Chemoattractant-Induced Changes in Surface Expression and Redistribution of a Functional Ligand for P-Selectin on Neutrophils

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Adhesion between platelets and neutrophils is mediated through the interaction of P-selectin on activated platelets with a carbohydrate-containing structure on neutrophils, and occurs under both static and shear conditions. Recent studies using flow chambers have shown that neutrophils become activated after binding to surface-adherent platelets expressing P-selectin. The objective of the present study was to investigate the effect of activation on the interactions of platelet P-selectin with its ligand on neutrophils. Flow cytometric analyses using P-selectin chimeras revealed that activation induced a rapid and marked reduction in chimeric binding, with levels of binding decreased by 71% after 15 minutes of stimulation with the chemotactic agent, FMLP. Using a visual assay of platelet-neutrophil rosetting, we showed that the P-selectin ligand was translocated and clustered at the uropod of neutrophils following the shape changes and polarization induced by chemotactic stimulation. Activated neutrophils bound to surface-adherent platelets also displayed the clustering of P-selectin ligand at the uropod, and these neutrophils detached from the platelets when a shear stress (2 dynes/cm²) was applied through the adhesion chamber. These results indicate that chemotactic stimulation of neutrophils induces changes in the surface expression and distribution of a biologically relevant ligand for P-selectin, and that these changes might influence the adhesive interactions occurring between neutrophils and activated platelets.

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COMPLEX INTERACTIONS between neutrophils and platelets, both key cellular elements of hemostatic and inflammatory processes, contribute to modulate host responses to vascular and tissue injury. Through the release of soluble mediators, neutrophils and platelets can enhance or inhibit each other’s functions in a regulated manner, although uncontrolled responses can also lead to vascular thrombosis, ischemia, and tissue damage. For example, thrombin-activated platelets can enhance neutrophil production of the reactive oxygen radical, superoxide anion, an event that could contribute to trigger an acute inflammatory reaction at the site of a vascular lesion.

Adhesion of activated platelets to neutrophils facilitate these interactions, bringing the two cell types to close proximity. P-selectin on the platelet surface and its counterreceptor on neutrophils mediate this adhesion. Studies on the identity of the P-selectin ligand(s) have identified glycoproteins carrying the oligosaccharide structure sialyl LewisX as likely candidates. Among them, a 120-kD dimeric sialylated glycoprotein selectively recognized by P-selectin has been isolated from neutrophils and HL-60 cells. The polypeptide component of this glycoprotein is identical to that of P-selectin glycoprotein ligand (PSGL-1), a newly described mucin-like transmembrane glycoprotein. PSGL-1 was recently shown to mediate adhesion of neutrophils to P-selectin under both static and shear conditions. A 160-kD glycoprotein requiring N-linked oligosaccharides for binding to P-selectin has also been recently reported. In addition, a role for L-selectin in neutrophil binding to P-selectin has been suggested by some studies.

Neutrophil-platelet interactions can occur under both static and flow conditions. Attachment of flowing neutrophils to surface-adherent platelets in vitro has been demonstrated, and is suggested as a possible way of enhancing neutrophil localization to a site of injury. Interestingly, some arrested neutrophils in contact for several minutes with surface-bound platelets exhibited signs of cellular activation, including changes of shape, upregulation of β₂ integrins CD11/CD18, and downregulation of L-selectin. It has been postulated that such activation might represent an important step in the process of neutrophil adhesion to platelets, comparable to the activation essential to neutrophil attachment and transmigration through vascular endothelium. However, little is known about the consequences of neutrophil activation on the platelet-neutrophil association, and on the fate of the P-selectin ligand after activation.

The objectives of the present study were therefore to investigate the effect of activation on the neutrophil ligand for P-selectin using two different sources of P-selectin, a P-selectin-IgG chimeric protein and P-selectin—expressing platelets, and to determine the physiological consequences of stimulation on neutrophil-platelet association using an in vitro system allowing analysis of such interactions under dynamic conditions of flow. These studies were performed with both human and canine cells.

