



REVIEW ARTICLE

The Pathophysiological Relevance of Monocyte-platelet Interactions in Inflammatory Disease

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Monocyte-platelet complexes are important in a number of chronic inflammatory diseases, including atherosclerosis, rheumatoid arthritis, and diabetes. Interactions between monocytes and platelets are predominantly mediated by divalent cation-dependent interactions between platelet P-selectin and P-selectin glycoprotein ligand-1 expressed on monocytes. After initial tethering, additional interactions involving CD40/CD40L and extracellular matrix metalloproteinase inducer are thought to induce firm adhesion through activation of β_2 integrins. The engagement of P-selectin glycoprotein ligand-1 on monocytes induces a series of complex signaling pathways, which regulate cytoskeletal arrangement, β_2 integrin activation, transcriptional activation of proinflammatory genes, and the activation of proinflammatory chemokines and cytokine expression. The induction of a proinflammatory phenotype in monocytes adherent to activated platelets promotes the inflammatory response and highlights the importance of monocyte-platelet complexes in inflammatory disease. Investigation into the molecular mechanisms governing monocyte-platelet interactions is integral to further understanding of these complexes and to the identification of novel therapeutic targets for the treatment of inflammatory diseases. The focus of this review, therefore, is to discuss mechanisms and functional consequences of monocyte-platelet complex formation.

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1. Introduction

Inflammatory responses are characterized by the recruitment of leukocytes into the site of injury or infection.¹ Leukocyte recruitment involves a coordinated series of cell adhesion and activation events, which include the initial tethering and rolling of cells along activated endothelium, followed by cell activation, diapedesis, and transmigration through the endothelium. The initial adhesion of leukocytes at sites of inflammation is mediated primarily by a group of endothelial cell adhesion molecules known as the selectins, which interact with glycoprotein ligands on leukocytes.^{2,3} There are three known selectins; L-selectin (CD62L), which is constitutively expressed on most leukocytes and mediates the initial tethering and rolling of cells to the endothelium. E-selectin (CD62E), which is expressed on activated endothelial cells, and finally P-selectin (CD62P), which is expressed by activated endothelial cells, following release from Weibel-Palade bodies and on the surface of activated platelets following its release from platelet α granules.^{4,5} P-selectin mediates the initial tethering and rolling of leukocytes to both the

inflamed endothelium and activated platelets, thus bringing them into close proximity with endothelium- or platelet-associated inflammatory cytokines and chemokines. Following the initial tethering of leukocytes via interactions with selectins, rolling and subsequent firm adhesion occurs by the activation of integrins on both leukocytes and the activated endothelium. Integrins are cell adhesion molecules that are composed of α and β subunits, which pair to form heterodimers.⁶ The β_1 and β_2 integrin subfamilies have a prominent role in leukocyte adhesion and arrest and specifically, the β_1 integrin very late antigen-4 (VLA-4, $\alpha_4\beta_1$) and the β_2 integrin lymphocyte function-associated antigen-1 (LFA-1) ($\alpha_L\beta_2$), whose function is regulated on leukocytes by cytokines and chemokines. Following the upregulation of integrins, additional “outside-in” signals are required to induce conformational changes that lead to integrin activation and allow high-affinity interactions with specific ligands. Recent studies of a novel form of leukocyte adhesion deficiency (LAD III) have identified a key role for the kindlin family of proteins in the regulation of integrin activation.⁷ The mechanism underlying integrin activation, leading to enhanced ligand binding, is now becoming clear. Activation of protein kinase C by diacylglycerol (DAG) and Ca^{2+} and the Ras-proximate-1 or Ras-related protein-1 (Rap1) guanine nucleotide exchange factor (calcium diacylglycerol-guanine nucleotide exchange factor 1) promotes Rap1 activation and recruitment to the plasma membrane via Rap1-guanine triphosphate molecule 1 Rap1-guanine triphosphate-

