P-selectin binding to P-selectin glycoprotein ligand-1 induces an intermediate state of αMβ2 activation and acts cooperatively with extracellular stimuli to support maximal adhesion of human neutrophils

Yan-Qing Ma, Edward F. Plow, and Jian-Guo Geng

Introduction

The recruitment of leukocytes to the site of inflammation entails a cascade of cellular adhesive events, which include tethering (initial attachment), rolling, firm adhesion, and transendothelial migration of the responding cells. Members of the selectin family of cell adhesion molecules expressed on the endothelial surface interact with their cognate glycoprotein ligands on leukocytes to mediate the tethering and rolling phase of leukocyte recruitment; that is, the weak adhesive interactions. Members of the β2 subfamily of leukocyte integrins, as well as other integrin subfamilies, recognize their cognate ligands to then mediate the firm adhesion (arrest) of leukocytes. The initial weak adhesion brings leukocytes into proximity of cytokines/chemoattractants displayed on or released from the activated endothelium, such as interleukin 8 (IL-8) and platelet-activating factor (PAF). These transduce signals through their G protein–coupled receptors that activate the integrins to induce the firm leukocyte adhesion to the endothelium. Such integrin-mediated firm adhesion can occur through direct ligand engagement or indirect, bridging mechanisms. For example, activated β2 integrins can bind various members of intercellular cell adhesion molecule (ICAM) family on endothelial cells to support leukocyte adhesion and transmigration. As an example of the bridging mechanism, integrin αMβ2 can recognize fibrinogen or fibrin deposited on the endothelial surface or at the sites of inflammation to promote the accumulation of leukocytes. Fibronectin engagement by activated αMβ2 is also one of the several mechanisms that contribute to formation of platelet-leukocyte conjugates, which are diagnostic of thrombotic events in vivo.

P-selectin, a member of the selectin family, is stored on the membranes of platelet α granules and endothelial Weibel-Palade bodies. On inflammatory and thrombogenic challenges, P-selectin rapidly translocates to the surface of these cells and contributes to the weak adhesion of leukocytes to stimulated endothelial cells and the heterotypic aggregation of activated platelets to leukocytes. A principal leukocyte ligand for P-selectin is P-selectin glycoprotein ligand 1 (PSGL-1), a disulfide bond-linked homodimer, with its G protein–coupled receptors that activate the integrins to induce the firm leukocyte adhesion to the endothelium. Such integrin-mediated firm adhesion can occur through direct ligand engagement or indirect, bridging mechanisms. For example, activated β2 integrins can bind various members of intercellular cell adhesion molecule (ICAM) family on endothelial cells to support leukocyte adhesion and transmigration. As an example of the bridging mechanism, integrin αMβ2 can recognize fibrinogen or fibrin deposited on the endothelial surface or at the sites of inflammation to promote the accumulation of leukocytes.
as several extracellular matrix proteins. Ligand recognition by αMβ2 is influenced by the activation state of the receptor. Integrin activation is induced by either a conformational change within each receptor, which increases apparent affinity for ligand, or integrin clustering, which enhances avidity for ligand. At the extreme, activation of an integrin allows it to engage soluble ligands, whereas binding to the unactivated integrin cannot be demonstrated. Such activation is induced physiologically by ligation of an agonist receptor, which initiates an intracellular pathway that ultimately transmits a signal to the cytoplasmic tails of the integrin. This signal, an inside-out signaling, is then transmitted across the membrane to activate the downstream signaling molecules.

