Intracellular heterogeneity in adhesiveness of endothelium affects early steps in leukocyte adhesion

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Submitted 3 June 2005; accepted in final form 30 January 2006

Mundhekar, Ameya N., Daniel C. Bullard, and Dennis F. Kucik. Intracellular heterogeneity in adhesiveness of endothelium affects early steps in leukocyte adhesion. Am J Physiol Cell Physiol 291: C130–C137, 2006; doi:10.1152/ajpcell.00261.2005.—Endothelial cell junctions are thought to be preferential sites for transmigration. However, the factors that determine the site of transmigration are not well defined. Our data show that the preferential role of endothelial cell junctions is not limited to transmigration but extends to earlier steps of leukocyte recruitment, such as rolling and arrest. We used primary mouse neutrophils and mouse aortic endothelium in a flow chamber system to compare adhesive interactions near endothelial cell junctions to interactions over endothelial cell centers. We found differences in both rolling velocity and arrest frequency for neutrophils at endothelial cell junctions vs. more central areas of endothelial differences were governed by adhesion molecule interactions, not local topography. Interestingly, the role of particular adhesion molecules depended on their location on the endothelial cell surface. Although ICAM-1 stabilized and slowed rolling over central areas of the cell, it did not influence rolling velocity over endothelial cell junctions. P-selectin and VCAM-1 were more important for rolling near endothelial cell junctions than E-selectin. This demonstrates that adhesive properties of endothelial cell junctions influence early events in the adhesion cascade, which may help explain how leukocytes are localized to sites of eventual transmigration.

endothelial cells; rolling; selectins; integrins

RECRUITMENT OF LEUKOCYTES is a multistep process, consisting of capture, rolling, firm adhesion, and transmigration (18). Each step depends on the previous one. For example, before they firmly adhere, leukocytes roll varying distances on stimulated endothelium (11) and are thought to integrate inflammatory signals, from both chemokines and adhesion molecule engagement, as they roll (12). Similarly, arrest precedes transmigration; thus the location of arrest can influence where a leukocyte exits a blood vessel. Although leukocytes have been observed to migrate transcellularly, that is, through the body of an endothelial cell, in general it is the endothelial cell junctions that are thought to be preferential sites for transmigration (1–3, 17). However, it is still not clear how earlier adhesive events influence the site of eventual transmigration. Neutrophils arrest preferentially near the site where they are to transmigrate (2, 3, 6), whereas, in at least one system, monocytes arrest more randomly and locomote to endothelial cell junctions before transmigration (16).

The present study was undertaken to investigate the role played by local variations in the adhesive properties of endothelial cells in early steps of the leukocyte recruitment cascade, with particular attention to junctional areas. To do this, primary mouse bone marrow neutrophil (BMN) adhesion to primary mouse aortic endothelial cells (MAECs) was examined in a flow chamber. To characterize neutrophil adhesive interactions near endothelial cell junctions and differentiate them from interactions over more central areas of the endothelial cell, we modified a single-particle tracking imaging system (10) to track neutrophil trajectories in a flow chamber. With this system, uncompressed, high-resolution images were collected at a time resolution of 33 ms and analyzed by a computer. Leukocyte positions were determined to within ±0.44 μm for each cell in each image (15) from cell centroids determined at subpixel resolution (5, 9) so that several accurate velocity measurements could be made as a neutrophil traversed a single endothelial cell.

We found differences in both rolling and stopping by neutrophils at endothelial cell junctions vs. more central areas of the endothelial cell. In addition, we found that the adhesive properties of endothelial cell junctions can influence the trajectory of rolling leukocytes. We have partially characterized these three distinct adhesive functions (capture, stopping, and influence on rolling trajectory) in terms of adhesion molecule interactions. Interestingly, there are no major surface features or variations in thickness near the cell junctions, so local topography does not seem to play a major role. A better understanding of the role of variations in endothelial cell adhesive properties across an individual endothelial cell can provide insights into regulation of leukocyte recruitment, with implications for normal host defense as well as a number of disease states.

