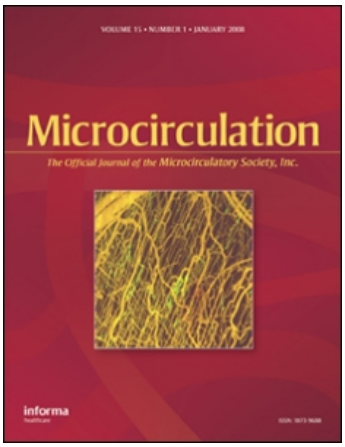

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Alexander Zarbock [ab]; Klaus Ley [a]
[a] Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA [b] Department of Anesthesiology and Intensive Care Medicine, University of Münster, Münster, Germany

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Neutrophil Adhesion and Activation under Flow

ALEXANDER ZARBOCK,*† AND KLAUS LEY*
*Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA;
†Department of Anesthesiology and Intensive Care Medicine, University of Münster, Münster, Germany

ABSTRACT

Neutrophil recruitment into inflamed tissue in response to injury or infection is tightly regulated. Reduced neutrophil recruitment can result in a reduced ability to fight invading microorganisms. During inflammation, neutrophils roll along the endothelial wall of postcapillary venules and integrate inflammatory signals. Neutrophil activation by selectins and chemokines regulates integrin adhesiveness. Binding of activated integrins to their counter-receptors on endothelial cells induces neutrophil arrest and firm adhesion. Adherent neutrophils can be further activated to undergo cytoskeletal rearrangement, crawling, transmigration, superoxide production, and respiratory burst. Signaling through G-protein-coupled receptors, selectin ligands, Fc receptors and outside-in signaling through integrins are all involved in neutrophil activation, but their interplay in the multistep process of recruitment is only beginning to emerge. This review provides an overview of signaling in rolling and adherent neutrophils.
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KEY WORDS: neutrophil, selectin, PSGL-1, integrin, chemokine

Polymorphonuclear neutrophils (PMN) belong to the innate immune system and constitute the main defense against invading bacteria and fungi. The recruitment of neutrophils out of blood vessels into injured tissue proceeds in a coordinated series of steps [13,90,57]. The activation and recruitment of neutrophils by different signals are tightly regulated. Defective leukocyte recruitment, such as that seen in leukocyte adhesion deficiency (LAD), leads to an inappropriate inflammatory response to injury or infection [5]. Patients with this disease suffer from recurrent bacterial infections and have a reduced life expectancy [5]. However, overwhelming neutrophil activation is also associated with tissue damage [67].

The classical neutrophil recruitment cascade comprises "capturing" ("tethering"), rolling, slow rolling, arrest, postadhesion strengthening, crawling, and paracellular or transcellular transmigration [57]. Capturing is the first contact between neutrophils and the endothelium of postcapillary venules mediated by selectins and their counter-receptors [41]. During this initial step, selectins as well as chemokines presented on the inflamed endothelium may initiate the activation of signaling pathways in neutrophils that regulate integrin adhesiveness. Binding of activated integrins to their counter-receptor leads, depending on the conformational state of the integrin, either to a reduction of the rolling velocity or arrest. However, the integrins are not only responsible for the attachment of neutrophils to the endothelium, but they are also able to transfer signals from the extracellular domain into the cell (i.e., outside-in signaling) [29]. These signals strengthen adhesion and induce superoxide production, respiratory burst, and transmigration.

Address correspondence to Klaus Ley, Division of Inflammation Biology, La Jolla Institute for Allergy & Immunology, 9420 Athena Circle Drive, La Jolla, CA 92037, USA. E-mail: klaus@liai.org

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ACTIVATION OF NEUTROPHILS BY SELECTINS: *IN VIVO* AND *IN VITRO* EVIDENCE

Selectins

The selectins are type I membrane glycoproteins composed of an amino terminal C-type lectin domain, a single epidermal growth factor (EGF)-like domain, two to nine short consensus repeat (SCR) domains, a membrane spanning region, and a cytoplasmic tail [41]. The family includes three molecules that display different patterns of expression and function.