Recent studies suggest that P-selectin binding to its counterreceptor PSGL-1 promotes αMβ2-dependent homotypic neutrophil aggregation and neutrophil-platelet conjugation. αMβ2-dependent adhesion of monocytes to vascular cell adhesion molecule 1 (VCAM-1), responses typically dependent on integrin activation. Hirata et al observed that engagement of PSGL-1 enhances tyrosine phosphorylation, activates mitogen-activated protein (MAP) kinase (ERK-1 and ERK-2) through MEK (MAP kinase kinase), and stimulates IL-8 secretion in neutrophils. Although these events are typically associated with integrin activation, functional activation of β2 integrins in human neutrophils was not detected. An earlier report also concluded that PSGL-1 ligation was not sufficient to activate the β2 integrins on human neutrophils. In addition, Blanks et al reported that P-selectin induced β2 integrin-mediated cell attachment to ICAM-1 by mouse but not human neutrophils. Thus, the relationship between PSGL-1 ligation and αMβ2 activation remains uncertain.

In the present study, we demonstrate that the binding of P-selectin to PSGL-1 results in a moderate clustering and a partial activation of αMβ2, thus enhancing adhesion and binding of human neutrophils to immobilized, but not to soluble, fibrinogen and the αMβ2 activation-specific monoclonal antibody (mAb) CBRM1/5. PAF or IL-8 acts in concert with P-selectin for further activation of αMβ2. In contrast to phorbol myristate acetate (PMA), no changes in the level of αMβ2 are observed. Hence, our data suggest that PSGL-1 engagement by P-selectin defines an intermediate state of αMβ2 activation, which can be further activated by extracellular stimuli for maximal adhesion of human neutrophils to cognate αMβ2 ligands.

Materials and methods

Proteins and antibodies

Recombinant human P-selectin immunoglobulin chimera (P-sel Ig) was prepared as previously described. Recombinant soluble human P-selectin (sP-selectin), which was previously characterized as a monomer, was purchased from R&D Systems (Minneapolis, MN). Recombinant human IL-8 was purchased from Calbiochem (La Jolla, CA). The endothelin levels in these reagents were less than 1 endothelin unit (EU) per microgram recombinant protein determined by the lumines amebocyte lysate (LAL) from Cape Cod. Plasminogen-depleted human fibrinogen was from Enzyme Research Laboratories (South Bend, IN). G1 (a leukocyte adhesion-blocking IgG1 mAb to P-selectin) and PS1 (a leukocyte adhesion-blocking IgG1 mAb to P-selectin) were prepared and characterized as before. KPL-1 (a leukocyte adhesion-blocking IgG1 mAb to PSGL-1) and PL2 (a leukocyte adhesion-blocking IgG1 mAb to PSGL-1) were kindly provided by Dr Rodger P. McEver (W. K. Warren Medical Research Institute, University of Oklahoma Health Sciences Center, Oklahoma City). Fab(‘)2 fragments of G1, PS1, and PL1 and Fab fragment of PL1 were prepared using ImmunoPure Fab(‘)2 and Fab preparation kits (Pierce, Rockford, IL). IB4 (a leukocyte adhesion-blocking mAb to β2 subunit), 44a (a leukocyte adhesion-blocking mAb to αM subunit), and OKM1 (a leukocyte adhesion-blocking mAb to αM subunit) were purchased from American Tissue Culture Collection (ATCC; Manassas, VA). CBRM1/5 (a mAb specific for an activation-dependent epitope on αM subunit) was a generous gift from Dr Timothy A. Springer (Center for Blood Research, Harvard Medical School, Boston, MA). Alexa Fluor 488–conjugated goat antibody to mouse IgG (H+L; cross-absorbed) was purchased from Molecular Probes (Eugene, OR). Alexa Fluor 488–conjugated fibrinogen was prepared using Alexa Fluor 488 protein-labeling kit (Molecular Probes). Human and mouse IgG, PAF, and PMA were purchased from Sigma (St Louis, MO).

