

Threshold Levels of Fluid Shear Promote Leukocyte Adhesion through Selectins (CD62L,P,E)

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Abstract. Leukocyte adhesion through L-selectin to peripheral node addressin (PNAd, also known as MECA-79 antigen), an L-selectin ligand expressed on high endothelial venules, has been shown to require a minimum level of fluid shear stress to sustain rolling interactions (Finger, E.B., K.D. Puri, R. Alon, M.B. Lawrence, V.H. von Andrian, and T.A. Springer. 1996. *Nature (Lond.)*. 379:266–269). Here, we show that fluid shear above a threshold of 0.5 dyn/cm² wall shear stress significantly enhances HL-60 myelocyte rolling on P- and E-selectin at site densities of 200/μm² and below. In addition, gravitational force is sufficient to detach HL-60 cells from P- and E-selectin substrates in the absence, but not in the presence, of flow. It appears that fluid shear-induced torque is critical for the maintenance of leukocyte rolling. K562 cells transfected with P-selectin glycoprotein ligand-1, a ligand for P-selectin, showed a similar reduction in rolling on P-selectin as

the wall shear stress was lowered below 0.5 dyn/cm². Similarly, 300.19 cells transfected with L-selectin failed to roll on PNAd below this level of wall shear stress, indicating that the requirement for minimum levels of shear force is not cell type specific. Rolling of leukocytes mediated by the selectins could be reinitiated within seconds by increasing the level of wall shear stress, suggesting that fluid shear did not modulate receptor avidity. Intravital microscopy of cremaster muscle venules indicated that the leukocyte rolling flux fraction was reduced at blood centerline velocities less than 1 mm/s in a model in which rolling is mediated by L- and P-selectin. Similar observations were made in L-selectin-deficient mice in which leukocyte rolling is entirely P-selectin dependent. Leukocyte adhesion through all three selectins appears to be significantly enhanced by a threshold level of fluid shear stress.

THE initial interaction of circulating leukocytes with the vessel wall during an inflammatory response consists in part of transient adhesive contacts (rolling) mediated by the selectin family of adhesion receptors (Ley and Tedder, 1995; Springer, 1995), and in the case of eosinophils and lymphocytes, the interaction also involves the VLA-4 integrins (Jones et al., 1994; Lusinskas et al., 1994; Sriramarao et al., 1994; Alon et al., 1995b; Berlin et al., 1995). As the leukocytes roll along the endothelium under the influence of fluid shear, adhesive bond formation in the contact patch is balanced by continuous bond breakage. Rolling adhesions retard the motion of leukocytes sufficiently that the inflammatory response is tightly localized at the site of infection or tissue damage. Selectin-dependent rolling interactions can be induced experimentally in vivo by either surgical trauma, histamine, or exposure of tissue to cytokines such as tumor necrosis factor-α

(TNF-α)¹. In the case of surgical trauma or histamine exposure (Asako et al., 1994; Kubes and Kanwar, 1994; Ley, 1994), P-selectin expression on the endothelium is upregulated, which accounts for the majority of leukocyte rolling. L-selectin-dependent rolling can be observed in vivo using cell lines transfected with L-selectin (Ley et al., 1993). On neutrophils, a high-affinity ligand for P-selectin, P-selectin glycoprotein ligand-1 (PSGL-1) has been shown in vitro and in vivo to mediate P-selectin-dependent leukocyte rolling (Moore et al., 1995; Norman et al., 1995). E-selectin expressed on vascular endothelium supports leukocyte rolling in vivo after administration of TNF-α and is coexpressed with P-selectin in the mouse after TNF-α treatment (Kunkel et al., 1996).

