Review

Molecular mechanisms of leukocyte recruitment in the inflammatory process

Klaus Ley *

University of Virginia Medical School, Department of Biomedical Engineering, Health Sciences Center, Box 377, Charlottesville, VA 22908, USA

Received 12 December 1995; accepted 6 February 1996

Abstract

The microcirculation constitutes the functional interface between the circulating blood and the interstitial space. To gain access to sites of inflammation, leukocytes must pass the endothelial barrier. The recruitment paradigm encompasses leukocyte margination, capture, rolling, activation, firm adhesion, and transmigration. Recent experimental work has shown that E-, L- and P-selectins and α4 integrins can mediate leukocyte rolling. Upon activation by chemokines, complement peptides or lipid mediators, firm adhesion is afforded by β1 and β2 integrins, Intercellular Adhesion Molecules (ICAMs) and Vascular Cell Adhesion Molecule-1 (VCAM-1). β1 and β2 integrins as well as Platelet-Endothelial Cell Adhesion Molecule-1 (PECAM-1) have been shown to be involved in transmigration. Intravital microscopic techniques have been instrumental in applying the conceptual advances of cell and molecular biology to the in vivo situation. This review focuses on the current understanding of the leukocyte recruitment paradigm as suggested by in vivo observations and in vitro model systems. The paradigm of neutrophil recruitment is presented to serve as a model for the recruitment mechanisms of other inflammatory cells.

Keywords: Neutrophils; Neutrophil activation; ICAM-1; Selectins; VCAM-1; Integrins; PECAM-1; Adhesion; Inflammation

1. Families of leukocyte-endothelial adhesion molecules

Leukocyte-endothelial adhesion molecules can broadly be grouped into four families. The selectins are a family of three carbohydrate recognizing transmembrane molecules, two of which, E- and P-selectin, are expressed on activated endothelium, and L-selectin is constitutively expressed on leukocytes [1,2]. An emerging family of selectin ligands is defined by their rich glycosylation via O-linked side chains [3,4] and N-linked carbohydrates [5]. Integrins are αβ-heterodimers which recognize extracellular matrix, cell surface glycoproteins and some soluble molecules such as fibrinogen and complement factor C3bi. All leukocytes express β2 (CD11/CD18) integrins, and eosinophils, monocytes and lymphocytes also express β1, β2 and α4 integrins on their surface. Most integrins require a conformational change to gain full adhesive function [6]. The largest family of endothelial adhesion molecules belongs to the immunoglobulin superfamily [7]. Some of the immunoglobulins can engage in homotypic interaction, while others serve as ligands for α4 and β2 integrins expressed on circulating lymphocytes and granulocytes. For details on the molecular structure and function, regulation of expression, and activation of adhesion molecules, the reader is referred to recent reviews [1-4,6,7].

2. Intravital microscopy

Intravital microscopy has been instrumental in dissecting the role individual adhesion molecules play in the various steps of leukocyte recruitment. Some transparent tissues such as the rat or rabbit mesentery [8] or omentum [9] can easily be exposed and mounted for microscopic observation of the living tissue. Most preparations require some surgical dissection, e.g. the hamster cheek pouch [10], the tenuissimus [11], cremaster [12] or spinotrapezius [13] muscle, or the spleen [14]. Observations in anesthetized animals can be made by using chronically im-
planted chambers like the rabbit ear chamber [15] or the mouse, rat or hamster dorsal skin window [16,17]. A few preparations require neither surgery nor anesthesia, i.e. the bat wing [18], the auricle of the hairless mouse [19], or the hindlimb skin of trained rats [20]. For the study of leukocyte adhesion, each approach has specific advantages such as ease of access, availability of reagents for the species of choice, and limitations, including acute tissue trauma, chronic inflammation, or poor optical resolution. Murine models of the microcirculation are becoming increasingly important, because transgenic and gene-targeted mice have become widely available and represent valuable tools to dissect the molecular mechanisms of the inflammatory process.