MATERIALS AND METHODS

Endothelial Cells

Endothelial cells purified from mouse aorta were isolated as previously described (7) and cultured in dishes precoated with 1% gelatin (Sigma, St. Louis, MO). The cells were maintained for 7 days in MCDB 131 medium (GIBCO, Grand Island, NY) supplemented with 10% FBS (HyClone, Logan, UT), 10 mg/l bovine brain extract (Biomedical Technologies, Stoughton, MA), 1,000 U/l heparin (American Pharmaceutical Partners, Los Angeles, CA), 1 mg/l hydrocortisone (Sigma), and 10 ml of penicillin (GIBCO). The cells were seeded in 8-cm² plastic tissue culture dishes (Corning, Corning, NY) coated with 1% gelatin (Sigma). The cells formed confluent monolayers 6–7 days after seeding and were used for experiments within 2–3 days of confluence.

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Leukocytes

Mouse BMNs were isolated from femurs of mice immediately preceding flow experiments, as follows. Freshly isolated femurs were flushed with ice-cold RPMI 1640 medium, and the effluent was filtered with a 100-μm strainer. Neutrophils were then isolated with Hypaque. Fifty million cells were loaded with fluorescent dye by 20-min incubation at room temperature with 1 μM BCECF (Molecular Probes, Eugene, OR) and suspended in HBSS (Sigma) at a concentration of 0.5 × 10⁹/ml. The cells were then washed once in HBSS, resuspended in HBSS at 10⁶ cells/ml, and loaded into a 25-ml syringe.

Flow Chamber Adhesion Assays

Eight hours before an experiment, a confluent monolayer of endothelial cells in tissue culture dishes was treated with 10 ng/ml of murine TNF-α (Sigma) for 8 h to activate the endothelium. A GlycoTech flow chamber insert and gasket (GlycoTech, Rockville, MD) were then inserted into the dish to form a laminar flow chamber that could be viewed on a microscope. Cells were injected into the flow chamber in HBSS at a controlled physiological shear stress of 2.5 dyne/cm² with a programmable syringe pump (KD Scientific, New Hope, PA). Cells were viewed on an Axiovert 100 microscope (Zeiss, Thornwood, NY) equipped with a charge-coupled device camera (model 300T-RC, Dage-MTI, Michigan City, IN) and viewed both as brightfield and fluorescent images. Video was recorded onto sVHS videotape, and selected sequences were digitized to TIF files with the Reality video editing package (Leitch, Florence, KY). Video images were analyzed to yield position measurements every 1/30 s with Metamorph software (Universal Imaging, West Chester, PA). Position measurements were processed by programs written for this purpose by D. F. Kucik to determine velocities, accelerations, and arrest durations. Counts of rolling and firmly adherent cells were determined by visual review of video sequences and confirmed by computer analysis.

Analysis of Adhesion and Localization

Definition of adhesion duration. In this study early, often transient, adhesion was of particular interest. When adhesion first develops, even cells that eventually become firmly adherent move slightly under shear stress. Therefore, to obtain an accurate and repeatable measure of duration of adhesion, an objective definition of adhesion must be used, especially when cells are observed at high time resolution. A cell was considered firmly adhered if its velocity fell below 5 μm/s for 5 video frames (0.16 s). Adhesion duration was defined as the time required to move 1 BMN diameter after stopping. In our system, this definition very effectively distinguished between what most investigators would consider rolling (where jerkiness results in brief stops of <0.16-s duration) and obvious stopping. It should be noted that the average duration of adhesion was ~2.5–7.5 s, depending on the location (see Fig. 2). Only events that occurred during the period of observation were quantified.

Definition of rolling. To distinguish cells that are rolling from those flowing at the lowest hydrodynamic velocity (close to the chamber wall) without adhesive rolling interactions, a critical velocity (V_crit) was calculated as described earlier (8). Rolling was then defined as cell motion that does not meet the criteria for adhesion (above) but is below V_crit. Average rolling velocity is the mean of the instantaneous velocities (measured at 1/30 s intervals) during the period that the cell is rolling. Rolling duration is the total time that the cell is rolling (number of frames × 0.033 s/frame). Only cells that were captured during the period of observation are counted in this measure. If a cell rolled intermittently (i.e., rolling was interrupted by either free flow or firm adhesion), only the frames during which the cell met the criteria for rolling were averaged. Cell velocities plotted in Fig. 4 are the result of an average of 71 velocity measurements per cell.