In vitro flow studies have demonstrated that all three selectins are capable of mediating leukocyte rolling and that leukocyte adhesion occurs at much higher fluid shear stresses than that mediated by β₂(CD18) integrins, consist-

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1. *Abbreviations used in this paper:* FucT, fucosyltransferase; OG, octylglucoside; PNAd, peripheral node addressin; PSGL, P-selectin glycoprotein ligand-1; TNF-α, tumor necrosis factor-α; Vcrit, critical velocity.

tent with *in vivo* observations (Kansas, 1996). Leukocyte attachment and rolling via selectins occurs *in vitro* in flow chamber assays at 10-fold higher wall shear stresses than adhesions mediated by β_2 integrin mechanisms (between 3.0 and 4.0 dyn/cm² wall shear stress versus 0.2 dyn/cm²). The relative effectiveness of selectin and β_2 integrin adhesion mechanisms has been attributed to differences in bond formation rates (Hammer and Apte, 1992; Tozeren and Ley, 1992) and, more recently, to the localization of L-selectin and PSGL-1 to the peaks of microvilli and membrane microfolds on the leukocyte surface and exclusion of β_2 integrins from these regions (Picker et al., 1991; Berlin et al., 1995; Moore et al., 1995).

While selectins support leukocyte adhesion under flow conditions, the β_2 integrins LFA-1 and Mac-1 (in the case of neutrophils and monocytes) are critical for leukocyte extravasation. The relative ineffectiveness of β_2 integrins at mediating leukocyte adhesion under flow conditions is balanced by the critical role they play in arrest and emigration of leukocytes through the vessel wall (Smith et al., 1989; von Andrian et al., 1991). In addition to strictly adhesive interactions, the β_2 integrins, like L-selectin (Wadell et al., 1994; Simon et al., 1995), may be involved in intracellular signal transduction (Fuortes et al., 1994).

Bond lifetimes of selectins appear to be on the order of seconds based on the magnitude of leukocyte rolling velocities observed *in vitro* and *in vivo*, suggesting that the binding of leukocytes to the vessel wall is unstable (Kansas et al., 1993; Kaplanski et al., 1993; Alon et al., 1995a; Zhao et al., 1995). Based on the hypothesis that selectin-mediated rolling requires the formation of new selectin bonds to balance dissociation, it was shown recently that a minimum level of fluid shear stress was required for adhesion through L-selectin (Finger et al., 1996). However, P- and E-selectin did not appear to require fluid shear to sustain adhesion in contrast to observations of L-selectin-mediated rolling. In the present study, we examined leukocyte rolling mediated by P-, E-, and L-selectin at defined site densities and showed that adhesion through both P- and E-selectin is promoted significantly by a threshold level of shear force as is the case for L-selectin-mediated adhesion. In addition, we show that P- and L-selectin-dependent leukocyte rolling in venules of the mouse cremaster muscle *in vivo* requires a minimum shear rate as indicated by a reduction of rolling leukocyte flux at low blood flow velocities in wild-type and L-selectin-deficient mice.

Materials and Methods

Monoclonal Antibodies

P23 murine antibody (mAb) to P-selectin was a generous gift of Drs. J.-G. Geng and D.C. Anderson (Upjohn Laboratories, Kalamazoo, MI) (Ma et al., 1994). BB11, a murine mAb to human E-selectin, was a generous gift of Dr. R. Lobb (Biogen, Inc., Cambridge, MA) (Lobb et al., 1991). DREG-56, a murine mAb against L-selectin, was a generous gift of Dr. T.K. Kishimoto (Boehringer Ingelheim, Danbury, CT) (Kishimoto et al., 1990).

