

# Leukocyte adhesion deficiency III: a group of integrin activation defects in hematopoietic lineage cells

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## Purpose of review

In the last 2–3 years our understanding of leukocyte adhesion cascades has increased, mainly in defining new pathways by which integrin activation occurs on circulating leukocytes recruited to sites of inflammation. While defects in the integrin structure (leukocyte adhesion deficiency (LAD) I) and in the selectin glycoprotein ligand biosynthesis (LAD II) have been described in the past few decades, a newly recognized defect in the activation of integrins (LAD III) was only recently delineated. The clinical manifestations and molecular basis of this syndrome and related cases will be reviewed.

## Recent findings

While in LAD I and II the defect in the adhesion cascade is restricted to leukocytes, all four cases of LAD III described to date also had defects in platelet aggregation. These patients suffered from recurrent bacterial infections and a severe bleeding tendency. All cases were reported to have activation defects in all major integrin subfamily members expressed in circulating leukocytes and platelets. In one case there was a defect in Rap1, which is a crucial protein in the inside-out and outside-in (ligand-induced) signaling underlying integrin activation mainly by cytokines. In this case, both chemokines and cytokines were unable to activate Rap1 leading to severe adhesive defects analyzed *in vitro*.

## Summary

While in LAD I and II the primary genetic defect is known, in the newly described LAD III the primary event leading to the defect is still unknown, despite a clear biochemical defect in Rap1 activation. The molecular basis or the defect in integrin activation may be different in the various cases described so far. It seems logical, however, to assume that in all reported cases, a key component of inside-out signaling to integrins activation is involved.

## Keywords

integrin, chemokine, Rap1, adhesion, lymphocytes

Curr Opin Allergy Clin Immunol 4:000–000. © 2004 Lippincott Williams & Wilkins.

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Current Opinion in Allergy and Clinical Immunology 2004, 4:000–000

## Abbreviations

<b>GEF</b>	guanine nucleotide exchange factor
<b>GPCR</b>	G-protein coupled receptor
<b>ICAM</b>	intercellular adhesion molecule
<b>LAD</b>	leukocyte adhesion deficiency
<b>TCR</b>	T-cell receptor
<b>VCAM</b>	vascular cell adhesion molecule

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## Introduction

During an inflammatory process, in order for leukocytes to arrive at sites of infection or injury, they must adhere to the blood vessel endothelium, emigrate through it and move towards their final destination in the inflamed tissue. The process of adhesion is a complex one and involves several crucial steps. Once endothelial activation occurs, selectins, specialized lectin-like adhesion molecules, are expressed and will bind loosely to circulating leukocytes expressing specific glycoprotein ligands for these endothelial selectins. This rolling phase is followed by rapid activation, primarily by chemokines, of various integrins expressed in inactive states on most circulating leukocytes. In-situ activated integrins will bind firmly to endothelial ligands such as intercellular adhesion molecules (ICAMs) or vascular cell adhesion molecule-1 (VCAM-1), which are expressed constitutively or inducibly on various endothelial beds. Sensing chemoattractant substances within the surface of blood vessel cells or in the inflamed area, the leukocytes will transmigrate the endothelial barrier and arrive at their site of function [1]. Recently, our understanding of the various phases of the adhesion cascade has increased tremendously. Several new molecules involved in the activation of leukocyte integrins have been described [2•,3•], transmigration was shown to involve previously unknown mechanisms regulated by integrins, chemokines and shear flow signals [4] and fundamental discoveries in the structural basis of integrin activation have recently been made [5].

One of the best ways by which molecular players in this process can be identified is through 'experiments of nature', when a defined mutation in a specific pathway can lead to disease. Although 'knock out' techniques have provided keen insights into human pathologies, the human in-vivo consequence of a particular defect can be best substantiated by rare inherited disorders. Human syndromes resulting from deficiency or impaired function

of integrins and other adhesion or cytoskeletal molecules have contributed to critical insights into their biological roles [6]. More than 20 years ago, genetic mutations in the gene encoded by the  $\beta 2$  subunit (CD18) of the integrin was found. These mutations, collectively named leukocyte adhesion deficiency (LAD) I, have been reported in over 300 patients and are associated with severe bacterial infections and marked leukocytosis [7]. In addition, LAD I can arise from point mutations in normally or partially expressed CD18, resulting in LAD I like syndrome [8]. A second LAD syndrome, LAD II, has been linked to defective biosynthesis of key fucosylated glycans, which comprise the carbohydrate ligands for both endothelial and leukocyte selectins [9]. Glanzmann thrombasthenia, which is a rare bleeding disorder, is due to mutations in the  $\beta 3$  integrin subunit. This leads to dysfunction in platelet aggregation, but does not affect leukocyte adhesion.