Definition of cell junctions. Cell junctional areas were defined as the center of the dividing line between two cells plus 1 leukocyte diameter on each side; the rest of the endothelial cell was considered the cell center.

Definition of deviation. Rolling cells were scored as deviating if at least once during their transit across the field of view they moved more than 2 cell diameters from the path predicted by the direction of flow and continued in a direction other than that of the flow for >0.5 s.

Microscopy of Endothelial Cell Monolayer Sections

MAEC monolayers were grown to confluence in 30-mm tissue culture dishes, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 1 h, rinsed in buffer, postfixed in 1% osmium tetroxide for 1 h, rinsed in buffer, dehydrated through an ascending series of ethanol to 100%, and embedded in Embed 812 resin (Electron Microscopy Sciences, Hatfield, PA). One-micrometer-thick sections were stained with 2% toluidine blue.

Function-Blocking Experiments

Wild-type MAECs were treated with 10 ng/ml TNF-α for 8 h before the experiment. During the last hour of TNF-α treatment, function-blocking anti-P-selectin, anti-VCAM-1, anti-E-selectin, or combinations of these antibodies (all from Pharmingen, San Diego, CA) were added to a final concentration of 10 μg/ml. The dish was then assembled into a flow chamber as above, and flow chamber adhesion assays were performed, with the initial buffer flow washing away excess antibody.

RESULTS

Visualization of Endothelial Cells

To preserve the integrity of the monolayer, MAECs were grown to confluence and allowed to form junctions before being mounted in an in vitro flow chamber in their original culture dish. Antibody labeling of cell junctions for flow experiments was avoided to prevent possible blocking of adhesion sites (if membrane proteins at the junctions were labeled) or perturbations of junction integrity or monolayer morphology (if the cytoplasmic face of the junction were labeled). Rather, by substituting a low angle of incidence light source for the microscope condenser, we were able to produce contrast sufficient to estimate the location of cell junctions such that there was good agreement among independent observers. A sample image is presented in Fig. 1. It is, of course, possible that with this system what appear to be junctions to independent observers are, in fact, some other cell feature. Because these areas have specialized adhesive properties (as demonstrated below), however, we consider it more likely that they are junctions than some other linear cell feature.

Preferential Adhesion of Neutrophils to Endothelial Cell Junctions

We examined localization of adhesion under flow conditions that mimic the shear stress inside blood vessels. Mouse primary BMNs were perfused over MAECs at a physiological shear rate of 2.5 dyne/cm², and adhesion to cell junctional areas vs. cell centers was assayed. When cells arrested, the length of stop time was quantified according to criteria described in MATERIALS AND METHODS and location was classified as junctional or nonjunctional (as defined in MATERIALS AND METHODS), providing an objective method of quantifying transient stops.
Conclusions about altering adhesive properties of the junctions.

Enhance contrast. Fluorescent labeling of cell junctions was avoided because of microscope condenser was replaced by custom illumination designed to analysis. To facilitate estimation of endothelial cell junction locations, the mounted on an inverted microscope, and images were recorded for adhesion

Previously described in MATERIALS AND METHODS in the entire trajectory (note that only preceded by the junctions is small compared with total cell area, even though the area represented by the junctions is small compared with total cell area (Fig. 2A). In addition, adhesion duration, defined as the time required to move 1 BMN diameter after stopping (because even “firmly adherent” cells often display some minor motion under shear stress), was assessed for all adherent cells. Adhesion durations were significantly longer at cell junctions compared with elsewhere on the endothelial cell (Fig. 2B). In general, when cells resumed movement, they rolled many micrometers before stopping again, regardless of the location of stopping (data not shown).