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interacting adaptor molecule (RIAM).^{8,9} Subsequent recruitment of talin to the RIAM/Rap1 complex facilitates stabilization of high-affinity integrin confirmation by bringing the complex into contact with the β subunit of integrins. Together, these mechanisms act to promote leukocyte recruitment through interactions with specific counter receptors expressed on the activated endothelium and platelets.¹

The upregulation of P-selectin expression on activated endothelial cells and platelets results in the recruitment of leukocytes through the Type I membrane protein, P-selectin glycoprotein ligand-1 (PSGL-1), which is a disulphide-linked homodimer that is constitutively expressed on the leukocyte cell surface.^{3,10} PSGL-1-mediated binding of leukocytes to activated endothelium and platelets has been reported to facilitate their recruitment to sites of inflammation and promote leukocyte activation. Platelets are known to bind via PSGL-1 to different types of leukocytes but have been identified as binding preferentially and more stably to monocytes compared with polymorphonuclear (PMN) leukocytes.^{11–13} This review will focus on the mechanisms and functional consequences of monocyte-platelet complex formation.

Monocytes can be separated into two major subsets depending on their expression of the cell surface markers CD14 and CD16.^{14,15} CD14⁺⁺CD16⁻ monocytes are referred to as “classical” monocytes and are known to express CCR2, CD62L, CD64, and interleukin (IL)-10. CD14⁺ CD16⁺ monocytes or “nonclassical” monocytes, in contrast, are thought to be proinflammatory and are characterized by their expression of human leukocyte antigen-DR, CX3CR1, and their high production of tumor necrosis factor- α (TNF- α).^{16–20} Investigations into the platelet-binding capacity of each subset have so far presented contradictory results, with our group showing that there are similar levels of platelet binding to both classical and nonclassical monocytes.¹³ In contrast, Boudjeltia et al.²¹ reported that platelet binding was much higher to CD14⁺CD16⁺ monocytes than CD14⁺⁺CD16⁻ monocytes. It is possible that the differences in these results may reflect variations in the methodologies used.^{13,21}

Monocyte-platelet complexes have been identified as being present in a number of chronic inflammatory diseases, including atherosclerosis,^{22,23} type I diabetes,²⁴ rheumatoid arthritis, and systemic erythematosis.²⁵ Interactions between monocytes and platelets were initially suggested to represent a surrogate measure of platelet activation;²⁶ however, there is growing evidence that the cross talk between monocytes and activated platelets promotes the activation and modulation of monocyte behavior, and that these interactions at sites of injury and infection may function to further promote the inflammatory response. Specifically, it has been widely demonstrated that interactions between monocytes and activated platelets induces a proinflammatory phenotype in the monocytes, characterized by the activation of the transcription factor nuclear factor- κ B (NF κ B) and the subsequent secretion of inflammatory cytokines and chemokines, such as TNF- α , IL-1 β , IL-8, and monocyte chemoattractant protein-1 (MCP-1) from monocytes.^{13,27–29} Furthermore, it has been shown that upon adhesion to activated platelets, monocytes secrete additional inflammatory mediators, including matrix metalloproteinase-9 (MMP-9)³⁰ and superoxide anions.³¹ The molecular mechanisms of monocyte-platelet interactions may therefore play a crucial role in regulating leukocyte function and the progression of inflammatory disease.

2. Monocyte-platelet Interactions

Monocyte binding to platelets is predominantly mediated by interactions between platelet P-selectin and PSGL-1 expressed on monocytes. This interaction is dependent on the presence of divalent cations, as demonstrated by the ability of the calcium-