3. Multistep paradigm of inflammatory cell recruitment

The characteristic steps taken by leukocytes entering an area of inflammation were originally observed and described by Cohnheim in the nineteenth century [21]. The initial molecular interactions between white blood cells and the endothelium are transient and reversible adhesion causing rolling. The first indication that distinct sets of adhesion molecules might mediate leukocyte rolling and firm adhesion came from an early intravital microscopic study [22], which established that β2 integrins were necessary for adhesion, but not for rolling. Antibodies blocking L-selectin [23,24] or P-selectin [17,25] were shown to inhibit leukocyte rolling in various preparations. The transition from rolling to firm adhesion of leukocytes requires their activation through soluble or surface-bound mediators, which was very elegantly shown in a reconstituted in vitro system by Lawrence and coworkers [26,27]. This observation inspired the now classical ‘three-step’ paradigm of inflammatory cell recruitment: rolling—activation—adhesion [28,29]. These three steps are preceded by initial capturing of leukocytes from flowing blood [30], and followed by transendothelial migration, penetration of the basement membrane, and migration in the interstitial space (Fig. 1). The multitude of adhesion receptors, chemokines, other soluble activators and their respective receptors, and restricted expression on certain types of leukocytes, endothelial cells and other cells produces a large array of combinatorial possibilities, selectively directing lymphocytes, neutrophils, eosinophils, basophils, and monocytes to specific organs and to sites of inflammation [7].

4. Margination

In venules, leukocytes tend to move in a position close to the endothelial surface rather than in the central blood

---

Fig. 1. Flow chart showing the sequence and decision points in leukocyte recruitment. As the leukocyte enters the postcapillary venule (top left), it will marginate. Leukocytes are captured from the blood stream via L- and P-selectin (not shown in diagram). Dependent on selectin expression, rolling will occur, which allows the leukocyte to respond to various pro-inflammatory mediators. Leukocyte activation increases integrin affinity for ligands and causes firm adhesion. The signals necessary for transmigration are not understood. Note that leukocyte passage through a postcapillary venule has three possible outcomes: transmigration, return to the circulation as a resting cell, or as an activated cell. The rolling mechanism tends to preserve leukocytes in the non-activated state, yet able to respond to potential surface-bound or soluble activators.
stream. This is caused by a passive rheological phenomenon called margination. Margination depends on the interaction between individual red and white blood cells in small venules [31] and a secondary process requiring red blood cell aggregation [32,33]. Rheological margination does not appear to be rate-limiting for inflammation, however. Intravital microscopy shows that leukocyte rolling often is initiated when a leukocyte exits from a capillary with a diameter smaller than its own. There is no known inflammatory deficiency that is due to insufficient rheological margination.

5. Capture

The interaction between leukocytes and endothelial cells is initiated by selectin-mediated capture [30]. L-selectin appears to be critical for this process, because antibodies blocking the function of L-selectin inhibit leukocyte rolling in many in vivo models [23,24,34–37], in which rolling is also P-selectin dependent [17,25,38–40]. This suggests that L-selectin and P-selectin may both be required in sequential steps of the recruitment process. L-selectin-transfected pre-B cells have been introduced into rat mesenteric venules via intrarterial catheters and were seen to start rolling approximately 20 min after exteriorization of the rat mesentery [41,42]. Leukocyte rolling was also observed using a different L-selectin-transfected cell line in IL-1-treated rabbit mesentery [43]. These cell lines lack expression of ligands for E- or P-selectin, suggesting that L-selectin can mediate some attachment in vivo independent of the other selectins. For this function, binding specificity is clearly conferred by the lectin domain of L-selectin [44], and cytoskeletal anchorage is required [45].

The time course of L-selectin-dependent rolling after trauma shows that functional expression of L-selectin ligand(s) on venular endothelium occurs later than expression of P-selectin. Rolling of L-selectin transfectants is less efficient than neutrophil rolling, because a lower percentage of L-selectin transfectants [41–43] roll at higher rolling velocities [41] than neutrophils in the same venules. In mice lacking P-selectin expression, L-selectin-dependent rolling can be observed, but it occurs at low efficiency and high velocity [38]. Taken together, these data suggest that L-selectin preferentially promotes capture from the flow (rapid bond formation), and that these bonds dissociate more easily than P-selectin bonds. Recent in vivo findings show that naive T-lymphocytes also require L-selectin to initiate interaction with specialized high endothelial venules in gut-associated lymphatic organs called Peyer’s patches, but sustained rolling is mediated by $\alpha_4$ integrins in this tissue [34].