Preferential Rolling Along Endothelial Cell Junctions

From hydrodynamic considerations, cells are expected to roll in a straight line in a flow chamber. In our system, however, deviation from straight-path rolling occurred frequency as cells altered their path to roll along cell junctions. To quantify this, we observed all cells that rolled into the field of view for a 1-min period and determined whether there was at least one deviation from straight-path rolling (as defined in MATERIALS AND METHODS) in the entire trajectory (note that only a fraction of the cells met our criteria). Sample trajectories are depicted in Fig. 3A. As expected, the number of rolling cells per minute decreased with increasing shear stress (Fig. 3B). The fraction of total rolling cells that deviated from straight-path rolling, however, was similar at the four physiological shear stresses tested (Fig. 3C). Thus these two different measures of endothelial cell adhesive function (capture and trajectory guidance) are differentially affected by shear stress.

Endothelial Topography Is Not Consistent with Localization to Endothelial Cell Junctions

Among the factors that might cause rolling neutrophils to deviate from a straight path is endothelial cell topography. Deviations from flatness near cell junctions might obstruct leukocyte motion, perturb the fluid flow, or otherwise decrease rolling velocity or alter the path of a leukocyte. Topography that might be expected to produce aberrant motion near endothelial cell junctions is illustrated in Fig. 4A. To determine the topography of endothelial monolayers under our experimental conditions, we grew endothelial cells in a tissue culture dish exactly as is done for flow experiments and then embedded and sectioned the monolayer (still on the dish). We then examined the sections on a light microscope to determine the cross-sectional shape of the monolayer (Fig. 4B). This analysis demonstrates that the endothelial cell monolayer used in our flow experiments is very thin and flat near cell junctions. The major deviation from flatness is a thickening at the center of the cell to accommodate the nucleus. The prediction from these pictures is that any topography-induced changes in velocity or deviations from a linear trajectory would likely occur near the endothelial cell nucleus, rather than at endothelial cell junctions. There are no variations in thickness or other prominent surface features near the cell junctions, and the distance between nuclei and endothelial cell junctions varies widely, so localization to the narrow region of endothelial cell junctions and velocity changes there are not predicted by cell topography.

Decreased Velocity of Rolling at Endothelial Cell Junctions

Videos of individual rolling leukocytes were divided into segments of rolling at cell junctions vs. rolling over central portions of the cell and analyzed by computer. Rolling velocities were determined 30 times per second for each segment. In

Fig. 1. Monolayer as visualized in flow chamber. Primary mouse aortic endothelial cell (MAEC) monolayers were prepared as described in MATERIALS AND METHODS and assembled into an in vitro flow chamber. The chamber was mounted on an inverted microscope, and images were recorded for adhesion analysis. To facilitate estimation of endothelial cell junction locations, the microscope condenser was replaced by custom illumination designed to enhance contrast. Fluorescent labeling of cell junctions was avoided because of concerns about altering adhesive properties of the junctions.

Fig. 2. Preferential adhesion of neutrophils to endothelial cell junctions. A: mouse primary bone marrow neutrophils (BMNs) were perfused over primary MAECs for 1 min at 2.5 dyn/cm², and adhesion at endothelial cell junctions vs. over cell centers was assessed. Significantly more BMNs adhered within 1 cell diameter of cell junctions than elsewhere on the endothelial cell (*P < 0.01; Mann-Whitney test). B: duration of adhesion (as defined in MATERIALS AND METHODS) was assessed at endothelial cell junctions vs. elsewhere on the endothelial cell. Duration of adhesion was significantly longer near endothelial cell junctions (*P < 0.01; Mann-Whitney test). Data are combined from 3 separate experiments on different days.
the example in Fig. 5A, which depicts velocity fluctuations of a single rolling cell, in most (but not all) cases velocity decreased as rolling cells encountered cell junctions (indicated by arrows). Although not every rolling cell slowed at every endothelial cell junction, when data from over 50 cells were combined a significant difference was found between velocities at cell junctions and those over more central areas (Fig. 5B). ICAM-1 Regulates Rolling Velocities Only Over Cell Centers

Recently, endothelium-expressed ICAM-1 has been shown to mediate slow rolling velocity of a variety of leukocytes, both in vivo and in vitro (8, 15, 19). Although not classically considered a rolling receptor, ICAM-1 cooperates with other adhesion molecules to stabilize rolling of leukocytes (15). To determine whether ICAM-1 could account for the slower rolling velocity of neutrophils at cell junctions, rolling veloc-
Neutrophil rolling velocities were similar at endothelial cell junctions, regardless of the presence or absence of ICAM-1. However, over cell centers, neutrophils rolled at a much higher velocity in ICAM-1-deficient endothelial cells (Fig. 6). This higher rolling velocity was consistent with transient, intermittent detachment and millisecond-scale “jumping,” as described earlier (8). Thus stabilization of leukocyte rolling by ICAM-1 cannot account for the lower velocity at cell junctions.

