrins and sialyl Lewis X (sLe^x), a fucosylated oligosaccharide present on P-selectin glycoprotein 1 and other selectin ligands], and functional analysis of leukocytes and platelets. LAD, leukocyte adhesion deficiency; PMN, polymorphonuclear leukocyte.

FIGURE 2.

The leukocyte adhesion cascade: traditional and new features. PMN interactions are used here to illustrate the leukocyte adhesion cascade; specific molecular interactions vary between leukocytes of different types in leukocyte–endothelial interactions. Early studies established the concept of a cascade of PMN tethering and rolling involving selectins and selectin ligands (1), leukocyte activation and inside-out signaling of β_2 integrins (2), tight adhesion and arrest resistant to shear (3), and emigration of adherent leukocytes across the endothelium in response to inflammatory signals [3,10]. In-vivo experiments complemented in-vitro models and confirmed that similar events occur in postcapillary venules and other vessels. Studies of PMNs from patients and animal models indicate that there is defective rolling in LAD-II (step 1), defective integrin activation and consequent tight adhesion in LAD-III (step 2), and defective tight adhesion in LAD-I (step 3). Each defect impairs the ability of PMNs to transmigrate to sites of extravascular infection or injury (step 4). More recent studies, many involving genetically altered mice, have refined the cascade paradigm, and added nuances to the stepwise events illustrated here (reviewed in [8,9,11,12[■]]). For example, β_2 integrins, particularly $\alpha_L\beta_2$, mediate early slow rolling of PMNs, in addition to later tight adhesion and arrest [8,13[■]]. Furthermore, there is evidence that engagement of PSGL-1 on PMNs can trigger inside-out signaling of β_2 integrins; thus, this is a second mechanism for β_2 integrin activation in addition to localized signaling of PMNs by endothelial presentation of activating agonists recognized by G-protein-coupled receptors [3,8,10]. This was first indicated by potentiation of β_2 integrin activation triggered via G-protein-coupled receptors when selectin ligands on human PMNs were engaged by purified P-selectin [14] and more recently supported by studies in murine models of inflammation [15]. Additional recent experiments demonstrate that PMN rolling on P-selectin or E-selectin can partially activate $\alpha_L\beta_2$ by engaging PSGL-1 [8,9,13[■],16–18]. Another new feature of the adhesion cascade is evidence for intraluminal crawling of monocytes and PMNs mediated by binding of $\alpha_M\beta_2$ to ICAM-1, occurring after rolling and arrest and prior to emigration [8,11]. Intraluminal gradients of chemokines may form, contributing to differential activation of β_2 integrins on PMNs [11]. New facets of extravasation of PMNs and transmigration through subendothelial matrix mediated by β_1 and β_2 integrins have been identified [8,11,12[■],19[■]]. Although it is not known that all of these newly identified events occur in the targeting and emigration of human PMNs in clinical infection and

inflammation, it is clear that there are intricate features of adhesive interactions mediated by selectin ligands and integrins on PMNs that may be disrupted in a complex fashion in LAD syndromes. The adhesion and signaling paradigms for monocytes and other leukocytes [12[■], 20] are also likely to be further refined. LAD, leukocyte adhesion deficiency; PMN, polymorphonuclear leukocyte; PSGL-1, P-selectin glycoprotein 1.

FIGURE 3.

Inside-out signaling of integrins on leukocytes and platelets is disrupted by *FERMT3* mutations and KINDLIN-3 deficiency in LAD-III. (a) In LAD-III, there is defective signal transduction (starburst) from G-protein-coupled receptors and other surface receptors to integrins of the β_1 , β_2 , and β_3 classes on leukocytes and platelets, leading to impaired inside-out signaling of integrins and consequent functional defects. There is a parallel defect in inside-out signaling of integrins $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$ on platelets from patients with LAD-III. (b) An example of defective adhesion of PMNs from an LAD-III patient in a static assay is illustrated. This figure is redrawn from data in [35]. In LAD-III there is impaired adhesion of PMNs to endothelial cells and to immobilized ligands for β_1 or β_2 integrins (fibronectin, fibrinogen, ICAM-1, others) when the leukocytes are stimulated with agonists recognized by G-protein-coupled receptors [N-formylmethionylleucylphenylalanine (FMLP); platelet-activating factor (PAF); interleukin 8 (IL-8)]. Defective tight adhesion of LAD-III PMNs, triggered when they are rapidly activated by agonists that are presented by inflamed endothelial cells [3,10], has been documented under conditions of flow [37]. This was identified as a defining characteristic of LAD-III [38]. (c) Mutations in *FERMT3* have been found in all LAD-III patients in whom genotyping has been reported. Identified mutations at the nucleotide level in primary leukocytes or Epstein-Barr virus-transformed lymphocytes are illustrated. These include: nonsense (a), splice site (b), insertion (c), nonsense (d), missense (e), deletion (f), splice site (g), nonsense (h), nonsense (i), splice site (j), and nonsense (k) mutations. A hotspot in exon 12 involving 24 nonsense alleles in 12 families of Turkish origin (Anatolia) indicates a founder effect [28^{***}]. Sequence in exons 7–13 and exons 14–15 encode the split F₂ domain and the F₃ subdomain of KINDLIN-3, respectively, which appear to be functionally important in molecular interactions of KINDLIN-3 with integrin β subunit cytoplasmic tails [44–46,47^{***}]. Nevertheless, no KINDLIN-3 protein has been detected in primary leukocytes or platelets from any of the LAD-III patients reported to date. See [28^{***},30,38–40,45,48–52] for details and genotype–phenotype correlations. A new mutation in exon 15 has recently been reported in abstract form [53]. Organization of the

FERMT3 gene and KINDLIN-3 domain structure has recently been reviewed [28[■],54]. LAD, leukocyte adhesion deficiency; PMN, polymorphonuclear leukocyte.

