Interaction of Platelets, Leukocytes and the Endothelium*

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Key Words

Multicellular interaction · Platelets · Leukocytes · Endothelium

Summary

Interactions between the vessel wall and platelets or leukocytes were traditionally thought to be separate cellular adhesive contributions to either coagulation or inflammation. Observations in biological systems and the recent discovery and characterization of the family of junctional adhesion molecules (JAMs) have now clarified that both systems are closely related. This, in retrospect, seems not surprising, as shear stress in the circulation places comparable dynamic and mechanical constraints on the adhesion of both leukocytes and platelets. This review will briefly summarize the molecular basis of the interaction between leukocytes and the endothelium, leukocytes and platelets and platelets and endothelial cells. The main emphasis will be on current data supporting the relevance of JAMs, GPIIb/IIIa and the Mac-1 integrin in these multicellular interactions.

Schlüsselwörter

Multizelluläre Interaktion · Thrombozyten · Leukozyten · Endothelium

Zusammenfassung

Im Zusammenspiel zwischen Gefäßwand und Thrombozyten oder Leukozyten wurden Adhäsionsprozesse dieser Zellen traditionell als eigenständige Beiträge zur Gerinnung oder Entzündung betrachtet. Beobachtungen in verschiedenen biologischen Systemen und, mehr noch, die Entdeckung und Beschreibung der Familie der so genannten JAMs (junctional adhesion molecules), haben dazu beigetragen, dass wir heute beide Systeme als eng verwandte Mechanismen ansehen müssen. Zurückblickend betrachtet ist das nicht überraschend, wenn man bedenkt, dass zur Adhäsion beider Zelltypen aus dem Blutstrom heraus vergleichbare mechanische und dynamische Widerstände überwunden werden müssen. Diese Übersichtsarbeit fasst die wesentlichen molekularen Grundlagen der Interaktion zwischen Leukozyten und Endothelzellen, Leukozyten und Thrombozyten sowie Thrombozyten und Endothelzellen zusammen. Der Schwerpunkt liegt dabei auf jüngsten Veröffentlichungen, die die Bedeutung der JAMs, des Plättchenglykoproteins IIb/IIIa und des leukozytären Integrins Mac-1 unterstreichen.

Introduction

Interactions between the vessel wall and platelets or leukocytes were traditionally thought to be separate cellular adhesive contributions to either coagulation or inflammation.

*Dedicated to Prof. Dr. Peter Hanfland, Bonn, on the occasion of his 65th birthday.

Platelets, in response to vessel wall injury, adhere to components of the extracellular matrix (ECM), which triggers sudden platelet activation and platelet plug formation. Initial responses are followed by coagulant activity and the formation of fibrin-containing thrombi that occlude the site of injury. These events are crucial to prevent posttraumatic blood loss, but they are also a major pathomechanism in arterial thrombosis. Leukocytes, in response to inflammation, role and arrest

on endothelial cells and finally emigrate from the vessel into the surrounding tissues, where they fight pathogens and contribute to the re-establishment of tissue integrity. However, they can also cause deleterious effects if the regulatory mechanisms are altered, and the defense mechanisms of the leukocytes turn against the organism's own tissue.

Although these principles of either coagulation or inflammation are still valid in general, a strong linkage between them has emerged within the last years. This linkage has been established on the basis of i) *in vitro* and *in vivo* observations, including leukocyte rolling and arrest on activated platelets, platelet rolling on activated endothelial cells and formation of platelet-leukocyte aggregates in flowing blood, and ii) the dissection of molecular mechanisms that allow platelets, leukocytes and endothelial cells to interact. These experimental data revealed that cell adhesion molecules that were originally thought to exclusively contribute to either inflammation or coagulation are indeed involved in both responses. Retrospectively, this seems not surprising, as shear stress in the circulation places comparable dynamic and mechanical constraints on the adhesion of both leukocytes and platelets to stationary vascular surfaces.

