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## Review Article

# Kindlin-3 in the immune system

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**Abstract:** Kindlin-3 is a member of the kindlin family of focal adhesion proteins which bind to integrin beta-chain cytoplasmic domains to regulate integrin function. In contrast to kindlin-1 and kindlin-2 proteins, kindlin-3 is expressed mainly in the hematopoietic system. Mutations in kindlin-3 result in the rare genetic disorder, leukocyte adhesion deficiency type III (LAD-III), which is characterized by bleeding and recurrent infections due to deficient beta1, beta2 and beta3 integrin activation in platelets and leukocytes. Here, we review the role of kindlin-3 in integrin activation and in different immune cell functions.

**Keywords:** Kindlin, integrin, adhesion, LAD-III, T cell

### Introduction

Integrins of the beta2-family (alphaLbeta2; CD11a/CD18; LFA-1, alphaMbeta2; CD11b/CD18; Mac-1, alphaXbeta2; CD11c/CD18 and alphaDbeta2; CD11d/CD18 integrins) play a vital role in leukocyte adhesion and migration *in vivo*. These receptors are exclusively expressed on leukocytes and bind to ligands such as intercellular adhesion molecules (ICAMs) on other leukocytes and endothelial cells, as well as soluble proteins such as the complement protein iC3b. beta2-integrins are essential for the firm adhesion of leukocytes to endothelial cells in the blood vessels during shear flow conditions [1]. They have also been reported to be important for neutrophil activation, T cell-dendritic cell contact formation and T cell killing of target cells. In addition, alphaM-beta2 and alphaXbeta2-integrins are important receptors for complement (iC3b) and mediate phagocytosis of complement-coated particles. A low or absent expression of beta2-integrins causes leukocyte adhesion deficiency type I (LAD-I), a rare genetic condition where leukocytes (especially neutrophils) cannot exit the blood stream, resulting in recurrent bacterial infections and fungal infections and delayed wound healing, showing the crucial importance of these receptors in immunity [2].

Leukocytes also express other integrins, such as alpha4beta1-integrin (VLA-4), which plays important roles in lymphocyte trafficking through binding to ligands such as vascular cell adhesion molecule-1 (VCAM-1).

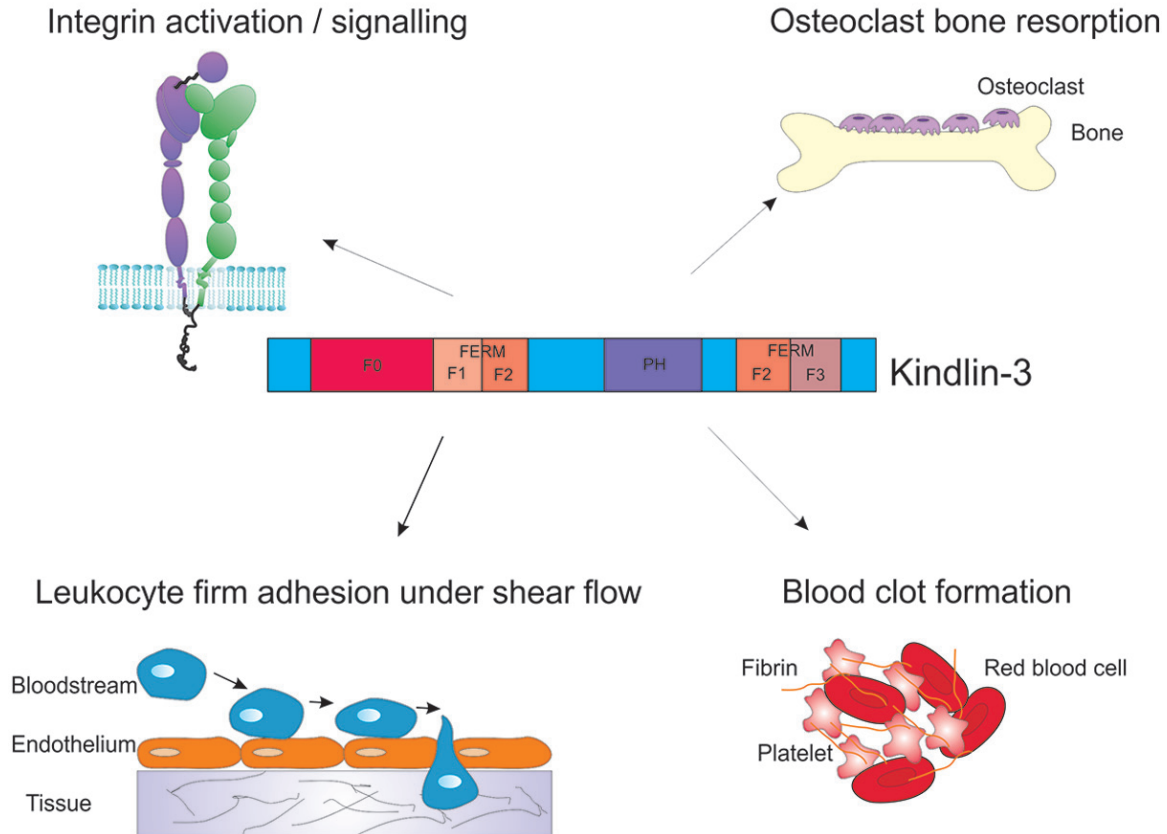
The alphallbbeta3 integrin is expressed on platelets and binds to ligands such as fibronectin, fibrinogen, von Willebrandt factor and thrombospondin. This integrin is important for platelet aggregation, and deficiencies in the function of this receptor are associated with Glanzmann thrombasthenia, a bleeding disorder [3].