Isolation of P-Selectin, E-Selectin, and Peripheral Node Addressin

P-selectin was purified from outdated platelets (American Red Cross, Richmond, VA) by immunoaffinity chromatography. Briefly, outdated platelets were lysed in Tris-saline-azide (TSA, 0.025 M Tris-HCl, 0.15 M

NaCl, 0.02% azide, pH 8.0) + 5 mM EDTA + 1% Triton X-100 in the presence of protease inhibitors aprotinin (10 μ g/ml; Sigma Chemical Co., St. Louis, MO) and leupeptin (5 μ g/ml; Sigma Chemical Co.). P23 (anti-P-selectin) was coupled to Sepharose (CNBr-activated 4B; Pharmacia Biotech AB, Uppsala, Sweden) (Ma et al., 1994), the platelet lysate was passed over the P23 column twice, and P-selectin was eluted with acetate buffer (50 mM, pH 3.0) containing 1% octylglucoside (OG) (Ma et al., 1994). The elutant was neutralized with 1 M Tris, pH 9.0, 1% OG (15% vol/vol). Site densities of adsorbed P-selectin were determined by a radioimmunoassay using the P-selectin mAb G1 (Geng et al., 1990). G1 mAb was iodinated using Iodobeads (Pierce, Rockford, IL) following manufacturer's instructions to a specific activity of 7 μ Ci/ μ g. Site densities were determined by saturation binding, assuming binding of one IgG molecule per antigen molecule. The site densities were determined in triplicate twice (see appropriate figure legend).

Recombinant E-selectin was purified from lysates of CHO cells transfected with wild-type human E-selectin cDNA (Lobb et al., 1991). BB11 was coupled to Sepharose at a concentration of 2.7 mg/ml (bed vol 3.5 ml). The CHO cell pellet was lysed in TSA + 5 mM EDTA + 1% Triton X-100 in the presence of protease inhibitors aprotinin (10 μ g/ml) and leupeptin (5 μ g/ml) and passed over the BB11 column. After washing with TSA + 1% OG, E-selectin was eluted with a glycine buffer (100 mM, pH 3.0, 1% OG) and neutralized with 1 M Tris, pH 9.0, 1% OG (15% vol/vol). Site densities of adsorbed E-selectin were determined by a radioimmunoassay using the E-selectin mAb BB11 iodinated to a specific activity of 5 μ Ci/ μ g. Site densities were determined by saturation binding in triplicate twice. MECA-79 antigen or peripheral node addressin (PNAd) (a generous gift of Drs. U.H. von Andrian and E.C. Butcher (Center for Blood Research, Inc., Boston, MA and Stanford University, Palo Alto, CA, respectively) was purified from human tonsil lysates as previously described (Berg et al., 1991).

Leukocyte Isolation and Cell Lines

K562 cells were stably cotransfected with PSGL-1 cDNA (Sako et al., 1993) and FucT-VII cDNA (Natsuka et al., 1994) as described elsewhere (Snapp et al., 1997). The Jurkat T lymphoma line and HL-60 cell line was cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY) with 10% FCS (Atlanta Biologicals, Atlanta, GA) and antibiotics in 5% CO₂. 300.19 cells transfected with L-selectin were cultured in RPMI 1640 + 10% FCS supplemented with 5 μ M mercaptoethanol. T lymphocytes and neutrophils were isolated from citrate anticoagulated whole blood by dextran sedimentation (Dextran T500; Pharmacia Biotech AB) followed by density separation over Ficoll-Hypaque (Histopaque 1077; Sigma Diagnostics, St. Louis, MO). T lymphocytes were separated from monocytes and B cells by sequential plastic and nylon wool absorption as previously described (Lawrence et al., 1995).

Laminar Flow Assays

Purified P-selectin, E-selectin, or PNAd was adsorbed to polystyrene cover slides cut from bacteriological petri dishes (model 1058; Falcon Plastics, Cockeysville, MD), which were then fitted onto a parallel plate flow chamber (Lawrence et al., 1994). The preparations were diluted with TSA (pH 8.0) as indicated and incubated on the coverslide for 2 h at room temperature (22°C). Nonspecific adhesion of leukocytes to the substrate was blocked with 1% HSA (American Red Cross, Washington, D.C.) in TSA for 30 min at 22°C.

