Integrin-mediated leukocyte adhesive interactions
Regulation by haemostatic factors

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Summary
Leukocyte recruitment to sites of inflammation, infection or vascular injury is a complex event that is orchestrated by a tightly coordinated sequence of interactions between leukocytes and cells of the vessel wall, especially endothelial cells. These interactions are controlled by the expression and activation of various adhesion receptors and protease systems. This review will focus on novel aspects of the regulation of integrin-dependent leukocyte adhesion by haemostatic factors. Here, so-called non-haemostatic properties of endogenous proteins such as high molecular weight kininogen, urokinase receptor, urokinase, as well as plasminogen and its cleavage product angiotatin in leukocyte adhesion and transmigration will be summarized. The crosstalk between haemostatic factors and inflammatory reactions may contribute to a better understanding of inflammatory vascular disorders and to the development of novel therapeutical concepts.

Adhesive mechanisms of leukocyte recruitment

As an immediate inflammatory response to infection or injury, leukocytes emigrate from the blood stream towards extravascular sites. The recruitment process of leukocytes consists of a highly coordinated sequence of adhesive interactions between leukocytes and the vascular endothelium, including

● initial tethering and rolling,
● leukocyte activation,
● firm adhesion and locomotion at the endothelium, and
● trans-endothelial migration.

Selectins, and integrins, as well as counter-receptors of the Ig superfamily that are spatio-temporally modulated by chemotactic tracts, act in a cooperative manner and provide the molecular platform for the regulation of leukocyte emigration from the vasculature to the site of inflammation (62, 63). Initial attachment and progressive leukocyte rolling depends on the interaction between endothelial cell E-selectin and P-selectin with their carbohydrate ligands on leukocytes (11, 62). Moreover, the firm adhesion of leukocytes to the endothelium is predominantly mediated by members of the β2-integrin family

– LFA-1 (αLβ2, CD11a/CD18) and
– Mac-1 (αMβ2, CD11b/CD18) as well as by

– β1-integrins, especially VLA-4 (α4β1), that interact with endothelial counter-receptors such as ICAM-1 or VCAM-1, respectively (11). The efficient interaction between integrins and their counter-receptors or ligands requires changes in both the expression level and the state of activation of these molecules. In particular, activation of integrins occurs either by avidity modulation (e.g. by clustering the integrins on the membrane due to the presence of co-receptors especially in cholesterol-rich lipid rafts), or by affinity modulation involving conformational changes (e.g. by inside-out signalling reactions). In addition, pro-inflammatory cytokines upregulate the expression of ICAM-1 or VCAM-1 on endothelial cells and thereby increase the availability of integrin ligands (44, 62, 63). The critical role of these molecules for leukocyte adhesion is underlined by studies with mice deficient in one or more of these components (26). The importance of β2-integrins for the inflammatory response is also revealed by the phenotype of a rare inherited defect in men known as leukocyte adhesion deficiency type I (2).
During the final step of leukocyte recruitment (diapedesis), leukocytes traverse the endothelium, while the endothelial permeability barrier is maintained. Although leukocytes can cross the endothelial cell layer by transcytosis (27), the major route for a leukocyte to pass the endothelial barrier is through the cleft between two or three adjacent cells, which requires transient making and braking of multiple leukocyte-endothelial cell contact sites. Leukocyte transendothelial migration is controlled by homophilic and heterophilic interactions between leukocyte and endothelial cell receptors, including integrins, platelet-endothelial cell adhesion molecule-1 (PECAM-1) (35), members of the junctional adhesion molecule family (JAM) (12, 25) as well as CD99 (44, 57). In contrast to rolling and adhesion, the underlying molecular mechanisms of leukocyte transmigration are not well understood.

