Role of shear forces and adhesion molecule distribution on P-selectin-mediated leukocyte rolling in postcapillary venules

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Kim, Michael B., and Ingrid H. Sarelius. Role of shear forces and adhesion molecule distribution on P-selectin-mediated leukocyte rolling in postcapillary venules. Am J Physiol Heart Circ Physiol 287: H2705–H2711, 2004. First published August 26, 2004; doi:10.1152/ajpheart.00448.2004.—Rolling on the venular endothelium is a critical step in the recruitment of leukocytes during the inflammatory response. P-selectin is a key mediator of leukocyte rolling, which is an early event in the inflammatory cascade; this rolling is likely to be directly regulated by both local fluid shear forces and P-selectin site densities in the microvasculature. However, neither the spatial pattern of P-selectin expression in postcapillary venules nor the effect of local expression patterns on rolling behavior in intact functional venules is known. We investigated the influence of local shear forces and the spatial distribution of endothelial P-selectin in intact blood perfused post capillary venules in anesthetized mice using intravital confocal microscopy, high temporal resolution particle tracking, and immunofluorescent labeling. We demonstrated a shear-dependent increase in average leukocyte rolling velocity that was attributable to a shear-dependent increase in the occurrence of transient leukocyte detachments from the endothelial surface: translational velocity during leukocyte contact with the vessel wall remained constant. P-selectin expression was not different in venules with characteristically different shear rates or diameters but varied significantly within individual venules. In postcapillary venules, regions of high P-selectin expression correlated with regions of slow leukocyte rolling. Thus the characteristically variable leukocyte rolling in vivo is a function of the spatial heterogeneity in P-selectin expression. The study shows how the local hydrodynamic forces and the nonuniform pattern of P-selectin expression affect the behavior of interacting leukocytes, providing direct evidence for the local variation of adhesion molecule expression as a mechanism for the regulation of leukocyte recruitment.

leukocyte recruitment; leukocyte adhesion cascade; adhesion molecule expression in vivo; venular hemodynamics

LEUKOCYTE RECRUITMENT during the inflammatory response is initiated by selectin-dependent tethering and rolling on the endothelial surface. A critical role for P-selectin, particularly in mediating rolling during the early phase of inflammatory responses, has been demonstrated in studies using P-selectin knockout mice (17, 22) or function blocking antibodies (5, 15, 19). Regulation of the leukocyte rolling response is critical to the overall regulation of the inflammatory cascade, because it serves as a precursor to adhesion and transmigration, all of which are necessary for an effective inflammatory response.

Selectin-mediated leukocyte rolling is characterized by transient adhesion and detachment that has been described as a series of ratchet-like steps (27). Reported values of leukocyte rolling velocity cover a wide range, even in well-defined in vitro systems (8); P-selectin-mediated rolling is typically described as highly variable. The erratic nature of leukocyte rolling is even more prominent in vivo, where the velocity and concentration of rolling leukocytes in postcapillary venules can vary with position and time (6, 16). This raises the important question of what determines this variability, and its impact on expected leukocyte-endothelial interactions. Factors that are likely to be primarily responsible for this variability in leukocyte rolling behavior are variations in shear-related binding kinetics due to local differences in shear forces in vivo and localized variations in adhesion molecule expression levels. Understanding the contributions of each of these to leukocyte rolling behavior in vivo is an essential step in understanding the manifestation of the inflammatory response in the native blood-perfused venular environment.

Shear dependence of leukocyte rolling has been reported both in isolated cell systems (8, 18) and in vivo (4, 13). The shear dependence of selectin bond kinetics (2, 3) suggests that leukocyte rolling velocity should be predictable from local shear forces. However, wide ranges of translational velocities can be observed for a single rolling leukocyte in a constant shear environment (6), suggesting the involvement of additional influences on leukocyte rolling behavior. Importantly, the effects of local shear forces on leukocyte rolling behavior in vivo are somewhat controversial in that some studies report a strong flow dependence of rolling velocity (4, 13), whereas elsewhere it has been reported that while mean rolling velocity varies approximately linearly over a wide range of shear rates, there is in fact substantial variability in velocity at any given shear rate and relative insensitivity to flow at lower shear stresses (6).