Since integrins are expressed on most circulating leukocytes and platelets in largely inactive states, they need to undergo in-situ activation by numerous signals in order to avidly bind their vascular and extravascular ligands. Until recently, no genetic defect in this activation process was described. We have recently described a new adhesion defect in which severe abnormalities in integrin activation by chemokines and lipid chemoattractants were observed [10\*\*]. This new LAD showed significant similarities to three previous cases, commonly referred to as LAD I variant (Table 1) [11]. All four cases had similar clinical symptoms, characterized by severe recurrent infections, bleeding tendency and marked leukocytosis. Notably, although integrin expression and structure were intact, all had defects in leukocyte (or platelet) integrin activation by either chemoattractants, T-cell receptor (TCR) ligation or agonists to major inside-out activators of integrins such as phorbol esters. As structural aberrations in these activation-defective hematopoietic cell lineage integrins were not reported, we proposed that they should be termed LAD III rather than LAD I variants [12\*].

### Activation of the integrins at endothelial contacts: a multistep adhesive cascade

The migration of myeloid and lymphoid cells from the vascular lumen to the tissues involves a series of sequential molecular interactions between the leukocyte and its endothelial cell targets. Selectin binding initiates these adhesion cascades, mediating leukocyte capture and rolling. This reversible motion allows the newly recruited leukocyte to encounter chemokines, and finally arrest on the endothelial cell and emigrate to extravascular tissues. The principal players in this process are integrin members which constitute a large family of surface glycoproteins and are composed of highly conserved  $\alpha$  and  $\beta$  subunits. Integrin activation at endothelial sites occurs primarily in response to intracellular signals by external stimuli such as cytokines or chemokines. This activation process, referred to as 'inside-out signaling' ultimately increases integrin adhesiveness. Although several molecules were found to be important in a variety of inside-out activation processes, their mode of action in the vasculature and in extravascular tissues is far from being understood [13\*]. Integrin activation processes occur through two modes: affinity modulation, which affects the strength of an individual integrin–ligand bond by conformational changes transmitted from the cytoplasmic tails to the extracellular domain of integrins [14]; and avidity modulation, through which integrin cell surface diffusion and clustering are modified. Integrin clustering is thought to enhance adhesion by increasing the number of integrin–ligand bonds at a specific point on the cell surface [15]. A third mode of activation may be the result of outside-in activation, through integrin rearrangements induced by ligand binding [5,13\*].

Chemokines, which are small polypeptides with chemotactic activity for leukocytes, are the major in-situ regulators of integrin activity on leukocytes interacting with vessel walls or migrating across the extracellular matrix [16]. Chemokines are generally displayed on activated endothelial cells along with selectins or selectin

**Table 1. Clinical, cellular and biochemical aspects of recently reported leukocyte adhesion deficiency cases (LAD III)**

	1	2	3	4
Bacterial infections	+	+	+	+
Bleeding tendency	+	–	+	+
Leucocytosis	+	–	+	+
Consanguinity	+	–	– <sup>a</sup>	+ <sup>a</sup>
Expression of structurally intact integrins	+	–	+	+
Intrinsic integrin avidity to ligand (activated by cations or mAbs)	Intact	Impaired	Intact	Intact
Expression of L-selectin and selectin ligands	–	+	ND	+
Chemotaxis or motility	ND	Impaired	Normal	Normal
Integrin inside-out defect	–	+	+	+
Defect in GPCR triggered integrin adhesiveness	$\beta 2, \beta 3^b$	$\beta 1, \beta 2, \beta 3^b$	$\beta 1, \beta 2, \beta 3^b$	$\beta 1, \beta 2, \beta 3^{b,c}$
Acquisition of leukocyte activation epitopes by GPCRs	Impaired <sup>d</sup>	ND	ND	Normal <sup>d</sup>

GPCR, G-protein coupled receptor; ND, not determined. <sup>a</sup>Sibling died from the same symptoms before the age of 1 month. <sup>b</sup> $\beta 3$  defects observed in patient derived platelets. <sup>c</sup>Aker *et al.* (unpublished). <sup>d</sup>CBRM1/5 or 327C in agonist-stimulated neutrophils. Adapted from Alon [12\*].

ligands. Thus, when a rolling leukocyte adheres and rolls over the endothelium, it can recognize the chemokines via its seven transmembrane spanning G-protein coupled receptors (GPCRs), which elicit an integrin activation signal by activating heterotrimeric G proteins, mainly of the Gi family. This process is extremely rapid and can occur in subseconds, the time frame of individual adhesive contacts engaged by a continuously rolling leukocyte [17].