L-selectin is expressed on almost all circulating leukocytes and is involved in lymphocyte homing [75] and leukocyte recruitment to sites of inflammation [57]. Following activation of leukocytes,

L-selectin can be shed by proteolytic cleavage near the cell surface. A disintegrin and metallopeptidase (ADAM)-17 and at least one other enzyme are involved in constitutive and activated L-selectin shedding [85]. E-selectin expression is limited to inflamed endothelial cells and is induced at the level of transcription, as inhibitors of either transcription or translation inhibit E-selectin expression [10]. P-selectin is inducibly expressed on activated endothelium and platelets. P-selectin is stored preformed in the α-granules and Weibel-Palade bodies of platelets and endothelium, respectively. Following activation, P-selectin is rapidly expressed at the cell surface as a result of fusion of these granules with the plasma membrane. Further, P-selectin expression on endothelium is also regulated transcriptionally [31], but the regulation is different in mice and humans [107]. In many assays, P-selectin is the dominant selectin in mice, but it is not clear whether this is also true in humans.

Capturing and rolling of neutrophils, which greatly facilitate subsequent arrest and recruitment, are mediated by selectins. Indeed, triple-selectin knockout mice [16] have a severe defect in neutrophil recruitment and other defects. All three selectins can mediate rolling, but the rolling behavior of neutrophils on the selectins is different. In venules of the cremaster muscle, the rolling velocity of leukocytes on L-selectin (130 μm/s) [39] is faster than the velocity on P-selectin (40 μm/s) [39], whereas E-selectin mediates slower rolling (3–7 μm/s) [48,109]. *Ex vivo* data suggest that the simultaneous presence of E- and P-selectin has a synergistic effect, with P-selectin increasing the number of rolling cells and E-selectin reducing rolling velocity [87]. This mechanism may explain why neutrophil recruitment is enhanced when both selectins are expressed. However, knocking out the *Sele* gene encoding E-selectin has little effect on neutrophil recruitment [50] and knocking out *Selp* only delays recruitment by two to four hours [60].

In addition to the direct interaction of neutrophils with the endothelium, neutrophils can also be recruited by "secondary capturing" [110]. PSGL-1 on free-flowing neutrophils can bind to P-selectin presented by adherent platelets [18] and L-selectin on free-flowing neutrophils can interact with PSGL-1 presented by adherent leukocytes [22] or leukocyte-derived fragments [88].

Counter-receptors

P-selectin glycoprotein ligand (PSGL)-1 is a homodimeric mucin-like 220–240-kDa glycoprotein that consists of an extracellular, transmembrane, and cytoplasmatic domain [61]. It is expressed on all leukocytes and is mainly located in lipid rafts on the top of microvilli [1]. PSGL-1 can bind L- [88], P- [66], and E-selectin [105]. The post-translational modifications of PSGL-1 are important for optimal selectin-binding capacity. PSGL-1 requires α2,3-sialylated and α1,3-fucosylated core2 O-glycans to bind P-selectin [61]. Core2 N-acetylglucosaminyl transferase-I is required to increase the binding affinity of PSGL-1 to P- and L-selectin [21], whereas the sulfation of tyrosine residues near the N-terminus optimizes the binding of PSGL-1 to P-selectin [61]. E-selectin binding to PSGL-1 requires sialylated and fucosylated O-glycans but not tyrosine sulfation [61]. The manipulation of the core-type protein glycosylation of PSGL-1 by eliminating the polypeptide N-acetylgalactosamine transferase-1 reduces the binding capacity of PSGL-1 to P- and E-selectin *in vitro* and *in vivo* under flow [96]. Due to the differences in the amino-acid sequence of the extracellular domain and glycosylation pattern of mouse and human PSGL-1 [61,106], the binding affinities and, consequently, the signaling characteristics of the two molecules might be different.