chelating agent EDTA to dramatically reduce the ability of monocytes to bind to platelets.^{13,23,28,32} Furthermore, the importance of the interaction between P-selectin and PSGL-1 has been highlighted through antibody inhibition studies, which revealed that the blockade of either P-selectin or PSGL-1 resulted in the attenuation of monocyte binding to platelets.^{13,23,33,34} For example, monocyte binding to platelets was significantly reduced by the addition of the function blocking PSGL-1 monoclonal antibody (mAb), PL-1, to monocyte-platelet complexes, but not by the functionally inert PSGL-1 mAb PL-2, emphasizing the importance of PSGL-1 in this interaction.^{13,23,33} Furthermore, the addition of the F(ab')₂ fragment of an mAb specific for P-selectin was also found to significantly inhibit monocyte binding to platelets.^{13,33} It is interesting to note that mAb blockade of P-selectin consistently resulted in greater inhibition of monocyte-platelet binding compared with PSGL-1 blockade. This suggests that there may be additional receptors expressed on monocytes that are capable of binding this selectin,²³ specifically receptors expressing the correct carbohydrates, such as sialylated CD15 (Lewis X), which is expressed on monocytes and has been identified as being able to bind P-selectin on platelets.³⁵ Although EDTA and EGTA were able to significantly attenuate monocyte binding to unactivated platelets in healthy donors, a small percentage of monocytes were still capable of binding to platelets, suggesting that there may be additional divalent cation-independent mechanisms involved in monocyte-platelet interactions.^{13,23} Furthermore, EDTA was unable to completely inhibit monocyte-platelet interactions in donors with acute coronary syndromes, in contrast to healthy donors. One possibility is that there is an increase in divalent cation-independent monocyte-platelets adhesion in disease, which may be a result of monocyte activation and the subsequent upregulation of additional adhesion receptors.²³ Overall, these data suggest that whilst the interaction between P-selectin and PSGL-1 is critical in the formation of monocyte-platelet complexes, there are potentially other ligands involved that play an additive role in adhesion strengthening and subsequent signaling events.

Following the initial tethering of monocytes to platelets by P-selectin PSGL-1 binding, additional cell-surface interactions are thought to play a role in stabilizing monocyte-platelet complexes. For example, firm adhesion is thought to occur through the regulation of integrins on the cell surface of monocytes. Specifically, the initial interactions between P-selectin and PSGL-1 have been shown to induce the expression and function of the very late antigen-4 and macrophage-1 antigen (Mac-1) (α M β 2) integrins on the cell surface of monocytes.³⁶ Several additional studies have also highlighted a role for integrins in monocyte-platelet interactions. For example, blocking antibodies specific for Mac-1 were capable of inhibiting monocyte-platelet binding.³⁷ The binding of monocytes to thrombin receptor-activating peptide-activated platelets was also shown to result in increased expression of the α M subunit of Mac-1 on monocytes.¹³ Furthermore, it has also been demonstrated that monocyte adherence to endothelial bound platelets is dependent on the expression of Mac-1 by the monocytes.³⁸ Antibody inhibition studies have identified glycoprotein 1b α (GP 1b α) expressed on platelets as being a ligand for Mac-1 on monocytes. Monoclonal antibodies against either Mac-1 or GP 1b α were able to inhibit human acute monocytic leukemia cell line THP-1 cell adhesion to purified GP 1b α . Furthermore, 293 cells expressing Mac-1, but not LFA-1, were capable of binding to GP 1b α , and this was inhibited by specific mAbs.³⁷ Junctional adhesion molecule (JAM)-3 expressed on platelets was also identified as an important ligand for Mac-1, with inhibition studies suggesting that JAM-3 and GP 1b α act together to form the predominant ligands for Mac-1.³⁹ However, GP 1b α and JAM-3 are not the only ligands that have been identified for Mac-1, and additional ligands include fibrinogen,^{40–42}

intercellular adhesion molecule-2,⁴³ and high-molecular-weight kininogen.⁴⁴ Despite evidence supporting a role for β_2 integrins in monocyte-platelet complexes, the exact role of Mac-1 remains controversial, with some reports suggesting that blockade of Mac-1 has no effect on monocyte-platelet binding.³³