MATERIALS AND METHODS

Cell isolation. Blood was collected by venipuncture from healthy adults and from healthy adult mixed-breed dogs in anticoagulant (citrate phosphate dextrose; Abbott Laboratories, North Chicago, IL). Neutrophils were isolated by dextran sedimentation of erythrocytes followed by Ficoll-Hypaque gradients, as previously described. Neutrophils were resuspended in Dulbecco’s phosphate-buffered saline (PBS) Gibco, Grand Island, NY), pH 7.4, containing 0.2% glucose. Platelet-rich plasma (PRP) was isolated by successive centrifugations of the citrated blood for 3 minutes at


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Platelet counts were determined using a Coulter Counter (Model ZBI; Coulter Electronics Inc, Hialeah, FL). PRP was used within 20 to 30 minutes of preparation. For the static platelet-neutrophil binding assay, platelets were isolated from PRP by gel filtration over a Sepharose 2B column (Pharmacia LKB Biotechnology, Piscataway, NJ) in modified Tagen-HEPES buffer, as previously described.11

Monoclonal antibodies. The monoclonal antibody (mAb) directed against a functional epitope of human P-selectin, G1, was kindly provided by Dr Rodger P. McEver (University of Oklahoma Health Sciences Center, Oklahoma City, OK). mAbs MD3 and MD6, both anticanine P-selectin, were generated in our laboratory. mAb MD3 has previously been shown to recognize a nonfunctional epitope of canine P-selectin,27 and mAb MD6 binds to a functional epitope.28 Anti-FeRII and anti-FeRIII mAbs were a generous gift from Dr Michael P. Tosi (Case Western Reserve University School of Medicine, Cleveland, OH). The anti-CD18 mAb R15.7 was kindly provided by Dr R. Rothlein (Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT). The anti-CD11b mAb Leu-15 (tagged with phycoerythrin [PE]) and the anti-L-selectin mAb Leu-8 (tagged with fluorescein) were obtained from American Type Culture Collection (Rockville, MD). All antibodies belong to the IgG, isotype, and contain the same regions as the first chimera plus an additional complement binding protein domain.

P-selectin-IgG chimeras. Two P-selectin chimeras were used. One was a generous gift from Dr Susan R. Watson (Genentech Inc, San Francisco, CA), for which the construction and characterization have been previously described.29 It included the lectin domain, the epidermal growth factor–like domain, and one complement binding protein domain of human P-selectin combined with the Fc portion of human IgG. The other P-selectin chimera was kindly provided by Dr Takashi K. Kishimoto (Boehringer Ingelheim Pharmaceuticals) and contained the same regions as the first chimera plus an additional complement binding protein domain.

Immunofluorescence flow cytometry. Binding of P-selectin–IgG chimera to human and canine neutrophils was assessed by flow cytometry. For these experiments, neutrophils were incubated with PBS only (resting) or were stimulated with a chemotactic factor (10 ng/mL FMLP for 5, 15, or 30 minutes at 37°C. At the end of the stimulation period, the P-selectin–IgG chimera (1:2 dilution of tissue culture supernatant or 20 μg/mL purified chimera) was added, and cells were incubated for 15 minutes at room temperature. For blocking experiments with the anti-P-selectin mAb, the chimera was preincubated for 15 minutes at room temperature with mAb G1 (40 μg/mL) or with an irrelevant isotype-matched control antibody before being added to the cells. For experiments controlling for FeRII and FeRII binding, neutrophils were preincubated for 10 minutes at room temperature with 10 μg/mL each of F(ab’2); fragments of mAb 4.3 (anti-FeRII mAb) and mAb 3G8 (anti-FeRIII mAb) before addition of the P-selectin chimera. After washing the cells twice with PBS, a PE-labeled goat antihuman IgG (Jackson ImmunoResearch Laboratories Inc, West Grove, PA) was added for 15 minutes at room temperature. Cells were washed twice with PBS, erythrocytes were lysed using the Becton-Dickinson lysing reagent, and cells were fixed in 1% paraformaldehyde. All samples were analyzed on a FACSscan (Becton Dickinson, Mountain View, CA).