The conserved cytoplasmic tail of PSGL-1 comprises 63 amino acids and interacts with cytoskeletal proteins [61]. Proteins of the ERM (ezrin-moesin-radixin) family link the juxtamembrane region of the cytoplasmatic tail of PSGL-1 with the cytoskeleton in the uropod of migrating cells [4,97]. Further, the interaction between the proteins of the ERM-family with the cytoplasmatic tail of PSGL-1 is important for the formation of protrusive membrane structures [12]. In addition to the interaction with ERM proteins, a juxtamembrane region of 18 amino acids forms a constitutive complex with Nef-associated factor 1 (Naf1), which is involved in P-selectin-induced signaling through PSGL-1 [100]. A recent study has identified a new molecule interacting with the cytoplasmatic tail of PSGL-1 [79]. The selectin ligand interactor, cytoplasmic-1 (SLIC-1; human ortholog of the mouse sorting nexin 20), binds phosphoinositides and targets PSGL-1 to endosomes, but does not participate in PSGL-1-induced signaling or leukocyte recruitment [79].

The E-selectin ligand, ESL-1, is a 150-kDa glycoprotein, which is localized in the Golgi apparatus and on the cell surface of leukocytes [91]. In contrast to PSGL-1 and L-selectin, ESL-1 is not located on the tips of microvilli [91]. ESL-1, which can bind E-selectin *in vitro* and *in vivo*, is carrier of the HECA452 carbohydrate epitope, and sialic acid and fucose are required for achieving E-selectin binding capacity [92,35,55].

In addition to PSGL-1 and ESL-1, neutrophils express other E-selectin ligands, including CD44 [35,42], macrophage antigen (Mac)-1 ($\alpha_M\beta_2$) [17,112], and other unknown and poorly characterized ligands [74,3]. Further, L-selectin from human, but not from mouse, neutrophils is able to bind E-selectin [114,73]. Sialic acid on L-selectin is necessary for the binding to E-selectin [114].

Signaling Events and Consequences *In Vitro*

Several lines of evidence show that neutrophil binding to P-selectin *in vitro* leads to the activation of neutrophils. Isolated human neutrophils stimulated with paraformaldehyde-fixed platelets, P-selectin-IgG fusion protein, or antibody against PSGL-1 show enhanced tyrosine phosphorylation [23]. Stimulation of murine bone-marrow-derived neutrophils with P-selectin-IgG or cross-linking PSGL-1 with complete antibodies or $F(ab')_2$ fragments leads to an increased production of reactive oxygen intermediates [11] and Mac-1 activation, which in turn, leads to increased binding of Mac-1 to ligands [1]. A recent study dissected the proximal signaling pathway following P-selectin engagement. *In vivo* and *in vitro* data demonstrated that dimeric, but not monomeric, purified soluble mouse P-selectin and recombinant mouse P-selectin receptor-Ig fusion protein, which included the lectin domain, the epidermal growth factor domain, and the first four and part of the fifth complement-like repeat domains of mP-selectin fused with the heavy chain of mouse immunoglobulin G, induce integrin activation on leukocytes with a subsequent increase of leukocyte adhesion to fibrinogen and ICAM-1 [100]. These findings suggest that PSGL-1, like growth-factor receptors [6], requires dimerization. It is unknown whether dimeric P-selectin binds to two P-selectin binding sites in the same PSGL-1 dimer, or whether it induces clustering of adjacent PSGL-1 dimers. In response to PSGL-1 engagement, Src family kinases are activated, which in turn, phosphorylate Naf1 following the stimulation of isolated human neutrophils or 293 cells cotransfected with PSGL-1 and Naf1 with mP-selectin-Ig [100]. This is necessary to recruit and activate the phosphoinositide-3-OH kinase p85-p110δ heterodimer and, subsequently, induce downstream signaling [100].