Further cell surface molecules thought to be involved in monocyte-platelet binding include CD40 expressed on monocytes and CD40 ligand (CD40L, CD154) expressed by activated platelets. The role of CD40-CD40L interactions in monocyte binding to platelets remains undetermined, with some groups suggesting that the blockade of either CD40 or CD40L had no effect on monocyte-platelet interactions.^{24,45} One possibility is that CD40-CD40L interactions are not of sufficient affinity to mediate platelet monocyte binding in the absence of additional molecular interactions. However, the involvement of the CD40/CD40L dyad in the induction of proinflammatory mediators, such as chemokines, cytokines, MMPs, tissue factor, and adhesion molecules, suggest that it is possible that CD40-CD40L interactions could provide additional signals that influence monocyte behavior through the induction of a proinflammatory phenotype.^{46–49}

Additional molecular interactions may also play a role in monocyte-platelet binding and further influence monocyte function. The immunoglobulin superfamily protein extracellular matrix metalloproteinase inducer (EMMPRIN, CD147) is expressed by activated inflammatory cells, including monocytes,⁵⁰ and has been shown to bind platelet glycoprotein VI (GPVI). EMMPRIN may be involved in the induction of matrix metalloproteinases in a number of different cell types.⁵¹

Another important receptor in the induction of monocyte-platelet interactions is triggering receptor expressed on myeloid cells-1 (TREM-1), through its ability to induce the expression of inflammatory chemokines, cytokines, and adhesion molecules.⁵² It is expressed on both monocytes and PMN.⁵² The ligand for TREM-1 has been identified as being present on activated platelets and has been shown to interact with TREM-1 on neutrophils, inducing neutrophil activation.⁵³ The involvement of TREM-1 and its ligand in platelet-neutrophil interactions provide evidence for the potential involvement of interactions between TREM-1 and TREM-1 ligand in the stabilization of monocyte-platelet interactions.

3. Signaling Pathways Downstream of PSGL-1

In addition to its role as an adhesion molecule, there is increasing evidence that PSGL-1 also functions as a signaling molecule, with the binding of P-selectin to PSGL-1 having been shown to initiate several different signaling pathways (Figure 1). These pathways have been shown to regulate cytoskeletal rearrangement,^{54,55} the activation of integrins,⁵⁶ the transcription of proinflammatory genes,⁵⁷ and chemokine and cytokine secretion.^{27,28} An increased understanding of the signaling pathways that are initiated following the interaction of monocytes with activated platelets will help to further understand the relationship between these cells and could also identify new potential therapeutic targets to help treat inflammatory disorders.

The 69 amino acid cytoplasmic tail of PSGL-1 is highly conserved over different species, suggesting that it carries important functions.⁵⁸ It has been shown to associate with ezrin and moesin, proteins of the ezrin/radixin/moesin (ERM) family, which function to link the cell membrane to the actin-cytoskeleton⁵⁵ (Figure 1). Specifically, the interaction between PSGL-1 and the amino-terminus of moesin has been shown to be important for redistribution of PSGL-1 to the uropod of activated cells.^{59–61} The redistribution of PSGL-1 helps facilitate interactions with other cells and promotes recruitment of activated cells to sites of inflammation.⁶² PSGL-1 redistribution has been reported to promote leukocyte interactions with platelets, and the association of PSGL-1 with the

ERM proteins influences cytoskeletal regulation and may strengthen the interaction of PSGL-1 with its ligand P-selectin and monocyte-platelet interactions.⁶³

In addition to their role as cytoskeletal linker molecules, ezrin and moesin have also been identified as important adaptors in the interaction between PSGL-1 and the spleen tyrosine kinase, Syk.⁵⁷ Syk associates indirectly with the cytoplasmic tail of PSGL-1 through its interaction with an immunoreceptor tyrosine-like activation motif (ITAM)-like motif in the N-terminus of moesin (Figure 1). Engagement of PSGL-1 with P-selectin results in the tyrosine phosphorylation of the ITAM-like sequences on moesin, which in turn results in the binding of Syk to moesin and its subsequent activation. The Src family kinases have been shown to phosphorylate ITAMs and are known to act upstream of Syk; it therefore remains to be determined whether they have a role in phosphorylation of the ITAM-like domains on moesin and in the activation and recruitment of Syk to the cytoplasmic tail of PSGL-1.^{64,65} The interaction between Syk and moesin has been identified as being essential for the association of Syk with PSGL-1 and in the