Platelet-neutrophil binding assay under static conditions. The binding of fixed stimulated platelets to resting or chemotactically stimulated neutrophils was determined using an assay previously described.15 Briefly, gel-filtered platelets (10^6 platelets/mL) were incubated with human thrombin (0.2 U/mL) for 10 minutes at room temperature and fixed with paraformaldehyde (1%) for 60 minutes at room temperature. Fixation was stopped by addition of Tris (250 mmol/L)/glycine (500 mmol/L) for 15 minutes. Platelets were washed three times with PBS and resuspended in PBS at a concentration of 5 × 10^6 platelets/mL. An aliquot of fixed platelets was incubated with isolated neutrophils (platelet:neutrophil ratio, 50:1) for 30 minutes at room temperature. For experiments examining the effect of cell activation, neutrophils were stimulated at 37°C with a chemotactic agent (10 mmol/L FMLP for human neutrophils and 1% zymosan-activated dog serum [ZADS] for canine neutrophils), and aliquots of the mixed cell preparations were fixed at various time intervals in glutaraldehyde (1%). Preparations were examined using a Leitz (Rockleigh, NJ) Diaplan differential interference contrast microscope with a 100 × oil objective.

Platelet-neutrophil adhesion assay under flow conditions. Neutrophil adhesion to and detachment from surface-adherent platelets under conditions of flow was assessed using a parallel plate flow chamber maintained at 37°C, as previously described.13 Briefly, 35-mm polystyrene tissue culture dishes (Corning, Corning, NY) were coated with type I collagen from bovine skin (0.25 mg/mL; ICN Biochemicals, Cleveland, OH) for 60 minutes at 37°C. Platelets in PRP (2 to 3 × 10^6 platelets/mL) were incubated on the collagen-coated surface for 45 minutes at room temperature, and nonadherent platelets were removed by decanting the PRP. Platelet preparations were mounted in the parallel plate flow chamber and perfused for 2 to 3 minutes with PBS only. Isolated neutrophils (1 × 10^6 cells/mL) were then perfused through the chamber at a wall shear stress of 2 dynes/cm². After 2 to 3 minutes, the flow was stopped by turning off the pump, and neutrophils were allowed to interact with the platelet-covered surface under static conditions. After a few minutes, flow was reinitiated at a wall shear stress of 2 dynes/cm², and neutrophil interactions with adherent platelets were observed under phase-contrast microscopy (Diaphot-TMD; Nikon Inc, Garden City, NY) using a 40 × objective and recorded on videotape. Digitized frames (Pretax System Inc, Houston, TX) of videotaped experiments were used to determine the number of neutrophils in contact with platelets.

Scanning electron microscopy. Mixed suspensions of neutrophils and activated platelets prepared as described earlier were fixed in cold PBS containing 10% glutaraldehyde, postfixed in PBS containing 1% osmium tetroxide, and dehydrated in a graded ethanol series. Cells were critical-point–dried, and preparations were mounted on aluminum stubs, sputter-coated with gold, and examined in a JEOL 6100 scanning electron microscope.

Statistical analysis. The data were analyzed using one-way analysis of variance followed by multiple comparisons of means with the Tukey-Kramer method, and statistical significance was set at P < .05. All values are given as the mean ± SEM.