Engagement of L-selectin can also lead to the activation of neutrophils. Early studies demonstrated that L-selectin engagement by antibodies or ligand mimetics increase intracellular calcium levels, induces tyrosine phosphorylation, superoxide production, and production of Interleukin (IL)-8 and tumor necrosis factor alpha (TNF)-α [53,98]. Although cross-linking of L-selectin by antibody leads to increased Mac-1 adhesiveness [3], L-selectin-dependent rolling of isolated human neutrophils on peripheral-node addressin and ICAM-1 in a parallel-plate flow chamber at a shear stress of 1.8 dyn/cm^2 is not sufficient to induce neutrophil arrest under flow [51]. This apparent discrepancy suggests that L-selectin cross-linking may be necessary, but not sufficient for signaling. Most of the studies showing neutrophil activation used intact monoclonal antibodies to L-selectin. These antibodies may also engage and activate Fc receptors and induce neutrophil activation through a combination of L-selectin and Fc-receptor-mediated signaling. Some studies used $F(ab')_2$ fragments of L-selectin antibodies and cross-linked them by secondary $F(ab')_2$ fragments [33] in order to stimulate neutrophils. This approach excluded Fc-receptor engagement, but still induced protein tyrosine phosphorylation [99].

In vitro stimulation of human neutrophils with soluble recombinant human E-selectin, lacking the transmembrane and cytoplasmic domains and the last two consensus repeats, for 15 minutes induces an increased β_2-mediated adhesion (76), tyrosine phosphorylation-dependent superoxide release [77], and polarization without affecting whole-cell deformability, as measured by filter assay [76]. Although soluble E-selectin did not induce calcium mobilization in isolated human neutrophils by itself *in vitro*, the elevation of intracellular calcium concentration lasted longer in the presence of E-selectin following chemokine stimulation [77,62]. This effect is mediated by Src-kinase- and PI(3)K-dependent activation of store operated calcium entry [62]. Further, *in vitro* data with isolated human neutrophils and E-selectin transfected 300.19 cells show that the formation of heterotypic aggregates, p38 MAPK phosphorylation, and surface upregulation

of integrins are shear stress dependent [34]. E-selectin engagement under shear-stress conditions induces calcium influx in human neutrophils [80]. However, these studies do not address the question of which E-selectin ligand is responsible for the observed effects. E-selectin engagement under shear-stress conditions also induces the redistribution and clustering of L-selectin and PSGL-1 to the trailing edge of human neutrophils [78]. *In vivo*, CD44 was found to be required for the redistribution of the adhesion molecules in a p38-dependent manner [35] (see Table 1). The redistribution and clustering of these adhesion molecules may provide an additional platform for capturing circulating leukocytes, which in turn, enhances leukocyte recruitment through cell-cell-interactions [88].

Using a new autoperfused flow chamber system [15], which allows the investigation of neutrophils in whole blood on different substrates, demonstrated that E-selectin engagement activates LFA-1 and induces an intermediate affinity state of LFA-1, which transiently binds to ICAM-1 and reduces the rolling velocity on E-selectin and ICAM-1 without inducing arrest [109] (Figure 1). This E-selectin signaling pathway is PSGL-1 and Syk-dependent [109] (see Table 1).

In contrast to the autoperfused flow chamber system, where rolling is observed at 6.0 dyn/cm^2 and more, flow chamber experiments with isolated human neutrophils on E-selectin (site density of up to 885 sites/μm^2) do not show neutrophil-substrate interactions at shear stresses above 3.6 dyn/cm^2 [52]. Neutrophils in whole blood interact with E-selectin *in vivo* under higher wall shear stress conditions [48,109], but additional molecules may contribute. Further, parallel-plate flow chambers with isolated human neutrophils show that neutrophils, in the absence of chemoattractants, adhere to L cells coexpressing E-selectin and ICAM-1 [84]. This may be caused by the isolation procedure