It is beyond the scope of this manuscript to review all multicellular adhesive interactions between platelets, leukocytes and the vessel wall. Rather, a brief summary of key events will be given focussing on the contribution of certain molecules, namely of junctional adhesion molecules (JAMs), platelet glycoprotein Ib α (GPIb α) and the leukocyte integrin Mac-1, which appear next to others at the coagulation/inflammation interface and take part in the interaction of platelets, leukocytes and the endothelium.

Leukocytes and Endothelial Cells

Leukocytes tether to endothelial cells at sites of inflammation and subsequently roll on the vessel wall. In inflammation, recruitment of leukocytes usually occurs in vessels with low-shear blood flow, particularly in postcapillary venules. However, leukocytes can also adhere to the endothelium of larger vessels, especially at vessel bifurcations, where the shear rate is decreased. After a phase of continuous movement, leukocytes arrest, spread and finally emigrate between endothelial cells into the surrounding tissue (fig. 1a).

Tethering and rolling are mainly mediated by interactions between selectins and carbohydrate ligand structures, both on the leukocyte and the endothelial cell [1–3]. L-selectin is expressed on most types of leukocytes. E-selectin and P-selectin are expressed on endothelial cells, with E-selectin being exclusively expressed on endothelium and P-selectin being also found on platelets. The interactions between leukocyte L-selectin and endothelial carbohydrates (such as MadCAM-1) on the one hand and endothelial P-selectin and leukocyte carbohydrates (such as PSGL-1) on the other hand, are mainly re-

Fig. 1. Adhesive interactions of leukocytes with stationary vessel surfaces: **A** Adhesion of leukocytes to activated endothelial cells. **B** Adhesion of leukocytes to activated platelets covering a vessel wall injury.

sponsible for recruiting leukocytes from the blood stream to the surface of the endothelium. Subsequently, endothelial E-selectin and carbohydrates on the leukocyte (such as ESL-1) slow down the rolling velocity of the leukocyte [4]. After L-selectin and the carbohydrate selectin ligands on the leukocyte surface have bound to their corresponding partners on the endothelial surface, signals are generated within the leukocyte, resulting in leukocyte integrin activation, which occurs through integrin clustering (avidity modulation) or through conformational changes (affinity modulation) [3]. This activation process may be enhanced further by additional stimuli, such as locally released chemokines [5].

Adhesive integrins on the leukocyte surface encompass members of the $\beta 2$ -family, namely $\alpha M\beta 2$ (or Mac-1) and $\alpha L\beta 2$ (or LFA-1), and the $\alpha 4\beta 1$ -integrin VLA-4. Once activated, these integrins bind to corresponding adhesion molecules on the endothelial cell, including ICAM-1, ICAM-2 and VCAM-1. In addition, leukocyte integrins do also interact with P-selectin and E-selectin, resulting in a further decreased rolling velocity and, finally, stable adhesion of the leukocyte [6]. More recent observations indicate that members of the family of JAMs are also important in mediating stable adhesion of leukocytes to the endothelium. JAMs contribute to a number of crucial cellular functions, including interactions between cells of the same type (e.g. endothelial cells), where JAMs participate in the formation of tight junctions, as well as interactions between cells of different types (e.g. leukocytes and endothelial cells), where JAMs support adhesive processes. The first member of the JAM family identified at endothelial tight junctions was JAM-A [7]. Under inflammatory conditions, JAM-A is redistributed from intercellular contacts to the apical surface of the endothelium [8], already suggesting a relevance of JAM-A in recruiting leukocytes to inflamed tissues. Subsequently, endothelial JAM-A was shown to bind to LFA-1 on the leuko-

Table 1. Participation of junctional adhesion molecules in multicellular interactions

JAM	Cellular distribution	Counter-receptors	Published participation
JAM-A	EC N, MO, T PL	LFA-1 JAM-A	leukocyte adhesion to EC transmigration PL adhesion to EC
JAM-B	EC	VLA-4 JAM-B JAM-C	leukocyte adhesion to EC
JAM-C	EC B, T, NK PL	Mac-1 JAM-C	leukocyte adhesion to EC transmigration leukocyte/PL interaction