RESULTS

Binding of a P-selectin–IgG chimera to resting and stimulated neutrophils. To investigate the effect of activation on the interaction of P-selectin with its ligand, binding of a P-selectin–IgG chimera to resting and to stimulated neutrophils was assessed by flow cytometry. Experiments were performed using P-selectin chimeras from two different sources, and results showed that both chimeras produced the same pattern of binding. When incubated with unstimulated neutrophils, the P-selectin–IgG chimera bound to human neutrophils (Fig 1) but not to canine neutrophils, suggesting that differences in the neutrophil structure recognized by P-selectin exist between species. In light of this observation, subsequent studies using a P-selectin chimera were performed on preparations of human neutrophils. To control for the possibility of Fc receptor binding of the chimeras, experiments were conducted with neutrophils that had been...
preincubated with anti-FcRII and anti-FcRIII F(ab')2 fragments before addition of P-selectin chimeras. Results showed that pretreatment of neutrophils with blocking anti-FcR antibodies had no influence on chimera binding (fluorescence of anti-FcR−treated cells, 108.6% of control untreated cells), indicating that chimeras were not interacting with neutrophils through their Fc portion. To further confirm specificity of the binding, the P-selectin chimera was preincubated with the blocking anti-P-selectin mAb G1 (40 ng/mL) before being added to the neutrophils. Preincubation with mAb G1 almost totally inhibited binding of the chimera to neutrophils (−−).

Since activation results in changes in surface expression and/or conformational changes of some molecules on neutrophils,10,24 including possibly the structure recognized by P-selectin, we next looked at the binding of the P-selectin chimera to neutrophils stimulated with the chemotactic peptide FMLP (10 nmol/L). Incubation of the cells with the chimera alone without FMLP stimulation caused only a slight activation of the neutrophils as measured with anti-Leu-15 and anti-Leu-8 antibodies (26.5% increase in CD11b/CD18 levels and no change in L-selectin levels).

Chemotactic stimulation with FMLP resulted in reduced levels of binding of the P-selectin chimera versus levels observed with unstimulated neutrophils (Fig 2). A time-course study showed a rapid decrease in binding, with levels diminished by 32% ± 4% after 5 minutes and by 71% ± 6% after 15 minutes of stimulation. Light-microscopic observation of neutrophils with a fluorescence microscope confirmed decreased levels of binding of the P-selectin chimera (data not shown). These results indicate that chemotactic stimulation induces rapid changes (downregulation or conformational changes) in the structure of neutrophils interacting with P-selectin.

Platelet binding to stimulated neutrophils under static conditions. To help elucidate the mechanisms involved in changes of the neutrophil ligand for P-selectin associated with activation, the next experiment was conducted. Using fixed thrombin-activated platelets as a source of P-selectin, an assay of platelet-neutrophil binding was performed to
visualize the interactions of P-selectin with neutrophils following chemotactic stimulation.

Incubation of fixed activated platelets with neutrophils for 30 minutes resulted in the binding of numerous platelets to the neutrophils (Table I), with a random distribution of platelets over the surface of neutrophils (Fig 3a and d). Either single platelets or small platelet aggregates were observed on the surface of neutrophils (Fig 4A). This phenomenon was observed with both human and canine cell preparations.

Addition of a chemotactic factor (FMLP for human cells and ZADS for canine cells) to the mixed cell suspensions resulted both in morphologic changes of the neutrophils and in redistribution of platelets on their surface. Shortly (within 30 seconds) after addition of the chemotactic factor, the neutrophil started to change shape, assuming an irregular appearance, often with one side of the cell beginning to ruffle. Although platelets were still observed binding to the neutrophil surface, the previously random distribution was replaced by convergence to one side of the cell, in most cases to the side opposite the area of ruffled membrane (Fig 3b and e). After 5 minutes of chemotactic stimulation, the majority of neutrophils had assumed a bipolar morphology with formation of a pseudopod and a uropod. By this time, all platelets had moved to one pole of the cells (Fig 3c and f) and could be observed as small aggregates binding to the uropod (Fig 4B). No changes in the number of neutrophils with bound platelets or in the number of platelets per neutrophil were present after 5 minutes of stimulation (Table I). However, at 90 minutes of chemotactic stimulation, many neutrophils were round again, while some still displayed a somewhat irregular shape (data not shown). By that time, most neutrophils (~80%) had no platelets attached to their surface, suggesting that they might have shed those previously bound to their uropod.