A growing body of evidence implicates the Ras-related GTPase, Rap1, as a key regulator of integrin activation via both TCRs as well as GPCRs [18\*]. Stimulation of the TCR or chemokine receptors triggers the activation for Rap1 through poorly defined pathways. These signaling pathways promote loading of GTP onto Rap1 by activating one or more Rap1 guanine nucleotide exchange factors (GEFs). The GTPase-activating protein can reverse this process and thus inactivate and terminate Rap1 activities [19]. Rap1 was found to be crucial in various aspects of leukocyte as well as endothelial and stromal cell adhesion [20]. In its active form (GTP-Rap1), integrin function is enhanced probably by modulating both integrin affinity and clustering and thereby enhancing ligand-induced integrin activation [21]. Furthermore, Rap1 has unique abilities to trigger cell polarization (Fig. 1) [22\*\*]. Recently, it was found that activated Rap1 engages a cytoplasmic protein, RAPL, which in turn will bind to the intracytoplasmic tail of the integrin and will activate it [2\*\*].

### Leukocyte adhesion deficiency syndromes I-III

LAD syndromes include disorders in which a specific defect in the adhesion cascade occurs. LAD I is due to a genetic defect in the  $\beta$  subunit (CD18) of the integrin. Hundreds of patients have been identified and the hallmarks of the syndrome are delayed separation of the umbilical cord, recurrent severe bacterial infection without pus formation, marked leukocytosis and the absence or marked decrease in CD18 expression on the leukocyte surface (Table 2). In the severe form the patient will not survive childhood and the only curative therapy is bone marrow transplantation. In two patients with a milder form of the disease, point mutations which disrupted integrin function rather than expression were described [8].

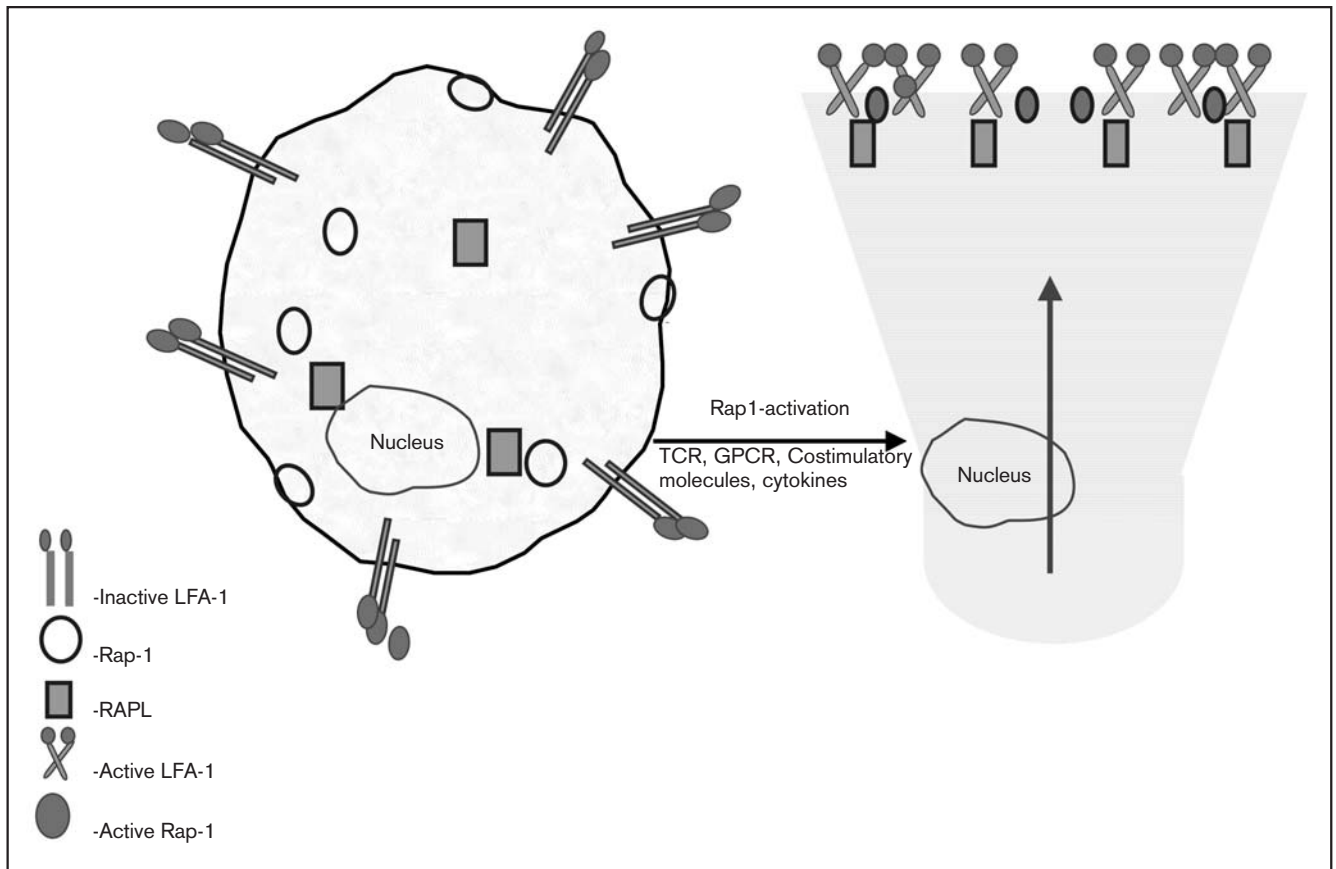
LAD II is a rare condition, described so far in only six children and is characterized by recurrent infection, growth and mental retardation, the rare Bombay blood group and the absence of sialyl lewis x (CD15a), the ligand for selectin, on leukocytes (Table 2) as well as on endothelial cells. These patients suffer from infection to a lesser degree compared with those with LAD I and have a defect in the first phase of the adhesion cascade,