Table 1. Known signaling pathways during leukocyte recruitment

	Ligand	Receptor		Biological effect
Rolling	E-selectin	PSGL-1	Syk	Forcing LFA-1 in the intermediate affinity conformation, mediating slow rolling (119)
	E-selectin	CD44	p38	redistribution of L-selectin and PSGL-1 to the rear trail of neutrophils, this probably mediates secondary recruitment of neutrophils (35,78)
	E-selectin	?	intracellular Ca^{2+}	? (80)
	P-selectin	PSGL-1	Src → Naf-	Activation of Mac-1, which - supports leukocyte adhesion to fibrinogen and ICAM-1 (100)-
	?	L-selectin	shedding	regulation of rolling velocity, inducing of arrest and transmigration (32, 33)
Arrest	chemokines	GPCR	Gai_2 +Gβγ → PLC → IP_3+DAG → Ca^{2+} → calDAG-GEFI → Rap1	Integrin activation and leukocyte arrest (108, 71, 14, 37, 8, 82)
Post-Arrest	?	PSGL-1	Moesin/Ezrin → Syk	Activation of transcription Factors (97)
	ICAM-1	integrins	Src → ITAM → Syk → WASp → Vav	Superoxide production and degranulation, post-adhesion strengthening (28, 25, 65, 2, 113, 26)

The shown signaling pathways may be activated by several inputs and converge or interact with each other. Interactions that are depicted as dashed lines may be indirect. $\alpha_i\beta\gamma$, G-protein subunits; PLC, phospholipase C; Naf-1, Nef-associated factor 1; Src, Src-family kinases; Mac-1, macrophage antigen; calDAG-GEF-1, calcium-diacylglycerol guanine nucleotide exchange factor I; Rap1, Ras-related protein 1; Syk, spleen tyrosine kinase; IP_3, inositol triphosphate; PI3K, phosphotidylinositol 3-kinase; Ca^{2+}, calcium; GPCR, G-protein-coupled receptor; LFA-1, lymphocyte function antigen-1; ICAM-1, intercellular adhesion molecule-1; PSGL-1, P-selectin glycoprotein ligand.