EC = Endothelial cells, N = neutrophils, MO = monocytes,
T = T lymphocytes, PL = platelets,
B = B lymphocytes, NK = natural killer cells.

cyte surface [9]. LFA-1 is an established adhesive receptor on various leukocyte subsets and binds to ICAM-1 and ICAM-2 [10]. Ostermann et al. [9] were able to demonstrate that the interaction between endothelial JAM-A and LFA-1 on leukocytes enhances the adhesion of T cells to endothelial cells and supports the transmigration of both T cells and neutrophils across the endothelium, thus establishing an important contribution of JAM-A to leukocyte-endothelial cell interaction.

Besides LFA-1, VLA-4 is an important integrin for leukocyte-endothelial cell interaction, mainly by binding to ICAM-1 and VCAM-1 on the endothelial cell [11]. However, it has recently been demonstrated that VLA-4 can also bind to the second member of the JAM family – JAM-B – which is also expressed on endothelial cells [12]. In addition, JAM-B constitutes a counter-receptor for JAM-C, which is expressed on certain leukocyte subpopulations [13, 14]. Thus, the VLA-4-mediated association with JAM-B on the endothelium is possibly enhanced by the co-expression of VLA-4 and JAM-C on the leukocyte, and both leukocyte surface molecules can bind to endothelial JAM-B in order to stabilize the leukocyte-endothelium interaction. The importance of JAM-C expression on endothelial cells in the process of adhesion will be discussed in the next paragraph.

The fact that leukocyte integrins (such as Mac-1 and VLA-4) do have a number of already established counter-receptors, does not implicate that JAM-mediated processes are of minor importance. Quite the reverse – as regulatory processes in inflammation are highly complex, JAM-mediated interactions may help to provide to the specificity that is required to attract a certain leukocyte subpopulation to its appropriate site of action (table 1). The same might be true for a number of other leukocyte subpopulation markers which, although known for years, have not yet been allocated to a certain function.

Leukocyte Emigration

Although leukocytes are able to cross the endothelial layer by transcytosis [15], the main route for a leukocyte from inside the vessel to the tissue is through the cleft between adjacent cells, preferably at tri-cellular corners [16]. On their way, leukocytes need to cross 2 major barriers, namely tight junctions (zonula occludens) and adherens junctions (zonula adherens). Tight junctions are the most apical barrier that has to be crossed. They are very close intercellular adhesive contacts and include a least 3 types of transmembrane proteins: occludin, claudin and JAMs. The second barrier, built up by adherens junctions, is mainly formed by cadherins which promote homophilic cell-cell contacts. In order to allow leukocytes to pass through the inter-endothelial cleft, endothelial cells express a number of molecules that relieve and control the leukocyte passage, of which VE-cadherin, PECAM-1 (platelet-endothelial cell adhesion molecule 1) and CD99 are well characterized. VE-cadherin is part of the adherens junctions and functions as a central gate-keeper in inter-endothelial passage, since its blockage increases neutrophil extravasation in vivo [17]. PECAM-1 is expressed on both leukocytes and the endothelium, and mediates homophilic interactions. Monoclonal antibodies against PECAM-1 block transendothelial migration [18]. However, animal models suggest a somewhat redundant function of PECAM-1, as PECAM-1^{-/-} mice have only minor defects in leukocyte recruitment [19]. CD99 is expressed on all leukocytes and on lateral inter-endothelial contacts. Antibodies against CD99 block transendothelial migration, but only after leukocytes have migrated deeply into the inter-endothelial cleft [20], indicating a predominant function in the very late phase of transmigration.

In contrast to the above mentioned molecules, JAMs seem to play a dual role in the interaction between leukocytes and the endothelium, as they do not only participate in leukocyte recruitment by supporting leukocyte adhesion, but also in transmigration.

