Leukocyte – endothelial interactions in inflammation

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Abstract

At sites of inflammation, infection or vascular injury local proinflammatory or pathogen-derived stimuli render the luminal vascular endothelial surface attractive for leukocytes. This innate immunity response consists of a well-defined and regulated multi-step cascade involving consecutive steps of adhesive interactions between the leukocytes and the endothelium. During the initial contact with the activated endothelium leukocytes roll along the endothelium via a loose bond which is mediated by selectins. Subsequently, leukocytes are activated by chemokines presented on the luminal endothelial surface, which results in the activation of leukocyte integrins and the firm leukocyte arrest on the endothelium. After their firm adhesion, leukocytes make use of two transmigration processes to pass the endothelial barrier, the transcellular route through the endothelial cell body or the paracellular route through the endothelial junctions. In addition, further circulating cells, such as platelets arrive early at sites of inflammation contributing to both coagulation and to the immune response in parts by facilitating leukocyte–endothelial interactions. Platelets have thereby been implicated in several inflammatory pathologies. This review summarizes the major mechanisms and molecules involved in leukocyte–endothelial and leukocyte–platelet interactions in inflammation.

Keywords: endothelial cells  ●  adhesion  ●  leukocytes  ●  platelets  ●  inflammation

Introduction

The characteristic steps taken by leukocytes to extravasate from blood to the site of inflammation caused by either exogenous or endogenous stimuli have been recognized and summarized for about two decades as the ‘three-step’ paradigm of inflammatory cell recruitment that involved rolling, activation and adhesion. Extensive research in this field has resulted in the expansion of the three-step leukocyte adhesion cascade to include further adhesive processes between leukocytes and the endothelium, such as the slow rolling, the locomotion or crawling as well as the transendothelial migration [1–10]. The interaction between the leukocytes and the endothelium comprises a variety of adhesive and migratory molecular events including low affinity transient and reversible rolling adhesions, integrin-dependent firm adhesive interactions and migratory events of the leukocytes through the endothelium and beyond that, such as the penetration of the basement membrane, and migration in the interstitial space [5, 11].

Adhesion molecules

Leukocyte–endothelial adhesion molecules can be grouped into three families (Table 1): (1) Selectins are a family of three carbohydrate-recognizing molecules, of which E-selectin is expressed on the activated endothelium, P-selectin is expressed on platelets and the endothelium, and L-selectin is constitutively expressed on leukocytes [12]. Several studies engaging antibody blockade of selectins demonstrated the participation of selectins in leukocyte...
Selectin ligands are glycoproteins that are rich in glycosylation of O-linked and N-linked carbohydrates [15, 16]. (2) Integrins are heterodimers comprising an α- and β-chain and can recognize multiple ligands including proteins of the extracellular matrix, cell surface glycoproteins as well as complement factors and soluble components of the haemostatic and fibrinolytic cascade [17–19]. Leukocytes express integrins of the α₄β₁ family (CD11/CD18). In addition, several leukocyte subpopulations express α₈β₇, α₅β₁ and α₄β₇ integrins on their surface. Integrins require conformational changes to gain full adhesive function [17, 20]. (3) The major integrin ligands involved in leukocyte adhesion belong to the immunoglobulin superfamily [21] and include intercellular cell adhesion molecules (ICAM) 1–5, vascular cell adhesion molecule-1 (VCAM-1), as well as the junctional adhesion molecules (JAMs) [4, 5], that are expressed on endothelial and other cells. Further important adhesion receptors of the immunoglobulin superfamily involved in leukocyte recruitment are the platelet–endothelial cell adhesion molecule-1 (PECAM-1) [22] and endothelial cell adhesion molecule (ESAM) [23].