Neutrophil preparation

Fresh human blood was collected from healthy, adult volunteers with their informed consent into acid-citrate-dextrose (ACD; 38 mM citric acid, 75 mM trisodium citrate, and 100 mM dextrose; 1:2 ACD to whole blood). After removal of platelet-rich plasma by centrifugation of the whole blood at 100g at 22°C for 15 minutes, the cell pellet was diluted with ice-cold phosphate-buffered saline (PBS), pH 7.4, to original blood volume and layered onto one-third volume of Ficoll-Paque Plus (Amersham Biosciences, Piscataway, NJ) and centrifuged at 200g at 18°C for 12 minutes. The supernatant and interface were removed, and ice-cold PBS was added to restore the original volume. The cell suspension was then mixed with one-third volume of 6% dextran (Sigma) in PBS, and the erythrocytes were allowed to settle at 22°C for 30 minutes. The top layer was collected and was centrifuged at 500g at 4°C for 5 minutes. After hypotonic lysis of residual erythrocytes with cold H2O, neutrophils were resuspended in ice-cold PBS at about 10 × 10^6 /mL for immediate use. The purity of neutrophils was routinely more than 95% and viability was greater than 96% measured by trypan blue exclusion. The endotoxin levels in all buffers used were less than 0.03 EU/mL determined by the LAL method. Approval for these studies was obtained from the Cleveland Clinic Foundation institutional review board. Informed consent was provided according to the Declaration of Helsinki.

Adhesion assay

Fibrinogen (20 μg/mL) in PBS was immobilized on the 96-well tissue culture plates (0.1 mL/well; Costar, Cambridge, MA) at 4°C overnight. The wells were postcoated with 0.5% polyvinyl alcohol (Sigma) at 22°C for 1 hour and washed twice with PBS. Neutrophils were resuspended at 2 × 10^6/mL in Hanks balanced salt solution, pH 7.4, 1.2 mM CaCl2, and 0.8 mM MgCl2 supplemented with 1% bovine serum albumin (HBSS/Ca/Mg/BSA). The cells were incubated with various testing agents, such as 10 μg/mL human IgG, P-sel Ig chimera, sP-selectin, mouse IgG, KPL-1 mAb, PL1 mAb, PL2 mAb, PL1 Fab, PL1 Fab(‘)2, 300 nM PAF, 0.3 μg/mL IL-8, or 8 nM PMA, for 2 minutes and then added to the coated wells (0.1 mL/well). For antibody inhibition experiments, 10 μg P-sel Ig chimera was preincubated with 20 μg G1 or PS1 Fab(‘)2, or 300 nM HBSS/Ca/Mg/BSA for 22°C at 15 minutes. Alternatively, neutrophils were preincubated with 15 μg/mL mouse IgG, mAb 44a, and mAb IB4. After incubation at 37°C for 25 minutes in a 5% CO2 humidified atmosphere, nonadherent cells were carefully removed by washing 3 times with PBS. The adherent cells were quantified using a myeloperoxidase (MPO) assay as described previously. MPO activity was converted to neutrophil numbers using a standard curve that was generated by measurements of enzyme activity derived from a series dilution of known numbers of neutrophils.
To test the effect of P-selectin on αMβ2 activation, we analyzed changes in the adhesion of human neutrophils to immobilized fibrinogen. Freshly isolated human neutrophils were added together with various concentrations of P-sel Ig chimera to fibrinogen-coated wells of tissue culture plates. After incubation at 37°C for 25 minutes, unbound cells were removed by washing with PBS, and bound neutrophils were quantified on the basis of their MPO activities. As shown in Figure 1A, P-sel Ig chimera enhanced neutrophil adhesion to fibrinogen in a concentration-dependent manner. A maximal effect was observed at 10 μg/mL. Over the course of 12 such experiments, the increment in neutrophil adhesion to fibrinogen induced by this concentration of P-sel Ig chimera was increased by about 3-fold although there was considerable variability among donors.