### Kindlin-3 binds integrin beta-chain cytoplasmic domains and regulates integrin function

Integrins are regulated by the binding of cytoplasmic factors, such as talin, filamin, 14-3-3 proteins and kindlin to their intracellular tails, and these interactions are of crucial importance for integrin function [1, 4, 5].

The kindlin family of focal adhesion proteins consists of three members. Kindlin-1 is expressed mainly in epithelial cells and keratinocytes, and mutations in kindlin-1 lead to skin blistering, skin atrophy and periodontal disease in patients with Kindler Syndrome [6]. Kindlin-2 is ubiquitously expressed and kindlin-2 knock-

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**Figure 1.** Schematic diagram of kindlin-3 functions in cells. The integrin binding site is in the F3 subdomain of kindlin-3.

out mice are embryonic lethal at the preimplantation stage [7]. Kindlin-2 is necessary for myogenesis and myocyte elongation [8, 9], and mice with kindlin-2 deficiency (kindlin-2 +/- animals) display defective angiogenesis and a leaky and immature vasculature [10].

In contrast to kindlin-1 and kindlin-2, kindlin-3 is expressed mainly in hematopoietic cells [11], although expression in endothelial cells has also been described [12]. Kindlin-3 contains a FERM domain and a PH-domain and adopts an elongated conformation which is similar to that of talin's head domain [13] (**Figure 1**).

Recently, it was discovered that kindlin-3 is mutated in the rare genetic disorder, leukocyte adhesion deficiency type III (LAD-III). Like LAD-I patients, LAD-III patients suffer from recurrent infections, but in addition, the patients have a bleeding phenotype due to deficient platelet integrin activation [14-16]. In addition, the patients may suffer from osteopetrosis [15], due to deficient integrin-mediated osteoclast

bone resorption [17]. Kindlin-3 binds to beta1, beta2 and beta3 integrin cytoplasmic domains and regulates integrin functions *in vivo*. The kindlin binding site in the integrin beta2-chain consists of the membrane-distal NPXY-motif [5] and a TTT-domain in the beta2-integrin tail [13, 18]. The kindlin binding site does not overlap with the binding site for talin, another important integrin regulator which binds to the membrane-proximal NPXY-motif [5, 18]. However, the kindlin-3 binding site does overlap with binding sites for other integrin interactors, eg filamin, a negative regulator of integrins [4] and 14-3-3 proteins, which bind to phosphorylated beta2-integrins and appears to regulate Rac-1 signalling and cell spreading downstream of the integrin [4, 19, 20]. In addition, sorting nexin 17, a protein which regulates integrin recycling has been reported to bind to a similar site in beta1-integrins [21]. Whether there is competition between these proteins for the binding site in beta2-integrins, and what the functional consequences of such competition would be is unknown at present.

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**Table 1.** Comparison of talin and kindlin-3 functions in integrin regulation and the immune system

	Talin -/-	Kindlin-3 -/-	References
Integrin conformation	Low affinity conformation only	Low and intermediate affinity conformations	[27]
Integrin outside-in signalling	Impaired	Impaired	[17]
Lymphocyte development	? (embryonic lethal)	Impaired	[5, 35]
Leukocyte rolling under shear flow	Impaired	Normal	[18, 27]
Leukocyte firm adhesion/spreading	Impaired	Impaired	[5, 18, 28]
T cell activation (spleen)	Impaired	Normal	[18, 28]

Kindlin-3 knockout animals die early after birth due to severe bleeding because of a deficiency in  $\alpha$ IIb $\beta$ 3 integrin activation and platelet aggregation [22]. Study of kindlin-3 deficient neutrophils have revealed an essential role for kindlin-3 in the regulation of neutrophil adhesion to endothelial cells under shear flow *in vivo* [5], and studies of other (human and mouse) immune cell types has revealed a similar role for kindlin-3 in other leukocytes, such as B cells [23], CD4 T cells and effector T cells [18, 24, 25] and NK cells [26] (**Figure 1**).