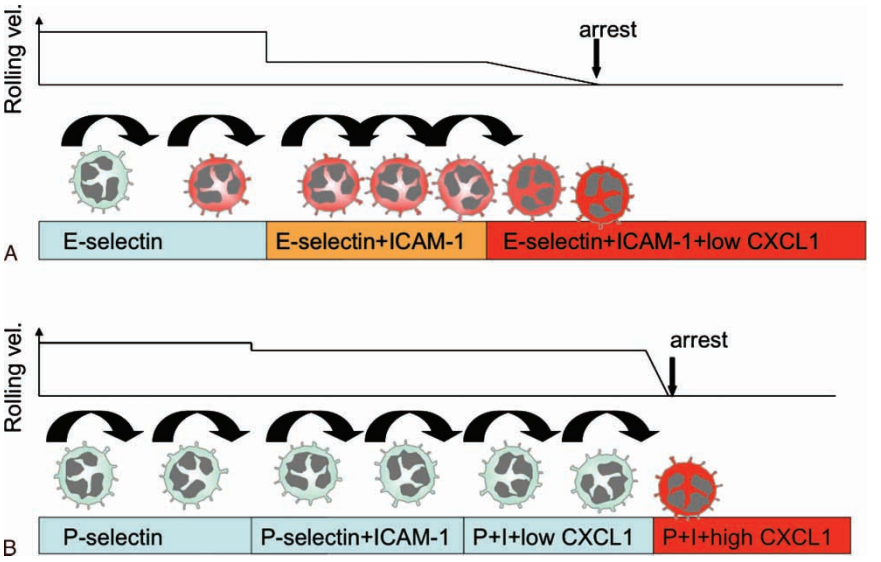

Figure 1. Two modes of neutrophil arrest: slow rolling versus immediate arrest. **A.** Neutrophils rolling on E-selectin pick up activating signals through P-selectin glycoprotein ligand (PSGL-1) and spleen tyrosine kinase (Syk), resulting in the partial activation of lymphocyte function antigen-1 (LFA-1) to the extended conformation with closed headpiece, indicated by the pink color. As soon as a neutrophil rolling on E-selectin encounters a surface with E-selectin and intercellular adhesion molecule-1 (ICAM-1) (orange), the rolling velocity immediately decreases because LFA-1 now engages ICAM-1. This is schematically represented in the velocity trace on top. Even a small amount of a CXCR2 ligand, such as immobilized CXCL1 (red), leads to arrest. **B.** Neutrophils rolling on P-selectin show little evidence of LFA-1 activation. Their rolling velocity changes little when ICAM-1 becomes available. A low dose of CXCL1 coimmobilized with P-selectin and ICAM-1 (P+I) cannot induce arrest, but a high dose can (red).

known to activate neutrophils and induce increased expression of Mac-1 and decreased surface expression of L-selectin [30,24,47]. The differences seen in studies using whole blood on recombinant proteins, compared with studies using isolated neutrophils on transfected L cells, may also be due to the expression of other adhesion molecules and/or cytokines by L cells. In addition, species differences between human and mouse neutrophils may explain some of the differences.

Signaling Events and Consequences During Neutrophil Rolling

In the absence of additional stimuli, such as other selectins, cytokines, and chemokines, P-selectin binding can prime neutrophils, but not fully activate integrins and induce arrest. These data are supported by *in vivo* experiments in uninflamed dermal microvessels [103] and flow chamber experiments that showed that the rolling velocity of neutrophils in whole blood is reduced on P-selectin and ICAM-1, compared to P-selectin alone [109,15]. However, in the presence of other proinflammatory stimuli, P-selectin acts synergistically and contributes to full integrin activation [100]. Elimination or blocking of P-selectin by gene targeting or antibody reduces neutrophil recruitment into the peritoneal cavity following thioglycollate injection. However, whether this reduction of neutrophil recruitment is only caused by a reduction of capturing or by decreasing the signaling input remains to be elucidated.

The activation of neutrophils by L-selectin engagement is also important *in vivo*. Blocking L-selectin shedding by a hydroxamic acid-based protease inhibitor increases L-selectin expression on the surface of neutrophils and augments signal input through L-selectin [33]. These changes were associated with a reduced rolling velocity [32], increased the "smoothness" of rolling, enhanced arrest, and transmigration [33] (see Table 1). Interstingly, this influence of L-selectin shedding on rolling velocity is cell specific, since increased L-selectin surface expression on T-lymphocytes does not influence the rolling velocity [27]. However, subphysiological L-selectin levels on T-lymphocytes increased the rolling velocity *in vitro* and *in vivo* as well as reduced homing to lymph nodes [27]. These data suggest that there is a threshold density of L-selectin on T-lymphocytes that is required for optimal homing to

peripheral lymph nodes. Elimination of ADAM17, which is involved in activated L-selectin shedding, by gene targeting increases the presence of L-selectin on the surface of neutrophils and enhances neutrophil rolling, arrest, and recruitment in a peritonitis model (K. Ley, E. Raines, J. Tang, A. Zarbock, unpublished observation). These data suggest that L-selectin has an important signaling role in neutrophil activation and recruitment. This may partially explain the substantial neutrophil recruitment defect seen in L-selectin-deficient mice [95], which is more severe than would be expected from the small contribution of L-selectin to neutrophil rolling.

Slow rolling *in vivo* can be induced by the injection of TNF-α and requires E-selectin and the engagement of β_2 integrins [48,40], as blocking both integrins involved in slow rolling, Mac-1 and LFA-1, increases leukocyte rolling velocity [20]. In contrast to the requirement of PSGL-1 for E-selectin-mediated slow rolling in the autoperfused flow chamber system, Hidalgo and colleagues showed that the rolling velocity on P- and E-selectin under inflammatory conditions *in vivo* is also dependent on CD44 [35].