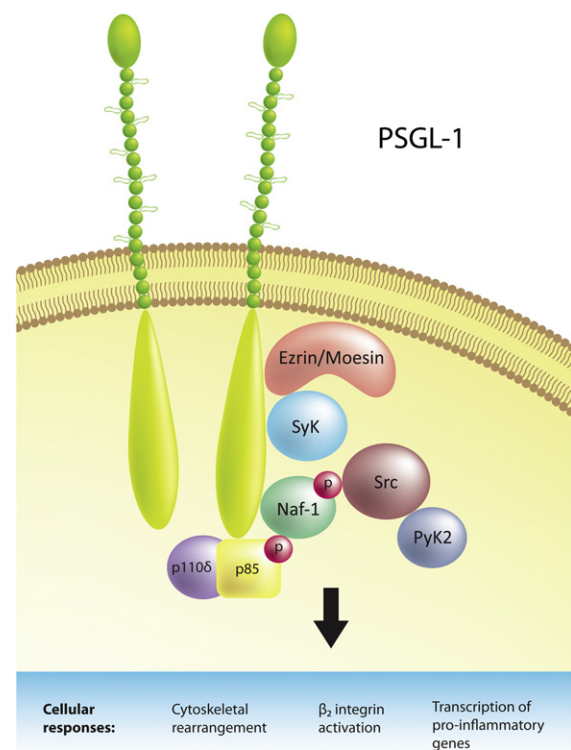


Figure 1 Signaling pathways downstream of PSGL-1: Engagement of PSGL-1 results in the activation of a series of complex signaling pathways that regulate a number of important cellular processes. The interaction between the cytoplasmic tail of PSGL-1 and the amino-terminus of the ezrin/radixin/moesin protein moesin has been shown to be important for the redistribution of PSGL-1 to the uropod of leukocytes and facilitates their interaction with platelets. Moesin also plays an important role as an adaptor in the interaction between PSGL-1 and the spleen tyrosine kinase Syk. Syk interacts with an immunoreceptor tyrosine-like activation motif-like motif on moesin, facilitating the interaction between Syk and the cytoplasmic tail of PSGL-1. Phosphorylation of Syk results in the induction of expression of early immediate activation genes such as *c-fos* and has been implicated in the activation of β_2 integrins in neutrophils. The activation of the Src family kinases (SFK), downstream of PSGL-1, leads to the activation of β_2 integrins via the phosphorylation of the focal adhesion kinase-related protein, Pyk2. The Src kinases are also involved in the activation of Naf-1, which is constitutively associated with the carboxyl terminus of PSGL-1. Following activation, Naf-1 phosphorylates the p85/p110δ heterodimer of PI3K, which has been shown to activate the integrins macrophage-1 antigen and lymphocyte function-associated antigen-1 downstream of PSGL-1. PSGL-1 = P-selectin glycoprotein ligand-1

phosphorylation of Syk following engagement of PSGL-1, highlighting the role of moesin as an important adapter for the interaction between PSGL-1 and Syk.⁵⁷ Phosphorylation of Syk results in the induction of expression of early immediate activation genes such as *c-fos*, which was abrogated in the presence of both a pharmacological inhibitor of Syk activation and an N-terminus ITAM mutant of moesin, underlining the importance of these events in the signaling downstream of PSGL-1.⁵⁷ The activation of Syk downstream of PSGL-1 engagement has also been implicated in the activation of the β_2 integrins on neutrophils, and therefore in promoting their recruitment to sites of inflammation.⁶⁶ Through the induction of gene expression and the activation of integrins, Syk is therefore capable of inducing signaling pathways that facilitate leukocyte activation and recruitment and thus helping to promote and strengthen the inflammatory response.

