Neutrophils Roll on Adherent Neutrophils Bound to Cytokine-induced Endothelial Cells via L-Selectin on the Rolling Cells
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Summary
Specific arrest of neutrophils in venules is central to their rapid accumulation during local
inflammatory responses. Initial neutrophil rolling on endothelium is mediated by leukocyte L-selectin
and the inducible vascular adhesion proteins P- and E-selectin. This rolling is a prerequisite for
endothelial-dependent neutrophil arrest. Here we describe rolling of neutrophils on the surface
of previously arrested neutrophils and demonstrate that this interaction involves L-selectin exclusively
on rolling cells. The adherent neutrophil support of L-selectin–dependent neutrophil rolling in
vivo can promote continuous and augmented leukocyte recruitment at sites of previous neutrophil
accumulation.

The recruitment of leukocytes to sites of acute inflammation is controlled by sequential interactions that eventually lead to migration of the cell from the blood into the affected tissue. Blood-borne neutrophils recognize the endothelium of the vascular bed within the inflamed tissue, stop on the luminal vessel wall while within the blood flow, migrate between the endothelial cells lining the vessel, and accumulate in the extravascular tissue within ~10 min (1–7).

Intravital microscopy shows that the initial contact between endothelial cells and leukocytes leads to a reversible adhesive interaction that results in rolling of leukocytes along the vessel wall. Members of the selectin family of C-type, lectinlike, adhesion proteins expressed on the plasma membrane of leukocytes (L-selectin) and inflamed endothelium (E- and P-selectin), help regulate these rolling events and cause the leukocyte to slow below the rate of blood flow. If appropriate activating factors are presented on the endothelium or are released from acute inflamed tissue during rolling, rapid signaling events will occur in neutrophils which lead to upregulation of their adhesiveness. These events promote neutrophils to adhere tightly via activated integrins to the vascular endothelium after which they migrate into the underlying tissue (8–12).

In vitro systems have been used to show that monolayers of activated endothelial cells, E- or P-selectin transfected fibroblasts, or isolated vascular selectins support neutrophil rolling under shear (13–15). However, during the course of inflammation in vivo, inflammatory leukocytes rapidly accumulate on the vascular wall. This irregular layer of adherent cells creates a barrier that prevents the interaction between newly arriving leukocytes and the inflamed endothelium. We demonstrate here that adherent neutrophils like activated endothelial cells can directly support neutrophil rolling, thus providing a mechanism for continued leukocyte recruitment throughout the inflammatory process.

Materials and Methods
Recirculating Loop Neutrophil–Neutrophil Human Umbilical Vein Endothelial Cell Capillary Tube Shear Assay (15). In experiments observing human umbilical vein endothelial cell (HUVEC)–bound neutrophil–neutrophil rolling, freshly isolated or second to sixth passage human umbilical cord endothelial cells (factor VIII and low density lipoprotein [LDL]-receptor positive) were grown to confluence (24–72 h) in endothelial cell growth media (EGM; Clonetics Corp., San Diego, CA) on the lower interior wall of capillary tubes (1.36-mm internal diameter x 2-cm-long sterile thin wall borosilicate glass; Drummond Scientific Corp., Broomall, PA) after plating at 3 x 10^6/ml. The endothelial cells were then stimulated with 10 ng/ml IL-1 (Immunex Corp., Seattle, WA) for 4 h at 37°C, followed by up to 2 h incubation in fresh IL-1-free EGM media (37°C). The IL-1–treated HUVECs expressed high levels of E-selectin (determined by flow cytometry; data not shown) and were used in shear experiments at 4–6 h after stimulation. Human blood leukocytes collected into sodium citrate–containing Vacutainers® (Becton Dickinson & Co., Mountain View, CA) were separated on Ficoll (Histopaque-1119; Sigma Chemical Co., St.