JAM-A was found to be expressed at inter-endothelial contacts and shown to participate in the transmigration of monocytes in vivo [7]. Subsequently, the association between JAM-A and typical members of tight junctions, including occludin and cingulin, was demonstrated [21, 22]. As discussed above, JAM-A participates in adhesive interactions between leukocytes and endothelial cells by binding to the leukocyte integrin LFA-1. Thus, it is well possible that JAM-A is supportive in steering the rolling leukocyte into the inter-endothelial cleft. However, this concept needs further evaluation as somewhat contradictory results have been reported recently. In an in vitro model of neutrophil transmigration employing human umbilical vein endothelial monolayers under shear, JAM-A did neither contribute to adhesion nor to transmigration [23]. In contrast, in a model of liver re-perfusion injury in JAM-A^{-/-} mice, the number of transmigrating neutrophils was

Fig. 2. Molecular mechanism of leukocyte-platelet interaction under flow: **A** In a first step, platelets adhere to subendothelial structures, comprising of collagen, VWF and fibronectin via their specialized surface receptors (GPIb-IX-V, GPIIb/IIIa, α IIb β 3, α 5 β 1). Upon platelet activation, P-selectin is up-regulated on the platelet surface. **B** Leukocytes are recruited via P-selectin/PSGL-1 interaction from the blood stream to the platelet surface, where they start to roll. **C** Following PSGL-1-mediated signaling within the leukocyte, integrins, predominantly Mac-1 (α M β 2), become activated, which allows direct and indirect binding to GPIIb/IIIa on the platelet surface. **D** Additional interaction between Mac-1 and JAM-C enables leukocytes to stably adhere to the platelet surface.

clearly reduced, but again there was no evidence that JAM-A does support neutrophil rolling or adherence on postischemic endothelial cells [24].

Only limited information is available regarding JAM-B. It is expressed on vascular and lymphatic endothelium, where it contributes to lymphocyte homing [25]. However, further details of the molecular mechanism await elucidation with the question of whether JAM-B/JAM-C interaction [14] participates in leukocyte transmigration being of special interest.

The third member of the JAM family, JAM-C, was previously shown to promote leukocyte transmigration when ectopically expressed on endothelioma cell lines [26]. JAM-C is also expressed by human endothelial cells, where it localizes at inter-endothelial junctions [27]. Important insight into its functions was obtained by employing recombinant soluble JAM-C. Whereas neutrophil adhesion to non-stimulated endothelial cells was not blocked in the presence of soluble JAM-C, neutrophil transmigration through the endothelial monolayer was inhibited in the presence of soluble JAM-C. Importantly, soluble JAM-C was also effective in preventing neutrophil extravasation in an *in vivo* model of acute peritonitis in mice [27]. Our observations are well in accordance with the idea that Mac-1/JAM-C interaction contributes to leukocyte transmigration. These findings were confirmed in a very recent publication employing transgenic mice overexpressing JAM-C on their endothelium [28]. Leukocyte recruitment into inflamed tissues was shown to be increased in several models of inflammation, and a predominant effect of JAM-C on neutrophil rather than on monocyte recruitment was observed. In addition, leukocyte adherence, but not leukocyte rolling, was enhanced. Taken together, there is now evidence that JAM-C might be up-regulated or re-distributed upon inflammatory stimulation of the endothelium and that it supports firm adhesion of rolling leukocytes by

interacting with Mac-1 as well as contributing to leukocyte transmigration across the epithelium.