the selectin-mediated rolling phase. The primary defect is a genetic mutation in the specific transporter of fucose from the cytoplasm into the Golgi apparatus. Thus, glycans which incorporate fucose like CD15a or the H antigen (Bombay), are not expressed on myeloid cells. This is a general defect not confined to hematopoietic or endothelial cells, and thus causes severe growth and mental retardation. In one case fucose administration improved both the clinical state as well as the adhesion abnormality.

The newly described LAD III syndrome is also very rare, reported in only four patients all of whom had similar clinical symptoms characterized by severe recurrent infections, bleeding tendency and marked leukocytosis with normal expression of CD18 and CD15a. In three of the cases consanguinity was reported, suggesting an autosomal recessive type of inheritance. While in LAD I and II only a specific molecule was involved in adhesion, CD18 and CD15a respectively, in LAD III  $\beta 1$ ,  $\beta 2$  and  $\beta 3$  integrins are affected and thus both leukocytes and platelets are defective in their ability to adhere and hence a tendency for bleeding accompanied the severe recurrent infections. In all cases integrin expression and structure were intact, but marked defects in integrin activation by physiological inside-out stimuli were observed. In the patient reported by McDowall *et al.* [11] neutrophils, lymphocytes and platelets showed defective functional activity of  $\beta 2$  and  $\beta 3$  as well as  $\beta 1$  integrins. Various inside-out signals failed to enhance platelet aggregation or binding of soluble fibrinogen, or neutrophil adhesion or T-cell adhesion to various  $\beta 2$  and  $\beta 1$  integrin ligands such as ICAM-1 and fibronectin. Furthermore, agonists that directly stimulate integrin activity bypassing inside-out signaling were able to reconstitute integrin-dependent adhesion, suggesting that hematopoietic cells from this patient expressed structurally intact integrins [23].

In the case reported by Alon *et al.* [10\*\*], activation of all major leukocyte integrins by endothelial displayed chemokines or chemoattractants was severely impaired. Although LAD III leukocyte rolling on the endothelial surface was normal, they failed to arrest on endothelial displayed chemoattractants. The key defect in this case was attributed to a genetic loss of integrin activation by rapid chemoattractant-stimulated GPCR signals [24]. Talin is crucial for integrin activation and anchorage to the actin cytoskeleton [25], yet it is not restricted to cells of the hematopoietic lineage and is therefore unlikely to be mutated in LAD III syndromes [26]. Since loss of integrin or Rap-1 function in mice results in embryonic lethality, 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. In all reported cases of LAD III,

Figure 1. Rap-1 increases affinity, outside-in signaling and polarization of integrins



GPCR, G-protein coupled receptor; LFA-1, lymphocyte function associated antigen-1; TCR, T-cell receptor.