**P-Selectin and VCAM-1 Combine to Localize Rolling BMNs to Endothelial Cell Junctions**

Although no major rolling receptors are localized exclusively to endothelial cell junctions, differences in functional activity might occur because of presentation, clustering, cytoskeletal anchoring, or other local properties. Therefore, the role of adhesion molecules in preferential rolling along the endothelial cell junction was assessed functionally. The major endothelial rolling receptors tested were P-selectin, E-selectin, and VCAM-1. Endothelial cell monolayers were preincubated with function-blocking antibodies before flow experiments, and the effect of antibody blockade on rolling cell trajectory was determined at a number of different physiological flow rates. For each experiment, three fields were examined for each blocking antibody at each shear stress. In each case, the trajectories for all cells entering the field during the period of observation were assessed according to the criteria for deviation (see MATERIALS AND METHODS). Data from multiple experiments were combined. Blocking individual adhesion molecules had no significant effect on the number of BMNs that attached and rolled on the endothelium at any of the flow rates measured, demonstrating redundancy in adhesion molecule capture and rolling functions (Fig. 7A). However, deviation from straight-line rolling to follow cell junctions was significantly reduced by blockade of either VCAM-1 or P-selectin (Fig. 7B). Blocking both simultaneously resulted in an additive effect, most pronounced at low shear stresses. In contrast, blocking E-selectin had no significant effect on rolling trajectories.

**DISCUSSION**

Endothelial cell junctions are well recognized as preferential sites for extravasation; however, much less is known about their role in earlier steps of the adhesion process. Previous studies showed that neutrophils arrest preferentially near endothelial cell junctions under flow conditions (1, 6, 20), but in those studies events preceding firm adhesion, such as rolling velocity changes, rolling path deviations, and brief arrests, were not quantified. On the basis of detailed observations of rolling neutrophils on cultured endothelial cells, we hypothesized that heterogeneity in the adhesive properties of endothelial cell junctions may play a role in preferential neutrophil arrest near these sites.

**Fig. 6.** ICAM-1 expression affects rolling velocity over endothelial cell centers but not over junctional areas. BMN flow chamber adhesion assays were done with either wild-type or ICAM-1-deficient (−/−) endothelial cells (from knockout mice) as the adhesive substrate. Rolling cell velocities (measured 30 times/s) on track segments over junctional areas vs. endothelial cell central areas were compared for both endothelial cell types. Velocities over junctional areas (filled bars) were similar regardless of whether the endothelial cells expressed ICAM-1, but over central areas (gray bars) rolling was much faster on the ICAM-1-deficient endothelial cells. Significant differences (Mann-Whitney test) are indicated. Data are combined from 3 separate experiments on different days.
lial cells might influence these early events in adhesion. This might have consequences for the site of eventual arrest and transmigration.

A key to the current studies was the ability to quickly digitize images of sufficient quality to readily identify endothelial cell junctions at a time resolution that provides several images of each rolling neutrophil as it traverses each endothelial cell. Using computer analysis to accurately track cell motions, along with objective measures of adhesive functions (defined in MATERIALS AND METHODS), we were able to compare adhesive behavior near cell junctions to that over more central areas of the endothelial cell.