Abbreviations used in this paper: HUVEC, human umbilical vein endothelial cell.
Louis, MO) at room temperature after dilution in DMEM Ca²⁺, Mg²⁺ free (Irvine Scientific, Santa Ana, CA). Neutrophils from this isolation (>90% pure population by flow cytometry) were resuspended in Ca²⁺, Mg²⁺ containing DMEM (Irvine Scientific) buffered with 20 mM Hepes (Meditech, Inc., Herdon, VA) (complete DMEM) after centrifugation and infused via a sterile three-way stopcock injection port at a concentration of 2 × 10⁶ cells/ml into a media-prewashed recirculating silicone tubing loop (1.2-mm internal diameter size 14 [model 96400-14; Cole-Parmer Instrument Co., Chicago, IL]) in which the HUVEC-containing capillary tube was integrated. The capillary tube was then positioned upon an inverted microscope stage (Nikon Diaphot, Melville, NY) maintained at 37°C and a variable speed peristaltic pump with a Masterflex 6-roller pump head (model 7519-10; Cole-Parmer Instrument Co.). The 6-roller pump head generated smooth physiological flow (2.0 dynes/cm² shear stress) that was immediately started. At 2 dynes/cm² shear stress, the recirculation time for one circuit of the 3-ml loop was 44.9 s. Neutrophil viability was determined by trypan blue exclusion before infusion and after termination of the assays and did not change.

Antiselectin blocking mAbs (WAPS-12.2, anti-P-selectin, provided by R. A. Warnock and E. C. Butcher); DREG 56 [16] and EL-246 [17], anti-L- and E-selectin) were infused via a sterile T-extension (Abbott Hospitals, Inc., North Chicago, IL) into the recirculation loop at a final concentration of 100 μg/ml to observe their blocking effect. LPS levels were determined to be <2 pg/ml (at a 10 μg/ml mAb concentration) for all mAbs tested. Continuous high resolution phase contrast video recordings (30 frames/s rate [National Television Standards Committee standard] via a 0.7× video coupler and 20× phase contrast objective (Nikon) using a color CCD-TV camera (Sony, Tokyo, Japan) and a four head shuttle wheel VHS video recorder [Sony]) of the flow-induced neutrophil-HUVEC rolling and subsequent neutrophil-neutrophil rolling were made for later analysis.

Recirculating Loop Neutrophil–Neutrophil Capillary Tube Shear Assay. For experiments examining neutrophil–neutrophil rolling on neutrophils bound to glass, borosilicate glass capillary tubes (identical to those described above) were precoated with freshly collected human serum at 37°C for 2-4 h before the addition of freshly Ficoll-isolated (as above) human peripheral blood neutrophils (10⁷ cells/ml). After allowing the neutrophils to statically adhere for 10 min at 37°C in complete DMEM, the capillary tube was integrated into the recirculating loop (as described above) and flow was initiated with freshly infused neutrophils from the same neutrophil cell isolate. The freshly infused neutrophils established a rolling interaction with adherent neutrophils which was recorded for off-line analysis.

Anti-L-selectin mAbs DREG 56, DREG 110 (16), and EL-246 were infused into the recirculating loop at a final concentration of 100 μg/ml for mAb blocking experiments (concentrations of DREG 56 as low as 10 μg/ml also completely blocked rolling). Similar concentrations of HERMES-3 (anti-CD44) (18), R15.7 (anti-CD18) (T. K. Kishimoto and R. Rothlein, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT), and HECA-452 (anti-CLA) (19) were also tested. Again, LPS levels were determined to be <2 pg/ml (at a 10 μg/ml mAb concentration) for mAbs used and no activation as a consequence of mAb treatment was observed as determined by measurement of both L-selectin and CD-18 level on the surface of pre- and post-mAb treated cells (data not shown).

Neutrophils were exposed to chemical inhibitors of rolling both in suspension and while bound, by treating with 5 × 10⁻⁶ M FMLP (Sigma Chemical Co.) in complete DMEM or with 1 U/ml/10⁷ cells neuraminidase (Sigma Chemical Co.) in acetate buffer (50 mM NaOOAc, 154 mM NaCl, pH 6.5, 4 mM CaCl₂; final concentrations) by preincubation of capillary tube neutrophil monolayers (20 min at 37°C for both FMLP and neuraminidase) or by direct infusion into the recirculating loop, treating neutrophil cell suspensions and capillary monolayers during the course of the flow experiment. DMEM or acetate buffer (pH 6.5), respectively, were infused into the loop as controls. Neutrophils treated with neuraminidase for examination of rolling behavior on neutrophil monolayers (as above) showed unaltered L-selectin and CD-18 levels by flow cytometry and thus indicated no induced activation as a consequence of this treatment (data not shown).