In addition to the possible contributions of local variations in shear-dependent behavior (24), leukocyte rolling interactions with the venular wall will necessarily be influenced by the presence of the appropriate adhesion molecule on the endothelial surface. Observations of clustering of rolling leukocytes in localized vessel regions and local similarities in rolling velocity in different venules have led to the suggestion that selectin expression might differ in different venular regions (6, 16). Variability in adhesion molecule expression has been reported between vessels of different organ systems (7) and in whole mount preparations of mouse cremaster microvasculature (10). These studies suggest that regions of variable adhesion molecule (selectin) expression are inherent to the intact microvasculature and that such differences in selectin density could locally alter the behavior of interacting leukocytes. To date, this hypothesis has not been tested directly.

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Thus the goals of this study were twofold. The first was to determine how the erratic nature of rolling behavior is related to the fluid shear forces experienced in the native venular environment, and the second was to test whether the spatial distribution of P-selectin in these vessels varied in a way that could directly contribute to leukocyte rolling events in vivo.

MATERIALS AND METHODS

Animals. All procedures were approved by the institutional review board of the University of Rochester. Male C57Bl/J mice (Jackson Laboratories), between 10 and 14 wk old and weighing 25–35 g, were prepared for intravital microscopy of the cremaster muscle as previously described (12, 13). Animals were anesthetized with pentobarbital sodium (75 mg/kg) and surgically prepared for microscopy, which included a tracheotomy, catheterization of the jugular vein, and exteriorization of the cremaster muscle. The muscle was superfused with physiological saline solution, and both the tissue temperature and animal anesthetic level were monitored throughout the experiment. At the completion of protocols, animals were euthanized by anesthetic overdose.

Intravital microscopy. Images of venules were acquired at 30 frames/s using an Olympus BX50WI microscope through an Olympus PlanFl immersion objective (×40, 0.80 numerical aperture). Images were recorded to 3/4-in. videotape (SONY VO9600) for off-line analysis. Bright-field images used to track leukocyte interactions with the venular wall were acquired with a charge-coupled device (CCD) video camera (Dage MTI, CCD725S). Fluorescence images were acquired through a Nipkow disk scanning confocal head (Yokogawa) connected to an intensified CCD camera (XR Mega10, Solamere Technology Group) using a 20-mW argon laser (National Laser) to provide excitation at 488 nm; laser power and camera gain settings were held constant for all image acquisition. In protocols where images of leukocyte interactions were matched to fluorescence images in the same venule, bright-field images were collected through the confocal light path using the XR Mega 10 intensified CCD camera.

Immunofluorescence labeling. P-selectin, expressed on the endothelial surface of intact blood-perfused microvessels, was labeled by localized perfusion using micropipette cannulation. Cannulating micropipettes were pulled from borosilicate capillary tubes (WPI) and beveled on an extra fine grinding wheel (Sutter) to create a needle-like tip with an 8–12-μm-diameter opening. Pipettes were filled with the appropriate solutions and connected to a pressure reservoir. A hydraulic micromanipulator (Narishige) was used to position the sharpened pipette and cannulate an arteriole feeding the microcirculatory network of the cremaster muscle, thus allowing the complete perfusion of cremaster microvessels with the pipette contents. When necessary, a blunted glass microoccluding rod was placed using a second micro manipulator and was used to reduce blood flow into the cannulated arteriole to eliminate the presence of blood from the perfused solution. A primary monoclonal antibody against P-selectin (50 μg/ml, RB40.34, BD Biosciences) was used instead of the primary antibody in the dual-antibody perfusion sequence: these controls showed no detectable fluorescent labeling above background (mean intensity of the sampled control tissue was 25.4 ± 2.8 vs. 24.8 ± 2.9 grayscale units sampled on a glass surface without the tissue). To further define the overall variability inherent in confocal imaging of the immunofluorescent labeled P-selectin, we also imaged P-selectin that had been uniformly coated onto an artificial surface and then labeled with the primary and secondary antibodies as described above. The standard deviation of P-selectin intensity imaged as described above was 11.6% of the mean pixel intensity.