Activation-dependent neutrophil detachment from surface-adherent platelets. Using an in vitro system with a parallel plate flow chamber, we have previously shown that canine neutrophils can arrest on collagen-attached platelets under flow conditions and that this firm adhesion is a P-selectin–mediated phenomenon. The same system was used in the present study to evaluate P-selectin–dependent neutrophil-platelet interactions in a detachment assay.

For this assay, suspensions of neutrophils were perfused through the chamber containing a platelet-covered surface at a shear stress of 2 dynes/cm². As we observed previously, many of the following neutrophils started interacting with the platelets, and eventually stopped completely on the monolayer. The majority of attached neutrophils appeared spherical, with only occasional cells showing morphologic signs of activation. After a few minutes, flow was stopped to allow neutrophil-platelet interactions under static conditions, and this resulted in increased numbers of neutrophils in contact with platelets. Most of those neutrophils (>80% of adherent cells) rapidly started to display signs of activation such as shape change and polarization (Fig 5A). Polarized neutrophils were observed to bind to the platelets through their uropod (Fig 6). To evaluate detachment of these cells, flow was resumed through the chamber at 2 dynes/cm² and the behavior of attached cells was monitored. Results showed that a high percentage of neutrophils (40.5% ± 8.4% and 51.6% ± 9.8% of human and canine cells, respectively) quickly detached from the platelets and were washed away by the flow (Figs 5B and 7). Moreover, a number of neutrophils that had changed shape were observed to be round
FIG 4. Binding of thrombin-activated canine platelets to resting and chemotactically stimulated canine neutrophils. Platelets were isolated from canine PRP by gel filtration, activated with thrombin, and fixed in 1% paraformaldehyde. Fixed platelets were coincubated with isolated canine neutrophils for 30 minutes at room temperature before activation with 1% ZADS. Scanning electron microscopy of mixed suspensions of platelets and activated platelets shows (A) random distribution of platelets on the surface of unstimulated neutrophils, and (B) clustering of platelets at the uropod on neutrophils after stimulation with a chemotactic factor for 5 minutes (original magnification $\times$ 10,000).

again within 40 seconds (Fig 8), remaining firmly attached to the platelets despite reestablishment of the flow. Those neutrophils and some neutrophils that had not become activated during arrest of the flow stayed firmly attached to the platelets.

DISCUSSION

Using in vitro systems that simulate biologically relevant conditions of dynamic flow, recent studies have demonstrated that flowing neutrophils can be recruited to a site of vascular injury or inflammation by surface-adherent platelets, and that this phenomenon is dependent on P-selectin expressed at the platelet surface. In two of these studies, morphologic evidence of neutrophil activation (shape changes and pseudopodal extensions) were observed after they had arrested on the platelets. However, the consequences of such activation on the interactions of P-selectin with its ligand on neutrophils are unclear. Results of the present study reveal that neutrophil stimulation induces a membrane redistribution of the ligand for P-selectin, leading to its translocation to the uropod of the cell. Such concentration of ligand at the uropod of the cell could modify binding interactions between the two cell types, and might be involved in the detachment of adherent neutrophils from a platelet surface after they have become activated.

