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Novel aspects in the regulation of the leukocyte adhesion cascade

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Abstract

Leukocyte recruitment plays a major role in the immune response to infectious pathogens and during inflammatory and autoimmune disorders. The process of leukocyte extravasation from the blood into the inflamed tissue requires a complex cascade of adhesive events between the leukocytes and the endothelium including leukocyte rolling, adhesion and transendothelial migration. Leukocyte-endothelial interactions are mediated by tightly regulated binding interactions between adhesion receptors on both cells. In this regard, leukocyte adhesion onto the endothelium is governed by leukocyte integrins and their endothelial counter-receptors of the immunoglobulin superfamily. The present review will focus on novel aspects with respect to the modulation of the leukocyte adhesion cascade.

The multistep process of leukocyte recruitment

Leukocyte recruitment is crucial in the course of infection, in inflammatory disorders, such as atherosclerosis, as well as in autoimmune diseases, such as in psoriasis, rheumatoid arthritis, vasculitis and chronic lung diseases (1). Leukocyte extravasation comprises several adhesive steps including (i) the initial selectin- dependent rolling and tethering of the leukocytes, (ii) the chemokine-induced leukocyte activation, (iii) the integrin-mediated firm adhesion and (iv) the transendothelial migration of leukocytes, which can take place in both a paracellular and a transcellular manner. Finally leukocytes migrate in the extracellular matrix (1-11).

Rolling adhesions function as a “brake” to slow down the flowing leukocytes. The transient and reversible rolling interactions between leukocytes and the endothelium are mediated by weak binding between the E-, P- or L-selectin with their carbohydrate ligands, such as P-selectin glycoprotein-1 (PSGL-1) (12). PSGL-1 on leukocytes binds to both endothelial E- and P-selectin. P-selectin is stored in Weibel-Palade bodies, and inflammatory stimuli induce its rapid exposure on the apical endothelial surface, whereas E-selectin is newly synthesized upon pro-inflammatory stimulation (12). Besides mediating rolling and tethering, the P-selectin / PSGL-1 and the E-selectin / PSGL-1 interactions play also an important role in the slow rolling process (13,14). Recent evidence has pointed to the involvement of integrins, and particularly of the β 2-integrin lymphocyte function antigen-1 (LFA-1) in slow rolling adhesions that promote firm arrest from rolling (15,16). Thus, the slow rolling process represents the transition between rolling and firm adhesion, where these two adhesive events functionally merge.

During the step of chemokine-induced activation, the low-affinity, selectin-dependent interaction is transformed into the high-affinity, integrin-mediated firm adhesion of leukocytes

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to the endothelium by activating signals derived from chemokines (17). The firm arrest of leukocytes to endothelial cells is mediated by interactions between leukocyte integrins, such as VLA-4 ($\alpha 4\beta 1$), $\alpha 4\beta 7$ -integrin, Mac-1 ($\alpha M\beta 2$) and LFA-1 ($\alpha L\beta 2$), and their endothelial counter-receptors of the immunoglobulin superfamily, such as the intercellular adhesion molecules (ICAM), the vascular cell adhesion molecule-1 (VCAM-1), or the receptor for advanced glycation endproducts (RAGE), which are usually upregulated on the inflamed endothelium (3,18,19). Whereas VCAM-1 interacts with VLA-4, $\beta 2$ -integrins (including LFA-1 and Mac-1) bind to ICAM-1 and ICAM-2 (3,18,20). ICAM-1 and ICAM-2 are the mostly studied $\beta 2$ -integrin ligands on the endothelium; ICAM-3, ICAM-4, and ICAM-5 are expressed in leukocytes, red cells and brain neurons, respectively (20-24).

After their firm adhesion, leukocytes move slowly over the endothelial cell surface using their integrins Mac-1 and LFA-1, a process called crawling or locomotion (8,25) until they reach an area appropriate for transmigration. Transendothelial migration (also designated as diapedesis) may take place at the intercellular junctions, i.e. in a paracellular manner, or through the endothelial cell body, i.e. in a transcellular manner (26-28). The preference of the one pathway over the other is not clear and may depend on the level of the proinflammatory stimulation of the endothelial cells (29). The process of transmigration has drawn a lot of attention recently. Here, we are going to briefly summarize the players of transmigration, since this process was recently reviewed elsewhere (11).

Besides their importance in leukocyte-endothelial adhesion, ICAM-1 and ICAM-2 participate in leukocyte transendothelial migration as well. During diapedesis, ICAM-1 has been identified in microvilli-like projections that form a “cuplike” structure that surrounds leukocytes migrating through the endothelial cell body (28). In addition, endothelial ICAM-1 co-localizes with the ring-like cluster of LFA-1 at the interface between the transmigrating leukocyte and endothelial junctions (27). The involvement of ICAM-2 in neutrophil diapedesis *in vivo* was demonstrated by intravital microscopy studies engaging blocking antibodies to ICAM-2 as well as with ICAM-2-deficient mice (30).

Endothelial adherens and tight junctions represent the major barrier for the transmigrating neutrophil in the paracellular pathway (31). The major constituent of adherens junctions is VE-cadherin, which is the predominant gatekeeper for the passage of leukocytes. VE-cadherin disappears transiently from the junctions during transmigration (32), whereas blockade of VE-cadherin increases the rate of neutrophil extravasation *in vivo* (33). In contrast, the junctional adhesion molecules (JAM) and the JAM-related endothelial selective adhesion molecule (ESAM) are found in tight junctions of endothelial cells but also in circulating cells and belong to the immunoglobulin superfamily (34-36). A conserved motif in JAMs mediates their homophilic interaction (37,38), however, JAMs have also the propensity to act as counter-receptors for leukocyte integrins: JAM-A, JAM-B, and JAM-C bind to LFA-1, VLA-4 and Mac-1, respectively (39-41). In addition, the interaction between JAM-C and JAM-B has been established (42,43). Inhibition experiments as well as experiments with genetically modified mice have suggested a role of JAM-A and JAM-C in leukocyte recruitment *in vitro* and *in vivo*, especially at the step of diapedesis (44-48). The exact mechanism by which JAMs regulate leukocyte recruitment requires further investigation. This mechanism seems to be rather complex, as besides their homophilic and heterophilic interactions, JAMs can regulate the endothelial barrier. In particular, JAM-C and ESAM can disrupt VE-cadherin-mediated adherens junctions through the regulation of signaling pathways that involve small GTPases such as RAP1 or RhoA (49,50), and these pathways may also participate in the modulation of leukocyte diapedesis.