Data Analysis. Rolling cells were quantified by manual counting from moving and frozen videotape frames on the video monitor and reported as the number of neutrophils rolling on the bound neutrophil monolayer within the 350 × 250-μm video-microscopic field as a function of time. Initial measured rolling, reported in the figures, was defined as greater than 10 neutrophils/video-field rolling on adherent neutrophils. Rolling speed was measured by following individual neutrophils frame by frame (1/30th of a s/frame) and recording distance vs. time rolling and reported as micrometers rolled per second on the bound neutrophil monolayer within the 350 × 250-μm video-microscopic field using an Apple Macintosh Quadra 660AV computer (14-inch diagonal Apple RGBAV monitor).

Results and Discussion. Using a closed-loop shear assay, we have established that IL-1-treated HUVECs expressing E-selectin (20) strongly support neutrophil rolling which is blocked by anti-E-selectin mAbs (15). In these analyses, we noted that neutrophils avidly arrest on the HUVECs, forming a secondary monolayer of neutrophils (Fig. 1, A–C) that can be disrupted by anti-CD18 mAb treatment (15), and that subsequently, these bound neutrophils themselves support rolling of newly arriving neutrophils (circles, Fig. 1, D–F). Neutrophils rolling on HUVECs (Fig. 1 G), when treated with a functional blocking anti-P-selectin mAb, experienced no decrease in either neutrophil–HUVEC or neutrophil–neutrophil rolling, indicating no role for P-selectin in either interaction (circles, Fig. 1 H). Formation of the bound neutrophil monolayer on the HUVECs continued, as shown by the large number of uncircled, bound neutrophils (Fig. 1 H). However, when treated with anti-L-selectin, all forms of rolling stopped (Fig. 1 I). A similar treatment using the functional blocking anti-L- and anti-E-selectin mAb (EL-246) prevented initial neutrophil rolling and formation of the bound neutrophil monolayer on HUVECs (data not shown).

The analysis of an individual neutrophil–neutrophil rolling sequence is demonstrated in Fig. 2 A. Overlapped video frames, five frames apart, represent 3 s of real time (numbered at 0, 1, 2, and 3 s) and contain a single highlighted neutrophil. Arrows indicate the direction of motion. This neutrophil established a variable speed rolling interaction (demonstrated by the varying widths of each video frame displayed) initially on the underlying monolayer and subsequently on the bound neutrophils. It followed a nonlinear rolling path (Fig. 2 B, in black) during which it jumped across several gaps between the bound neutrophils (white arrows, A; gaps in the black
Figure 1. Video-frame stills of the shear-dependent accumulation of neutrophils on an IL-1-activated HUVEC monolayer and initiation of neutrophil-neutrophil rolling followed by antibody inhibition (12). The HUVEC monolayer (A) supported the shear-dependent onset of neutrophil-HUVEC rolling at 1 min after beginning flow (B), and under constant shear at 3 min, neutrophils arrested on the HUVECs to form a monolayer capable of supporting subsequent neutrophil rolling (C). Individual neutrophils, denoted by circles in (D–F) at the 3-min assay time point, rolled across the HUVEC-bound neutrophil monolayer; starting and intermediate positions are shown by black dots, 0- and 1-s positions (E and F). Rolling neutrophils moved at velocities well below those observed for noninteracting cells. Dashed lines and solid arrows represent the distance traveled in 1 s (in this experiment averaging 64 μm/s at 2.0 dynes/cm² (E and F); noninteracting cells moved too rapidly to appear as clearly defined objects on the video-frame stills). (G–I) Effects of treatment with antiselectin mAbs were examined during neutrophil-neutrophil rolling. (G) Untreated neutrophil-endothelial cell and neutrophil-neutrophil interactions before infusion of antiselectin mAbs. (H) Anti-P-selectin blocking mAb (WAPS 12.2, 100 μg/ml) had no effect on either neutrophil-endothelial cell rolling or neutrophil-neutrophil rolling 2.5 min after injection (rolling cells denoted by circles with short arrows). This was in great contrast to the effect of the anti-L-selectin mAb (DREG56 100 μg/ml) and the anti-L, anti-E-selectin mAb (EL-246, 100 μg/ml) both of which blocked, within 2 min, all neutrophil-endothelial cell and neutrophil-neutrophil rolling interactions without release of adherent cells (I).

rolling track, B) to eventually roll once again onto the underlying monolayer and arrest. From these rolling sequences, neutrophil behavior, rolling speed, and numbers of rolling cells were easily determined and quantified.