FIG 5. Activation of platelet-bound neutrophils after stopping the flow. Platelets adherent to a collagen-coated surface were mounted in a parallel plate flow chamber and perfused for 2 to 3 minutes with PBS only. Isolated neutrophils were then perfused through the chamber at a wall shear stress of 2 dynes/cm². Many flowing neutrophils started interacting with the platelets, and eventually stopped completely on the monolayer. (A) After 2 to 3 minutes, flow was stopped by turning off the pump, and neutrophils were allowed to interact with platelets under static conditions. Increased numbers of neutrophils came in contact with platelets. Many neutrophils displayed signs of activation such as shape change and polarization (arrows show two examples). (B) After a few minutes, flow was reinitiated (2 dynes/cm²), and many neutrophils that had displayed morphologic signs of activation were washed away by the flow. Note that the same area of the flow chamber is shown in (A) and (B).
Flow cytometry analyses showed that P-selectin chimeras containing the lectin domain, the epidermal growth factor–like domain, and one or two complement binding protein domains of human P-selectin recognized in a specific manner (binding could be blocked by an anti–P-selectin antibody) a structure on unstimulated human neutrophils, but not on canine cells. This species-specific binding suggests that differences in the neutrophil determinant recognized by P-selectin exist between species. This finding was unexpected in light of the fact that canine P-selectin displays a high degree of homology at the amino acid level with human P-selectin, especially in the lectin domain (80%) and the epidermal growth factor–like domain (93%), two regions recently shown to play a direct role in ligand recognition by P-selectin. It would therefore be reasonable to assume that the ligands on human and canine neutrophils are also structurally close. Indeed, Lenter et al recently observed binding of human HL60 cells to a murine P-selectin chimera, and conservation of the amino acid sequence between human and murine P-selectin is also high (overall identity, 79%).

Stimulation of neutrophils with a chemotactic agent (FMLP) rapidly resulted in decreased levels of binding of the chimera. This finding is in contrast to earlier studies reporting that activation does not change the number of high-affinity binding sites for P-selectin. The reason for this difference is unclear. However, our results are in agreement with a recent report showing that activation of whole blood causes an immediate increase in neutrophil-platelet conjugates, followed by a decline in the percentage of conjugates over 30 to 60 minutes. The investigators concluded that neutrophil activation decreases expression of the ligand for P-selectin. The decreased expression observed in our study could be due to shedding of the ligand, as is the case for L-selectin on activated neutrophils. Alternatively, it could result from reinternalization of the ligand inside the neutrophil or from a conformational change.

To further investigate the fate of the P-selectin ligand after stimulation, we used an assay of platelet-neutrophil binding that allowed visualization of the events following activation of neutrophils. Since binding of activated platelets to neutrophils is mediated by P-selectin, visualization of platelet-neutrophil associations is a direct reflection of P-selectin–ligand interactions. This assay revealed that neutrophil shape changes associated with chemotactic stimulation are accompanied by a membrane redistribution of the ligand for P-selectin. From an initial random distribution over the entire surface of neutrophils, the platelets were rapidly moved to one side of the ruffled cell and eventually clustered at the uropod. Lorant et al also recently showed the surface redistribution and clustering of P-selectin–coated beads to the uropod of activated neutrophils. A similar redistribution of adhesion molecules in association with activation and shape changes of neutrophils has been reported for CD11b/CD18.
Fig 8. Reversal of shape change of platelet-bound neutrophils after reinitiation of the flow. Platelets adherent to a collagen-coated surface were mounted in a parallel plate flow chamber, and isolated neutrophils were perfused through the chamber at a wall shear stress of 2 dynes/cm². After 2 to 3 minutes, the flow was stopped by turning off the pump, and neutrophils were allowed to interact with platelets under static conditions. (A) Many neutrophils displayed signs of activation such as shape change and polarization (arrows show two examples). (B) After flow was reinitiated, some neutrophils that had displayed morphologic signs of activation remained attached to platelets but reversed to a spherical shape. The time interval between the two photographs was 10 seconds.

bodies, Francis et al. described the transport of CD11b/CD18 (Mac-1) from a uniform distribution on unstimulated cells to the uropod during cell locomotion. In agreement with these studies, Hughes et al. showed the transport of CD11b/CD18 to the uropod in neutrophils stimulated with stepwise increases in chemotactic factors, and demonstrated that the newly expressed CD11b/CD18 at the leading edge of the cell is subsequently used during adherence-dependent migration. Considering this similarity in behavior, it is reasonable to speculate about a possible association between the P-selectin ligand and the CD11b/CD18 integrin. A role for P-selectin in activating CD18-dependent phagocytosis of unopsonized zymosan has recently been shown, and the researchers hypothesized that it could result from a direct interaction of CD18 and P-selectin or its receptor.