Data acquisition and analyses. Videotaped sequences were digitized to 8-bit tiff images using a CG-7 frame grabber (Scion) on an Apple G4 computer and analyzed with NIH Image (version 1.61). The hydrodynamic critical velocity as defined by Ley and Gaechtgens (20) was used to identify rolling leukocytes. A rolling leukocyte was defined as one that was seen to be translating along the venular wall in contact with the endothelium and was moving more slowly than the critical hydrodynamic velocity. Translational velocity for both leukocytes and fluorescent flow tracers (0.5 μm diameter, Fluorescein, Polysciences, injected through a jugular catheter in tracer quantity) was calculated from displacements over defined time intervals. Newtonian wall shear rate was calculated as $8V_{avg}/D$ from the measured bead velocities ($V_{avg}$) and vessel diameter ($D$) (12, 13, 20). Bead velocities were also used to calculate the critical velocity as above. P-selectin expression levels were analyzed (using NIH Image) from confocal images of axial cross sections of each labeled vessel. Fluorescence intensities were sampled for $2 \times 5$-μm regions of interest drawn around a region of vessel wall that was in clear focus. These were expressed relative to a fluorescence standard solution (0.1 mg/ml FITC-albumin, Sigma-Aldrich) that was continuously perfused into the microvasculature by micropipette cannulation after capture of all P-selectin-labeled images. The FITC-albumin standard solution completely filled each sampled microvessel; the images were used to normalize for vessel specific attenuation of the fluorescence signal due to localized variability in the optical properties of the tissue. Intensity measurements of the fluorescence standard were acquired for each sampled vessel in a region of interest located in the center of the vessel at least 5 μm away from the labeled vessel wall.

Statistics. Statistical tests were performed using Graphpad Prism (version 3.0) to undertake t-tests, ANOVA, linear regression, or correlation analyses as appropriate. Differences were considered to be significant when $P < 0.05$.

RESULTS

The dominant role of P-selectin in mediating the observed leukocyte rolling was verified using a function blocking monoclonal antibody (RB40.34, BD Biosciences). Intravenous infusion of 10 μg of antibody reduced leukocyte rolling by 96%, consistent with previous reports (19, 25) implicating P-selectin as the primary mediator of early rolling events.