A well established player in transmigration is platelet endothelial cell adhesion molecule-1 (PECAM-1), which is a member of the immunoglobulin superfamily and is expressed both on

platelets and leukocytes as well as at the interendothelial junctions (51,52), and can interact in a homophilic fashion to promote leukocyte transendothelial migration (53-55). PECAM-1 recycles in vesicular structures between the junctions and the subjunctional plasmalemma, and is thereby targeted to the area where leukocyte transmigration takes place (56). Besides its direct involvement in transmigration, PECAM-1 acts as a signaling receptor, as it contains two immunoreceptor tyrosine-based inhibitory motifs (57,58) and its homophilic ligation also induces the upregulation of the laminin receptor $\alpha 6 \beta 1$ -integrin on transmigrating neutrophils, thereby promoting the subsequent penetration of the basement membrane by these cells (59).

Regulation of integrin-mediated leukocyte adhesion

The major importance of integrin-mediated adhesion of leukocytes for the immune response is suggested by the immunodeficiency observed in the leukocyte adhesion deficiency syndrome (LAD I) in men lacking $\beta 2$ -integrins (18,60), as well as by several studies engaging mice deficient in one or more leukocyte integrins (61-63). On the other hand, leukocyte integrins represent important therapeutic targets for disease, especially for autoimmune disorders. Blocking monoclonal antibodies against VLA-4 and LFA-1 are effective therapeutic strategies in multiple sclerosis and psoriasis, respectively (64,65). Thus, understanding the regulation of integrin-mediated leukocyte adhesion as well as the characterization of exogenous and endogenous inhibitors of the leukocyte adhesion cascade is of major importance and will be the further focus of this review.

Leukocyte arrest on endothelial integrin ligands after variable periods of selectin-mediated rolling requires the proper activation of the integrins (10,66-68). The two major mechanisms regulating the adhesive activity of integrins involve changes in their affinity and their valency. The regulation of integrin affinity for their respective ligands is mediated by conformational changes of the integrin subunits (10,69,70). An equilibrium of at least 3 different conformations of integrins exists on the cell surface: low, intermediate and high affinity conformations (71-77). The transition from the low affinity to the intermediate and high affinity conformation can be induced by separation of the cytoplasmic tails of the α - and β -subunits (76,78). This separation is mediated by inside-out integrin signalling. Regulation of integrin valency involves changes of integrin distribution on the cell surface and clustering (10,79).

Whereas both affinity and valency changes are involved in the regulation of leukocyte arrest (adhesion) on the endothelium (10,66), regulation of affinity changes is the predominant mechanism for the induction of firm arrest/adhesion of leukocytes on vascular endothelium (3,10,80,81). Inside-out signaling mediates the transition of the low affinity to the high affinity state. The contact of rolling leukocytes with the endothelium allows leukocytes to sense chemokines, presented on the apical endothelial cell membrane via heparan sulphate proteoglycans (17), which triggers integrin activation. In vitro systems resembling this process suggested that rapid triggering of integrin-dependent adhesion of leukocytes can be mediated by G-protein coupled receptors activated by immobilized chemokines (10,82,83), allowing leukocyte integrins to bind to their endothelial ligands (10,66). The chemokine-induced signaling leading to integrin activation and subsequent firm leukocyte arrest happens in an immediate rather than in a stepwise successive manner (83). A key event transducing integrin-activating signals in leukocytes downstream of GPCR is the activation of PLC (84). PLC signaling subsequently induces activation of CALDAG-GEFI, a guanine exchange factor activating the small GTPase Rap1. GTP-bound (active) Rap1 rapidly stimulates integrin affinity and integrin-dependent adhesiveness through effector proteins such as RAPL and RIAM (10,85-94). Moreover, RhoA is involved in mediating the chemokine-induced integrin activation in leukocytes (81,95) (Figure 1).

Additional pathways may be involved in the regulation of $\beta 2$ -integrin activity in leukocytes. In this regard, the cytoplasmic tails of the α and β chains of the integrin can be phosphorylated during inside-out signaling activation (96-98). LFA-1 is constitutively phosphorylated on the α L-chain Ser-1140, which is important for the Rap1 pathway of integrin affinity regulation (96). In contrast, the phosphorylation of the β -chain of LFA-1 occurs upon cell stimulation, e.g. phosphorylation of T758 is induced by phorbol ester or downstream of T cell receptor activation (99) and can mediate cytoskeletal interactions of the integrin with the multifunctional adaptor proteins of the 14-3-3 family, thereby modulating LFA-1-mediated cell adhesion and spreading (96,100,101). Further interactions between the integrin cytoplasmic tail and cytoskeletal proteins have been implicated in leukocyte adhesion. Talin is a central downstream effector in integrin activation. Talin associates with the cytoplasmic tail of the integrin and participates in a complex with the Rap1 effector, RIAM transmitting the integrin-activating Rap1 signals (102,103). The binding of talin to the NPXY motif of the cytoplasmic tails of β -integrin subunits leads to separation of the cytoplasmic tails resulting in conformational rearrangements of integrin extracellular domains thereby inducing the intermediate or high integrin conformations (78,104,105) (Figure 1).

Furthermore, selectin-mediated rolling of neutrophils can induce integrin activation. Binding of PSGL-1 with E-selectin induces the intermediate affinity conformation of LFA-1, which participates in both rolling and adhesive interactions (15). The PSGL-1-dependent activation of LFA-1 requires signaling pathways involving Fgr, DAP12, FcR γ and Syk kinase (16,106).

Outside-in integrin signaling i.e. signaling following integrin ligation, is transduced through the cytoplasmic tails of the integrins and mediates the stabilization of initial adhesion. This pathway also designated as ligand-induced post-adhesion strengthening is relevant for the sustained adhesion of leukocytes onto the vascular endothelium and resistance to shear stress (107). Vav1 and Vav3 have been implicated in this process, since Vav1/Vav3-double deficient neutrophils displayed reduced sustained adhesion and spreading despite their normal arrest onto ICAM-1 under flow (108). Similarly, WASP-deficient neutrophils displayed normal rolling, integrin activation and firm arrest, but reduced resistance to detachment under flow (109). Furthermore, the src-like kinases Hck and Fgr are required for $\beta 2$ -integrin-mediated outside-in signaling (107) (Figure 2).