The Src family kinases have also been shown to be important downstream of PSGL-1 in the activation of β_2 integrins on leukocytes,⁶⁷ promoting firm adhesion to activated platelets and further suggesting that PSGL-1 plays a key role in that activation of the β_2 integrins.^{56,68,69} Experiments involving the use of pharmacological inhibitors and genetic targeting of Fgr, Hck, and Lyn have highlighted the importance of these Src kinases in leukocyte-platelet interactions. For example, the addition of the Src kinase inhibitor, PP2, to platelet-PMN cell aggregates resulted in a decrease in the activation and adhesive function of Mac-1 on PMN.^{68,69} Importantly, the Src kinases do not appear to affect initial binding between leukocytes and platelets suggesting that the Src kinases are not involved in P-selectin-PSGL-1 interactions.⁵⁶ The focal adhesion kinase-related protein, Pyk2, has been identified as a potential downstream mediator of Src kinase activity and has been reported to be tyrosine phosphorylated in human leukocytes bound to activated platelets. The phosphorylation of Pyk2 was reduced by a P-selectin mAb and was further inhibited by an anti- β_2 integrin mAb. Pyk2 phosphorylation was also inhibited with the Src kinase inhibitor PP2 and was significantly attenuated in PMNs from Fgr^{-/-} and Hck^{-/-} mice compared with wild-type PMNs. Inhibition of Pyk2 activity with tryphostin A9 (AG17) inhibited neutrophil-platelet interactions but did not affect Src kinase phosphorylation.⁵⁶ Overall, these results strongly suggest a mechanism in which Src kinase activity is required downstream of PSGL-1 and that Pyk2 acts downstream of the Src kinases in mediating Mac-1 activation.⁵⁶ Through the induction of firm adhesion, the Src kinases are therefore potentially capable of stabilizing and promoting monocyte-platelet interactions within the inflammatory response.

Interestingly, recent work has also suggested a role for the Src kinases in the activation of Nef-associated factor-1 (Naf-1), which also plays a role in signaling downstream of PSGL-1.⁷⁰ The cytoplasmic tail of PSGL-1 has been found to constitutively interact with Naf1. Deletion mutants in which the carboxyl terminus of the cytoplasmic tail of PSGL-1 was truncated "stepwise" were used to identify the domains involved in the interaction between PSGL-1 and Naf-1. Identification of the residues involved in binding established that Naf-1 interacts with a region of PSGL-1 that is distinct from the residues that are critical for binding of ezrin and moesin.⁷⁰ As previously discussed, the binding of P-selectin to PSGL-1 has been shown to activate the Src kinases, including Lyn and Hck. Activated Src kinases were identified as being responsible for the phosphorylation of Naf1, which then recruits and activates the p85-p110 δ heterodimer of phosphoinositide-3-OH kinase (PI3K). Naf1 is thought to act as an adapter downstream of PSGL-1, facilitating interactions between PSGL-1 and PI3K. The importance of the Src kinases in the activation of Naf1 was demonstrated using pharmacological inhibition of Src kinases, which prevented Naf1 phosphorylation and the association of p85 with PSGL-1. PI3K

activates a variety of signaling pathways, regulating cell adhesion, growth, migration, proliferation, and apoptosis and is therefore central in many inflammatory processes.⁷¹ In acting downstream of PSGL-1, PI3K has been shown to activate the leukocyte integrins Mac-1 and LFA-1, therefore promoting firm adhesion required for the progression of the inflammatory response (Figure 1). Furthermore, the identification of PI3K as a signaling molecule acting downstream of PSGL-1 provides further molecular targets for therapeutic intervention in pathogenic inflammation.

Signaling through PSGL-1 by activated platelets has also been shown to induce the immediate early response gene COX-2.⁷² COX-2 induces the production of prostaglandins and has therefore been implicated in the progression of a number of inflammatory diseases, including atherosclerosis.⁷³ Initial interactions between PSGL-1 and P-selectin induce stable interactions between monocytes and platelets, which is sufficient for the induction of COX-2 mRNA, following 18 hours of cocubation. COX-2 protein expression, however, requires additional factors, one of which is IL-1 β , which is produced by both platelets and monocytes. The induction of COX-2 following the interaction of monocytes with activated platelets further supports a critical role for these complexes in the promotion of inflammatory responses and identifies new modes of action for established pathways in the treatment of inflammatory disease.