At sites of vascular injury, where the endothelial cell lining has been denuded, deposited platelets and fibrin may promote leukocyte infiltration as well (39). Reminiscent of the leukocyte-endothelium interactions, a sequential adhesion process including leukocyte attachment to and transmigration across surface-adherent platelets, has been proposed: platelet-P-selectin-mediated initial tethering and rolling of leukocytes through P-selectin glycoprotein ligand-1 (PSGL-1) is followed by Mac-1-dependent firm adhesion and transplatelet migration (41). In this regard, Mac-1 interacts with a number of counter-receptors on the surface of platelets such as ICAM-2, integrin αIIbβ3-associated fibrinogen (24, 67), glycoprotein Ibα and junctional adhesion molecule-C (JAM-C) (54, 59). In addition, Mac-1 mediates the adhesion of leukocytes to the deposited fibrinogen matrix (11). This review will focus on the regulation of integrin-dependent leukocyte adhesion in inflammation by haemostatic factors. In particular, the crosstalk between the urokinase receptor, urokinase and integrins determines the activation and strength of β2-integrin interactions, whereas high molecular weight kininogen and angiotatin (fragments of plasminogen) serve as potent endogenous anti-adhesive/anti-inflammatory components (Fig. 1).

**Urokinase receptor**

Integrins associate via their extracellular domain with coreceptors or other transmembrane proteins often in cholesterol/sphingolipid-rich lipid rafts of the plasma membrane. These interactions regulate integrin avidity and affinity and thereby allow the control and modulation of ligand binding and cellular signaling (inside-out and outside-in) necessary for effective adhesiv processes to occur. Besides so-called integrin-associated proteins, such as the tetraspan family of membrane proteins (e. g. CD47, CD98) (38), different glycosyl-phosphatidylinositol(GPI)-anchored proteins, including CD14, CD59 and the urokinase receptor (uPAR) have been shown to physically associate with various integrins on different cell types (48).

The uPAR is a highly glycosylated 55–60 kDa protein (4) consisting of three homologous domains anchored to the plasma membrane by a GPI moiety. This GPI-anchor entails uPAR with a relatively high lateral mobility in the plasma membrane and predisposes uPAR to concentrate in lipid rafts, characteristic glycosphingolipid- and cholesterol-rich membrane microdomains that exhibit a lower fluidity than the surrounding plasma membrane and thereby enable the preassembly of multireceptor adhesion complexes and signaling molecules (29, 34). Many cell types including circulating blood leukocytes, endothelial and vascular smooth muscle cells, fibroblasts and bone marrow cells, as well as a variety of neoplastic cells express uPAR on their surface (3), and its expression level on cells strongly correlates with their migratory and invasive potential (47).

Upon enzymatic removal of uPAR from the plasma membrane or its inactivation integrin-dependent adhesion is prevented by 50–75 % (40). Due to its interaction with various β2-integrins on leukocytes (13, 51, 60), uPAR regulates β2-integrin-mediated adhesion.

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**Fig. 1** β2-integrin mediated leukocyte adhesion to endothelial cells. Leukocyte adhesion is mediated by interactions between the β2-integrins LFA-1 and Mac-1 on leukocytes and their major counter-receptor ICAM-1 on endothelial cells. Leukocyte-endothelial interaction is regulated by haemostatic factors:

- **(A)** Activation of β2-integrins by urokinase receptor (uPAR): The GPI-anchored uPAR associates with β2-integrins in a cis fashion and is essential for β2-integrin activation.
- **(B)** Urokinase (uPA) binds to both, uPAR with its aminoterminal fragment (ATF) and to Mac-1 with its kringle and proteolytic fragment (K + P). This multiple binding enables formation of a multiprotein complex on the surface of leukocytes that regulates cell adhesion and plasminogen activation.
- **(C)** Angiotatin (kringles K1–4) interacts with Mac-1 thereby blocking Mac-1-dependent leukocyte adhesion to endothelial cells.
- **(D)** High molecular weight kininogen (HK), and particularly its domain 5, binds to Mac-1, thereby blocking the interaction between Mac-1 and ICAM-1.