### Table 1 Players in leukocyte extravasation

| Adhesion molecule | Synonyms | Binding partner | General role | Reference |
|-------------------|----------|----------------|-------------|-----------|
| α₄β₁              | VLA-4, CD49d/CD29 | VCAM-1, fibronectin | Adhesion | [129] |
| α₄β₇              |          | MAdCAM-1       | Adhesion    | [130] |
| α₅β₂              | LFA-1, CD11a/CD18 | ICAM-1,-2,-3, JAM-A | Adhesion, slow rolling | [65, 131–133] |
| α₄β₂              | Mac-1, CD11b/CD18, CR3 | iC3b, ICAM-1,-2, heparin, fibrinogen, vitronectin, kininogen, JAM-C, RAGE | Adhesion | [68, 119, 134–139] |
| α₂β₂              | p150.95, CD11c/CD18 | iC3b, fibrinogen, JAM-C |         | [140] |
| α₂β₁              | CD11d/CD18 | ICAM-3, Fibrinogen, vitronectin | Adhesion | [141, 142] |
| JAM-A             |          | JAM-A, LFA-1   | Adhesion, transmigration | [62, 65] |
| JAM-B             |          | VLA-4, JAM-B, JAM-C | | [66, 143] |
| JAM-C             |          | JAM-B, JAM-C, Mac-1 | Adhesion, transmigration | [62, 143] |
| P-selectin        |          | PSGL-1, Sialyl-Lewis<sup>x</sup> | Cell rolling | [144–146] |
| E-selectin        |          | Sialyl-Lewis<sup>x</sup> | Cell rolling | [147] |
| L-selectin        |          | CD34, MAdCAM-1 | Cell rolling | [148, 149] |
| CD31              |          | CD31 | Transmigration | [77] |
| CD99              |          | CD99 | Transmigration | [82] |
| ESAM              |          | ESAM | Transmigration | [23] |

**Leukocyte margination, capture and cell rolling**

The very initial contact of the leukocyte with the vascular wall is determined by simple flow dynamics. Leukocyte margination is defined by the flow of leukocytes in a position close to the endothelial surface rather than in the central blood stream, depends on the interaction between individual red and white blood cells, and is enhanced in small postcapillary venules, which represent the main location for leukocyte recruitment [24]. Margination is rather a passive phenomenon and it is not entirely clear whether it is a rate-limiting step in inflammatory cell recruitment.

The initial process of active leukocyte recruitment is the tethering or rolling of leukocytes describing the initial selectin-mediated interaction between leukocytes and endothelial cells [25]. Antibody blockade of selectins inhibited leukocyte rolling in vivo in multiple studies [13, 26]. An apparent synergism between L-selectin and the vascular endothelial selectins exists [27]. Selectins bind to carbohydrate ligands, and leukocyte rolling can be inhibited by charged carbohydrates [28]. Moreover, P-selectin<sup>−/−</sup> mice show no leukocyte rolling in vivo [29]. The most important ligand for selectins is the glycoprotein P-Selectin Glycoprotein Ligand-1 (PSGL-1), which is present as a homodimer on leukocytes and can bind to both P-selectin and E-selectin [30, 31]. Interestingly, PSGL-1 ligation in neutrophils by both P-selectin and E-selectin can result in activation of integrins, thus providing a link between rolling and the subsequent integrin-mediated firm adhesion [31, 32]. This interplay between the PSGL-1-selectin interaction and LFA-1 activation is important for slow rolling of neutrophils. It has been shown that an ITAM-dependent pathway involving the Src-family kinase Fgr and the ITAM-containing adaptor proteins DAP12 and FcγRgamma mediates the signalling events downstream of PSGL-1 that are required to initiate...
neutrophil slow rolling [32]. The importance of the rolling process as a required step in the leukocyte recruitment cascade is underlined by a congenital pathology called leukocyte adhesion deficiency II (LAD II). Patients suffering from this rare disease reveal a congenital defect in fucose processing and cannot produce functional fucosylated selectin ligands [33]. Neutrophils from patients with this syndrome are defective in rolling [33], associated with severe recurrent bacterial infections.

**Activation and adhesion of leukocytes**

During the process of leukocyte rolling the contact of leukocytes with the luminal endothelial surface allows leukocytes to effectively 'sense' the endothelial surface-bound chemokines. Chemokines are dramatically induced by inflammatory mediators [34]. Chemokines secreted from an inflamed environment can be transcytosed through the endothelium and presented on the endothelial cell surface associated with proteoglycans [35, 36]. These surface-deposited chemokines are presented to rolling leukocytes. By interaction with the G-protein coupled chemokine receptors on leukocytes chemokines induce intracellular signals leading to inside-out integrin activation and firm leukocyte adhesion as well as to shape change and pseudopod formation [35]. These shape changes are associated with the conversion of G-actin to F-actin enabling the cell to enter the adhesion and later the transmigration process.

