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Review Article

Platelet-leukocyte crosstalk: Linking proinflammatory responses to procoagulant state

Mehran Ghasemzadeh [a,b], Ehteramolsadat Hosseini [b,*]

[a] Australian Centre for Blood Diseases, Monash University, Melbourne, Australia
[b] Blood Transfusion Research Centre, Iranian Blood Transfusion Organization, Tehran, Iran

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ABSTRACT

Platelet activation is known to be associated with the release of a vast array of chemokines and proinflammatory lipids which induce pleiotropic effects on a wide variety of tissues and cells, including leukocytes. During thrombosis, the recruitment of leukocytes to activated platelets is considered an important step which not only links thrombosis to inflammatory responses but may also enhance procoagulant state. This phenomenon is highly regulated and influenced by precise mutual interactions between the cells at site of vascular injury and thrombi formation. Platelet-leukocyte interaction involves a variety of mediators including adhesion molecules, chemokines and chemoattractant molecules, shed proteins, various proinflammatory lipids and other materials. The current review addresses the detailed mechanisms underlying platelet-leukocyte crosstalk. This includes their adhesive interactions, transcellular metabolisms, induced tissue factor activity and neutrophil extracellular traps formation as well as the impacts of these phenomena in modulation of the proinflammatory and procoagulant functions in a reciprocal manner that enhances the physiological responses.

Contents

Introduction

Interactions between platelets and leukocytes are important for innate immunity, inflammation and allergic responses. Dysregulation of these interactions is also relevant to a range of human diseases, including atherosclerosis, acute myocardial infarction, ischemic stroke, acute respiratory distress syndrome and allergic airways disease [1–3].

Classically, leukocytes rolling and adhesion to inflamed endothelium is primarily mediated by selectins expressed by endothelial cells. However, platelets also play a critical role in providing an adhesive surface for secondary leukocyte recruitment. This phenomenon is best illustrated in acute lung injury (ALI) [4–6], where endothelial cells of alveolar capillaries lacking Weibel–Palade bodies [7] and P-selectin expression [8,9] are able to recruit a large number of neutrophils promoting inflammatory responses [10].

Leukocytes can bind to activated platelets and transmigrate through a platelet monolayer [11]. Within hours of vascular injury, leukocytes become enmeshed in platelet thrombi and/or transiently form a monolayer on top of adherent or aggregated platelets [12,13]. By depositing chemokines on activated endothelium [14,15] or by direct interactions

* Corresponding author at: Blood Transfusion Research Centre, Iranian Blood Transfusion Organization Bldg, Hemmat Exp. Way, Next to the Milad Tower, Tehran, 14665–1157, Iran. Tel.: +98 912 1599012; fax: +98 21 88060717.
E-mail address: e.hosseini10@yahoo.com.au (E. Hosseini).

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with leukocytes [16], platelets can enhance leukocyte recruitment to inflamed endothelium. Thereby promoting either physiological events like limited proinflammatory and procoagulant responses or pathological state, such as development and progression of atherosclerosis. All these events occur through an intimate cellular crosstalk which might be precisely regulated and controlled or otherwise could lead to an uncontrolled situation creating life-threatening health problems.

To better understand the mediators and mechanisms involved in this mutual crosstalk, we first briefly reviewed the classical recruitment of leukocyte to site of inflammation and injury. Then we focused on platelet-leukocyte interaction as a second key player which can link inflammation to hemostasis.

Leukocyte adhesion and activation

Neutrophils are the most frequent leukocytes rapidly recruited to the site of injury or inflammation. Therefore, to demonstrate the classical patterns of leukocyte adhesion and activation, the functions of neutrophils are represented here in detail [17,18]. The recruitment of polymorphonuclear leukocytes (PMNs) to sites of inflammation, follows a distinct multistep pattern. During recruitment, PMNs become activated and subsequently release various mediators which may affect other cells in the surrounding tissues. The activation and recruitment of neutrophils is closely regulated by different signals, with defective leukocyte recruitment leading to an inappropriate inflammatory response to injury or infection [19]. On the other hand, exaggerated and uncontrolled neutrophil activation is associated with tissue damage [20]. The classical neutrophil recruitment cascade consists of "capturing" ("tethering"), rolling, slow rolling, arrest, post-adhesion strengthening (firm adhesion), crawling and paracellular or transcellular transmigration [21].