CHEMOKINE-INDUCED ARREST

During inflammation, different cell types, including endothelial cells, leukocytes, platelets and other cells, produce and release a broad range of chemokines and other chemoattractants. Some of these proinflammatory mediators circulate in the plasma, others are only found in the inflammatory tissue, and yet others are presented on endothelial cells. The Duffy antigen receptor for chemokines (DARC) participates in transcytosing chemokines from the tissue to the luminal surfaces of endothelial cells [63]. DARC has a serpentine structure with seven transmembrane domains, like other chemokine receptors, but is not G-protein coupled and has no known signaling mechanism [69]. It exhibits a broad specificity, binding members of both CC and CXC classes of chemokines [68,93]. Elimination of DARC by gene targeting leads to a change of the spatial distribution of chemokines in the tissue [111] and, in some models, to reduced neutrophil recruitment into the tissue following injury [111,59]. Glycosaminoglycans (GAGs) are also known to bind and present chemokines [64]. These molecules are negatively charged polysaccharides and are thought to bind chemokines by electrostatic interactions [49]. GAG binding is required for efficient recruitment of leukocytes by chemokines [38].

Binding of chemokines to G-protein-coupled receptors (GPCRs) on neutrophils induces the activation of intracellular signaling pathways, which activates integrins almost instantaneously [56,108]. The activated integrins mediate arrest by binding to immunoglobulin superfamily members expressed on endothelial cells [46]. The rapid activation of integrins downstream of GPCR engagement is referred to as inside-out signaling.

Neutrophils express four β_2 integrins, where Mac-1 and LFA-1 are most relevant for neutrophil arrest in the systemic circulation. As presented above, selectins are able to modulate integrin adhesiveness and mediate slow rolling, while activation of GPCR is a more rapid mechanism to induce neutrophil arrest. Engagement of chemokine and other chemoattractant GPCRs with their respective ligands rapidly regulates integrin adhesiveness. The adhesiveness may be influenced by the regulation of the affinity and the avidity of the integrin [45,54,58]. An upregulation of the "affinity" is associated with a conformational change, which increases ligand binding and decreases ligand dissociation. The different integrin conformations are associated with at least three affinity states (e.g., low-, intermediate-, and high-affinity states) [82], but additional states may exist. Studies on LFA-1 showed that inside-out signaling by GPCR activation leads to a conformational change of the integrin with upregulation of its affinity. The integrin undergoes rearrangement from a bent low-affinity conformation to an extended high-affinity conformation, which is associated with the exposure of the ligand-binding pocket [58]. Stimulation of neutrophils with chemokines only activates a small fraction of integrins on the surface of the cells [19] and the regulation is dynamic. The regulation of the integrin affinity is a critical step in chemokine-induced arrest under flow [71].

Only a few steps in the chemokine-induced integrin-activation pathway in neutrophils are known. Most studies of GPCR-induced integrin activation were done in lymphocytes and monocytes. Due to the differences in chemokine receptor, integrin, and

signaling molecule expression in these cells, it is likely that different leukocyte subtypes do not use the same signaling pathways and molecules. Activation of GPCR leads to dissociation of the Gα-subunit from the Gβγ-complex. The elimination of the $G\alpha_{i2}$-subunit in neutrophils leads to an almost complete loss of chemokine-induced arrest *in vivo* and *in vitro* [108]. The Gβγ-complex is able to activate phospholipase C (PLC) [14], which in turn, hydrolyzes phosphatidylinositol 4,5-biphosphate to produce inositol triphosphate and diacylglycerol. However, it is not known which β- and γ-subunits are involved in PLC activation. Neutrophils express five different β-subunits and 12 γ-subunits [104]. It was demonstrated that PLC is involved in chemokine-induced arrest and $\alpha_4\beta_1$ integrin affinity upregulation in a monocyte-like cell line [37]. The involvement of PLC in chemokine-induced arrest was also confirmed for primary neutrophils [108] (see Table 1). PLC activity leads to increased IP_3 concentration, which triggers Ca^{2+}-release from the endoplasmic reticulum,", whereas diacylglycerol activates some isoforms of protein kinase C. The Gβγ-complex can also activate other molecules including P-Rex-1 [102] and PI3Kγ [36], which is not directly involved in chemokine-induced arrest. PI3Kγ-deficient mice have normal chemokine-induced arrest under flow, but show a defect in postadhesion strengthening [86].