Chemokines are grouped into four major subfamilies depending on the presence or absence of intervening amino acids between the first two N-terminal cystein residues and are thereby designated as CC, CXC, CX3C or C chemokines. Besides secreted chemokines, CX3CL1 (fractalkine) and CXCL16 represent the transmembrane chemokines [35, 36]. CXCL8 (Interleukin-8, IL-8) plays an important role for neutrophil activation as IL-8 receptor–/– mice have 12-fold elevated systemic neutrophil counts and are severely impaired in their ability to recruit neutrophils into thioglycollate-induced peritonitis [37].

Binding of chemokines to their receptors on leukocytes results in the inside-out signalling activation of leukocyte β1-integrins and the β2-integrins LFA-1 and Mac-1, that mediate firm arrest of leukocytes [38] (Fig. 1A). In humans, leukocyte adhesion deficiency I (LAD-I) is caused by the absence of β2 integrins resulting in impaired leukocyte recruitment and as a consequence recurrent, life-threatening bacterial infections [39, 40]. In mice, β2-integrin deficiency I (LAD-I) is caused by the absence of β2 integrins resulting in impaired leukocyte recruitment and as a consequence recurrent, life-threatening bacterial infections [39, 40]. In mice, β2-integrin deficiency I (LAD-I) is caused by the absence of β2 integrins resulting in impaired leukocyte recruitment and as a consequence recurrent, life-threatening bacterial infections [39, 40]. Neutrophils use both LFA-1 (CD11a/CD18;αLβ2) and Mac-1 (CD11b/CD18;αMβ2) for adhesion as shown in different animal models [18, 42, 43]. LFA-1 and Mac-1 bind to endothelial ICAMs such as ICAM-1 and ICAM-2 [44]. ICAM-1 and ICAM-2 are constitutively expressed, and ICAM-1 expression is further increased after endothelial activation [45, 46]. In contrast, endothelial VCAM-1 is recognized by β-1 integrin receptors predominantly found on lymphocytes and monocytes [47]. This pathway of adhesion appears to be responsible for immune functions that occur in the absence of β2 integrins in LAD-I patients [48]. The adhesive activity of integrins is regulated by alterations...
in integrin affinity and integrin valency, the former being mediated by conformational changes of the integrin subunits and the latter involving changes of integrin distribution on the cell surface [9, 20, 49–51].

Transmigration

Transmigration of leukocytes through the vascular endothelium can take place in a paracellular or transcellular manner [6, 10, 52]. The major determinant in the paracellular pathway is the endothelial intercellular junctions, as changes in the integrity of the endothelial barrier in postcapillary venules affect inflammatory cell recruitment [4, 53, 54]. Two types of interendothelial junctions are relevant for the transmigration process [55]. Tight junctions (zonula occludens) are apically located and contain three types of transmembrane proteins, occludin, claudins, and junctional adhesion molecules (JAMs). These transmembrane molecules are linked to the actin cytoskeleton via interaction with molecules containing PDZ domains, such as ZO-1 [4]. Hierarchically, the most important determinant of the endothelial barrier is the adherens junctions that are formed by the homophilic interaction of VE-Cadherin [56]. VE-cadherin acts as a gatekeeper for the passage of leukocytes and inhibition of VE-cadherin increases the permeability of endothelial-cell monolayers and the rate of neutrophil extravasation in vivo [57]. In vitro studies indicate that VE-cadherin gaps may form transiently during leukocyte diapedesis [58]. The function of VE-cadherin to regulate the endothelial barrier or leukocyte transmigration can be modulated by phosphorylation of the cytoplasmic tail of VE-cadherin, at tyrosines 658 and 731 or at Ser 665 [59, 60], which can be stimulated by ICAM-1-mediated neutrophil adhesion to endothelial cells [61].

In addition, leukocyte transmigration involves homophilic and heterophilic interactions between adhesion receptors on the leukocyte and the endothelium [4, 10, 22, 62]. Junctional adhesion molecules (JAMs) belong to the immunoglobulin superfamily consisting of two extracellular Ig-like domains [4]. Besides interacting in a homophilic manner [63, 64], JAMs are engaged as counter-receptors for leukocyte integrins. JAM-A has been shown to interact with LFA-1 [65], JAM-B binds to VLA-4 [66] and JAM-C interacts with Mac-1 [67, 68]. The function of JAM-A in leukocyte diapedesis in vivo has been demonstrated by antibody inhibition experiments [69] as well as by evaluation of JAM-A-deficient mice showing that JAM-A on neutrophils as well as on endothelial cells participates in neutrophil extravasation [70, 71]. JAM-C can function as a heterophilic binding partner of integrin Mac-1. The JAM-C/Mac-1 interaction was found to mediate a firm platelet–neutrophil interaction [68]. In addition, soluble JAM-C or antibodies to JAM-C blocked neutrophil transmigration through endothelial cells, whereas accumulation of neutrophils in vivo was enhanced by endothelial-specific overexpression of JAM-C in mice [72, 73]. Thus, JAMs are important receptors determining leukocyte migration across the endothelial barrier [4, 74]. In addition, JAM-C also acts to antagonize VE-cadherin-dependent interendothelial adhesion thereby promoting the disruption of the endothelial barrier by a mechanism involving the small GTPase Rap1 [75].