Further evidence promoting the concept of L-selectin-mediated capturing of neutrophils comes from in vitro studies. Established neutrophil rolling on purified E-selectin is not affected by L-selectin antibody treatment, but L-selectin is required in the same assay when the cells have to attach to the E-selectin-containing surface from the free-flowing state [46]. The concept of a special role for L-selectin in capturing is reinforced by the recent finding that localization of L-selectin to the tips of microvolds (‘microvilli’) on the neutrophil surface is required for effective attachment in a flow chamber [47], but that the topographical position of L-selectin does not influence rolling velocity or detachment rate once rolling has been established. When during the course of inflammation the microvascular endothelium becomes covered with leukocytes, newly arriving neutrophils may attach to already adherent leukocytes. In an in vitro model system, this has been shown to occur via an L-selectin-dependent mechanism [48].

The sequential requirement for L-selectin (capture) and P- and/or E-selectin (rolling) can also explain the apparent synergism observed between L-selectin and the vascular selectins seen in many models [38,49,50] including the striking defect of inflammatory cell recruitment in L-selectin- [38,51,52] as well as in P-selectin-deficient mice [38,39,53]. It should be emphasized, however, that P-selectin at high levels of expression appears able to function both as a capturing and a rolling receptor. This is suggested by the absence of a detectable defect of leukocyte rolling in L-selectin-deficient mice immediately after surgical tissue exteriorization [38,51], an intervention known to promote expression of P-selectin [54].

6. Rolling

Leukocyte rolling can be inhibited by charged carbohydrates [55–57]. This finding led to the proposal that the selectins, carbohydrate-binding molecules, may be involved in mediating rolling [58,59]. Early studies by Tangelder and Arfors [60] using an L-selectin antibody were negative. Subsequent work provided clear evidence that early leukocyte rolling in vivo is dependent on P-selectin, based on observations showing that P-selectin antibodies block both constitutive (in skin) and trauma-induced (in other organs) rolling in vivo [17,25,38]. Endothelial cell stimulation with secretagogues can induce an increase of leukocyte rolling in vivo, which is P-selectin dependent [39,40]. At reduced shear rates, a monoclonal antibody to P-selectin has been shown [61] to increase the velocity of rolling leukocytes in cat mesenteric venules without affecting rolling leukocyte flux, the number of rolling leukocytes passing each venule per unit of time. Moreover, P-selectin antibody pretreatment prevents the rise of rolling leukocyte flux induced in rat mesenteric venules caused by hypoxanthine/xanthine oxidase, a superoxide anion radical-generating system, but leaves basal leukocyte rolling unaffected [62]. The insensitivity to P-selectin antibodies of ‘basal’ leukocyte rolling seen in venules of exteriorized tissues indicates that P-selectin-independent mechanisms of leuko-
cyte rolling are operative in vivo at least under some experimental conditions. Gene-targeted mice lacking expression of P-selectin show no leukocyte rolling immediately after exteriorization of the mesentery [39] or cremaster muscle [38], but rolling is induced within 60–120 min after tissue trauma. Similarly, rolling in mesenteric venules is largely P-selectin dependent initially, while L-selectin-dependent mechanisms are prominent at later times [41,42].

Cell lines that can bind to P-selectin (e.g., HL-60 promyelocytes) but do not express L-selectin roll in rat mesenteric venules within less than 10 min of exteriorization, but their rolling efficiency decreases within 30–60 min, suggesting that endothelial expression of P-selectin rapidly increases and subsequently decreases after exposure of the mesentery [41,42]. Rolling of HL-60 cells can be blocked completely by neuraminidase treatment which removes sialic acid residues from their surface, or by O-sialoglycoprotease, an enzyme which cleaves glycoproteins with clusters of O-linked carbohydrates [42]. One such glycoprotein is P-Selectin Glycoprotein Ligand-1 (PSGL-1), which is present as a homodimer on all lymphocytes, monocytes, and neutrophils [63]. Myeloid-cell specific glycosylation is required for P-selectin binding activity of PSGL-1 [63,64]. PSGL-1 accounts for all or most of the P-selectin-dependent rolling of myeloid cells in vitro [63]. Similarly, P-selectin-dependent rolling of neutrophils or HL-60 cells in vivo is almost completely blocked by pre-treatment of these cells with a blocking monoclonal antibody [72].