To verify the specificity of this effect, that is, the roles of P-selectin and αMβ2, P-sel Ig chimera was added together with antibodies to their cell surface receptors. When P-sel Ig chimera was preincubated with F(ab’)2 fragments of G1 (as a function blocking IgG1 mAb to P-selectin) or PS1 (a nonblocking IgG1 mAb against P-selectin), the increment in neutrophil adhesion was blocked selectively by G1 (Figure 1B). Similarly, when neutrophils were preincubated with IB4 (a mAb to the integrin-β2 subunit that blocks leukocyte adhesion) or 44a (a blocking mAb to the integrin-αM subunit), the increment in neutrophil adhesion induced by P-sel Ig chimera was completely inhibited, whereas the mouse IgG had no detectable effect. This inhibition pattern indicates that (1) P-selectin specifically increases neutrophil adhesion and (2) the augmented adhesion is mediated by αMβ2.

To evaluate the role of PSGL-1, the ligand for P-selectin, in the observed response, experiments were performed with anti–PSGL-1 mAbs. However, addition of KPL-1 and PL1, 2 blocking mAbs to PSGL-1, or PL2, a nonblocking PSGL-1 mAb, all increased neutrophil adhesion to fibrinogen by about 2-fold even in the absence of P-sel Ig chimera (Figure 2). This increment was blocked by IB4, confirming β2-integrin involvement. Control mouse IgG did not enhance cell adhesion (Figure 2). Thus, the mAb interaction with PSGL-1 is sufficient to induce functional up-regulation of αMβ2.

To exclude that the observed effects of P-sel Ig chimera were due to traces of endotoxin in the reagents, we tested the direct effect of lipopolysaccharide (LPS) on neutrophil adhesion to fibrinogen. Concentrations as high as 10 EU/mL LPS (>10-fold higher than in the reagents) did not increase the adhesion of responding cells (data not shown).

**Results**

**P-selectin increases αMβ2-mediated adhesion of neutrophils to fibrinogen**

Flow cytometric assay

Neutrophils, resuspended at $2 \times 10^6$/mL in HBSS/Ca/Mg/BSA, were incubated with 10 μg/mL human IgG, P-sel Ig chimera, 300 nM PAF, 300 ng/mL IL-8, or 8 nM PMA at 22°C for 2 minutes followed by 3 μg/mL mAb IB4 or CBRM1/5 and an Alexa Fluor 488–conjugated goat antibody to mouse IgG (cross-absorbed; 1:1000 dilution) at 37°C for 25 minutes in a 5% CO₂-humidified atmosphere. Alternatively, neutrophils were incubated with 60 μg/mL Alexa Fluor 488–conjugated fibrinogen. The cells were centrifuged through a cushion of fetal calf serum and resuspended in 1% paraformaldehyde in PBS. Cell-bound antibodies or fibrinogen were detected by fluorescent-activated cell sorting (FACScan; Becton-Dickinson, San Jose, CA).

Confocal microscopy

Freshly isolated human neutrophils were incubated with buffer alone, 10 μg/mL human IgG, P-sel Ig chimera, 300 ng/mL IL-8, 8 nM PMA, 300 nM PAF, or 300 nM PAF plus 10 μg/mL P-sel Ig chimera in HBSS/Ca/Mg/BSA at 37°C for 25 minutes. The cells were fixed with 4% paraformaldehyde for 15 minutes at 22°C. After washing 3 times with PBS, samples were incubated with IB4 or OKM-1 mAb (10 μg/mL) in PBS/BSA (PBS supplemented with 1% BSA) and an Alexa Fluor 488–conjugated goat antibody to mouse IgG (cross-absorbed; 1:1000 dilution) at 22°C for 30 minutes. Following washing with PBS, the cells were mixed with the same volume of Vectashield mounting medium (Vector Laboratories, Burlingame, CA) containing DAPI (4’,6-diamidino-2-phenylindole) and dropped onto positively charged slides followed by mounting coverslips. The samples were then sealed with nail polish and observed under a confocal laser scanning microscope (Leica TCS SP2; Heidelberg, Germany) using HCX PL APO lens at 63 × 8 magnification, numerical aperture at 1.4, and Leica Confocal Software version 2. The relative fluorescence intensity was calculated using ImagePro Plus (version 4.5; Media Cybernetics, Silver Spring, MD).