As expected (and commonly seen during the isolation of neutrophils from the blood of human donors), neutrophil aggregation occurred to varying degrees and was observed in many of the video sequences. The aggregation was the result of the physiological state of the donor and/or caused by the isolation procedure. The neutrophil aggregates also showed the ability to interact and roll over the surface of the bound neutrophil monolayer. Sporadic interactions of these irregular aggregates composed of highly variable numbers of neutrophils, with the neutrophil–endothelial cell monolayer, mirrored the behavior of individual neutrophils. Treatment with anti-L-selectin mAbs completely blocked this interaction, but failed to disrupt the neutrophil aggregates present and suspended in the flow (data not shown). Because aggregates exhibited behavior similar to that of isolated cells and because their presence was variable from experiment to experiment, neutrophil aggregates were excluded from the quantitative analyses reported below.

To exclude the possibility of a contribution of the HUVECs in this neutrophil–neutrophil rolling interaction, we tested whether neutrophils bound to serum-coated glass could support neutrophil rolling. In both systems (serum and endothelial cell bound), rolling occurred under a shear stress of 0.5–2.0 dynes/cm² (determined by the Hagen-Poiseuille equation), levels that have been defined for leukocyte–endothelial cell rolling in vitro and in vivo (13). Rolling velocities of neutrophils rolling on adherent neutrophils had a mean value of 88.4 μm/s ± 30.1 (SD), over the entire course of the experiments, as compared with 16.6 μm/s ± 24.6 (SD) for neutrophils rolling directly on HUVECs. Both values were within the lower range of velocities reported for neutrophil rolling in vivo (10–840 μm/s) (21). The number of neutrophils rolling on the bound neutrophil monolayers initially increased over time (Fig. 3 A). Maximal rolling occurred within 4–6 min.
Figure 2. Behavior of an individual neutrophil rolling on several groups of arrested neutrophils that facilitated its eventual arrest on the underlying monolayer. Early in the establishment of the neutrophil monolayer, A shows a single neutrophil rolling as the neutrophil interacts with bound neutrophils in 19 individual computer-digitized and overlapped video frames. Individual frames shown in A and B are demarcated by vertical black lines and labeled with the total number of elapsed frames from right to left (in the space between A and B). The total sequence shows a single highlighted neutrophil with arrows indicating the direction of motion and represents 3 s of real time. The rolling cell is labeled at the 0-, 1-, 2-, and 3-s time points (A). White arrows indicate discontinuities in rolling where the neutrophil jumps from bound neutrophil to bound neutrophil along its track of motion. (B) The track of the rolling neutrophil across the field is shown as a discontinuous black line with gaps indicating jumps from one bound neutrophil to another. The image of the rolling neutrophil has been removed by computerized digital frame subtraction to allow visualization of the bound neutrophils with which the rolling cell was interacting.

After infusion of the neutrophil cell suspension into the recirculating loop (14–16 min after the onset of formation of the tube-bound neutrophil monolayer) and was maintained for ~8–10 min. After 10–15 min, the number of rolling cells decreased dramatically (Fig. 3 A). Infusion of additional suspended neutrophils into the rolling assay after 9 min did not result in a renewed rolling increase (Fig. 3 B), demonstrating that the decreased neutrophil-neutrophil rolling interaction was due to changes in the adherent neutrophil monolayer. Considerable shape change of the bound neutrophils occurred in correlation with the decreased rolling (data not shown). These results indicate that the bound neutrophils can directly support neutrophil rolling.