Inhibitors of the leukocyte adhesion cascade

Several exogenous microbial-derived inhibitors of the leukocyte adhesion cascade have been described. For example, the canine hookworm (*Ancylostoma caninum*)-derived neutrophil inhibitory factor (NIF) as well as the filamentous hemagglutinin (FHA) of *Bordetella pertussis* bind to Mac-1 integrin and potently inhibit Mac-1-dependent leukocyte recruitment (110-112). In addition, *Staphylococcus aureus* contains several anti-adhesive and anti-migratory proteins that can interfere with multiple steps of host inflammatory cell recruitment, which have been recently reviewed (113). The staphylococcal superantigen-like protein-5 directly interacts with PSGL-1 thereby interfering with leukocyte rolling (114), whereas the staphylococcal extracellular adherence protein binds ICAM-1 resulting in potent inhibition of ICAM-1 interactions with $\beta 2$ -integrins thereby preventing leukocyte recruitment *in vitro* and *in vivo* (115-118).

In contrast, less is known about endogenous inhibitors of the leukocyte adhesion cascade. Recent work from our lab has identified Developmental Endothelial Locus-1 (Del-1, *Edil3*) as a potent inhibitor of leukocyte recruitment. Del-1 is a secreted protein expressed by embryonic and some adult endothelial cells (119). Earlier studies implicated a role for Del-1 in angiogenesis (120-126), however, the Del-1-deficient mice are viable, fertile and display no obvious vascular defects (127). Del-1 consists of three epidermal growth factor repeats at its

N-terminus and two discoidin I-like domains at its C-terminus (119). Interestingly, Del-1 is expressed in immunoprivileged tissues such as the brain and the eye as well in the lung vessels of adult mice (128). Del-1 is secreted by endothelial cells and most likely associates with the endothelial surface and matrix (125,128,129). Recent studies shed light to a novel function of Del-1 as an inhibitor of LFA-1-dependent inflammatory cell adhesion (128). Specifically, Del-1 was shown to associate with LFA-1, as murine neutrophils bound to Del-1 in a LFA-1-dependent manner. These findings were corroborated by experiments with LFA-1-transfected cells and solid-phase binding assays involving Del-1 and the I-Domain of LFA-1 locked in the open high affinity conformation. Thus, Del-1 is a LFA-1-integrin ligand. However, in contrast to the classical LFA-1 ligand ICAM-1, Del-1 functioned to antagonize LFA-1-dependent adhesion to the endothelium (128). Immobilized Del-1 promoted only weak adhesion of leukocytes under physiologic flow conditions, whereas both soluble Del-1 as well as Del-1 co-immobilized with ICAM-1 inhibited the LFA-1-dependent adhesion of neutrophils to ICAM-1 under flow (128). *In vivo* intravital microscopy studies revealed that increased numbers of leukocytes adhered to postcapillary venules of Del-1^{-/-} mice as compared to wild type mice under both baseline conditions and upon TNF α stimulation (128). In addition, a significant reduction of the rolling velocity of leukocytes accompanied by an increase in the slow rolling fraction of leukocytes was observed in Del-1^{-/-} mice, which is consistent with the function of LFA-1 as mediator of slow rolling of leukocytes (16). Furthermore, enhanced neutrophil recruitment in LPS-induced lung inflammation was observed in Del-1-deficient mice, which was reversed in Del-1/LFA-1-double deficient mice, thereby demonstrating that Del-1 specifically interferes with LFA-1-dependent leukocyte recruitment (128). Several possibilities could explain the anti-adhesive actions of Del-1 and are currently under investigation. First, secreted Del-1 may compete with ICAM-1 for binding to LFA-1. Second, Del-1 may act as an allosteric antagonist of LFA-1 inhibiting the conformational activation of LFA-1. However, we found no inhibitory effect of soluble Del-1 on LFA-1 affinity, as assessed by the induction of reporter epitopes on LFA-1, such as mAb24 epitope (Choi et al., unpublished data and (83)). Third, in contrast to other LFA-1 ligands, the binding of Del-1 to LFA-1 may be insufficient to induce post-adhesion strengthening or may negatively affect intracellular signaling pathways involved in the adhesion cascade. Fourth, Del-1 may also inhibit LFA-1-dependent leukocyte adhesion by affecting expression of ICAM-1. Indeed, we found increased ICAM-1 expression in Del-1^{-/-} endothelial cells and murine lung tissues under baseline conditions however, this phenomenon was overcome upon pro-inflammatory stimulation (128). It is not clear to what degree the regulation of ICAM-1 expression by Del-1 contributes to the anti-inflammatory activities of Del-1. Although further investigations are required to clarify the mechanism of action of Del-1, Del-1 is an important endogenous inhibitor of inflammatory cell adhesion and homing.

Interestingly, the expression of Del-1 in endothelial cells and in mice was reduced upon proinflammatory stimulation (128), suggesting that inflammatory stimuli lead to a switch with increased expression of inflammation-promoting adhesion receptors (such as ICAM-1 or VCAM-1) and to reduced expression of inflammation inhibiting signals (such as Del-1). Extensive analysis by mass spectroscopy suggested the absence of soluble Del-1 from murine plasma (Choi et al., unpublished data). In addition, the discoidin domains of Del-1 protein contribute to its deposition in the extracellular matrix, a process that may involve interactions of Del-1 with glycosaminoglycans (129). Thus, Del-1 immobilized on the vascular endothelium most likely acts as a local and not systemic inhibitor of leukocyte adhesion, i.e. Del-1 acts in an autocrine / paracrine manner in the tissues that express it. However, one could envision that applying a form of soluble Del-1 appropriate to circulate in the plasma may be a novel therapeutic approach to inhibit inflammatory cell recruitment in disease.