Variability in leukocyte velocity during rolling. The measured translational velocity for leukocytes undergoing rolling behavior can vary widely as a function of time and position in any observed vessel. To define the breadth of variability in this translational velocity, measurements were made from displacement samples at 100-ms intervals for the population of rolling leukocytes observed during a 2-min interval. The frequency distributions for the sampled minimum and maximum velocities across the observed population show that translational velocities ranged from 0 to >200 μm/s for this representative rolling population (Fig. 1A). This variability was also demonstrated by the velocity trajectory of a representative cell (Fig. 1B), which illustrates the transient high velocity translations and periodic pauses that typify P-selectin-mediated leukocyte rolling. Importantly, we found that the variability in velocity can be related to identifiable local regions of the venular endothelium, as demonstrated in Fig. 1C, which shows a compilation of the average velocity for all leukocytes at each axial location as a function of position along the sampled length of vessel wall. Along the 200-μm sample length of a
that we typically observed in these venules. As illustrated in Fig. 1, leukocyte rolling actually consists of a variety of behaviors ranging from transient pauses to intermittent periods of high velocity translations. We hypothesized that the local fluid shear environment could affect the overall average velocity during rolling either by altering the rolling velocity during periods of consistent contact between the leukocyte and the endothelial surface or, alternatively, by increasing the occurrence of periods of high velocity translations during which the leukocyte transiently loses contact with the endothelium and translates rapidly along the wall. Displacements of 256 rolling leukocytes were tracked at 33-ms intervals over a 1-s period in 13 postcapillary venules from 7 mice. Newtonian wall shear rates sampled for each vessel ranged from 112 to 381 s\(^{-1}\) for native flows in these venules. In addition to measuring the overall average translational velocity (from the total displacement during a 1-s sampling interval), we also measured a “continuous contact” translational velocity from the displacements that occurred while the leukocyte was observed to be directly interacting (i.e., in contact) with the endothelial surface. This continuous contact velocity excluded leukocyte displacements that were associated with transient high velocity periods during which the cell exceeded twice the average velocity for all sampled cells. During this latter interval, leukocytes are translating along the vessel wall in the flowing hydrodynamic streamline and are therefore assumed not to be undergoing the P-selectin-mediated interactions that occur during the continuous contact displacements. There was a statistically significant increase in the overall average velocity with increasing wall shear rate (\(r^2 = 0.56, P < 0.005\)), whereas, in contrast, the relationship between continuous contact translational velocity and wall shear rate was not statistically significant (Fig. 2A). Comparisons between the two pools of velocity values showed that the continuous contact translational velocity was significantly lower than the overall average velocity (27 ± 2 vs. 39 ± 4 \(\mu\)m/s, means ± SE, paired \(t\)-test, \(P < 0.001\)) with the only difference between the two measures being the inclusion of the transient high velocity displacements that occur while the leukocyte is not in continuous contact with the endothelium. Thus our data show that the effect of an increase in shear forces appears to be specifically related to the high velocity translations during transient detachment rather than being a reflection of variability in the leukocyte behavior during P-selectin-mediated rolling.

The shear-dependent elevation of the overall average rolling velocity could also result from an increase in the frequency of transient leukocyte detachments rather than from changes in the velocity of leukocytes that are maintaining consistent contact with the endothelial wall. We therefore compared leukocyte displacements in different shear environments. In Fig. 2B, we show the behavior of three typical leukocytes: this provides further evidence that leukocyte velocity is insensitive to shear rate during P-selectin-mediated interactions with the wall. Figure 2B shows the trajectories of representative leukocytes in three shear conditions (113, 214, and 323 s\(^{-1}\)) and illustrates that the velocities were not significantly different at 28 ± 3, 28 ± 3, and 32 ± 3 \(\mu\)m/s, respectively (one-way ANOVA, \(P > 0.5\)) except during the period of detachment that is observed only for the leukocyte in the highest shear rate condition. These data illustrate that the local wall shear rate in the venule does not exert a significant influence on the consis-
...detachment occurs when the shear rate is in the region of 250 s⁻¹, to a point where nearly all rolling leukocytes exhibited transient detachments at the highest observed wall shear rates (>300 s⁻¹). These data clearly show that elevations in local wall shear rate are related to an increase in the occurrence of transient detachments. In turn, it is these transient detachments that account for the erratic nature of leukocyte rolling; the velocity of leukocytes during P-selectin-mediated interaction is relatively invariant, as illustrated in Fig. 2B. From these observations, we hypothesized that variable leukocyte rolling behavior was likely to be related to differences in P-selectin expression level or its variable spatial distribution in different local regions of the venule.

**P-selectin expression in postcapillary venules.** To address whether there is a direct relationship between P-selectin-mediated leukocyte rolling and the localized expression patterns of P-selectin on vascular endothelium, we first asked whether P-selectin expression varied significantly in different regions of the microcirculation. Using immunofluorescent labeling and confocal microscopy, we undertook detailed characterizations of the spatial distribution of P-selectin in the intact blood-perfused microvasculature. Immunofluorescence labeling of P-selectin on the luminal surface of the endothelial membrane revealed a punctate pattern (Fig. 3) that is consistent with that observed in cultured endothelial cells (5, 9, 26). P-selectin expression was detectable only in venules and not in true capillaries or arterioles, consistent with observations on perfusion-fixed microvascular networks (10).