To determine the receptor mechanisms of this interaction, we further tested the effects of inhibitors on neutrophil/neutrophil rolling. Rolling interactions were first established, then various antibody or chemical inhibitors were infused into the closed-loop system. Similar results were obtained with neu-
Figures 3. Numbers of neutrophils demonstrating rolling on bound neutrophil monolayers as a function of time. Neutrophils were allowed to bind firmly to serum-coated tubes (13) for 10 min at 37°C, forming an adherent monolayer, then new neutrophils were passed over the monolayer under flow and the number of rolling cells were followed over time. (A) Results from three typical experiments. Neutrophil-neutrophil rolling, identical to that seen with HUVEC and supported by neutrophil monolayers, was observed to have a finite time course after binding of the monolayer forming neutrophils, rapidly increasing to peak at 4–6 min after infusion of cells (14–16 min after initiating the binding of adherent neutrophils) followed by a decline to baseline 7 min later. As demonstrated (B) in two experiments duplicating the same protocol as in (A), fresh neutrophils (from the original isolation held at 15°C) were infused after the total decay of the initial neutrophil-neutrophil rolling interactions. No new peak in the number of rolling cells was observed, indicating that decreased rolling was due to secondary changes in the adherent cells.

Figures 4. The effects of treatment with antiadhesion molecule mAbs after the onset of initial measured neutrophil-neutrophil rolling. (A) Blocking effects of anti-L-selectin mAb (DREG 110, DREG 56, and EL-246) treatment when infused into the recirculating loop after initiation of neutrophil-neutrophil rolling. In all three experiments, mAbs were infused at the 3-min time point after neutrophil infusion during the increasing phase of neutrophil rolling interactions; the existing rolling and the initiation of new neutrophil-neutrophil rolling rapidly declined to baseline within 2 min in all cases. (B) Results of three independent antiadhesion molecule mAbs tested for their blocking effects on neutrophil-neutrophil rolling in parallel with anti-L-selectin mAb controls. As before, these mAbs were infused during the increasing phase of neutrophil-neutrophil rolling interaction. The data plots for anti-CD44, anti-CD18, and anti-CLA mAbs demonstrated that they had no effect on neutrophil-neutrophil rolling as shown by representative plots of three replicate experiments. The anti-L-selectin mAb results, shown as pooled data with standard error bars, demonstrated reproducibly complete blocking.

Neutrophils bound to cytokine-activated endothelial cells or serum-coated tubes; more detailed analyses were conducted using serum-coated capillary tubes. The rolling cells were monitored for 4 min after injection into the loop—well before their normal decline in rolling described above. Within 1–2 min after the infusion of the anti-L-selectin mAbs DREG 56, DREG 110, and EL-246, rolling was completely inhibited (Fig. 4 A). In separate experiments using the same anti-L-selectin mAbs, pretreatment of neutrophils before their infusion inhibited all rolling interactions (data not shown). Antibodies directed against CD44, cutaneous leukocyte antigen (CLA), or CD18 were infused without effect (Fig. 4 B). Experiments examined for 10–20 min showed the normal decline in the number of rolling cells, which was not accelerated by the CD44, CLA, or CD18 antibodies (data not shown). Treatment with R15.7 (anti-CD18 mAb) did cause a loss in the number of HUVEC and serum-coated tube-adherent neutrophils by 20 min, demonstrating that the antibody was functional (data not shown). HECA-452 (anti-CLA mAb) was also tested at an increasing range up to 400 μg/ml in one experiment where no effect on rolling was observed (data not shown).

Inhibition of rolling by anti-L-selectin could have been due to an effect on the rolling neutrophils, the adherent neutrophils, or both. To distinguish between these possibilities, experiments were done to determine whether the adherent or rolling cells were inhibited by the anti-L-selectin mAbs. A
rolling interaction was established and blocked 2 min later by the addition of DREG 56. The loop system was then flushed with media to remove the free DREG 56 mAb. New, untreated neutrophils were then added to the loop. As shown (Fig. 5 A), the neutrophil monolayer, after DREG 56 treatment and washing, supported renewed rolling of nonantibody-treated neutrophils. These results show that the DREG 56 anti-L-selectin mAb inhibited rolling by blocking L-selectin function on the rolling cells. The same results were seen with other anti-L-selectin mAbs (data not shown). In separate experiments using DREG 56, pretreatment of the suspension of neutrophils before their infusion inhibited all rolling interactions, whereas DREG 56 pretreatment of the neutrophil monolayer had no effect (Fig. 5 B). These results show that the DREG 56 anti-L-selectin mAb inhibited rolling by blocking L-selectin function on the rolling cells.