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Role of P-selectin multimerization in αMβ2 activation

Because P-sel Ig chimera and PSGL-1 mAbs are dimers, they may cross-link PSGL-1. Because native P-selectin, isolated from human platelets and endothelial cells exists as dimeric and oligomeric forms even at the detergent concentrations well above the critical micelle concentration,29,30 such cross-linking of PSGL-1 has clear biologic relevance. To test whether cross-linking of PSGL-1 was important for αMβ2 activation, we examined the capacity of monomeric sP-selectin to activate the integrin. In contrast to the multimeric P-sel Ig chimera, monomeric sP-selectin (10 μg/mL) did not enhance neutrophil adhesion to immobilized fibrinogen (Figure 3A). Moreover, when the F(ab')2 fragment of anti-human IgG antibody (Fc specific) was added together with P-sel Ig chimera, the combination induced more extensive adhesion than P-sel Ig chimera alone, consistent with a role of cross-linking of PSGL-1 in αMβ2 activation (Figure 3A). As controls, sP-selectin added with the anti-human IgG antibody failed to stimulate neutrophil adhesion to fibrinogen (data not shown). To further verify that PSGL-1 cross-linking is necessary for αMβ2 activation, we compared the activity of F(ab')2 and Fab fragments of PL1 to stimulate neutrophil adhesion to fibrinogen. As shown in Figure 3B, the former but not the latter reagent induced cell adhesion. Together, these data suggest that engagement of PSGL-1 alone is not sufficient for αMβ2 activation, but engagement and cross-linking of PSGL-1 by dimers or higher multimers of P-selectin or mAbs to PSGL-1 will induce activation of the integrin.

Consequences of PSGL-1 ligation with P-selectin on αMβ2

We next characterized the basis for the functional up-regulation of αMβ2 on treatment of neutrophils with P-sel Ig chimera. Enhanced adhesion to fibrinogen may arise from changes in expression levels or activation of the integrin by either affinity or avidity modulation. To assess whether P-sel Ig chimera increased the expression level of αMβ2, FACS analyses were performed on neutrophils with or without P-sel Ig chimera treatment using mAb IB4. This mAb to β2 subunit reports on the level of αMβ2. As shown in Figure 4A, P-sel Ig chimera failed to increase αMβ2 expression. Treatment of neutrophils with PMA did enhance mAb IB4 reactivity, providing a positive control. To assess changes in activation, mAb CBRM1/5 was used, which reacts with an activation-dependent epitope on the αM subunit. Whereas treatment of the cells with PMA resulted in up-regulation of the CBRM1/5 epitope in FACS analyses, stimulation with P-sel Ig chimera did not (Figure 4A). In view of these results, we also tested whether we could detect the binding of soluble fibrinogen to αMβ2 by FACS. As shown in Figure 4B, fibrinogen bound poorly to nonstimulated neutrophils or to neutrophils stimulated with P-sel Ig chimera. However, when the cells were stimulated with PMA, binding of soluble fibrinogen could be detected. Furthermore, in examining the adhesion of neutrophils to immobilized fibrinogen, we observed that soluble fibrinogen inhibited the interaction of PMA-stimulated, but not P-sel Ig chimera-stimulated, neutrophils (Figure 4C). Taken together, these data suggest that (1) nonstimulated neutrophils express αMβ2 in a state that it can neither adhere to fibrinogen nor bind it as a soluble ligand; (2) P-sel Ig chimera induces activation of αMβ2 to a state where it is competent to support neutrophil adhesion to immobilized, but not soluble, fibrinogen; and (3) PMA can lead to an αMβ2 activation state, in which the integrin can mediate fibrinogen recognition as both soluble and adhesive ligand.
To validate and generalize these observations, we tested whether P-sel Ig chimera could induce binding of neutrophils to immobilized mAb CBRM1/5. As shown in Figure 4D, stimulation of neutrophils with P-sel Ig chimera supported binding to CBRM1/5. This interaction was inhibited by the blocking P-selectin mAb, G1, but not by the nonblocking mAb, PS1. Thus, P-sel Ig chimera can induce αMβ2 activation to a state where insoluble but not soluble activation-dependent ligands can be recognized.