The influence of shear forces on P-selectin expression levels was addressed by quantifying relative immunofluorescence intensity of labeled P-selectin in two hydrodynamically distinct classes of venules. To do this, P-selectin intensity measurements were normalized to intensity measurements of an intensity standard solution, as described in MATERIALS AND METHODS. These normalized measures of P-selectin expression were acquired in two categories of venules differentiated by size, position in the branching network, and shear forces: smaller diameter venules that were within two branching generations of true capillaries and that experienced low shear forces (low shear venules) versus larger venules that were more than two branching generations distal to true capillaries (high shear venules). Low shear venules were significantly smaller (19.8 ± 1.0 vs. 44.0 ± 2.0 μm) and exhibited lower wall shear rates (70.1 ± 9.8 vs. 113.3 ± 12.1 s⁻¹) and lower flow velocities (356 ± 55 vs. 1,254 ± 157 μm/s) than high shear venules (n = 46 low shear and 26 high shear venules). Comparison of normalized P-selectin levels between these two classes of venules showed no significant difference (1.15 ± 0.07 vs. 1.25 ± 0.10, n = 25 and 15, low shear vs. high shear) despite significant differences in wall shear rate, suggesting that, overall, P-selectin expression in intact postcapillary venules is not significantly affected by the prevailing hydrodynamic forces.

Even though generalized differences in P-selectin expression were not observed across vessels experiencing different wall shear rates, the high spatial detail available from the in vivo labeling procedure combined with confocal observations showed that there was considerable local variability in P-selectin expression within an individual venule (Fig. 4). Regions of high P-selectin expression in venules displayed fluorescence intensities more than twofold greater than regions of low expression (Fig. 4, bottom). Typically, the regions of high

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**Fig. 2.** A: overall average leukocyte velocity increases significantly with increasing shear rate (solid line). In contrast, no statistically significant trend was observed for the part of the total population that maintains consistent contact with the wall. B: leukocyte velocity (slope of the displacement versus time) for 3 representative leukocytes, each sampled in venular regions with different shear environments, showing that velocity remains consistent except where there is a high velocity translation associated with detachment from the endothelial surface. This occurs for the cell experiencing the highest wall shear rate (○). C: fraction of leukocytes undergoing detachments from the endothelium and translation in the free flow stream increases with wall shear rate. The spline fit line for leukocyte detachment rate sampled in venular regions with different characteristic shear rates shows that the rapid increase in the rate of detachment occurs when the shear rate is in the region of 250 s⁻¹.
versus low fluorescence intensity in situ were on the order of 50 μm in length or less. Furthermore, the axial length dimension of the high expression regions that we observed is qualitatively consistent with the regions of slow leukocyte rolling identified earlier using the high temporal resolution analyses of P-selectin-mediated rolling (Fig. 1C). By extension, we infer that the regions of slow leukocyte rolling might be directly attributable to the observed regions of high P-selectin expression.

To address this, we directly compared the relationship between leukocyte rolling behavior and P-selectin expression patterns in a venule that was representative of the population studied: to do so, we used a combination of immunofluorescent labeling and leukocyte tracking. To do this, the amount of time that a vessel wall region was occupied by a leukocyte (inversely proportional to its rolling velocity) was measured for successive 5-μm lengths of the vessel wall by tracking all rolling leukocytes in a specified 200-μm length of the vessel wall for 2 min. Immediately after the collection of these leukocyte rolling data, the observed venule was labeled for P-selectin using dual micropipette cannulation of the intact blood-perfused venule as described in MATERIALS AND METHODS. We were thus able to match directly the time that a particular region of the vessel wall was occupied by rolling leukocytes with the corresponding P-selectin expression level in that same 5-μm length. The resulting analysis (Fig. 5) shows, for this typical venule, that there was a consistent relationship between P-selectin expression levels and the time that that local region of the venular wall was occupied by leukocytes, indicating that the regions of slowing rolling were those where P-selectin expression was elevated, suggesting that rapid shear-dependent axial translation of leukocytes occurred in those regions where P-selectin expression was lower. For all observed wall regions, the leukocyte-occupied time correlated significantly with P-selectin labeling intensity ($r = 0.58$, $P < 0.005$), further supporting our hypothesis that the variable spatial distribution of P-selectin was a determinant of the observed variability in leukocyte rolling behavior.