We found that not only did neutrophils accumulate at endothelial cell junctions, but, even if they did not arrest, rolling over cell junctions was slower and brief stops were more common and of longer duration. As a result, the neutrophils spend relatively more time near cell junctions than elsewhere on the endothelial cell. There are several potential consequences of this. First, the effect of chemokines or other signals can be enhanced, because the neutrophil has more time to accumulate and integrate them. Second, slower motion of the neutrophil might encourage the formation of multiple selectin bonds, reinforcing adhesion and making it more likely that the cell will arrest completely near cell junctions. Third, slowing may increase the probability of engagement of $\beta_2$-integrins. It has been demonstrated that efficiency of firm adhesion through $\beta_2$-integrins decreases as shear increases, and this was interpreted as suggesting a requirement for a collisional contact duration of at least 5 ms (13, 14). Thus slowing or transient arrest near endothelial cell junctions may provide the time necessary for stronger, integrin-mediated bonds to develop.

Areas of increased adhesiveness would be predicted not only to affect stopping but also to alter the path of a rolling cell. In a dynamic process in which adhesive bonds are constantly being made and broken, a cell will tend to move toward an area of higher bond density, because the equilibrium there is shifted toward bond formation. In cell migration, this causes locomoting cells to move to areas of higher extracellular matrix density (a process known as haptotaxis). Similarly, rolling cells should also deviate toward areas where bonds are more readily formed. This constitutes a third adhesive function, distinct from both capture of flowing cells and subsequent stopping. In our system, rolling neutrophils frequently deviated from straight-line rolling to track along endothelial cell junctions.

It was important to distinguish whether velocity variations and deviations from straight-line rolling at endothelial cell junctions were due to differential adhesion or other factors, such as endothelial cell topography. For example, surface features and height variations in the right locations in a monolayer could serve to guide a rolling leukocyte to junctional areas. Variations in endothelial cell height at cell junctions could also influence the hydrodynamic forces exerted on a rolling cell. As demonstrated by sectioning of the monolayer, though, our MAEC preparations were very flat in the junctional regions, with height variations only near the very center of the cell, over the nucleus (Fig. 3). Although perturbation of fluid flow over the cell nucleus might potentially result in effects at a distance, it is unlikely that this could explain our observations, because the distance from the nucleus to cell junctions varies widely, yet deviations from straight-line rolling occurred almost exclusively over a narrow region at the junctions. Therefore, it might be predicted that, although topographical features might force a rolling cell to detour around an endothelial cell nucleus, there should be no preference for the narrow region within 10 μm of the junctions (as junctional areas were defined in this study), as we observed.

Of course, our micrographs cannot entirely rule out a role for topography in determining the path of a rolling leukocyte. It is possible that small features below the resolution of light microscopy could influence fluid flow and, in turn, leukocyte trajectory. Such features might only be detectable by other methods, such as atomic force microscopy. Also, in other systems, such as many endothelial cell lines, the cells are not as flat, and topography may play a role there. Our point is only that in our system the cells are very flat near the junctions, yet the cells deviate from straight-line rolling. Antibody blockade of specific adhesion molecules affects this, so adhesion clearly plays a role. Although the height of nuclei may be underestimated because of the plane of section, this only strengthens the conclusion that the major height variations are near the nucleus, not near cell junctions. In fact, cells did not appear to alter their path over nuclei either, where topographical differences were pronounced, suggesting that adhesive forces dominate over topographical features in determining a rolling cell’s path.

Adhesion molecule interactions were clearly responsible for deviations from straight-line rolling near endothelial cell junctions. Rolling along endothelial cell junctions could be significantly inhibited by antibody blockade of P-selectin or VCAM-1, but not E-selectin. Furthermore, blockade of P-selectin and VCAM-1 were additive, particularly at lower shear rates. This indicates a functional role for these two adhesion molecules in the increased adhesivity of endothelial cell junctions, supporting the idea that velocity and trajectory changes at endothelial cell junctions are due to adhesive forces rather than topographical considerations.

It should be noted that deviation from a straight path is shear dependent in the control condition and with blockade of E-selectin, but with antibody blockade of VCAM-1 and P-selectin, residual deviation still occurs, but shear dependence is lost (Fig. 6). The adhesion molecules responsible for this relatively shear-independent influence on trajectory remain unidentified. The adhesion molecule specificity certainly argues against a simple hydrodynamic model, however.