Two studies in mice and humans convincingly demonstrated that the guanine nucleotide exchange factor (GEF) CalDAG-GEFI is involved in chemokine-induced neutrophil arrest [71,8] (see Table 1). Calcium, diacylglycerol, and perhaps other factors are required for full activation of CalDAG-GEFI. Activated CalDAG-GEFI can subsequently activate the small GTPase RAP1/2 that is involved in chemokine-induced arrest [82,44]. The molecule that links Rap1 with the integrin in neutrophils is still unknown. It has been shown in platelets and other cell types that talin1 interacts with the cytoplasmic tail of the β-chain of integrins and modulates the conformational change of $\alpha_{IIb}\beta_3$ [101,43,94]. The selective disruption of the talin1 gene in mouse platelets leads to spontaneous hemorrhage, pathological bleeding, impaired $\alpha_{IIb}\beta_3$-mediated platelet aggregation, and β_1 integrin-mediated platelet adhesion [72,70]. Whether talin1 is also involved in chemokine-induced arrest and/or selectin-mediated integrin affinity regulation in neutrophils remains to be seen.

STABILIZATION OF ADHESION AND FULL ACTIVATION OF NEUTROPHILS BY OUTSIDE-IN SIGNALING

Integrin binding to their ligands induces cell adhesion and generates intracellular signals that regulate cellular functions, including cell motility, phagocytosis, superoxide production, degranulation, proliferation, and apoptosis (i.e., outside-in signaling) [58]. The conformational changes of integrins induced by inside-out signaling (e.g., GPCR- and selectin-signaling) presumably participate and facilitate outside-in signaling. The most proximal signaling event during outside-in signaling is thought to be the activation of the Src family kinases. However, it is still unknown how these kinases are activated by integrin engagement. Elimination of the Src kinases, Hck, Fgr, and Lyn, expressed in neutrophils abolishes postadhesion strengthening, transmigration [28], superoxide production, and degranulation [25; for detailed information, see [7]. The activated Src kinases phosphorylate the ITAM-containing adaptor molecules, DAP12 and FcRgamma, which in turn, recruit and activate spleen tyrosine kinase (Syk) that subsequently initiates further downstream signaling, including respiratory burst [65] (see Table 1). The pathway shows similarities with T-cell, B-cell, and Fc-receptor signaling [2].

A defect of postadhesion strengthening was also found in mice lacking the downstream-signaling molecules, Wiskott-Aldrich Syndrome (WAS) protein [113], PI3kγ [86], and Vav1 and 3 [26]. Elimination of these molecules also affected other signaling pathways and functional outcome (86, 113, 26; for detailed information, see [9]. These data suggest that signals exchanged between neutrophils and other cells have important consequences for their phenotype. Most likely, interaction with extracellular matrix proteins induces further signaling. It is known that neutrophils that have undergone rolling, arrest, adhesion, and transmigration display a very different phenotype from blood neutrophils [89].

FURTHER PERSPECTIVE

Our knowledge of how shear stress acts on, and is transmitted into, neutrophils is very limited. It is unknown how different signaling pathways interact with each other. Understanding the different signaling pathways and how they interact may facilitate

the development of therapeutics that only modulate the desired function.

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