Another endogenous inhibitor of leukocyte recruitment is galectin-1 (130,131), that limits T-cell rolling and adhesion to activated endothelial cells under flow conditions. Galectin-1-

deficient mice displayed enhanced homing of T lymphocytes to mesenteric lymph nodes and in a model of delayed-type hypersensitivity (130), as well as enhanced IL-1 β -induced leukocyte recruitment in the cremasteric circulation (131). Moreover, neuron-expressed soluble ICAM-5 can bind to LFA-1 and causes inhibition of T lymphocyte and microglia activation, in a manner opposite to the pro-inflammatory action of ICAM-1 (24,132,133). Interestingly, both Del-1 and ICAM-5 are highly expressed in the brain tissue and may thereby contribute to its immune privilege.

Conclusions

Despite the significant progress in the knowledge regarding the leukocyte adhesion cascade in the recent years, there are still several gaps in our understanding. In particular, the relative importance of each of the adhesion receptors *in vivo* for tissue- and vascular bed-specific inflammatory cell recruitment needs to be established. In addition, identifying and understanding endogenous inhibitors of the leukocyte adhesion cascade will not only increase the complexity of the cascade, but may also open the venue for designing novel therapeutic approaches in order to treat patients with autoimmune and inflammatory disorders.

References

1. Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol* 2005;6:1182–1190. [PubMed: 16369557]
2. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 1994;76:301–314. [PubMed: 7507411]
3. Ley K, Laudanna C, Cybulsky MI, et al. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol* 2007;7:678–689. [PubMed: 17717539]
4. Rao RM, Yang L, Garcia-Cardena G, et al. Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall. *Circ Res* 2007;101:234–247. [PubMed: 17673684]
5. Keiper T, Santoso S, Nawroth PP, et al. The role of junctional adhesion molecules in cell-cell interactions. *Histol Histopathol* 2005;20:197–203. [PubMed: 15578438]
6. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood* 1994;84:2068–2101. [PubMed: 7522621]
7. Muller WA. Leukocyte-endothelial cell interactions in the inflammatory response. *Lab Invest* 2002;82:521–533. [PubMed: 12003992]
8. Schenkel AR, Mamdouh Z, Muller WA. Locomotion of monocytes on endothelium is a critical step during extravasation. *Nat Immunol* 2004;5:393–400. [PubMed: 15021878]
9. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. *Nat Rev Immunol* 2004;4:432–444. [PubMed: 15173832]
10. Kinashi T. Intracellular signalling controlling integrin activation in lymphocytes. *Nat Rev Immunol* 2005;5:546–559. [PubMed: 15965491]
11. Vestweber D. Adhesion and signaling molecules controlling the transmigration of leukocytes through endothelium. *Immunol Rev* 2007;218:178–196. [PubMed: 17624953]
12. McEver RP. Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biol* 2002;14:581–586. [PubMed: 12231353]
13. McEver RP, Cummings RD. Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest* 1997;100:S97–103. [PubMed: 9413410]
14. Smith ML, Olson TS, Ley K. CXCR2- and E-selectin-induced neutrophil arrest during inflammation *in vivo*. *J Exp Med* 2004;200:935–939. [PubMed: 15466624]
15. Salas A, Shimaoka M, Kogan AN, et al. Rolling adhesion through an extended conformation of integrin α L β 2 and relation to α I and β I-like domain interaction. *Immunity* 2004;20:393–406. [PubMed: 15084269]

16. Zarbock A, Lowell CA, Ley K. Spleen tyrosine kinase Syk is necessary for E-selectin-induced alpha (L)beta(2) integrin-mediated rolling on intercellular adhesion molecule-1. *Immunity* 2007;26:773–783. [PubMed: 17543554]
17. Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokines grammar for immune cells. *Annu Rev Immunol* 2004;22:891–928. [PubMed: 15032599]
18. Yonekawa K, Harlan JM. Targeting leukocyte integrins in human diseases. *J Leukoc Biol* 2005;77:129–140. [PubMed: 15548573]
19. Chavakis T, Bierhaus A, Al-Fakhri N, et al. The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment. *J Exp Med* 2003;198:1507–1515. [PubMed: 14623906]
20. Gahmberg CG, Valmu L, Fagerholm S, et al. Leukocyte integrins and inflammation. *Cell Mol Life Sci* 1998;54:549–555. [PubMed: 9676574]
21. Staunton DE, Dustin ML, Springer TA. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature* 1989;339:61–64. [PubMed: 2497351]
22. Fawcett J, Holness CL, Needham LA, et al. Molecular cloning of ICAM-3, a third ligand for LFA-1, constitutively expressed on resting leukocytes. *Nature* 1992;360:481–484. [PubMed: 1448173]
23. Bailly P, Tontti E, Hermand P, et al. The red cell LW blood group protein is an intercellular adhesion molecule which binds to CD11/CD18 leukocyte integrins. *Eur J Immunol* 1995;25:3316–3320. [PubMed: 8566017]
24. Gahmberg CG, Tian L, Ning L, et al. ICAM-5--a novel two-facetted adhesion molecule in the mammalian brain. *Immunol Lett* 2008;117:131–135. [PubMed: 18367254]
25. Phillipson M, Heit B, Colarusso P, et al. Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med* 2006;203:2569–2575. [PubMed: 17116736]
26. Nourshargh S, Marelli-Berg FM. Transmigration through venular walls: a key regulator of leukocyte phenotype and function. *Trends Immunol* 2005;26:157–165. [PubMed: 15745858]
27. Shaw SK, Ma S, Kim MB, et al. Coordinated redistribution of leukocyte LFA-1 and endothelial cell ICAM-1 accompany neutrophil transmigration. *J Exp Med* 2004;200:1571–1580. [PubMed: 15611287]
28. Carman CV, Springer TA. A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. *J Cell Biol* 2004;167:377–388. [PubMed: 15504916]
29. Yang L, Froio RM, Sciuto TE, et al. ICAM-1 regulates neutrophil adhesion and transcellular migration of TNF-alpha-activated vascular endothelium under flow. *Blood* 2005;106:584–592. [PubMed: 15811956]
30. Huang MT, Larbi KY, Scheiermann C, et al. ICAM-2 mediates neutrophil transmigration in vivo: evidence for stimulus specificity and a role in PECAM-1-independent transmigration. *Blood* 2006;107:4721–4727. [PubMed: 16469869]
31. Bazzoni G, Dejana E. Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. *Physiol Rev* 2004;84:869–901. [PubMed: 15269339]
32. Shaw SK, Bamba PS, Perkins BN, et al. Real-time imaging of vascular endothelial-cadherin during leukocyte transmigration across endothelium. *J Immunol* 2001;167:2323–2330. [PubMed: 11490021]
33. Gotsch U, Borges E, Bosse R, et al. VE-cadherin antibody accelerates neutrophil recruitment in vivo. *J Cell Sci* 1997;110(Pt 5):583–588. [PubMed: 9092940]
34. Orlova VV, Chavakis T. Regulation of vascular endothelial permeability by junctional adhesion molecules (JAM). *Thromb Haemost* 2007;98:327–332. [PubMed: 17721614]
35. Bradfield PF, Nourshargh S, Aurrand-Lions M, et al. JAM family and related proteins in leukocyte migration (Vestweber series). *Arterioscler Thromb Vasc Biol* 2007;27:2104–2112. [PubMed: 17615384]
36. Weber C, Fraemohs L, Dejana E. The role of junctional adhesion molecules in vascular inflammation. *Nat Rev Immunol* 2007;7:467–477. [PubMed: 17525755]
37. Santoso S, Orlova VV, Song K, et al. The homophilic binding of junctional adhesion molecule-C mediates tumor cell-endothelial cell interactions. *J Biol Chem* 2005;280:36326–36333. [PubMed: 16118203]