Leukocytes and Platelets

Although leukocytes possess a diverse armament of surface molecules, leukocyte-platelet interaction widely resembles the interaction of leukocytes with the endothelium (fig. 1b). P-selectin (on endothelial cells) and PSGL-1 (on leukocytes) are known to mediate interactions between these cell types in order to recruit leukocytes from the flowing blood to the vessel wall. Accordingly, adherent platelets, which express large amounts of P-selectin on their surface [29], do also serve as a substrate for leukocyte adhesion and rolling. Beyond this, leukocytes do not only roll and arrest on but also transmigrate across surface-adherent platelets via a sequential action of P-selectin and β 2-integrins, perfectly resembling the above mentioned mechanism that is employed by leukocytes on endothelial cells [30]. Following PSGL-1-mediated rolling, leukocytes use the α M β 2 integrin (Mac-1) and, to a lower extent, α L β 2 integrin (LFA-1) to firmly adhere to platelets (fig. 2). Mac-1 has been demonstrated to directly bind GPIIb/IIIa – a member of the GPIb-IX-V complex – via its I domain [30]. The I domain of Mac-1 is related to the A1 domain of von Willebrand-factor (VWF), the main ligand of GPIIb/IIIa. However, evidence is accumulating that the A1 domain of VWF and the I domain of Mac-1 do not bind to the same part of GPIIb/IIIa. Besides a direct binding mechanism, it has also been demonstrated that Mac-1 and GPIIb/IIIa can be bridged by high molecular weight kininogen (HK) [32]. This indirect clustering doubles the number of available binding sites and augments platelet-leukocyte interaction.

JAMs are now recognized as important participants in leukocyte-endothelial interactions, and they also play a significant

Fig. 3. Molecular mechanism of platelet-endothelial cell interaction under flow: **A** Platelets can roll on endothelial cells via the interaction of P-selectin and PSGL-1 (left part) and via the interaction of GPIIb α with both, P-selectin and VWF (right part). **B** Stable adhesion of platelets on the endothelium is mediated via homophilic interactions between JAM-A, being expressed on both cell types (left part). The contribution of additional receptors, including platelet P-selectin/carbohydrate ligands on endothelium and JAM-C, awaits further clarification (right part).

role in the interaction between leukocytes and platelets. JAM-C, which is expressed on platelets, binds to the leukocyte integrin Mac-1 [33]. Data obtained under flow conditions indicate that the interaction between Mac-1 and GPIIb α is of higher importance under conditions of high shear, whereas the interaction of Mac-1 and JAM-C occurs under conditions of low shear [33, 34]. These findings may support the idea that following selectin-mediated fast rolling of leukocytes on platelets, the process is slowed down by Mac-1/GPIIb α interaction and, in a second step, Mac-1/JAM-C interactions induce leukocytes to adhere (fig. 2d). Further evidence for a major role of GPIIb α and JAM-C as platelet counter-receptors for Mac-1 comes from the use of platelets from patients with Bernard-Soulier syndrome that do express only very small amounts of GPIIb α [35]. Employing such platelets, neutrophil-platelet interaction was completely abolished in the presence of purified JAM-C [33]. So far, Simon et al. [31] have demonstrated that monocytes roll on immobilized GPIIb α fragment. However, it has not yet been demonstrated whether rolling of leukocytes on platelets through PSGL-1/P-selectin interaction can be converted to firm adhesion through Mac-1/GPIIb α and/or Mac-1/JAM-C interactions. However, as mentioned above, there is evidence that JAM-C expressed on endothelial cells supports leukocyte adhesion and transmigration, and the same might be the case with leukocytes and platelets.

A number of additional putative integrin ligands present on the platelet surface has also been suggested to support the adhesion of leukocytes, including fibrinogen attached to platelet α IIB β 3, ICAM-2 and glycosaminoglycans [36–39]. However, experimental evidence by others has questioned the relevance of several of these substrates. Neither the absence of β 3 from the platelet surface nor the use of α IIB β 3 antagonists prevented leukocyte accumulation on the surface of activated