Based on the illustrated specific interactions, the uPAR-β2-integrin complex is a potential target for therapeutic interventions, and the indicated endogenous fragments of haemostatic factors can very well serve as inhibitory substances in hyper-inflammatory situations.
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adhesion of leukocytes to the endothelium as well as the extracellular matrix (28). In vivo, the number of emigrated neutrophils into the inflamed peritoneum during the initial, acute inflammatory phase was dramatically (>50%) reduced in uPAR-deficient mice as compared to wild-type mice (40). Thus, uPAR is needed for inflammatory adhesive reactions and host defense in vivo. Moreover, zinc ions derived from degranulated platelets (42) induce conformational changes in uPAR leading to subsequent activation of β2-integrins, which is reflected by a two-to-three fold increase in leukocyte adhesion. Other divalent cations did not show this effect on uPAR-mediated β2-integrin-dependent leukocyte adhesion. Zinc ions did not affect β2-integrins directly, because they were ineffective in the absence of uPAR (40).

Urokinase

The ligation with urokinase (uPA) appears to prevent the mentioned crosstalk between uPAR and β2-integrins, since uPA partly inhibited leukocyte adhesion to endothelial cells (40). Moreover, Mac-1 on neutrophils also interacts with uPA thereby mediating neutrophil adhesion and migration to immobilized uPA. Within uPA, both the kringle and proteolytic domains are recognized by Mac-1, whereas the growth factor domain binds to uPAR (49) (Fig. 1). Within the αM subunit of Mac-1, the I domain interacts with uPA, whereas a distinct site of this integrin subunit interacts with uPAR (49).

The uPAR binding region in Mac-1 lies in the amino acid sequence 424–440, which comprises the entire upper loop sequence of the fourth repeat within the so-called b-propeller model (61). The direct Mac-1-uPA interaction may thereby explain the aforementioned inhibition by uPA of Mac-1-dependent leukocyte adhesion to fibrinogen and the endothelium. In particular, this inhibitory mechanism may be due to direct competition between uPA and ICAM-1 or fibrinogen for binding to Mac-1 rather than due to disruption of the Mac-1-uPAR interaction as previously interpreted (40). Thus, the recognition of uPA by Mac-1 allows for for-
mation of a multicontact trimolecular complex, in which a single uPA ligand may bind simultaneously to both uPAR and Mac-1 (Fig. 1). This complex could play an important role in the control of inflammatory cell migration and thrombolytic functions of leukocytes.

Plasminogen and angiostatin

The interaction between Mac-1 and the uPA/uPAR-system can be extended by demonstrating a specific interaction between Mac-1 and plasminogen (50), whereby Mac-1 regulates neutrophil-dependent fibrinolysis. In plasminogen activation assays, addition of purified Mac-1 to a single chain uPA/plasminogen mixture augmented the catalytic efficiency by 50-fold. Moreover, when plasminogen was added to phorbol-ester-stimulated neutrophils, both uPA and plasminogen were co-immunoprecipitated together with Mac-1. Thus, stimulated neutrophils have the ability to lyse fibrin clots in a Mac-1-dependent manner, and the underlying mechanism depends upon the direct and simultaneous binding of uPA and plasminogen to the β2-integrin (50). Plasminogen was also found to promote leukocyte adhesion in a Mac-1 and integrin α5β1-dependent manner (37), whereby the lysine-binding sites in the kringle-domains of plasminogen were indispensable for this activity.