**DISCUSSION**

In this study, we demonstrated that the variable spatial distribution of endothelial P-selectin in postcapillary venules has a direct effect on the characteristics of the local leukocyte interactions. We show, for P-selectin-mediated rolling, that the apparent shear dependence of the average leukocyte rolling velocity in vivo is primarily the result of a shear-dependent increase in the occurrence of transient detachments of the leukocyte from the endothelial surface. Leukocyte translational behaviors during rolling are closely associated with the localized spatial heterogeneity in P-selectin expression in postcapillary venules. Our study shows that leukocytes do not interact equally with all regions of the venular wall and, furthermore, that the regions of high interaction are not random but are directly determined by the spatial pattern of P-selectin expression. These data thus pose new questions about the influence of the observed localization of leukocyte rolling on the ensuing adhesion and transmigration steps of the inflammatory response.

**Leukocyte rolling velocity.** P-selectin-mediated leukocyte rolling and tethering are critical events for the propagation of the inflammatory response. Many studies have detailed biophysical aspects of bond formation between P-selectin and its primary counterligand, P-selectin glycoprotein ligand 1, including kinetic rate constants (3) and the effects of ligand valence (23). This raises questions about how P-selectin directly affects leukocyte interactions with the endothelial surface in a physiologically relevant environment, specifically postcapillary venules. Leukocyte rolling as usually measured in vivo describes a compilation of erratic behaviors that characterize the translation of transiently adhesive leukocytes in the sometimes variable environment of blood flow-induced shear forces. Thus a better understanding of the involvement of shear forces in the regulation of the rolling step of the leukocyte adhesion cascade clearly requires a more detailed analysis of rolling behavior. The mean rolling velocity that we report here is similar to mean velocities reported elsewhere in both venules...
and isolated cell systems (4, 6, 8, 18, 20). However, we show here that the average leukocyte rolling velocity can be further divided into qualitatively distinct components. In particular, separation of those interactions where the leukocyte is in continuous contact with the endothelial surface from those where the leukocyte appears to “skip” along the surface identifies the influences of shear forces versus localized adhesion molecule distributions that are hidden in the commonly reported values of average rolling velocity. Our data reveal two categories of translational behavior for rolling leukocytes: one consisting of a slow, steady translation where contact between the leukocyte and endothelium is maintained via P-selectin-mediated interactions, and the other consisting of a high velocity translation where direct contact with the endothelium is not apparent. We note that if different hemodynamic criteria were used to identify the rolling leukocyte population, that population would be slightly different from the one we studied; importantly, the division into two categories of translational behavior is independent of this consideration. Our study thus helps to clarify how the prevailing shear forces affect the rolling behavior of leukocytes under flow. Characterizations of shear-dependent selectin binding kinetics (1, 2) suggest that rolling velocity should vary with shear forces. This idea has been tentatively supported by in vivo observations of average velocity (Refs. 4, 6, 13, and 21 and the present study). However, we found that the mechanism underlying the apparent increase in average velocity was more subtle than first anticipated, because the fluid shear forces affected P-selectin-mediated rolling behavior primarily by increasing the occurrence of transient detachments rather than by affecting the translational velocity during consistent (P-selectin mediated) contact with the wall. These findings suggest that elevations in average rolling velocity with increasing shear forces are derived from two causal factors: the increase in the occurrence of transient detachments and the local flow velocity during the detachment, both leading to increased leukocyte translational velocity compared with periods where consistent endothelial contact is maintained. Notably, the occurrence of transient detachments increases rapidly at wall shear rates near 250 s⁻¹, which suggests that there is a threshold level of force where P-selectin bonds are not sustained by the site densities native to the vasculature. Our study shows that, overall, the time that an individual leukocyte will spend interacting with the venular wall is indeed a function of the shear environment in the sense that this will determine both the frequency and characteristic velocity of the skips that occur between the periods of P-selectin-mediated rolling interaction with the wall.