Enzymatic and chemical treatments were tested to further characterize the neutrophil–neutrophil rolling interaction. Removal of terminal sialic acid residues by infusion of neuraminidase (1 U/ml) into the loop completely inhibited rolling within 3 min (Fig. 6 A). Infusion of $5 \times 10^{-6}$ M FMLP, which causes rapid activation of neutrophils, also led to rapid inhibition of rolling (Fig. 6 A). Although an increase in the number of aggregates of neutrophils was observed, large numbers of individual noninteracting neutrophils were seen and recorded after FMLP treatment. Neuraminidase, but not FMLP treatment of the bound neutrophils, blocked their ability to support rolling (Fig. 6 B); however, neuraminidase treatment had no effect on rolling neutrophils (data not shown). The neuraminidase acetate buffer control had no effect on the rolling interaction. In contrast, FMLP treatment of the rolling cells alone blocked the interaction (data not shown). These results show that (a) activation of the rolling but not of the adherent cells leads to diminished rolling, consistent...
with shedding of L-selectin, and (b) terminal sialic acid residues on the adherent cell are required for the interaction, consistent with the predicted role of the sialylated Lewis\(^a\) carbohydrate ligands (22).

Our in vitro observations suggest that when neutrophils arrest on endothelium at sites of inflammation, they themselves can serve as adhesive substrates to support shear-dependent recruitment of additional neutrophils. Adherent neutrophils in vivo support neutrophil rolling which has been recently observed by intravital microscopy in both rabbit and mouse venules (Bargatze, R. F., and E. C. Butcher, unpublished observations). This interaction is important for the continued influx of inflammatory cells beyond the adhesive events that occur within the initial few minutes after induction of inflammation. It is interesting to note that neutrophil-neutrophil shear-dependent rolling like neutrophil-endothelial cell rolling is dependent on L-selectin on the rolling cell. The data suggest that the ligand(s) for L-selectin, likely comprised in part by the sialylated Lewis\(^a\) carbohydrate moiety on adherent neutrophils, is exquisitely regulated as rolling only occurs for a short time (10-14 min) after neutrophil binding and initial spreading. The ligand could be shed after the short interaction time frame as previously shown for L-selectin (16, 23) or internalized, or it could be modulated in its surface expression during neutrophil adherence-associated shape changes, making it unavailable to the rolling neutrophil. These results greatly extend those of Simon et al. (24) who showed that chemoattractant-induced neutrophil aggregation (examined in stirred suspension) can be blocked by anti-L-selectin mAb. Uniquely, in the flow-induced shear experiments reported here in vitro, neutrophil-neutrophil rolling-augmented recruitment to endothelium is only dependent on L-selectin expression on the rolling cell (not the bound neutrophil). In addition, anti-\(\beta_2\) integrin mAb antibodies, which block aggregate formation in suspension (24), have no effect on neutrophil-neutrophil rolling at 2 dynes/cm\(^2\). This is not surprising as the estimated shear in suspension experiments reported by Simon et al. (24) was <1 dyne/cm\(^2\), a level at which integrin interactions may play a primary role in cell adhesion interactions. We are currently studying the nature of the L-selectin ligand expressed by bound neutrophils to better compare these two L-selectin-dependent neutrophil-neutrophil interactions.

Effective blocking of inflammatory reactions by anti-L-selectin mAbs occurs in vivo (9, 25, 26). Indeed, anti-L-selectin mAbs are the most consistent antiselectin blocking reagents in models of peritonitis, and lung and dermal inflammation (27-31). Blocking by anti-L-selectin mAbs is thought to be due to inhibition of neutrophil-endothelial cell interactions. However, in virtually all in vitro inflammation and L-selectin-dependent models of neutrophil-endothelial cell adhesion, the blocking by anti-L-selectin mAbs only accounts for a portion of the observed adhesion (7, 15, 17, 32-35). Since neutrophil-neutrophil rolling is consistently completely blocked by anti-L-selectin mAbs in our experiments, the effectiveness of these mAbs in inhibiting inflammation in vivo may possibly result from blocking of this interaction. Thus, shear-dependent neutrophil-neutrophil interactions may provide a new target in the design of therapeutic inhibitors of inflammation. It is probable that these inhibitors would be effective in diverse inflammatory settings, because neutrophil-neutrophil rolling may occur during many if not all types of inflammation.