T lymphocytes and all cell lines were suspended in RPMI 1640 with 2% FCS (0.5 $\times 10^6$ cells/ml) for flow assays. Wall shear stress was calculated assuming a viscosity of assay buffer equal to water at room temperature (1.0 centipoise, 24°C). A critical velocity (V_{crit}) was measured based on the velocity of a noninteracting leukocyte in a shear flow near the wall of the flow chamber (Goldman et al., 1967). Leukocytes moving more slowly than the V_{crit} for the appropriate cell type were defined as rolling. To equalize cell delivery rates at wall shear stresses of 3 to 0.5 dyn/cm², the time of flow was increased with inverse proportion to the flow rate. To equalize cell delivery rates at wall shear stresses below 0.5 dyn/cm², fluxes of noninteracting cells were determined by counting the number of cells flowing over the substrate that were in the plane of focus.

Inverted Flow Chamber Assay (Leukocyte Release Assay)

In selected experiments, the flow chamber was inverted before the wall shear stress was lowered to take advantage of gravity sedimentation to prevent re-

attachment of HL-60 cells after their release from the E- or P-selectin-coated substrate. Once a population of HL-60 cells had attached and begun rolling on the immobilized selectin at 1.0 dyn/cm² wall shear stress, the flow chamber was inverted so that the rolling cells would then be on the upper wall rather than the lower wall. The effect of changes in wall shear stress was assessed once a stable population of HL-60 cells was established after 2 min. In this orientation, the influence of gravity sedimentation pulls an unbound or released cell away from the wall at ~1 μm/s, and consequently, the probability of reattachment is markedly diminished, which allows a clear differentiation between adherent and nonadherent HL-60 cells to be made even at wall shear stresses as low as 0.1 dyn/cm².

Intravital Microscopy

L-selectin-deficient mice were obtained from established colonies derived from gene-targeted founders described previously (Arbones et al., 1994). All mice used in this study were of a mixed 129/Sv × C57BL/6 background. Preceding the experiment, mice were anesthetized with an intraperitoneal injection of ketamine hydrochloride (Ketalar, Parke-Davis, Ann Arbor, MI; 100 mg/kg) after premedication (given intraperitoneal) with sodium pentobarbital (Nembutal; Abbott Laboratories, Abbott Park, FL; 30 mg/kg) and atropine (Elkins-Sinn, Inc., Cherry Hill, NJ; 1 mg/kg). The trachea, carotid artery, and jugular vein of the mice were cannulated for injection of supplementary anesthetic, blood pressure monitoring, and blood sampling. During the experiment, the mice were thermo-controlled using a small animal heating pad (model 50-7503; Harvard Apparatus, Inc., South Natick, MA). The cremaster muscle was prepared for intravital microscopy as described previously (Ley et al., 1995). During and subsequent to this procedure, the muscle was superfused with a thermo-controlled (37°C) bicarbonate-buffered saline as described (Kunkel et al., 1996). Microscopic observations were made between 10 and 120 min after surgical tissue exteriorization using an intravital microscope (Carl Zeiss, Inc., Thornwood, NY) with a saline immersion objective (SW 40, 0.75 NA). Venules with diameters between 15 and 80 μm were observed and recorded through a CCD camera system for approximately 60 s each. Centerline red blood cell velocity in the recorded microvessels was measured using a dual photodiode and a digital on-line cross-correlation program as described (Kunkel et al., 1996). The rolling leukocyte flux was determined by counting the number of leukocytes passing through a stationary plane perpendicular to the axis of each venule over a given time period. The total leukocyte flux through a given venule was estimated by the product of the systemic leukocyte count and the volumetric flow rate of blood through the venule, which was calculated using the microvessel diameter and the mean blood flow velocity assuming cylindrical geometry. The leukocyte rolling flux fraction is defined as the flux of rolling leukocytes as a percent of the total leukocyte flux and, because of this definition, is independent of variations in systemic leukocyte counts.