Mobilization of CD11b/CD18 from intracellular pools and translocation to the uropod contribute to motility of the cell, and could play an important role in extravasation of neutrophils. Similarly, the translocation of P-selectin ligand to the uropod could contribute to neutrophil motility and locomotion over a platelet-covered surface, for example, an injured vessel wall surface. To determine if this was the case, we used an adherence chamber that allows study of adhesive cell-cell interactions under precisely controlled conditions of flow. With this system, we observed that neutrophils started to display signs of activation and polarization only when interacting with surface-adherent platelets under static conditions (ie, when flow was turned off), but did not demonstrate migratory movements. However, when flow was applied through the chamber, neutrophils that were polarized did not remain attached to the platelets and were rapidly washed away by the flow. A number of bound activated neutrophils reversed to a spherical shape and remained attached to the platelets after initiation of the flow. These results suggest that collagen-adherent platelets release mediators capable of activating neutrophils, but that these soluble mediators are washed away under conditions of flow. Yeo et al. also observed morphologic signs of activation in the majority of platelet-bound neutrophils, but under conditions of flow. They proposed that contact activation or a surface-bound cytokine might play a role in neutrophil activation. The reasons for this difference are unclear, but might include differences in the flow system used and in the experimental shear stress applied through the chamber (<1 dyne/cm² in their report vs 2 dynes/cm² in this study). Washing away of soluble mediators would result in neutrophils reversing from a polarized to a round morphology, as described by Zigmond et al., who observed neutrophils changing in shape from polarized to round after they replaced a chemotactic peptide solution with a neutral solution.

The observation that polarized neutrophils were binding to surface-adherent platelets through their uropod also suggests that activation of bound neutrophils by mediators secreted by platelets induces translocation of P-selectin ligands to the uropod of the cell, as observed with platelets attached to chemotactically stimulated neutrophils in suspension. This concentration of P-selectin-ligand at the uropod of the cell could result in binding interactions too weak to keep neutrophils firmly attached to platelets when flow is reinitiated. Decreased adhesion of activated neutrophils to P-selectin has also been recently described. Under in vivo conditions of flow such as those found at areas of vascular injury, washing of the mediators secreted by adherent platelets could prevent neutrophil activation and preclude their detachment from the site of damage. The mechanisms responsible for the dissociation can only be speculative for the moment. Possible scenarios include shedding of the complex P-selectin-ligand as a whole (with shedding of a small part of membrane), dissociation of P-selectin from its ligand, leaving the latter on the neutrophil surface, or dissociation of P-selectin from its ligand but with reinternalization of the ligand. Downregulation of L-selectin occurs in response to neutrophil activation, and is part of an important cascade of events for neutrophil emigration. A recent report by Kahn et al. clearly demonstrated the existence of a cleavage site for L-selectin at a
membrane proximal site, leaving a 6-kD transmembrane fragment on activated neutrophils. A similar mechanism has also been reported for other leukocyte membrane proteins, including CD43 and tumor necrosis factor receptor.

Rapid loss of L-selectin expression has also been induced by cross-linking of surface L-selectin, a mechanism that could serve as a specific way of regulation dependent on the interaction of the adhesion receptor with its ligand.

Despite sustained interest and numerous investigations, much remains to be known about the neutrophil structure displaying high-affinity recognition for P-selectin on activated platelets and endothelial cells. Among the various glycoproteins proposed as potential ligands, PSGL-1 has recently been shown to be localized to microvilli on neutrophils and be required for the rolling of neutrophils on P-selectin at physiologic shear stresses. Results of the present study show that activation of neutrophils induces changes in surface expression and redistribution of the molecular determinant on neutrophils responsible for binding P-selectin on activated platelets (possibly PSGL-1), and suggest that this phenomenon can have direct consequences on adhesive interactions between the two cell types. Increased understanding of the biology of the P-selectin ligand will help in understanding the complex mechanisms regulating neutrophil-platelet interactions during hemostatic and inflammatory processes.

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