In summary, adherent neutrophils, like activated endothelial cells, support continuous neutrophil rolling by presentation of a novel L-selectin ligand as demonstrated in vitro by the consistent anti-L-selectin mAb blocking of this interaction. The demonstration of L-selectin-mediated neutrophil-neutrophil rolling in vitro suggests a new mechanism for leukocyte recruitment in vivo which could support augmented accumulation of neutrophils during inflammation.

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References

1. Smith, C.W. 1993. Endothelial adhesion molecules and their role in inflammation. Can. J. Physiol. Pharmacol. 71:76.
2. Bargatze, R.F., and E.C. Butcher. 1993. Rapid G protein-regulated activation event involved in lymphocyte binding to high endothelial venules. J. Exp. Med. 178:367.
3. Lasky, L.A. 1992. Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science (Wash. DC). 258:964.
4. Jutila, M.A. 1992. Leukocyte traffic to sites of inflammation. APMIS. 100:191.
5. Butcher, E.C. 1991. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell. 67:1033.
6. Stoolman, L.M. 1989. Adhesion molecules controlling lymphocyte migration. Cell. 56:907.
7. Springer, T.A. 1990. Adhesion receptors of the immune system. Nature (Lond.). 346:425.
8. Arfors, K.-E., C. Lundberg, L. Lindblom, K. Lundberg, P.G. Beatty, and J.M. Harlan. 1987. A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear accumulation and plasma leakage in vivo. Blood. 69:338.
9. von Andrian, U.H., J.D. Chambers, L. McEvoy, R.F. Bargatze, K.-E. Arfors, and E.C. Butcher. 1991. Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and leukocyte beta-2 integrins in vivo. Proc. Natl. Acad. Sci. USA. 88:7538.
10. Kishimoto, T.K. 1991. A dynamic model for neutrophil localization to inflammatory sites. The Journal of NIH Research. 3:75.
11. Zimmermann, G.A., S.M. Prescott, and T.M. McIntyre. 1992. Endothelial cell interactions with granulocytes: tethering and signaling molecules. Immunol. Today. 13:93.
12. Rot, A. 1992. Endothelial cell binding of NAP-1/IL-8: role in neutrophil emigration. Immunol. Today. 13:291.
13. Lawrence, M.B., and T.A. Springer. 1991. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell. 65:859.
14. Abbassi, O., T.K. Kishimoto, C.L. Lane, L.V. McIntyre, and C.W. Smith. 1993. Endothelium-leukocyte adhesion molecule-1 supports neutrophil rolling in vitro under conditions of flow. J. Clin. Invest. 92:2719.
15. Bargatze, R.F., S. Kurk, G. Watts, T.K. Kishimoto, C.A. Speer, and M.A. Jutila. 1994. In vivo and in vitro functional examination of a conserved epitope of L- and E-selectin crucial for leukocyte-endothelial cell interactions. J. Immunol. 152:5814.
16. Kishimoto, T.K., M.A. Jutila, and E.C. Butcher. 1990. Identification of a human peripheral lymph node homing receptor: a rapidly down-regulated adhesion molecule. Proc. Natl. Acad. Sci. USA. 87:2244.
17. Jutila, M.A., G. Watts, B. Walcheck, and G.S. Kansas. 1992. Characterization of a functionally important and evolutionarily well-conserved epitope mapped to the short consensus repeats of E-selectin and L-selectin. J. Exp. Med. 175:1565.
18. Jalkanen, S., R.F. Bargatze, J. de los Tojos, and E.C. Butcher. 1987. Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85-90-kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. J. Cell Biol. 105:983.