4. Activation of Monocytes Adherent to Activated Platelets

The binding of monocytes to activated but not unactivated platelets has been shown to induce the secretion of inflammatory cytokines and chemokines with important implications for monocyte function. Specifically, upon binding thrombin-stimulated platelets, monocytes increase secretion of both MCP-1, and the inflammatory cytokine TNF- α was observed.²⁸ Furthermore, it was also demonstrated that monocytes were able to secrete the neutrophil chemoattractant protein IL-8 in response to interaction with thrombin-stimulated platelets.^{27,28} Antibody inhibition studies highlighted the importance of P-selectin and PSGL-1 interactions in the induction of MCP-1 and IL-8. Blockade of P-selectin using a F(ab')₂ fragment of the P-selectin mAb G1 to cultures significantly attenuated MCP-1 release from monocytes. Similarly, the neutralization of monocyte PSGL-1 with the PL-1 mAb antibody also had a significant inhibitory effect. Interestingly, experiments in which monocytes were cultured on immobilized P-selectin strongly suggested that P-selectin alone was not sufficient for the induction of inflammatory mediators from adherent monocytes and that P-selectin acts in concert with additional signaling molecules. Further work identified the CC chemokine regulated upon activation normal T cell expressed presumed secreted (RANTES), which is released from activated platelets, as acting in concert with P-selectin in the induction of MCP-1 and IL-8 from monocytes adherent to activated platelets. The neutralization of RANTES was able to significantly attenuate MCP-1 secretion from monocyte-platelet complexes, further supporting its role in the induction of inflammatory mediators from monocytes. It is important to note that while the neutralization of RANTES was capable of abrogating MCP-1 release, it did not affect the formation of monocyte-platelet complexes, therefore suggesting that RANTES signaling occurs downstream of the binding of monocytes to platelets.²⁸ While RANTES has been implicated in the induction of inflammatory chemokines from monocyte-platelet complexes, the contribution of other inflammatory cytokines remains unknown.

Similarly, it has also been demonstrated that the interaction of monocytes with activated, but not nonactivated, platelets induces the expression of the proinflammatory cytokine, IL-1 β in addition to IL-8 and MCP-1.²⁹ Consistent with other findings, mAb blockade

of platelet P-selectin only partially abrogated the induction of IL-1 β , IL-8, and MCP-1, again suggesting that other molecules are involved in the production of inflammatory mediators by monocytes adherent to activated platelets. Overall these results highlight the importance of signaling through PSGL-1 and signaling pathways following monocyte adhesion to activated platelets but indicate that additional factors have a key role in determining the release of inflammatory mediators from these cells.

The stimulation of monocytes by thrombin-activated platelets has been shown to induce activation of the heterodimeric transcription factor NF κ B, a member of the Rel family of proteins, which is important in the expression of proinflammatory mediators, such as IL-1 β , IL-8, MCP-1, and TNF- α .^{74,75} NF κ B is located in the cytoplasm of unactivated cells but will rapidly translocate to the nucleus on cell stimulation. Several groups have clearly demonstrated that the interaction with activated, but not unactivated platelets, results in the nuclear translocation of NF κ B, where it binds to κ B sequences in the promoter and enhancer regions of immediate early genes.^{13,28} Additional support for the activation of NF κ B in adherent monocytes has come from data demonstrating that the binding of monocytes to activated platelets also results in the degradation of I κ B α , an inhibitor of NF κ B, when compared with monocytes alone and monocytes incubated with unactivated platelets. Together these data suggest that the interaction of monocytes with thrombin-activated platelets results in the activation of the transcription factor NF κ B, which induces the upregulation and expression of a number of proinflammatory cytokines and chemokines from monocytes.