To further characterize the activation state of αMβ2 induced by P-sel Ig chimera, we assessed the distribution of the integrin on the cell surface. Nontreated neutrophils or neutrophils exposed to human IgG showed a weak rim and punctuate staining with either mAb IB4 to the β2 subunit or mAb OKM1 to the αM subunit. The image intensity was adjusted to a point where the rim staining was not detectable but the punctuate staining was. Under this condition, mAb IB4 (Figure 5A,Bi) and mAb OKM1 (Figure 5A,Bii) showed only a weak punctuate staining pattern. In contrast, PMA stimulation induced considerably brighter and larger patches of staining (Figure 5A). With P-sel Ig chimera stimulation, an intermediate staining was observed (Figure 5A). The patches were larger and more abundant than those in control cells. However, they tended to be fewer and less intense than those treated with PMA (Figure 5A-B). These data are consistent with the induction of a variable degree of clustering of αMβ2 on the P-sel Ig chimera and PMA-stimulated neutrophils.

**Additive effects of P-selectin with PAF or IL-8 on αMβ2 activation**

In the sequence of reactions that mediate leukocyte adhesion to endothelium, engagement of P-selectin occurs in a microenvironment rich in other neutrophil stimuli, notably IL-8 and PAF. According to this, we considered whether stimulation of neutrophil adhesion by P-selectin was influenced by these mediators. As shown in Figure 6A, similar to P-sel Ig chimera, PAF or IL-8 (concentrations inducing maximal effects are shown) increased adhesion of neutrophils to fibrinogen. The extent of the increment in adhesion induced by PAF or IL-8 was similar to that induced by P-sel Ig chimera. In combination with these agonists, P-sel Ig chimera further enhanced the extent of adhesion to fibrinogen. In contrast to P-sel Ig chimera, PAF did enhance the binding of mAb IB4, indicating an increase in integrin expression and did induce
the activation epitope recognized by CBRM1/5 (Figure 6B). In contrast, IL-8 induced only a slight increment (~10% compared to control) in the binding of either IB4 or CBRM1/5, indicating that IL-8 did not significantly alter integrin expression or conformation (Figure 6B). Addition of soluble fibrinogen partially inhibited the adhesion of the neutrophils induced by PAF alone or PAF plus P-selectin Ig chimera but did not inhibit the adhesion supported by IL-8 alone or IL-8 plus P-selectin Ig chimera (data not shown).

When changes in integrin distribution on the cell-surface of αMβ2 were evaluated by mAb IB4 immunofluorescence staining (Figure 6C-D), PAF alone failed to induce an apparent change in the cell surface distribution of the integrin compared to nonstimulated cells. However, treatment with a combination of PAF plus P-selectin Ig chimera but did not inhibit the adhesion supported by IL-8 alone or IL-8 plus P-selectin Ig chimera (data not shown).

In this study, we have shown that ligation of PSGL-1 by P-selectin leads to increased susceptibility to infections.44 These data are consistent with the role of the pathway that we have defined in leukocyte biology. Studies using PSGL-1 tailless or cytoplasmic domain mutants in knock-in mice may provide a definitive answer to the biologic importance of these cooperative signaling events.

The binding of P-selectin to PSGL-1 reportedly enhances tyrosine phosphorylation, activates mitogen-activated protein kinases (MAP kinases; ERK-1 and ERK-2) through MAP kinase kinase (MEK), and stimulates IL-8 secretion in human neutrophils.31 In addition, neutrophil tethering mediated by E-selectin can activate β2 integrins through a MAP kinase signal transduction pathway.45 In contrast, cytokines and chemotactic agents, such as PAF and IL-8, are synthesized by endothelial cells or displayed on their cell surface. They bind to their G protein–coupled receptors (GPCRs) and induce signaling that impinges on integrin cytoplasmic domains for activation of β2 integrins.46-47 Consistent with these concepts, our data that P-selectin–induced intermediate state of αMβ2 activation can be further activated by PAF or IL-8 also indicate that P-selectin and PAF/IL-8 may activate β2 integrins through distinct signaling pathways.