19. Picker, L.J., S.A. Michie, L.S. Rott, and E.C. Butcher. 1990. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of HECA-452 epitope by benign and malignant T cells at cutaneous sites. Am. J. Pathol. 136:1053.
20. Bevilacqua, M.P., J.S. Pober, D.L. Mendrick, R.S. Cotran, and M.A. Gimbrone, Jr. 1987. Identification of an inducible endothelial-leukocyte adhesion molecule. Proc. Natl. Acad. Sci. USA. 84:9238.
21. Ley, K., M. Cerrito, and K.-E. Arfors. 1991. Sulfated polysaccharides inhibit leukocyte rolling in rabbit mesenteric venules. Am. J. Physiol. 260:H1667.
22. von Andrian, U.H., J.D. Chambers, E.L. Berg, S.A. Michie, D.A. Brown, D. Karolak, L. Ramezani, E.M. Berger, K.-E. Arfors, and E.C. Butcher. 1993. L-selectin mediates neutrophil rolling in inflamed venules through sialyl Lewis*-dependent and -independent recognition pathways. Blood. 82:182.
23. Kishimoto, T.K., M.A. Jutila, E.L. Berg, and E.C. Butcher. 1989. The Mac-1 and Mel-14 defined adhesion molecules are inversely regulated on neutrophils by chemotactic factors. Science (Wash. DC). 245:1238.
24. Simon, S.I., J.D. Chambers, E. Butcher, and L.A. Sklar. 1992. Neutrophil aggregation is $\beta_2$-integrin- and L-selectin-dependent in blood and isolated cells. J. Immunol. 149:2765.
25. Ley, K., P. Gashtgens, C. Fennie, M.S. Singer, L.A. Lasky, and S.D. Rosen. 1991. Lectin-like adhesion molecule-1 mediates leukocyte rolling in mesenteric venules in vivo. Blood. 77:2552.
26. Ma, X.L., A.S. Weyrich, D.J. Lefer, M. Buereke, K.H. Alber- tine, T.K. Kishimoto, and A.M. Lefer. 1993. Monoclonal antibody to L-selectin attenuates neutrophil accumulation and protects ischemic reperfusion cat myocardium. Circulation. 88:649.
27. Lewinsohn, D.M., R.F. Bargatze, and E.C. Butcher. 1987. Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes and other leukocytes. J. Immunol. 138:4313.
28. Jutila, M.A., L. Rott, E.L. Berg, and E.C. Butcher. 1989. Function and regulation of the neutrophil Mel-14 antigen in vivo: comparison with LFA-1 and Mac-1. J. Immunol. 143:3318.
29. Watson, S.R., C. Fennie, and L.A. Lasky. 1991. Neutrophil influx into an inflammatory site inhibited by a soluble homing receptor-IgG chimera. Nature (Lond.). 349:164.
30. Mulligan, M.S., and P.A. Ward. 1993. Role of selectins in acute inflammatory lung injury in rats. FASEB (Fed. Am. Soc. Exp. Biol.) J. 24, Pt II:A640. (Abstr.)
31. Sasaki, S., S. Sharar, L. Flaherty, J. Paulson, J. Harlan, and R. Winn. 1993. P-selectin blockade does not impair leukocyte host defense against bacterial peritonitis or soft tissue infection in rabbits. FASEB (Fed. Am. Soc. Exp. Biol.) J. 4, Pt II:A640. (Abstr.)
32. Picker, L.J., R.A. Warnock, A.R. Burns, C.M. Doerschuk, E.L. Berg, and E.C. Butcher. 1991. The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectin ELAM-1 and GMP-140. Cell. 66:921.
33. Spertini, O., G.S. Kansa, K.A. Reimann, C.R. Mackay, and T.F. Tedder. 1991. Function and evolutionary conservation of distinct epitopes on the leukocyte adhesion molecule-1 (LAM-1) that regulate leukocyte migration. J. Immunol. 147:942.
34. Moore, K.L., A. Vari, and R.P. McEver. 1991. GMP-140 binds to a glycoprotein receptor on human neutrophils: evidence for a lectin-like interaction. J. Cell Biol. 112:491.
35. Lecico, A., J. Mühloff, S. Isenmann, and D. Vestweber. 1993. Identification of a glycoprotein ligand for E-selectin on mouse myeloid cells. J. Cell Biol. 121:449.