The upregulation of integrin expression by monocytes further supports the concept that they are activated following the binding of activated platelets. As discussed above, monocytes have been shown to upregulate both β 1 and β 2 integrins following their binding to platelets, suggesting they acquire increased adhesive properties and therefore an activated proinflammatory phenotype on interactions with activated platelets.^{13,36,37}

The binding of monocytes to activated platelets has also been shown to induce the release of extracellular superoxide anions (O₂⁻) compared with binding unactivated platelets.³¹ The increased release of O₂⁻ from monocytes was found to be dependent on the interaction between P-selectin and its carbohydrate ligands, including PSGL-1, as demonstrated by inhibition of mAb specific for P-selectin and Sialyl Lewis X.³¹ Superoxide anions have been implicated in triggering tissue damage and promoting inflammatory reactions, and therefore the release of O₂⁻ from monocytes is likely to prolong the inflammatory response.

Further regulatory effects of platelet monocyte binding may occur when there is exposure of basement membrane components, for example when endothelial damage occurs. The interaction of monocytes with activated platelets together with the extracellular matrix protein, collagen, has been shown to induce the expression of MMP-9 by monocytes.³⁰ MMP-9 has been identified as being an important mediator of tissue injury and inflammation and is overexpressed in atherosclerotic plaques that are vulnerable to rupture.⁷⁶ The demonstration that monocytes interacting with collagen alone or activated platelets alone were unable to secrete MMP-9 suggests that the induction of MMP-9 differs from the induction of proinflammatory cytokines and chemokines. Interestingly, as with the production of other inflammatory mediators, blockade of PSGL-1 with neutralizing antibodies was unable to fully prevent MMP-9 synthesis, suggesting that other signaling pathways are involved. Furthermore, neutralizing antibodies against the β 1 integrin subunit, which mediates monocyte attachment to collagen, also attenuated MMP-9 synthesis, and although the addition of both sets of antibodies had an additive effect they were unable to completely abrogate MMP-9 release. This suggests that

additional, as yet unidentified, receptors are involved in the induction of MMP-9 from monocytes following interactions that are interacting with platelets and collagen.

Recent work has suggested a role for the platelet receptor EMMPRIN in the induction of MMP-9 from monocytes.⁷⁷ The binding of EMMPRIN on activated platelets to EMMPRIN expressed on monocytes was shown to be able to induce upregulation of MMP-9 by the monocytes and the activation of NF κ B through degradation of the I κ B α complex. Activation of NF κ B permitted secretion of inflammatory cytokines IL-6 and TNF- α and was prevented by pretreatment of monocytes with EMMPRIN-specific small interfering RNA.⁷⁷ Overall these results implicate the potential involvement of the platelet receptor EMMPRIN in the induction of MMP-9 and other proinflammatory mediators from monocytes adherent to activated platelets.

Taken together, these data clearly show that the binding of activated platelets by monocytes is able to induce monocyte activation and further the inflammatory response through the induction of proinflammatory mediators, including chemokines, cytokines, and cell adhesion molecules.

5. Conclusion

The interaction between PSGL-1 and P-selectin is critical for the formation of monocyte-platelet complexes. Following initial P-selectin-dependent tethering of monocytes, it appears that additional interactions involving CD40-CD40L and EMMPRIN act together to strengthen binding by promoting firm adhesion via integrins including Mac-1. The adhesion of monocytes to activated platelets results in the induction of a series of complex signaling pathways, which regulate cytoskeletal rearrangement, activate β 2 integrins, and induce the expression of inflammatory mediators, such as MCP-1, IL-1 β , IL-8, MMP9, and the release of superoxide O₂⁻. The induction of a proinflammatory monocyte phenotype following adhesion to activated platelets supports the idea that these interactions have important proinflammatory consequences. This concept is further supported by the presence of monocyte-platelet complexes in a number of important inflammatory diseases, including atherosclerosis, rheumatoid arthritis, and diabetes. Continued research into the molecular mechanisms governing monocyte-platelet interactions is integral to further understanding of these complexes and the identification of novel therapeutic targets for the treatment of inflammatory diseases.

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