38. Kostrewa D, Brockhaus M, D'Arcy A, et al. X-ray structure of junctional adhesion molecule: structural basis for homophilic adhesion via a novel dimerization motif. *Embo J* 2001;20:4391–4398. [PubMed: 11500366]
39. Santoso S, Sachs UJ, Kroll H, et al. The junctional adhesion molecule 3 (JAM-3) on human platelets is a counterreceptor for the leukocyte integrin Mac-1. *J Exp Med* 2002;196:679–691. [PubMed: 12208882]
40. Ostermann G, Weber KS, Zerneck A, et al. JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. *Nat Immunol* 2002;3:151–158. [PubMed: 11812992]
41. Cunningham SA, Rodriguez JM, Arrate MP, et al. JAM2 interacts with alpha4beta1. Facilitation by JAM3. *J Biol Chem* 2002;277:27589–27592. [PubMed: 12070135]
42. Arrate MP, Rodriguez JM, Tran TM, et al. Cloning of human junctional adhesion molecule 3 (JAM3) and its identification as the JAM2 counter-receptor. *J Biol Chem* 2001;276:45826–45832. [PubMed: 11590146]
43. Lamagna C, Meda P, Mandicourt G, et al. Dual interaction of JAM-C with JAM-B and alpha(M) beta2 integrin: function in junctional complexes and leukocyte adhesion. *Mol Biol Cell* 2005;16:4992–5003. [PubMed: 16093349]
44. Del Maschio A, De Luigi A, Martin-Padura I. Leukocyte recruitment in the cerebrospinal fluid of mice with experimental meningitis is inhibited by an antibody to junctional adhesion molecule (JAM). *J Exp Med* 1999;190:1351–1356. [PubMed: 10544206]
45. Corada M, Chimenti S, Cera MR, et al. Junctional adhesion molecule-A-deficient polymorphonuclear cells show reduced diapedesis in peritonitis and heart ischemia-reperfusion injury. *Proc Natl Acad Sci U S A* 2005;102:10634–10639. [PubMed: 16027360]
46. Khandoga A, Kessler JS, Meissner H, et al. Junctional adhesion molecule-A deficiency increases hepatic ischemia-reperfusion injury despite reduction of neutrophil transendothelial migration. *Blood* 2005;106:725–733. [PubMed: 15827135]
47. Chavakis T, Keiper T, Matz-Westphal R, et al. The junctional adhesion molecule-C promotes neutrophil transendothelial migration in vitro and in vivo. *J Biol Chem* 2004;279:55602–55608. [PubMed: 15485832]
48. Aurrand-Lions M, Lamagna C, Dangerfield JP, et al. Junctional adhesion molecule-C regulates the early influx of leukocytes into tissues during inflammation. *J Immunol* 2005;174:6406–6415. [PubMed: 15879142]
49. Orlova VV, Economopoulou M, Lupu F, et al. Junctional adhesion molecule-C regulates vascular endothelial permeability by modulating VE-cadherin-mediated cell-cell contacts. *J Exp Med* 2006;203:2703–2714. [PubMed: 17116731]
50. Wegmann F, Petri B, Khandoga AG, et al. ESAM supports neutrophil extravasation, activation of Rho, and VEGF-induced vascular permeability. *J Exp Med* 2006;203:1671–1677. [PubMed: 16818677]
51. Muller WA. Leukocyte-endothelial-cell interactions in leukocyte transmigration and the inflammatory response. *Trends Immunol* 2003;24:327–334. [PubMed: 12810109]
52. Newman PJ. The biology of PECAM-1. *J Clin Invest* 1997;100:S25–29. [PubMed: 9413397]
53. Muller WA, Weigl SA, Deng X, et al. PECAM-1 is required for transendothelial migration of leukocytes. *J Exp Med* 1993;178:449–460. [PubMed: 8340753]
54. Bogen S, Pak J, Garifallou M, et al. Monoclonal antibody to murine PECAM-1 (CD31) blocks acute inflammation in vivo. *J Exp Med* 1994;179:1059–1064. [PubMed: 8113674]
55. Liao F, Ali J, Greene T, et al. Soluble domain 1 of platelet-endothelial cell adhesion molecule (PECAM) is sufficient to block transendothelial migration in vitro and in vivo. *J Exp Med* 1997;185:1349–1357. [PubMed: 9104821]
56. Mamdouh Z, Chen X, Pierini LM, et al. Targeted recycling of PECAM from endothelial surface-connected compartments during diapedesis. *Nature* 2003;421:748–753. [PubMed: 12610627]
57. Hua CT, Gamble JR, Vadas MA, et al. Recruitment and activation of SHP-1 protein-tyrosine phosphatase by human platelet endothelial cell adhesion molecule-1 (PECAM-1). Identification of immunoreceptor tyrosine-based inhibitory motif-like binding motifs and substrates. *J Biol Chem* 1998;273:28332–28340. [PubMed: 9774457]