Another important adhesion molecule regulating leukocyte transmigration is PECAM-1, a member of the immunoglobulin superfamily consisting of six Ig domains that is expressed at the intercellular borders of endothelial cells as well on platelets, neutrophils, monocytes and some T cells [22]. Several studies suggest that endothelial transmigration of leukocytes is mediated by the homophilic interaction of platelet endothelial cell adhesion molecule 1 (PECAM-1, CD31) as shown by antibody blocking studies both in vitro and in vivo [22, 76–78]. Endothelial PECAM-1, which was found to act preferentially in interleukin (IL)-1β- but not TNF-α-induced inflammatory cell recruitment [79], recycles between the junctions and the subjunctional plasmalemma, and is targeted to the zone of active leukocyte transmigration [78]. Recently, CD177, a 58- to 64-kD glycosyl-phosphatidyl-inositol (GPI)-anchored glycoprotein expressed exclusively on neutrophils, was identified as a novel heterophilic adhesion ligand of PECAM-1 involved in neutrophil transendothelial migration [80]. Distal to the step of transmigration mediated by PECAM-1, a further molecule, CD99, which is expressed on both neutrophils and other leukocytes and at the interendothelial junctions, participates in leukocyte transmigration [81, 82].

Endothelial cell ICAM-1 has been implicated in transmigration. ICAM-1 can colocalize with ringlike LFA-1 clusters on leukocytes during transmigration. In addition, a ‘cuplike’ transmigratory structure containing ICAM-1-enriched microvilli-like projections was shown to surround transmigrating neutrophils during diapedesis [83, 84]. During neutrophil adhesion to endothelial cells, ICAM-1 ligation induces cytoskeletal remodelling associated with ICAM-1 clustering, a process that is dependent on cortactin [85]. Moreover, cortactin and its tyrosine phosphorylation are required for the clustering of ICAM-1 around transmigrating neutrophils [86].

Endogenous inhibitors of leukocyte adhesion

In contrast to the numerous adhesion receptors that have been identified to promote leukocyte–endothelial interactions, very little is known about functionally important endogenous inhibitors of leukocyte adhesion [5, 8, 10, 74]. Endogenous inhibitors exist in several aspects of inflammation and immunity, and function to attenuate exuberant inflammatory and immune activation [87, 88]. Recently, developmental endothelial locus-1 (Del-1) was identified as an endogenous inhibitor of the leukocyte
adhesion cascade. Del-1, a glycoprotein secreted by endothelial cells and associated with proteoglycans of the endothelial cell surface and/or with the extracellular matrix [89], has been previously implicated as an adhesive molecule regulating vascular remodelling in the context of angiogenesis [90]. Del-1 was shown to interfere with LFA-1-dependent leukocyte–endothelial interactions [91]. In particular, Del-1 deficiency resulted in markedly increased leukocyte adhesion and recruitment to inflamed tissues in vivo [91]. The exact mechanistic action of the inhibitory role of Del-1 in leukocyte recruitment requires detailed investigation. Soluble ICAM-5 has also been described to act as an inhibitor of LFA-1 and can decrease T lymphocyte and microglia activation in a manner opposite to the pro-inflammatory action of ICAM-1 [92]. Interestingly, the high expression of both Del-1 and ICAM-5 in the central nervous tissue may contribute to its immune privilege. Galectin-1 is another endogenous inhibitor of leukocyte recruitment. Galectin-1 inhibits T-cell rolling and adhesion to activated endothelial cells under flow conditions, whereas galectin-1-deficiency in mice induced increased homing of T lymphocytes to lymph nodes and enhanced leukocyte recruitment in the cremasteric circulation [93, 94].