Table 2. Leukocyte adhesion deficiency (LAD) syndromes

	LAD I	LAD II	LAD III
Clinical manifestation			
Recurrent severe infections	+++	+	+++
Neutrophilia			
basal	+	+++	++
with infection	+++	+++	+++
Periodontitis	++	++	?
Skin infection	++	+	++
Delayed separation of the umbilical cord	+++		+
Developmental abnormalities		+++	
Bleeding tendency			+++
Laboratory findings			
CD18 expression	↓↓↓ or absent	Normal	Normal
SLeX expression	Normal	Absent	Normal
Neutrophil motility	↓↓↓	↓↓	↓↓
Neutrophil rolling	Normal	↓↓↓	Normal
Neutrophil adherence	↓↓↓	↓	↓↓↓
Opsonophagocytic activity	↓	Normal	Normal
T and B cell function	↓	Normal	?

defects in avidity upregulation of at least two integrin subtypes were reported. In three out of the four cases, platelet GPIIb/β3 integrins failed to acquire high affinity in response to GPCR signals, suggesting a defect in

cytoplasmic factor that, normally, upon activation by G protein, rapidly alters integrin conformation and acquisition of high affinity. All these data point to a novel hematopoietic cell restricted factor, which is essential in the inside-out signaling for integrin activation, and is either absent or functionally impaired in LAD III. As discussed above, Rap1 is a crucial factor in the inside-out signaling of integrin activation in leukocytes and platelets. We have therefore looked at Rap1 activation in LAD III patient-derived lymphocytes [27•]. Although normal expression of Rap1 was observed, the basal GTP loading of Rap1 (activated form) was markedly attenuated in LAD III cells. This decreased basal GTP Rap1 had a profound effect on the ability of the VLA-4 integrin to bind to its ligand, VCAM-1, under shear flow, but did not reduce VLA-4 affinity to soluble VCAM-1.

Chemokines and phorbol ester (PMA) trigger Rap1 exchange of GDP to GTP within seconds. We therefore tested whether Rap1 derived from LAD cells exhibits defects in de-novo activation by the prototypic chemokine stromal cell-derived factor 1α. Despite normal

expression of its receptor, CXCR4, stromal cell-derived factor 1 activation of Rap1 was completely abolished. Strikingly, Rap1 activation by PMA remained intact [27••]. These findings rule out an intrinsic activation defect of the GTPase, which would also have attenuated Rap1 activation by PMA. The ubiquitous expression of Rap1 in most tissues, and its highly diverse functions in nonhematopoietic cells [28], make it unlikely that Rap1 is structurally mutated in any of the new LAD III cases. It should be mentioned that basal Rap1 expression was normal in another LAD III case [11], but no data were given regarding its activated form.

The results suggest that a GPCR-regulated Rap1 GEF activity essential for Rap1 activation and integrin avidity regulation is defective in LAD III hematopoietic lineage cells. We conclude that the possible cause of this defective Rap1 activation could be abnormality in Rap1-GEF like C3G [18•] or in a novel yet to be identified Rap1 GEF that accounts for the majority of hematopoietic integrin activation by GPCR agonists.

## Conclusion

LAD III may comprise a new family of genetic deficiencies in key adaptors implicated in rapid activation of all major integrins on hematopoietic cells. Thus, from a clinical aspect, it is unique for the combination of severe bacterial infection (leukocyte adhesion defect) together with a marked bleeding tendency (platelet aggregation abnormalities). From a mechanistic point of view, it is interesting that the LAD III syndrome represents a global yet hematopoietic lineage restricted inside-out signaling defect rather than integrin deficiency or structural aberration. It is not currently understood how basal (cytokine) and chemokine-triggered Rap1 activation is regulated, and so, identification of upstream regulators of Rap1, such as novel GEFs, may provide important clues to severe as well as milder forms of LAD III. Like LAD I and II, dissection of LAD III may provide useful genetic and biochemical markers and new therapeutic targets to selectively interfere with key inside-out and outside-in integrin activation processes of platelets and leukocytes, which regulate both their migration and effector functions in health and disease.

## Acknowledgement

The authors thank Dr S. Feigelson for editorial assistance.

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