Recently, the dogma that only selectins can mediate leukocyte rolling has been challenged by findings that certain integrins can support cell rolling in flow-chamber systems. Mammary carcinoma cells can roll on laminin via an αβ, integrin-dependent pathway [75]. Eosinophils can roll in cytokine-treated venules in vivo using α4 integrins [76]. Moreover, lymphocytic cell lines have been shown to be able to use αβ, integrin to roll on vascular cell adhesion molecule-1 (VCAM-1) [77–79] and αβ, integrin to roll on Mucosal Adressin Cell Adhesion Molecule-1 (MAdCAM-1), the main receptor for αβ, integrin in gut-associated lymphatic tissue [34,77]. This α4 integrin-dependent rolling occurs at much lower efficiency than typically seen in selectin-dependent systems as reflected by high rolling velocities.

In one report, a monoclonal antibody to InterCellular Adhesion Molecule-1 (ICAM-1) has been shown to partially inhibit leukocyte rolling in rat liver microvessels [80]. However, ICAM-1 alone cannot support leukocyte rolling as shown in reconstitution experiments in vitro [26]. Consistent with this notion, rolling in venules of the cremaster muscle is normal in ICAM-1-deficient mice [72], although these mice have marked deficiencies in various other inflammatory functions [81,82]. Interestingly, mice deficient for both ICAM-1 and P-selectin show a complete absence of leukocyte rolling for a much longer time period than mice lacking P-selectin alone [72]. These findings suggest that ICAM-1 may serve an auxiliary function in leukocyte rolling, the mechanism of which remains to be elucidated.

Several selectin antibodies have been effective at limiting leukocyte recruitment in a variety of inflammatory models, although the step at which leukocyte recruitment is blocked (i.e., rolling, firm adhesion or transmigration) cannot be directly investigated in these models [35–37,70,83–86]. Since this review is focused on the microcirculation, these findings will not be discussed here in detail.

7. Activation

Rolling leukocytes are in intimate contact with the endothelium for prolonged periods of time, allowing them to effectively sample the endothelial surface. Activated
endothelial cells produce chemoattractants which may be secreted or remain surface-bound. These include interleukin-8 (IL-8) and platelet-activating factor (PAF). The important role played by IL-8 is illustrated by the phenotype of mice in which the gene for the IL-8 receptor homologue was targeted and eliminated [87]. Not only do these mice have twelve-fold elevated systemic neutrophil counts, but they are also severely impaired in their ability to recruit neutrophils into thioglycollate-induced peritonitis. Based on these findings, IL-8 appears to be the most important endogenous neutrophil chemoattractant, although other factors such as complement fragment C5a, leukotriene B4 and platelet-activating factor are clearly important in various model systems [88,89].

Any of these mediators can activate neutrophils, initiating a cascade of intracellular events that can eventually lead to transmigration. Clearly, the β2 integrins LFA-1 and Mac-1 are activated by chemoattractants, which is reflected by a gain-of-function conformational change and expression of new epitopes detected by specific ‘reporter’ monoclonal antibodies [90,91]. In addition, stimulation of chemoattractant receptors causes conversion of G-actin to F-actin, making the cell more rigid and enabling it to migrate. L-selectin is known to be proteolytically lost from the leukocyte surface upon activation [92,93], but it is unclear whether physiological concentrations of chemoattractants in the inflamed microcirculation are sufficient to achieve L-selectin shedding. Transmigrated neutrophils found in the interstitial space are L-selectin low or negative [93], which leaves open the possibility that transmigration may contribute to L-selectin shedding. Activation-dependent transmigration appears to promote neutrophil degranulation. Interestingly, after transendothelial migration or treatment with cytochalasins neutrophils can express α4 and β2 integrin subunits on their surface, which are translocated from preformed stores in intracellular granules [94].