58. Pumphrey NJ, Taylor V, Freeman S, et al. Differential association of cytoplasmic signalling molecules SHP-1, SHP-2, SHIP and phospholipase C-gamma1 with PECAM-1/CD31. *FEBS Lett* 1999;450:77–83. [PubMed: 10350061]
59. Dangerfield J, Larbi KY, Huang MT, et al. PECAM-1 (CD31) homophilic interaction up-regulates alpha6beta1 on transmigrated neutrophils in vivo and plays a functional role in the ability of alpha6 integrins to mediate leukocyte migration through the perivascular basement membrane. *J Exp Med* 2002;196:1201–1211. [PubMed: 12417630]
60. Springer TA, Thompson WS, Miller LJ, et al. Inherited deficiency of the Mac-1, LFA-1, p150,95 glycoprotein family and its molecular basis. *J Exp Med* 1984;160:1901–1918. [PubMed: 6096477]
61. Ding ZM, Babensee JE, Simon SI, et al. Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. *J Immunol* 1999;163:5029–5038. [PubMed: 10528208]
62. Mizgerd JP, Kubo H, Kutkoski GJ, et al. Neutrophil emigration in the skin, lungs, and peritoneum: different requirements for CD11/CD18 revealed by CD18-deficient mice. *J Exp Med* 1997;186:1357–1364. [PubMed: 9334375]
63. Berlin-Rufenach C, Otto F, Mathies M, et al. Lymphocyte migration in lymphocyte function-associated antigen (LFA)-1-deficient mice. *J Exp Med* 1999;189:1467–1478. [PubMed: 10224287]
64. Steinman L. Blocking adhesion molecules as therapy for multiple sclerosis: natalizumab. *Nat Rev Drug Discov* 2005;4:510–518. [PubMed: 15931259]
65. Lebwohl M, Tying SK, Hamilton TK, et al. A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *N Engl J Med* 2003;349:2004–2013. [PubMed: 14627785]
66. Laudanna C, Alon R. Right on the spot. Chemokine triggering of integrin-mediated arrest of rolling leukocytes. *Thromb Haemost* 2006;95:5–11. [PubMed: 16543955]
67. Salas A, Shimaoka M, Chen S, et al. Transition from rolling to firm adhesion is regulated by the conformation of the I domain of the integrin lymphocyte function-associated antigen-1. *J Biol Chem* 2002;277:50255–50262. [PubMed: 12368274]
68. Salas A, Shimaoka M, Phan U, et al. Transition from rolling to firm adhesion can be mimicked by extension of integrin alphaLbeta2 in an intermediate affinity state. *J Biol Chem* 2006;281:10876–10882. [PubMed: 16505487]
69. Carman CV, Springer TA. Integrin avidity regulation: are changes in affinity and conformation underemphasized? *Curr Opin Cell Biol* 2003;15:547–556. [PubMed: 14519389]
70. Dustin ML, Bivona TG, Philips MR. Membranes as messengers in T cell adhesion signaling. *Nat Immunol* 2004;5:363–372. [PubMed: 15052266]
71. Shimaoka M, Xiao T, Liu JH, et al. Structures of the alpha L I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. *Cell* 2003;112:99–111. [PubMed: 12526797]
72. Takagi J, Petre BM, Walz T, et al. Global conformational rearrangements in integrin extracellular domains in outside-in and inside-out signaling. *Cell* 2002;110:599–511. [PubMed: 12230977]
73. Takagi J, Springer TA. Integrin activation and structural rearrangement. *Immunol Rev* 2002;186:141–163. [PubMed: 12234369]
74. Luo BH, Carman CV, Springer TA. Structural basis of integrin regulation and signaling. *Annu Rev Immunol* 2007;25:619–647. [PubMed: 17201681]
75. Kinashi T. Integrin regulation of lymphocyte trafficking: lessons from structural and signaling studies. *Adv Immunol* 2007;93:185–227. [PubMed: 17383542]
76. Nishida N, Xie C, Shimaoka M, et al. Activation of leukocyte beta2 integrins by conversion from bent to extended conformations. *Immunity* 2006;25:583–594. [PubMed: 17045822]
77. Kinashi T. Adhere upright: a switchblade-like extension of beta2 integrins. *Immunity* 2006;25:521–522. [PubMed: 17046677]
78. Kim M, Carman CV, Springer TA. Bidirectional transmembrane signaling by cytoplasmic domain separation in integrins. *Science* 2003;301:1720–1725. [PubMed: 14500982]
79. Bazzoni G, Hemler ME. Are changes in integrin affinity and conformation overemphasized? *Trends Biochem Sci* 1998;23:30–34. [PubMed: 9478133]
80. Chan JR, Cybulsky MI. Detection of high-affinity alpha4-integrin upon leukocyte stimulation by chemoattractants or chemokines. *Methods Mol Biol* 2004;239:261–268. [PubMed: 14573925]