platelets [40]. Thus, a contribution of fibrinogen, simultaneously attached to Mac-1 and α IIB β 3, to the process of leukocyte-platelet interaction seems unlikely. The same is true for ICAM-2 and LFA-1, because blocking antibodies against these molecules failed to interfere with the adhesion of neutrophils on a platelet layer [30]. Accordingly, P-selectin, GPIIb α and JAM-C constitute the major players on the platelet surface in platelet-leukocyte interaction. Additional evidence has also been gained recently by the use of human antibodies against Mac-1 [34]. In this paper, we demonstrated that the binding sites of the two Mac-1 counter-receptors on platelets, GPIIb α and JAM-C, are different, because the adhesion of neutrophils to GPIIb α was impaired by human antibodies, whereas neutrophil adhesion to purified JAM-C was not inhibited. Furthermore, human anti-Mac-1 inhibited platelet-neutrophil aggregates only under conditions of high shear but not of low shear. This is in accordance with the above suggested mechanism that following P-selectin-dependent interaction of platelets and leukocytes, GPIIb α /Mac-1 interactions stabilize aggregate formation under high shear rates, whereas JAM-C/Mac-1 interactions come into play once the shear is diminished. Finally, adjacent but different binding sites for GPIIb α and JAM-C on the Mac-1 integrin are commensurate with the discussed stepwise mechanisms in platelet-leukocyte interaction. A detailed mapping of JAM-C/Mac-1 interaction by surface plasmon resonance (SPR) analysis employing Mac-1 mutants has meanwhile confirmed these findings (manuscript in preparation).

Platelets and Endothelial Cells

Quiescent platelets are known to roll on endothelial cells upon stimulation with TNF- α [41], with an approximately 6- to 9-fold faster rolling velocity than leukocytes. This rolling mechanism is mainly mediated via P-selectin on endothelial cells and PSGL-1 and GPIIb α on the platelet membrane [42–44]. In addition to endothelial P-selectin, VWF is an important mediator of platelet translocation on the endothelium [45] (fig. 3).

The relevance of rolling platelets is only partially understood. Rolling platelets may constitute a kind of surveillance mechanism, making platelets quickly available for a rapid procoagulant response at sites of vascular injury [41]. However, there is stronger evidence from *in vivo* experiments suggesting a participation of rolling platelets in the generation of atherosclerotic lesions, probably by secreting proinflammatory cytokines and chemokines that affect leukocyte responses. Massberg et al. [46] were the first to demonstrate that platelets adhere to the vascular endothelium of carotid arteries in ApoE-deficient mice before manifest atherosclerotic lesions develop. An antibody against GPIIb α was able to limit atherosclerotic lesion formation and adhesion of leukocytes, indicating that platelet adhesion to endothelium indeed precedes further events in

the atherosclerotic process. As GPIb α -VWF interaction resembles one molecular mechanism that enables platelets to roll on endothelial cells, it is not surprising that VWF-/ApoE double-deficient mice are protected from atherosclerosis at branch points of arteries, which are known to be highly prone to lesion development in ApoE single-deficient mice [47]. Besides GPIb α -VWF, which probably has a restricted relevance in areas of high shear, P-selectin has gained attention as a relevant molecule in the formation of atherosclerotic lesions because, as described in detail above, it mediates interactions between leukocytes, platelets and the endothelium. Early reports have already stated that in certain situations, platelets may use P-selectin on their surface to roll by recognizing carbohydrate ligands on the endothelium, a mechanism that has been demonstrated to facilitate platelet-mediated lymphocyte delivery to high endothelial venules [48]. Later, the absence of P-selectin has been shown to inhibit leukocyte infiltration of atherosclerotic lesions in a mouse model, a process initially thought to be driven by the absence of P-selectin from the endothelium [49]. However, Huo et al. [50] were able to demonstrate that activated platelets exacerbate atherosclerosis in ApoE-null mice in a platelet P-selectin-dependent manner. Wild-type but not P-selectin-null platelets interacted with atherosclerotic arteries of ApoE-deficient mice, and led to substantial leukocyte adhesion.