Ligation of various adhesion receptors has been shown to afford neutrophil activation either in concert with [95,96] or independent of soluble activators [97–99]. Ligation is usually modeled by cross-linking receptors with specific monoclonal antibodies followed by secondary antibodies, a procedure prone to artifactual stimulation of Fc receptors constitutively expressed by neutrophils. The use of Fab'2 fragments for both cross-linking reagents can eliminate this potential artefact. Adhesion-molecule-mediated cell activation is well documented for β2 integrins in a variety of systems [95–97,99]. In addition, ligation of L-selectin [98] and binding of neutrophils to E-selectin [100,101] have been suggested to produce activating responses. On the other hand, trauma-induced and constitutive rolling in vivo clearly does not activate neutrophils, because no evidence of integrin activation and cell arrest is seen. Moreover, resting neutrophils roll on, but do not firmly attach to lipid bilayers containing both P-selectin and ICAM-1 [26].

8. Firm adhesion

Firm adhesion of neutrophils (‘sticking’) is largely CD18-dependent [22,102], although additional mechanisms appear to exist. Neutrophils use both Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18) for adhesion, the relative importance of which varies between animal species and the inflammatory stimulus. In patients with LAD-I, which is characterized by an absence of β2 (CD18) integrins and consequent inability to recruit neutrophils, severe pathologies including recurrent, life-threatening bacterial infections are seen [103]. In gene-targeted mice with reduced expression of CD18 (β2) integrins [104], firm leukocyte adhesion has been seen to be severely compromised, and thioglycollate-induced neutrophil recruitment is reduced by 50%. At least two of the β2 integrins, Mac-1 and LFA-1, bind to endothelial ICAM-1 [105,106], and LFA-1 also binds to ICAM-2 [107]. ICAM-1 and ICAM-2 are constitutively expressed, and ICAM-1 expression is further increased by inflammatory cytokines [108].

Lymphocytes, monocytes and eosinophils can bind to endothelial VCAM-1 via their α4 integrin receptors [109]. This pathway of adhesion appears to be responsible for immune functions that occur in the absence of β2 integrins in LAD-I patients [110]. VCAM-1 shows very little baseline expression, but is up-regulated by inflammatory cytokines for prolonged periods of time [111,112].

In several in vitro model systems, E-selectin [113] and P-selectin [114] have been shown to support firm adhesion. However, it is not clear whether this adhesion seen in static systems would be converted to a rolling interaction by the flow forces (shear stress) present in microvessels. Recent experiments with long-term (24 h) cytokine-stimulated endothelial cells suggest that other leukocyte adhesion mechanisms not mediated by the currently known adhesion molecules may exist (C W Smith, unpublished results).

9. Transmigration

Leukocyte transmigration requires PECAM-1 (CD31) as shown by antibody blocking studies both in vitro [115] and in vivo [116]. Recent investigations in the rat mesenteric microcirculation suggest that antibodies to PECAM-1 can also prevent neutrophils from crossing the endothelial basement membrane [117]. In several in vitro systems, β2 integrins have also been shown to be required for transmigration [118,119]. ICAM-1 on endothelial cells may be distributed in a way suitable to guide emigrating cells to inter-endothelial junctions [120,121]. However, since it is not even clear whether neutrophils use a transcellular or paracellular route for transmigration in vivo, the significance of this observation requires further consideration.
After transmigration, leukocytes move through the interstitial fiber matrix to reach bacteria, parasites, infected or dead cells, or debris. It is likely that adhesive interaction with extracellular matrix (ECM) proteins is required for this movement [122]. Many of the \( \beta_1 \) integrins expressed on activated lymphocytes are known receptors for ECM proteins [6]. Neutrophils do not express the full array of \( \beta_2 \) integrins [123] and may be able to use \( \beta_2 \) integrins to bind to ECM components [122,124]. After transmigration, neutrophils also can express significant amounts of \( \beta_1 \) integrins [94], which clearly can serve as ECM receptors. Currently, too little is known about the adhesive interactions in extravascular leukocyte migration to warrant conclusive statements.