Because kindlin-3 knockout animals die early after birth due to the bleeding phenotype, and because lymphocyte development is affected in these animals [5], it has remained unclear how kindlin-3 regulates  $\beta$ 2-integrins in lymphocytes *in vivo*. The development of a novel animal model, where the kindlin-3 binding site in the  $\beta$ 2-integrin has been mutated (TTT/AAA- $\beta$ 2-integrin knock-in mouse model), has confirmed the importance of kindlin-3 in regulation of leukocyte adhesion *in vivo*, as these animals display splenomegaly and neutrophilia [18]. Lymphocyte development is normal in these mice, but kindlin-3 binding to the  $\beta$ 2-integrin cytoplasmic domain is essential for chemokine-induced CD4+ T cell adhesion to ICAM-1 and endothelial cells under shear flow conditions and for normal lymphocyte recirculation and homing to lymph nodes. In addition, the integrin-kindlin interaction is necessary for effector T cell adhesion under flow conditions [18].

### Kindlin-3 is not necessary for all integrin-mediated functions in the immune system

Clues to how kindlin-3 regulates integrins have come from detailed investigations of integrin function in kindlin-3 deficient cells or cells where the interaction between kindlin-3 and the integrin has been abolished. Neutrophils deficient in talin are deficient in both rolling and arrest, whilst kindlin deficient neutrophils can

still mediate slow rolling [27], indicating that these different integrin regulators serve different roles in integrin activation (**Table 1**). Neither talin nor kindlin-3 deficient neutrophils were able to adopt the high-affinity conformation (extended/open I domain) of the LFA-1 integrin, whilst only talin -/- cells were deficient the first step of integrin activation to the intermediate affinity form (extended/closed I domain) [27].

We have recently shown that the integrin/kindlin interaction is not completely necessary for integrin-mediated effector T cell 2-dimensional migration on ICAM-1, or for  $\beta$ 2-integrin mediated rolling on ICAM-1 under shear flow conditions [18]. Atomic force microscopy measurements of early interactions between the integrin and its ligand have revealed that such early interactions in the absence of shear flow is normal [18]. Probably because of this, integrin-kindlin interactions is not essential for T cell activation in the spleen *in vivo*, which occurs in an environment without shear stress [18]. In contrast, talin is necessary for T cell activation *in vivo* [28]. In addition, recent studies have shown that kindlin-3 is not necessary for effector T cell diapedesis [25]. Interestingly, it is also not necessary for the induction of experimental autoimmune encephalomyelitis in mice, although it is necessary for recruitment of autoreactive effector T cells into the naïve CNS [24]. These different requirements for kindlin-3 in integrin regulation in effector T cells appear to reflect differences in ligand levels on resting and inflamed endothelium; kindlin-3 appears to be required only for adhesion to low, but not high, levels of integrin ligands ICAM-1 and VCAM-1. Collectively, these studies reveal the selective role of the integrin/kindlin interactions in regulating integrin-mediated adhesive functions (**Table 1**).

### Outside-in signaling

In addition to its role in integrin activation to mediate adhesion under shear flow conditions,

Kindlin-3 is thought to be involved in integrin outside-in signaling [29]. Integrin-mediated cell spreading is deficient in kindlin-3  $-/-$  neutrophils [5] and in effector T cells where the kindlin-3 binding site is mutated in the beta2-integrin [18]. In addition, integrin-mediated outside-in signaling through Syk, Erk and Akt is deficient in kindlin-3  $-/-$  osteoclasts [17], and targeted knockdown of kindlin-3 leads to reduced alphaMbeta2 (Mac-1)-mediated Syk and Vav signaling, and reduced Rac-1 activation in Mac-1-transfected K562 cells [30]. How kindlin-3 couples to these intracellular signaling pathways remains to be investigated in the future.

### Non-integrin related effects of kindlin deficiency

In addition to integrins, kindlin proteins have also been described to interact with binding partners such as integrin linked kinase (ILK) and migfilin and kindlin-3 has been described to interact with receptor for activated C kinase 1 (RACK1) [29, 31]. Interestingly, kindlin-3 deficient mice have been described to have abnormally shaped erythrocytes with disrupted membrane skeletons [32]. As erythrocytes don't have integrins, this finding indicates that kindlin-3 has functions in cells which are not integrin dependent. The molecular targets for such functions of kindlin-3 have so far not been described.

Integrin-independent functions have also been described for other kindlin-family members. Kindlin-2 binds directly to the clathrin heavy chain, which regulates clathrin-dependent vesicle trafficking in endothelial cells in an integrin-independent manner. Therefore, trafficking of CD39 and CD73, which are enzymes involved in ADP/AMP catabolism, is affected in Kindlin2 +/- mice [33]. Kindlin-2 has also been reported to bind to the TGF- $\beta$  type 1 receptor through its FERM domain and to Smad3 through its N terminus, which mediates this interaction in human kidney tubular epithelial cells [34]. Kindlin-1 and kindlin-2 have also been described to be found in the nucleus of cells; no such localization of kindlin-3 has so far been reported [6].

### Conclusions

It is by now well established that kindlin-3 plays a fundamentally important role in integrin regu-

lation in immune cells. Further research is now necessary to reveal the molecular details of this process, as well as defining non-integrin related protein partners for kindlin-3.

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### Disclosure of conflict of interest

None.

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