PSGL-1 is a homodimer covalently linked by a disulfide bond between a single extracellular cysteine in each subunit.17 The dimerization of PSGL-1 is functionally important because elimination of dimerization by mutation of the cysteine residue prevents the binding of P-selectin to PSGL-1–expressing cells48 and inhibits rolling of PSGL-1–expressing cells on immobilized P-selectin under flow.49 Consistent with an earlier observation PSGL-1 mAbs induce rapid phosphorylation,51 our data showing that PSGL-1 cross-linking by dimeric or higher oligomeric forms of P-selectin is obligatory for αMβ2 activation, indicate that the formation of PSGL-1 oligomers is essential for key signal transduction events. Such an oligomerization requirement is a common scheme in many signaling cascades in which ligand-induced dimerization or oligomerization is required (eg, various epidermal growth factors30 and GPCRs31). In this context, we should point out that native P-selectin, isolated from human platelets and endothelial cells exists as dimeric and oligomeric forms.39,40 Furthermore, using immunoelectron microscopy, we found that P-selectin molecules were not evenly distributed on the surface of human umbilical vein endothelial cells (HUVECs) following stimulation with tumor necrosis factor α (Y.-Q.M. and J.-G.G., unpublished observations, April 2002). Instead, the P-selectin molecules were clustered in sparse, but discrete patches, similar to the PSGL-1 patches we observed on neutrophils. Thus, the ligand for PSGL-1 on neutrophils may be presented in the multimeric state on the endothelium, which is requisite for αMβ2 activation.

Detailed structural studies of integrins at the atomic level have led to the suggestion that these adhesion receptors may exist in at least 3 conformational states: (1) an active or high-affinity state with an open conformation; (2) a transiently active, low-affinity state with an intermediate conformation; and (3) a resting, low or undetectable affinity state with a closed conformation.23,24 Both the open and intermediate conformations are competent for cell adhesion. However, the open conformation, but not the intermediate conformation, is critically required for the binding of soluble ligands. Accordingly, P-selectin–mediated adhesion of human neutrophils to immobilized, but not soluble, fibrinogen and mAb CBRM1/5 appears to suggest that P-selectin binding to PSGL-1 can only induce an intermediate state of neutrophil activation in which the functions of αMβ2 are partially but not fully enhanced. The finding that P-selectin does not trigger increased expression of
αMβ2 but potentiates αMβ2 activation cooperatively with PAF or IL-1 is consistent with this proposition. As an alternative explanation, the capacity of P-selectin to evoke adhesion but not soluble ligand recognition may mainly result from the avidity modulation due to receptor clustering, instead of the affinity modulation due to receptor conformational change. These possibilities are not mutually exclusive because evidence indicates that integrins within clusters undergo conformational activation. The intermediate state of αMβ2 induced by P-selectin may preclude occupancy of the integrin by an overwhelmingly large excess of soluble and potentially competitive ligands in the circulation and instead limit occupancy to the target ligands presented on the surface of the endothelium.2,3 This intermediate state of neutrophil adhesion induced by P-selectin/PSGL-1 interaction may be analogous to the adhesion of cells to matricellular proteins, including thrombospondins, tenascins, and SPARC,33 which allow cells to transit either to a nonadhesive or firmly adhesive phenotype, depending on the additional chemical and biophysical signals within their surrounding environments. For leukocytes such transitions to firm adhesion (arrest) or de-adhesion may be a process that is essential for transmigration. P-selectin–mediated activation of αMβ2 to an intermediate rather than a fully activated state may act as a physiologically important gatekeeper, fine tuning the fate of leukocytes during their trafficking and recruitment in vivo.

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