Because both substrates on the surface of endothelial cells – P-selectin and VWF – are recognized by platelet GPIb α , the GPIb-IX-V complex can be addressed as a central receptor in platelet-endothelial cell interactions. Correspondingly, a number of studies have been performed in order to identify variants of the GPIb-IX-V complex that may contribute to an elevated risk of atherothrombotic diseases. Because GPIb α bears a variable number of tandem repeat (VNTR) region within its stalk-like domain that may extend the VWF binding site from the platelet surface [51], this polymorphism has been studied intensively. Several smaller clinical studies described an association between a higher number of VNTR repeats and arterial thrombosis, but larger studies were unable to confirm these findings [52]. However, these studies neglect the fact that GPIb α is not expressed as a single molecule on the platelet surface, but assembles in a 2:2:2:1 complex, comprising GPIb α , Ib β , IX and V, respectively. Accordingly, in heterozygotes, 2 GPIb α molecules of different length can be present in GPIb-IX-V receptor, and this length disparity may have an impact on VWF binding. We have very recently finished a large study encompassing 1,545 individuals in whom myocardial infarction (MI) and coronary artery disease (CAD) were confirmed by coronary angiography (unpublished data). We were unable to identify any association between the GPIb α variants and the risk of CAD or MI, but comparison between heterozygotes and homozygotes revealed an overrepresentation of heterozygous individuals in the patient group. Thus, for the first time, we give evidence that length disparity of the two GPIb α isoforms within the complex represents a genetic risk

factor for atherothrombosis, with heterozygous individuals being at risk. Given this epidemiological evidence and the overall importance of GPIb α in mediating platelet-endothelial cell interaction, further analysis of the biological mechanism is urgently required.

Next to GPIb α , JAM-A might also be involved in mediating interactions between platelets and the endothelium. Babinska et al. [53] demonstrated that homophilic interactions between JAM-A expressed on platelets and JAM-A expressed on the endothelium support platelet binding. In this study, the authors suggest a role for JAM-A in the adhesion of platelets to cytokine-inflamed endothelial cells and thus in thrombosis and atherosclerosis, because JAM-A is redistributed from the inter-endothelial cleft to the apex of the cell upon stimulation [8]. This attractive possibility has not yet been further evaluated, and an additional contribution of JAM-C, which again is expressed on both cell types, also awaits further research. It is also still unclear whether JAM-A and/or JAM-C contribute to platelet-endothelial cell interaction under physiological (non-inflammatory) conditions.

A more detailed review regarding the role of platelets in atherothrombosis has, with a focus on mouse models, been published recently [54].

Multicellular Interactions in the Vasculature: Limitations and Perspectives

At present, established and newly gained insights into multicellular interactions in hemostasis and inflammation, as presented in this brief overview, answer only a limited number of questions. It has become clear that adhesion processes are closely related, and recent discoveries in the field of junctional adhesion molecules and Mac-1-dependent interactions have added substantially to our understanding. Leukocytes adherent to inflamed endothelium can recruit platelets from the blood stream, where they contribute to the inflammatory reaction or initiate coagulation. Vice versa, activated platelets adherent to endothelium can recruit leukocytes from the blood stream and transfer these leukocytes to the endothelium, where they can react with selectins, carbohydrate ligands and JAMs in order to firmly attach and finally transmigrate [55, 56]. One pathology in which these interactions play a pivotal role is atherothrombosis, as platelet and leukocyte recruitment to the altered arterial vessel wall are important contributors to neointima formation and disease progression [57]. However, much needs to be learned about how recruitment of certain leukocytes to a specific endothelium is regulated. Most data presented in this review deal with 'leukocytes' in general, but only little information is available on whether different expression patterns of adhesion molecules, e.g. JAMs, contribute to the recruitment of a specific subpopulation, e.g. neutrophils or T cells. The same aspect applies to endothelium of different provenance.

Furthermore, there is evidence that subcellular structures, such as microparticles, add to the complexity of the system. Platelets, leukocytes and endothelial cells can generate microparticles containing certain cellular adhesion molecules that allow further (indirect) interactions between these 3 cell types [58–60].

Further research points into two directions: discovery and characterization of new molecules that contribute to the complex interplay at the interface of inflammation and coagulation and dissecting the relevance of each of the newly defined

counter-receptors and ligands. Both, platelet and leukocyte proteomics and the use of knockout and transgene technologies will be necessary to follow these routes in the future.

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