Neutrophil capturing and rolling

Capturing and rolling of neutrophils represents the first events of neutrophil interaction with the endothelium, with an adhesive step mediated by the interaction of selectins with their counter-receptors [22]. Mice, where all the selectins (L, E and P) have been knocked out, demonstrate a severe defect in neutrophil recruitment [23]. Whilst P-selectin is expressed on the endothelial surface and platelets upon activation [24], L-selectin and E-selectin expression is restricted to circulating leukocytes and inflamed endothelium, respectively. These selectins are also involved in leukocyte recruitment to sites of inflammation [21,25]. The main counter-receptor for selectins is PSGL-1, which is expressed on all leukocytes [26] and can bind to L- [27], P- [28] and E-selectins [29]. Neutrophils also express the E-selectin ligand; ESL-1 [30] and other E-selectin ligands which appear to play a less important role in neutrophil capturing including CD44 [31] and Mac-1 ($\alpha_M\beta_2$) [32,33]. Given that selectins are expressed on different cell types, neutrophils can be also recruited by "secondary capturing" [34]. For example, PSGL-1 on free-flowing neutrophils can bind to P-selectin presented by adherent platelets, [35] or L-selectin on free-flowing neutrophils can interact with PSGL-1 presented by adherent leukocytes [36] or leukocyte-derived fragments [27].

Integrin priming during capturing and rolling of neutrophils on P-selectin positive surfaces

Several lines of evidence suggest that neutrophils become activated following P-selectin binding. The incubation of isolated human neutrophils, with paraformaldehyde-fixed platelets, a P-selectin-IgG fusion protein or an antibody against PSGL-1 is followed by the significant tyrosine phosphorylation of neutrophil proteins [37,38]. Stimulation of murine bone marrow-derived neutrophils with P-selectin-IgG or cross-linking PSGL-1 with either complete antibodies or F(ab')2 fragments towards this receptor induces ROS production [39] and Mac-1 activation. The latter in turn leads to increased binding of Mac-1 to its ligands [26]. Collectively, these observations provide evidence of the role of selectins not only as cell adhesion receptors, but also as cell signaling molecules.

While signaling events that follow the ligation of selectins by PSGL-1 are already described [26,40], it is noteworthy to mention that for full integrin activation in leukocytes, additional stimuli such as other selectins, cytokines and chemokines are also required (Fig. 1). In their absence, P-selectin binding can only prime the neutrophil integrin, a process which is not sufficient for the full arrest of the cell [40–42]. These observations suggest that P-selectin stimulation acts synergistically with proinflammatory stimuli such as cytokines, chemokines and chemoattractants to induce full integrin activation [40].

Chemokine-induced neutrophil arrest

During inflammation, endothelial cells, leukocytes, platelets and other cells produce and release a broad range of chemokines and other chemoattractants. PAF is one of the most important chemoattractant proinflammatory lipids which acts cooperatively with other extracellular stimuli and P-selectin to induce full integrin activation [43–45]. Some of these chemokines are washed off in the plasma, others are trapped in the inflammatory milieu, and/or presented on the surface of endothelial cells and other reactive cells. In this regard, several molecules have been identified to contribute to the transcytosis and immobilization of these molecules [46–48]. Activated platelets at sites of vascular injury secrete glycosaminoglycans that can induce the immobilization of chemokines and contribute to leukocyte adhesion and extravasation [49].

Following selectin-mediated integrin priming and slow rolling, the engagement of chemokines with G protein coupled receptors (GPCRs) on neutrophils induces an almost instantaneous activation of the integrins through the triggering of inside-out signaling [44,50]. The importance of GPCRs in this process is highlighted by the observation that the elimination of the $G\alpha_{i2}$ subunit of this receptor (using $G\alpha_{i2}^{-/-}$ neutrophils or lethally irradiated mice reconstituted with $G\alpha_{i2}^{-/-}$ bone marrow) has been shown to result in almost complete loss of chemokine-induced arrest *in vivo* and *in vitro* [44]. It should however be noted that, stimulation of neutrophils with extracellular stimuli, only activates a small fraction of integrins on the cell surface, while full integrin activation and firm cell adhesion is dependent on outside-in signaling triggered by the engagement of integrins with their specific ligands [51].

In general, leukocyte stable adhesion and migration at sites of inflammation is critically dependent on the leukocyte β_2-integrins ligation, including LFA-1 (CD11a/CD18 or $\alpha_L\beta_2$) and Mac-1 (CD11b/CD18 or $\alpha_M\beta_2$) with their specific ligands (as depicted in Fig. 1) [52,53]. This is consistent with the notion that Mac-1 plays the dominant role in promoting stable leukocyte-platelet interactions [35], the process that ultimately leads to neutrophil extravasation and has critical role in inflammatory responses.

Platelet-leukocyte mutual interactions

Recruitment of neutrophil to activated platelets

Whilst leukocyte recruitment to regions of tissue ischemia is primarily initiated by the direct adhesion of leukocytes to the surface of 'inflamed' endothelial cells [54–56], there is a growing body of evidence that platelets also participate in this process [57]. Experimental and clinical evidence has demonstrated that platelet thrombi are highly efficient at recruiting leukocytes from flowing blood [58], with the extent of leukocyte-thrombus interaction correlating with the degree of tissue inflammation, organ damage and clinical outcome [59].

During platelet activation, degranulation leads to P-selectin expression and the release of the abundant source of proinflammatory molecules that can regulate the adhesion and activation of leukocytes at the site of injury. These molecules include various chemokines and