Platelet–leukocyte crosstalk

Besides their well-established role as the first cellular response in the coagulation cascade [95, 96], platelets are intimately involved in inflammatory reactions largely because of their direct crosstalk with leukocytes [97]. Upon vascular injury and endothelial denudation, platelets adhere and aggregate via their contacts with the free subendothelial matrix. However, platelets also rapidly adhere to the activated vascular endothelium. Endothelial-adherent platelets promote further endothelial activation [98]. Indeed, platelets adhere to the vascular endothelium of the carotid artery in ApoE-deficient mice before the development of advanced atherosclerotic lesions [99–101].

The interaction of platelets with both leukocytes and endothelial cells provides an important process in inflammation [97, 102]. First, platelet adhesion on endothelial cells and the release of potent inflammatory and mitogenic substances by platelets can alter the adhesive, chemotactic and proteolytic properties of endothelial cells thereby supporting the adhesion and transmigration of leukocytes to the inflamed tissue [103, 104]. Second, activated platelets release a variety of growth factors, inflammatory cytokines and chemokines into their microenvironment that can further directly stimulate leukocytes [102]. For instance, platelets are a major source for the chemokine stromal cell-derived factor-1 (SDF-1) [105, 106], which supports leukocyte integrin activation and thereby primary adhesion of circulating leukocytes to the vascular endothelium [107]. Third, platelets can directly interact with leukocytes; the platelet–leukocyte/monocyte aggregates have been implicated in atherosclerotic lesion formation [99, 108, 109]. The platelet receptors P-selectin, GPIb and glycoprotein Ib/IIla contribute substantially to these inflammatory processes in inflammation and atherosclerosis [99, 108–111]. Fourth, via their direct interaction with both endothelial cells and leukocytes, platelets can serve as a bridge to promote leukocyte adhesion to the vascular wall [68, 99, 110, 112] (Fig. 1B). The mechanisms involved in the crosstalk between platelets and leukocytes are multiple. Platelet–leukocyte interactions can be mediated by both selectin-dependent and integrin-dependent adhesive interactions. In particular, P-selectin on platelets interacts with PSGL-1 on leukocytes [110, 113, 114]. A central leukocyte receptor mediating adhesion to platelets is the integrin Mac-1 [115, 116]. Mac-1 can interact with several platelets receptors. For example, the interaction between Mac-1 and glycoprotein Ib (GPIb) on platelets can mediate adhesive interactions between leukocytes and platelets [68, 115, 116]. Inhibition of the Mac-1/GPIb interaction has been implicated as a therapeutic target in several inflammatory diseases [115–119]. Another major ligand for leukocyte Mac-1 is platelet JAM-C, promoting recruitment of leukocytes and dendritic cells [67, 68]. Fibrinogen bound onto platelet glycoprotein Ib/IIla (αIIbβ3-integrin) may also serve as a binding site for leukocyte Mac-1, thereby modulating the recruitment of leukocytes to sites of inflammation by platelets [120, 121].

The relevance of leukocyte–platelet interactions is not restricted to chronic inflammatory disease, but is important in a variety of processes in immunity, including the immune response to bacterial infections. For instance, during the course of infections microbial contents trigger immune-mediated platelet activation and thrombus formation resulting in a proinflammatory and procoagulatory state of the infected tissue [122–124]. In an in vivo model of sepsis, Clark et al demonstrated that platelets stimulate the formation of extracellular traps by neutrophils that can engulf bacteria in the septic blood [125]. Platelets can also contribute to cytotoxic T-lymphocyte (CTL) mediated liver immunopathology independently of their procoagulant function [126]. In this study, platelet depletion reduced accumulation of virus specific CTLs in mouse models of acute viral hepatitis and subsequently liver damage [126]. On the other hand, platelets and their released growth factors are important for tissue regeneration [127, 128]. Using a mouse model of liver regeneration it was shown that platelet-derived serotonin is centrally involved in the initiation of liver regeneration [128].

Conclusions

In summary, the involvement of leukocytes in local or general inflammatory processes is self-evident. During the last decades, it has become clear that leukocyte recruitment involves a multi-step
cascade of adhesive events. Improved imaging techniques will delineate the importance of the different adhesive interactions for tissue-specific and disease-specific inflammatory cell recruitment. Moreover, the role of other non-classical inflammatory cells such as platelets in inflammatory processes is increasingly elucidated, which results in a more comprehensive and thorough understanding of inflammation. Given the major importance of inflammatory processes in infectious, inflammatory and autoimmune diseases, the detailed understanding of the leukocyte recruitment cascade is an important prerequisite for developing targeted therapeutic approaches in the aforementioned pathologies.

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