81. Giagulli C, Scarpini E, Ottoboni L, et al. RhoA and zeta PKC control distinct modalities of LFA-1 activation by chemokines: critical role of LFA-1 affinity triggering in lymphocyte in vivo homing. *Immunity* 2004;20:25–35. [PubMed: 14738762]
82. Grabovsky V, Feigelson S, Chen C, et al. Subsecond induction of alpha4 integrin clustering by immobilized chemokines stimulates leukocyte tethering and rolling on endothelial vascular cell adhesion molecule 1 under flow conditions. *J Exp Med* 2000;192:495–506. [PubMed: 10952719]
83. Shamri R, Grabovsky V, Gauguier JM, et al. Lymphocyte arrest requires instantaneous induction of an extended LFA-1 conformation mediated by endothelium-bound chemokines. *Nat Immunol* 2005;6:497–506. [PubMed: 15834409]
84. Hyduk SJ, Chan JR, Duffy ST, et al. Phospholipase C, calcium, and calmodulin are critical for alpha4beta1 integrin affinity up-regulation and monocyte arrest triggered by chemoattractants. *Blood* 2007;109:176–184. [PubMed: 16960156]
85. Ghandour H, Cullere X, Alvarez A, et al. Essential role for Rap1 GTPase and its guanine exchange factor CalDAG-GEFI in LFA-1 but not VLA-4 integrin mediated human T-cell adhesion. *Blood* 2007;110:3682–3690. [PubMed: 17702895]
86. Lafuente E, Boussiotis VA. Rap1 Regulation of RIAM and Cell Adhesion. *Methods Enzymol* 2005;407:345–358. [PubMed: 16757337]
87. Kinashi T, Katagiri K. Regulation of immune cell adhesion and migration by regulator of adhesion and cell polarization enriched in lymphoid tissues. *Immunology* 2005;116:164–171. [PubMed: 16162265]
88. Bos JL. Linking Rap to cell adhesion. *Curr Opin Cell Biol* 2005;17:123–128. [PubMed: 15780587]
89. Lafuente EM, van Puijenbroek AA, Krause M, et al. RIAM, an Ena/VASP and Profilin ligand, interacts with Rap1-GTP and mediates Rap1-induced adhesion. *Dev Cell* 2004;7:585–595. [PubMed: 15469846]
90. Kinashi T, Katagiri K. Regulation of lymphocyte adhesion and migration by the small GTPase Rap1 and its effector molecule, RAPL. *Immunol Lett* 2004;93:1–5. [PubMed: 15134891]
91. Shimonaka M, Katagiri K, Nakayama T, et al. Rap1 translates chemokine signals to integrin activation, cell polarization, and motility across vascular endothelium under flow. *J Cell Biol* 2003;161:417–427. [PubMed: 12707305]
92. Bos JL, de Bruyn K, Enserink J, et al. The role of Rap1 in integrin-mediated cell adhesion. *Biochem Soc Trans* 2003;31:83–86. [PubMed: 12546659]
93. Bos JL, de Rooij J, Reedquist KA. Rap1 signalling: adhering to new models. *Nat Rev Mol Cell Biol* 2001;2:369–377. [PubMed: 11331911]
94. Katagiri K, Hattori M, Minato N, et al. Rap1 is a potent activation signal for leukocyte function-associated antigen 1 distinct from protein kinase C and phosphatidylinositol-3-OH kinase. *Mol Cell Biol* 2000;20:1956–1969. [PubMed: 10688643]
95. Pasvolovskiy R, Grabovsky V, Giagulli C, et al. RhoA is involved in LFA-1 extension triggered by CXCL12 but not in a novel outside-in LFA-1 activation facilitated by CXCL9. *J Immunol* 2008;180:2815–2823. [PubMed: 18292502]
96. Fagerholm SC, Hilden TJ, Nurmi SM, et al. Specific integrin alpha and beta chain phosphorylations regulate LFA-1 activation through affinity-dependent and -independent mechanisms. *J Cell Biol* 2005;171:705–715. [PubMed: 16301335]
97. Fagerholm SC, Hilden TJ, Gahmberg CG. P marks the spot: site-specific integrin phosphorylation regulates molecular interactions. *Trends Biochem Sci* 2004;29:504–512. [PubMed: 15337124]
98. Fagerholm S, Morrice N, Gahmberg CG, et al. Phosphorylation of the cytoplasmic domain of the integrin CD18 chain by protein kinase C isoforms in leukocytes. *J Biol Chem* 2002;277:1728–1738. [PubMed: 11700305]
99. Hilden TJ, Valmu L, Karkkainen S, et al. Threonine phosphorylation sites in the beta 2 and beta 7 leukocyte integrin polypeptides. *J Immunol* 2003;170:4170–4177. [PubMed: 12682249]
100. Hibbs ML, Jakes S, Stacker SA, et al. The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 beta subunit: sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site. *J Exp Med* 1991;174:1227–1238. [PubMed: 1682411]

101. Choi EY, Orlova VV, Fagerholm SC, et al. Regulation of LFA-1-dependent inflammatory cell recruitment by Cbl-b and 14-3-3 proteins. *Blood* 2008;111:3607–3614. [PubMed: 18239087]
102. Han J, Lim CJ, Watanabe N, et al. Reconstructing and deconstructing agonist-induced activation of integrin α IIb β 3. *Curr Biol* 2006;16:1796–1806. [PubMed: 16979556]
103. Lee HS, Lim CJ, Puzon-McLaughlin W, et al. RIAM activates integrins by linking talin to Ras GTPase membrane-targeting sequences. *J Biol Chem*. 2008[Epub ahead of print]
104. Tadokoro S, Shattil SJ, Eto K, et al. Talin binding to integrin beta tails: a final common step in integrin activation. *Science* 2003;302:103–106. [PubMed: 14526080]
105. Vinogradova O, Velyvis A, Velyviene A, et al. A structural mechanism of integrin α (IIb) β 3 (3) “inside-out” activation as regulated by its cytoplasmic face. *Cell* 2002;110:587–597. [PubMed: 12230976]
106. Zarbock A, Abram CL, Hundt M, et al. PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR gamma to induce slow leukocyte rolling. *J Exp Med* 2008;205:2339–2347. [PubMed: 18794338]
107. Giagulli C, Ottoboni L, Cavegion E, et al. The Src family kinases Hck and Fgr are dispensable for inside-out, chemoattractant-induced signaling regulating beta 2 integrin affinity and valency in neutrophils, but are required for beta 2 integrin-mediated outside-in signaling involved in sustained adhesion. *J Immunol* 2006;177:604–611. [PubMed: 16785558]
108. Gakidis MA, Cullere X, Olson T, et al. Vav GEFs are required for beta2 integrin-dependent functions of neutrophils. *J Cell Biol* 2004;166:273–282. [PubMed: 15249579]
109. Zhang H, Schaff UY, Green CE, et al. Impaired integrin-dependent function in Wiskott-Aldrich syndrome protein-deficient murine and human neutrophils. *Immunity* 2006;25:285–295. [PubMed: 16901726]
110. Moyle M, Foster DL, McGrath DE, et al. A hookworm glycoprotein that inhibits neutrophil function is a ligand of the integrin CD11b/CD18. *J Biol Chem* 1994;269:10008–10015. [PubMed: 7908286]
111. Zhou MY, Lo SK, Bergenfeldt M, et al. In vivo expression of neutrophil inhibitory factor via gene transfer prevents lipopolysaccharide-induced lung neutrophil infiltration and injury by a beta2 integrin-dependent mechanism. *J Clin Invest* 1998;101:2427–2437. [PubMed: 9616214]
112. Rozdzinski E, Sandros J, van der Flier M, et al. Inhibition of leukocyte-endothelial cell interactions and inflammation by peptides from a bacterial adhesin which mimic coagulation factor X. *J Clin Invest* 1995;95:1078–1085. [PubMed: 7883955]
113. Chavakis T, Preissner KT, Herrmann M. The anti-inflammatory activities of *Staphylococcus aureus*. *Trends Immunol* 2007;28:408–418. [PubMed: 17681885]
114. Bestebroer J, Poppelier MJ, Ulfman LH, et al. Staphylococcal superantigen-like 5 binds PSGL-1 and inhibits P-selectin-mediated neutrophil rolling. *Blood* 2007;109:2936–2943. [PubMed: 17132726]
115. Rhee JS, Santoso S, Herrmann M, et al. New aspects of integrin-mediated leukocyte adhesion in inflammation: regulation by haemostatic factors and bacterial products. *Curr Mol Med* 2003;3:387–92. [PubMed: 12776993]
116. Chavakis T, Hussain M, Kanse SM, et al. *Staphylococcus aureus* extracellular adherence protein serves as anti-inflammatory factor by inhibiting the recruitment of host leukocytes. *Nat Med* 2002;8:687–693. [PubMed: 12091905]
117. Athanasopoulos AN, Economopoulou M, Orlova VV, et al. The extracellular adherence protein (Eap) of *Staphylococcus aureus* inhibits wound healing by interfering with host defense and repair mechanisms. *Blood* 2006;107:2720–2727. [PubMed: 16317095]
118. Xie C, Alcaide P, Geisbrecht BV, et al. Suppression of experimental autoimmune encephalomyelitis by extracellular adherence protein of *Staphylococcus aureus*. *J Exp Med* 2006;203:985–994. [PubMed: 16585266]
119. Hidai C, Zupancic T, Penta K, et al. Cloning and characterization of developmental endothelial locus-1: an embryonic endothelial cell protein that binds the α v β 3 integrin receptor. *Genes Dev* 1998;12:21–33. [PubMed: 9420328]
120. Penta K, Varner JA, Liaw L, et al. Del1 induces integrin signaling and angiogenesis by ligation of α v β 3. *J Biol Chem* 1999;274:11101–11109. [PubMed: 10196194]