These data are strengthened by our recent findings that angiostatin, a proteolytic fragment of plasminogen, serves as a ligand for the β2-integrin Mac-1 as well as for β1-integrins, thereby blocking cell adhesion (15). Angiostatin, entailing the kringle-domains K1–3 or K1–4 of plasminogen has originally been described as a potent inhibitor of angiogenesis in vivo (45, 46, 58). A specific interaction particularly of K4 of angiostatin with Mac-1 and K1–3 with integrin α4β1 was identified (15). K1–4 and the isolated K4 specifically inhibited Mac-1-dependent adhesion of neutrophils and myelomonocytic cells to immobilized fibrinogen or ICAM-1, whereas integrin α4β1-dependent adhesion to fibronectin and VCAM-1 was blocked by K1–4 and K1–3. Based on their interaction with Mac-1, K1–4 (and K4 to a lower extent) inhibited leukocyte recruitment in the in vivo model of thioglycollate-induced acute peritonitis, in which acute neutrophil emigration is predominantly mediated by Mac-1 and LFA-1. These data clearly define angiostatin as an anti-adhesive and anti-inflammatory factor for leukocytes. In addition, angiostatin inhibited tumor necrosis factor-a induced NFκB activation as well as tissue factor upregulation in a Mac-1-dependent manner. Since tissue factor is a major initiator of haemostasis after vascular injury, inhibition of its expression by angiostatin indicates that this plasminogen fragment serves a further yet unrecognized function as a local regulator of the initiation of blood coagulation. Compatible with this interpretation is the observation that tissue factor appears to block the angiostatin-mediated inhibition of endothelial cell proliferation (1, 15).

These findings are in accordance with the data that biologically active angiostatin can be generated, among other reactions, by neutrophil-derived elastase (55), and can interfere with neutrophil migration and chemokine-induced angiogenesis (5). In the inflamed tissue angiostatin might counteract the angiogenic potential of platelets (53) or inflammatory cells by inhibiting integrin-mediated leukocyte recruitment and activation. For example, platelets and monocytes are a source for vascular endothelial growth factor, basic fibroblast growth factor and metalloproteinases and can modulate angiogenesis and arteriogenesis, respectively (9, 53, 56). Moreover, neutrophils produce proangiogenic growth factors such as heparin-binding epidermal growth factor, vascular endothelial growth factor, interleukin-8 or tumour necrosis factor-a (7, 10, 64), and are an abundant source of matrix metalloproteinase-9, which controls the angiogenic switch during (tumour) neovascularization (6).

Thus, the overall contribution of platelets and inflammatory cells to angiogenesis regulation most likely depends upon a fine balance of proangiogenic and anti-angiogenic factors, which are secreted by recruited blood cells. Depending on the environmental stimuli, the anti-adhesive and anti-inflammatory action of angiostatin on leukocytes represents a novel negative feedback counteracting angiogenesis at a site of inflammation or vascular injury. Angiostatin might thereby be crucial in linking inflammation and angiogenesis under both physiologic conditions such as wound healing, and pathologic situation such as in rheumatoid arthritis, psoriasis, diabetic retinopathy or atherosclerosis. In a recently published study, administration of angiostatin reduced lesion progression and the infiltration of macrophages into atheromas (43), which might be not only an indirect consequence of reduced plaque neovascularization but also due to a direct effect of angiostatin on inflammatory cells.

High molecular weight kinogen

High molecular weight kinogen (HK) was initially identified as a non-enzymatic cofactor in the initiation of the contact system (22). HK is composed of six domains, and upon cleavage by kallikrein, the short-lived vasodilator peptide bradykinin is liberated from domain D4 thereby generating a two chained kinin-free molecule. This HKa lacks most of domain D4 and consists of a heavy (domains D1-D3) and a light chain (domains D5 and D6) (22).

Cellular binding sites for HK/HKα exist on endothelial cells, granulocytes as well as on platelets, and interactions are mediated by domains 3 and 5 (30, 33) with an absolute requirement for zinc ions. On endothelial cells the binding protein for globular C1q (denoted gC1qR) as well as uPAR and cytokine-1 were identified as binding proteins for HK (22, 31, 32). On granulocytes both, uPAR and the β2-integrin Mac-1 (CD11b/CD18) account for HK binding (16, 17, 65). Finally, on platelets, glycoprotein Ib as well as cell-associated thrombospordin serve as additional HK-binding factors (8, 18).

Although initially identified as a component of the contact system, HK appears to be associated with vascular injury, inflammation or activation of complement in humoral immune defense, whereby two chained kinin-free HKs may serve as an anti-adhesive.
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References


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