Spatial localization of interactions. Our velocity analyses showed that there were localized regions of consistent slow rolling and localized regions where rolling leukocytes were absent. As discussed, possible causes for these observations could be localized differences in shear forces and/or endothelial cell adhesion molecule levels; interestingly, Damiano and colleagues (6) reported that leukocyte adhesion energy varies locally in venules and hypothesized that this might reflect heterogeneity in P-selectin expression on the venular wall. Our data show that, indeed, differences in P-selectin expression can account directly for spatial differences in leukocyte rolling behavior.

Fig. 4. Longitudinal confocal section at the midplane of a vessel immunofluorescently labeled for P-selectin. The outlined regions show where the fluorescence was sampled. The intensity in each of these regions (I and II) is shown below, illustrating the localized differences in fluorescence intensity within a single sampled region in the venule. In the intensity plots, position refers to the location along the outlined region of vessel wall, with 0 at the top of the image and 125 at the bottom. Intensity is expressed in grayscale units. Scale bar = 20 μm.

Fig. 5. Fluorescence intensity (indicating P-selectin expression) variations along a 200-μm sampled length of a representative venule are plotted together with the time spent by a leukocyte at the matched local positions on the wall. There is a statistically significant correlation (P < 0.05) between the fluorescence intensity and the time that that region of the wall is occupied by leukocytes.
Interestingly, the length dimension of the regions of slow rolling velocity and, accordingly, high P-selectin expression generally match the characteristic axial length of a venular endothelial cell (~50 μm, J. C. Wojciechowski and I. H. Sarelius, unpublished observations), suggesting that an individual endothelial cell can exhibit dramatically different P-selectin expression compared with adjacent cells. We have also seen this localized difference in P-selectin expression among endothelial cells in histamine-stimulated human umbilical vein endothelial cells (11). The underlying cause of these localized differences in P-selectin levels is not obvious as cells in such close proximity are likely to experience similar levels of shear forces and inflammatory stimuli.

What might be the physiological consequences of these local variations in P-selectin expression? We note that on an even smaller length scale than one endothelial cell, P-selectin expression on the surface of individual cells is heterogeneous in that it appears punctate (Fig. 3), an observation reported earlier by others (9, 26). In preliminary modeling studies (14), we found that this punctate distribution supports slower leukocyte rolling than would occur if the same level of P-selectin was distributed uniformly on the endothelial cell surface, perhaps by optimizing local adhesion molecule surface density and hence bond formation and kinetics. We speculate that the P-selectin-rich venular regions that we describe in the present study may also support more efficient leukocyte capture in the venule or optimize later events (adhesion, transmigration) of the inflammatory cascade, for example, by altering adhesion molecule expression, junctional associations, or cytoskeleton arrangements in neighboring endothelial cells.

In summary, the behavior of rolling leukocytes that is mediated by P-selectin is directly related to the heterogeneous spatial expression pattern of P-selectin in intact postcapillary venules. Shear forces in this physiological environment affect the overall translational velocity of rolling leukocytes by increasing the occurrence of transient detachments from the endothelium at higher shear forces, thereby leading to brief periods of high velocity translation near the wall in the free fluid stream. These findings indicate that spatially localized elevations of adhesion molecule expression can regulate patterns of leukocyte rolling, a critical aspect of leukocyte recruitment in inflammation. This finding identifies an important mechanism by which leukocyte recruitment to the venular wall can be controlled in the in vivo environment.

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