121. Rezaee M, Penta K, Quertermous T. Del1 mediates VSMC adhesion, migration, and proliferation through interaction with integrin $\alpha(v)\beta(3)$. *Am J Physiol Heart Circ Physiol* 2002;282:H1924–1932. [PubMed: 11959660]
122. Hidai C, Kawana M, Habu K, et al. Overexpression of the Del1 gene causes dendritic branching in the mouse mesentery. *Anat Rec A Discov Mol Cell Evol Biol* 2007;287:1165–1175. [PubMed: 16302230]
123. Aoka Y, Johnson FL, Penta K, et al. The embryonic angiogenic factor Del1 accelerates tumor growth by enhancing vascular formation. *Microvasc Res* 2002;64:148–161. [PubMed: 12074641]
124. Zhong J, Eliceiri B, Stupack D, et al. Neovascularization of ischemic tissues by gene delivery of the extracellular matrix protein Del-1. *J Clin Invest* 2003;112:30–41. [PubMed: 12840057]
125. Ho HK, Jang JJ, Kaji S, et al. Developmental endothelial locus-1 (Del-1), a novel angiogenic protein: its role in ischemia. *Circulation* 2004;109:1314–1319. [PubMed: 14981004]
126. Fan Y, Zhu W, Yang M, et al. Del-1 gene transfer induces cerebral angiogenesis in mice. *Brain Res* 2008;1219:1–7. [PubMed: 18534562]
127. Hsu GP, Mathy JA, Wang Z, et al. Increased rate of hair regrowth in mice with constitutive overexpression of Del1. *J Surg Res* 2008;146:73–80. [PubMed: 17764695]
128. Choi EY, Chavakis E, Czabanka MA, et al. Del-1, an endogenous leukocyte-endothelial adhesion inhibitor, limits inflammatory cell recruitment. *Science* 2008;322:1101–1104. [PubMed: 19008446]
129. Hidai C, Kawana M, Kitano H, et al. Discoidin domain of Del1 protein contributes to its deposition in the extracellular matrix. *Cell Tissue Res* 2007;330:83–95. [PubMed: 17701220]
130. Norling LV, Sampaio AL, Cooper D, et al. Inhibitory control of endothelial galectin-1 on in vitro and in vivo lymphocyte trafficking. *Faseb J* 2008;22:682–690. [PubMed: 17965266]
131. Cooper D, Norling LV, Perretti M. Novel insights into the inhibitory effects of Galectin-1 on neutrophil recruitment under flow. *J Leukoc Biol* 2008;83:1459–1466. [PubMed: 18372340]
132. Tian L, Lappalainen J, Autero M, et al. Shedded neuronal ICAM-5 suppresses T-cell activation. *Blood* 2008;111:3615–3625. [PubMed: 18223167]
133. Zhang H, Casanovas JM, Jin M, et al. An unusual allosteric mobility of the C-terminal helix of a high-affinity α_L integrin I domain variant bound to ICAM-5. *Mol Cell* 2008;31:432–437. [PubMed: 18691975]

Figure 1. Inside-out signaling activation of leukocyte integrins

The contact of rolling leukocytes with the endothelium allows leukocytes to sense chemokines, presented on the apical endothelial cell membrane, which triggers integrin activation leading to the binding of the ligands on the endothelial cell surface and providing firm adhesion. Chemokines bind to their G-Protein coupled receptors (GPCR) thereby triggering the activation of PLC and CALDAG GEF. CALDAG GEF activates in turn the small GTPase Rap1, which associates with effector proteins such as RIAM and RAPL inducing integrin activation. Specifically, RIAM associates with the cytoskeletal protein talin, which binds to the cytoplasmic tail of β -integrin subunits leading to separation of the cytoplasmic tails. This results in conformational alterations of the extracellular domains of the integrins, which adopt the intermediate or high affinity conformation. Besides Rap1, also RhoA-signaling is involved in the chemokine-induced increase of integrin affinity in leukocytes.

Figure 2. Integrin outside-in signaling

Binding of integrins to their respective ligands triggers intracellular signaling leading to a further increase of adhesivity. This so-called ligand-induced post-adhesion strengthening is relevant for the sustained adhesion of leukocytes onto the vascular endothelium and resistance to shear stress. $PI3K_{\gamma}$, the src-like kinases Hck and Fgr, the GEF Vav1/Vav3 and WASP are implicated in the process of ligand-induced strengthening of adhesion.