Table 1

Clinical and cellular features in index patients with LAD-III

	Patient 1 (1997) [34]	Patient 2 (2001) [35]	Patient 3 (2003) [36]	Patient 4 (2003) [37]
Multiple infections	+	+	+	+
Bleeding tendency	+	+	+	+
Leukocytosis	+	+	+	+
Consanguinity	+	+	–	+
Familial occurrence	–	+	a	a
Expression of structurally intact integrins	+	+	+	+
Integrin function activated by cations or mAbs	Intact	Partial	Intact	Intact
Expression of L-selectin and selectin ligands	+	+	N.R.	+
Leukocyte chemotaxis or motility <i>in vitro</i>	Decreased PMN transmigration across endothelial monolayers	Decreased PMN chemotaxis in Boyden chamber assay	Intact migration of T cells on immobilized ICAM-1	Intact transmigration of T cells across endothelial cells and filters
Defect in integrin inside-out signaling	+	+	+	+
Defect in G-protein-coupled receptor (GPCR)-triggered integrin activation	$\beta 2$, $\beta 3$	$\beta 1$, $\beta 2$, $\beta 3$	$\beta 1$, $\beta 2$, $\beta 3$	$\beta 1$, $\beta 2$, $\beta 3$
Anemia (based on hematocrit or hemoglobin measurement)	+	+	N.R.	+
Splenomegaly and/or hepatomegaly	+	+	N.R.	N.R.
Detachment of umbilical cord	5 weeks	N.R.	1 week	N.R.
Ethnicity	Turkish (Anatolia)	Hispanic (U.S.)	Maltese	Arabic
Mutation in <i>FERMT3</i>	+	+	+	+

^aSibling with a similar syndrome died before the age of 1 month.

The table is adapted from [38] and a similar summary in [27]. Additional details and characteristics of these patients are reported in [27,30,39,40]. The mutations in *FERMT3* are reported in [30,39–41]. A sibling of patient 2 with a similar clinical presentation and the same *FERMT3* mutation is reported in [39]. Osteopetrosis was not reported in any of these index patients. N.R., not reported.

Table 2**Functional defects in primary leukocytes and platelets from patients with LAD-III**

Neutrophils	
Adhesion to endothelial cells and/or purified β_1 , β_2 ligands in response to agonists recognized by G-protein-coupled receptors (GPCRs) or phorbol myristate acetate (PMA) (static or shear assays)	[27,34–37,39,48,80]
Aggregation stimulated by GPCR agonists or PMA	[35,48]
Transmigration across resting and cytokine-stimulated endothelial monolayers	[34]
Chemotaxis in response to GPCR agonists	[35]
Spreading	[48,52]
Monocytes	
Adhesion of cells in mixed mononuclear cell or mixed leukocyte suspensions (percentage of monocytes not reported)	[49,51]
Adhesion, spreading, differentiation of isolated monocytes	[39]
T or B lymphocytes	
Spontaneous or stimulated adhesion to purified ICAMs, VCAM-1, fibronectin; adhesion to cytokine-stimulated endothelial cells under shear	[36,37,40,41,45,81]
Transendothelial migration under shear	[37]
Aggregation	[34,48]
Spreading on purified ICAM-1 or dendritic cells	[52,81]
Platelets	
Aggregation mediated by agonists that induce activation of integrin $\alpha_{IIb}\beta_3$	[34–36,48,51,52]
Soluble fibrinogen binding	[36,48,51]
Pac-1 antibody binding	[34,36]
Clot retraction	[35]
Adhesion and spreading on immobilized fibrinogen	[48,49,51]
Integrin $\alpha_2\beta_1$ -mediated degranulation	[51]
Integrin $\alpha_2\beta_1$ -mediated aggregation	[82*]

The table lists selected functional responses and is not comprehensive. Additional assays and details are also included in the cited reports. Also see Fig. 3. In some studies variations in functional responses were detected when cells were activated with phorbol esters compared to activation with GPCR agonists [52], but in most studies the results were concordant. Many studies also utilized immortalized lymphoblasts or T-cell clones derived from LAD-III patients and family members, or controls [34–37,45,48,51]. There can be differences in the responses of transformed lymphocytes versus those of primary cells [34,49,51], but the results have commonly been consistent with defective inside-out signaling of multiple integrins in experiments using immortalized or cloned cell lines. The platelet responses listed include functional activities that are classically defective in Glanzmann thrombasthenia (aggregation, fibrinogen binding, Pac-1 binding, and clot retraction [79]). In addition, altered function of integrin $\alpha_2\beta_1$ has been reported [51,82[■]], consistent with KINDLIN-3 deficiency (see text) and potentially contributing to severity of bleeding in LAD-III [82[■]]. Studies of platelet–monocyte and platelet–PMN interactions, which involve integrins and influence leukocyte targeting and hemostatic responses [25] in LAD-III, have not been reported.