### 10. Gene-targeted mice in leukocyte adhesion research

Important insights into the physiology of adhesion molecules have recently been obtained from studies in gene-targeted mice. Some adhesion molecules relevant in inflammation also play critical roles in embryogenesis (e.g., \( \alpha_4 \) integrins, VCAM-1) [125,126], resulting in embryonic lethality of homozygous mutants. However, most mouse strains lacking inflammatory adhesion molecules survive to adulthood, and various lines have been produced including strains that lack L- [51], E- [71], or P-selectin [39], ICAM-1 [81], \( \beta_2 \) integrins (CD18) [104], a combination of ICAM-1 and P-selectin [127], and a combination of P- and E-selectin [128]. Most of these mice have characteristic defects of inflammatory responses which are reflected in diminished neutrophil emigration in bacterial or thioglycollate-induced peritonitis, reduced delayed type hypersensitivity reactions, and decreased responses in other models. In addition, L-selectin-deficient mice are unable to recruit lymphocytes into peripheral lymph nodes which consequently are hypoplastic [51]. Gene-targeted mice lacking P-selectin, ICAM-1, CD18, or combinations thereof have elevated circulating neutrophil counts [129]. Taken together, these findings support the important role played by these adhesion molecules.

At the microcirculatory level, the defects seen in different gene-targeted mouse strains are quite distinct. The selectin-deficient mice generally show defects in leukocyte rolling, and firm adhesion is impaired in CD18-deficient and in ICAM-1-deficient animals ([38,129], and unpublished results). Interestingly, the defects in leukocyte rolling are quite characteristic for each of the selectin-deficient strains. In P selectin deficient mice, leukocyte rolling is completely absent immediately after tissue exteriorization, and some rolling is induced between 1 and 2 h [38,39]. By contrast, rolling is normal initially in L-selectin-deficient mice, but declines with time. Leukocyte rolling flux reaches about 30% of that seen in wild-type control mice at 1–2 h [38,51]. In P-selectin and ICAM-1 double mutant mice, no leukocyte rolling is detectable after tissue trauma [72].

Treatment with TNF-\( \alpha \) produces leukocyte rolling in P-selectin mutant mice [38] and induces some rolling in P-selectin/ICAM-1 double mutants [72]. Interestingly, no overt defect of leukocyte rolling or neutrophil recruitment is detectable in E-selectin-deficient mice, suggesting that E-selectin is not needed for the normal inflammatory response in the mouse [71]. However, in P-selectin/E-selectin double mutants, rolling is completely absent and cannot even be induced by TNF treatment, indicating that E-selectin becomes essential when P-selectin is not available [128]. As a result of this serious defect, E- and P-selectin double mutant mice suffer from severe spontaneous infections under vivarium conditions [128].

### 11. Conclusion

Evidence from antibody blocking, reconstitution, and gene targeting experiments indicates that leukocyte recruitment is initiated by the selectins, which are indispensable for effective adhesion in the presence of shear flow. This takes the form of capture, followed by rolling. Activation of rolling leukocytes is achieved by surface-bound and soluble chemokines and other chemoattractants. Integrins (\( \beta_1 \) for neutrophils, \( \beta_2 \) and \( \beta_1 \) for eosinophils, monocytes and lymphocytes) require activation in order to bind to their endothelial counter-ligands, ICAM-1, VCAM-1 and others. Transendothelial migration requires PECAM-1 function and \( \beta_2 \) integrins. Experimental or clinical elimination of selectin function (LAD-II, E-/P-selectin double mutant mice), the main endogenous neutrophil chemokine (IL-8 receptor homologue mutant mice), the \( \beta_2 \) integrins (LAD-I, CD18 mutant mice), or PECAM-1 (antibody blocking studies) all produce defects of neutrophil recruitment. This convincingly confirms the sequential nature of the adhesion cascade. Intravital microscopic studies are indispensable tools to investigate at which stage of the recruitment paradigm various adhesion molecules and chemoattractants are important in different organs and tissues.

### Acknowledgements

This review is in part based on original work supported by grants from NIH HL54136 and Deutsche Forschungsgemeinschaft DFG 573/3-2. I thank Dr. Keith Norman for valuable comments on the manuscript.

### References


[53] Schmidt EE, MacDonald IC, Groom AC. Interactions of leukocytes with vessel walls and with other blood cells, studied by high-resolution intravital videomicroscopy of spleen. Microvasc Res 1990;40:99–117.


[116] Guttner GC, Davis V, Li H, McCoy M, Sharp A, Cybulsky MI,