There are a number of possible mechanisms to cause increased receptor-specific adhesion over endothelial cell junctions. The simplest would be increased adhesion molecule concentration. P-selectin has also been shown to be relatively concentrated near cell junctions on human umbilical vein endothelial cells (HUVECs) (1). We were unable to determine whether this was also the case in our system, however, because, even though we were able to visualize P-selectin and other surface adhesion molecules in HUVECs and some mouse endothelial cell lines, in our primary MAECs the density of surface P-selectin was too low to reliably localize (data not shown). This was also the case with ICAM-1 and VCAM-1. Thus, although we cannot rule out concentration differences, we can only conclude that none of the adhesion molecules tested are so obviously concentrated at cell junctions that this can be readily demonstrated by immunofluorescence.

This observation has interesting implications regarding the minimum number of bonds needed to mediate efficient rolling,
because the knockout and antibody blocking experiments clearly demonstrated that the MAECs expressed sufficient adhesion receptors for good adhesive function. It should be noted, however, that higher adhesivity does not necessarily imply higher adhesion receptor density. The same effect might be achieved by localized higher receptor affinity states or by receptor clustering.

Our data are in good agreement with an earlier study examining leukocyte localization with intravital microscopy (20). Although those investigators quantified only firm adhesion (≥30 s), it was found that most adhesion was near or overlapping endothelial cell junctions. In that study, however, mathematical modeling indicated that, because of the elongated shape of the endothelial cells and the small size of the vessels, leukocytes would be likely to be near endothelial cell junctions because of geometric considerations alone. That is, the number and length of endothelial cell junctions in that system were such that even given random placement of leukocytes many would be near cell junctions (and this was more pronounced with smaller vessels). In the present study, we examined earlier events in adhesion (rolling, transient stopping) in a system with a geometry that was more amenable to separating adhesive interactions from those due to topographical considerations. Our data support the hypothesis that adhesive forces are dominant in both guiding leukocyte paths and determining the locations of early, transient stops.

Our model of inflammation was activation of endothelial cells for 8 h by TNF-α. This is a standard, widely used method of simulating inflammation and allows comparison with a large body of previous work. Inflammation, however, is a complex process and is dependent on both the identity of the stimulus and the time of exposure. Recently, it has been reported that in vitro systems, using TNF-α (21) or TNF-α in combination with chemokines (4), the likelihood of transmigration at cell junctions vs. central locations on HUVECs was time dependent. Time dependence was not tested in our study. Our conditions correlated well with intravital microscopy of mouse cremaster venules, however, where most cells adhere and eventually transmigrate at endothelial cell junctions (20), suggesting that the model of inflammation was valid. Whether adhesive properties of endothelial cell junctions change as a function of time and stimulus, though, especially in vivo, remains an important question.

Interestingly, the role of particular adhesion molecules depended on their location on the endothelial cell surface. For example, although ICAM-1 stabilized and slowed rolling over central areas of the cell, it did not influence rolling velocity over endothelial cell junctions, indicating that other adhesion molecule interactions dominate to limit velocity over cell junctions. In retrospect, this should not have been unexpected, because rolling velocities are governed by a complex mixture of simultaneously occurring adhesion molecule interactions, and cooperativity among these may not be uniform over the entire surface of an endothelial cell. These local variations in adhesive properties of endothelial cells have been underappreciated, however, in models of leukocyte-endothelial cell interactions and leukocyte recruitment.

Thus we find that endothelial cell junctions, in addition to their role as a site of extravasation, also form a specialized site with increased adhesive properties compared with the rest of the cell. A net effect of this is to guide rolling neutrophils to endothelial cell junctions, to slow their rolling motion while they are there, and to increase the frequency and duration of brief arrests. A greater fraction of rolling time spent near the endothelial cell junction, a site where soluble signals from the underlying tissues are likely to be localized, might increase the efficiency of signaling processes. P-selectin and VCAM-1 contribute to increased adhesiveness, although other adhesion molecules may be involved as well. If, as seems reasonable, early events contribute to subsequent firm adhesion and extravasation, modulation of adhesion specifically at endothelial cell junctions could constitute an additional level of regulation in the recruitment of the leukocytes.

REFERENCES