Results

Selectin-dependent Capture and Rolling Requires a Threshold Level of Shear Forces

Purified PNAd (also known as MECA-79 antigen) adsorbed to plastic supported L-selectin-mediated attachment and rolling of T lymphocytes under conditions of continuous fluid shear stress (Lawrence et al., 1995) that was also dependent on adsorbed PNAd concentration (Fig. 1 A). However, as defined by the criteria of 0.5 s of continuous rolling, a drop in adhesion was observed at wall shear stresses below 0.8 dyn/cm² at all concentrations of PNAd, consistent with a previous report (Finger et al., 1996). T lymphocyte rolling interactions became progressively more transient at wall shear stresses below 1.0 dyn/cm², with rolling interactions rarely lasting more than 0.5 s, leading to a reduction in the number of cells defined as bound.

To address the question of whether the requirement for hydrodynamic shear is a unique property of L-selectin, or of selectins in general, we examined HL-60 cell adhesion to purified P- and E-selectin under flow conditions (Fig. 1, B and C). HL-60 cells express ligands for P- and E-selectin

(Bevilacqua et al., 1987; Larsen et al., 1989) and roll in a manner identical to neutrophils (Moore et al., 1995) but offer a significant advantage over neutrophils in that L-selectin expression is low (Ley et al., 1995) and CD18 integrins are not functional (Bevilacqua et al., 1987), thus reducing any potential effect of leukocyte-leukocyte interactions that might either inhibit or enhance adhesion (Bargatze et al., 1994; Lawrence et al., 1994).

As determined by a quantitative radioimmunoassay, higher site densities of P-selectin correlated with increased adhesion of HL-60 cells under flow conditions. However, HL-60 cell adhesion to P-selectin at concentrations of 140 sites/μm² and below was significantly attenuated at wall shear stresses below 0.25 dyn/cm² (Fig. 1 B). The threshold wall shear stress required for optimal rolling on P-selectin was ~0.5 dyn/cm², half of that observed for L-selectin-dependent rolling, and was influenced by the site density of P-selectin.

HL-60 cell adhesion to purified E-selectin was examined over a similar range of wall shear stresses and site densities as was HL-60 cell adhesion to P-selectin (Fig. 1 C). Increased site densities of E-selectin correlated with increased adhesion and rolling of HL-60 cells. Consistent with observations of HL-60 cell interactions with P-selectin, HL-60 adhesion to E-selectin under flow conditions was significantly reduced at wall shear stresses below 0.25 dyn/cm², particularly at E-selectin densities below 200 sites/μm² (Fig. 1 C). The shear threshold for inhibition of HL-60 binding to E-selectin was slightly lower than that observed for P-selectin. Adhesion of HL-60 cells to E- and P-selectin was highly specific, as 100% of the rolling HL-60 cells could be detached by infusion of media with 5 mM EDTA and completely inhibited by mAbs G1 to P-selectin and BB11 to E-selectin, respectively (data not shown). Therefore, HL-60 cell adhesion to P- and E-selectin appears to be promoted by a threshold level of fluid shear, particularly at concentrations of selectin below 200 sites/μm². Neutrophils were also observed to have a similar pattern of reduced adhesion to isolated P- and E-selectin at site densities <200/μm² when wall shear stresses were lowered below 0.25 dyn/cm² (data not shown). Site densities of P- and E-selectin in vivo have not yet been determined. However, on cultured human endothelium, P-selectin expression has been reported to be 50 sites/μm² (Hattori et al., 1989), suggesting that the densities used in these studies may be physiologically relevant.

Capture of L-Selectin Transfectants on PNAd and of PSGL-1 Transfectants on P-Selectin Requires a Threshold Level of Fluid Shear

A murine B cell line 300.19 transfected with L-selectin was used to determine whether the fluid shear dependence of L-selectin-mediated rolling on PNAd was a unique property of T lymphocytes (Kansas et al., 1993). As with T lymphocytes, adhesion and rolling of 300.19-L-selectin transfectants on PNAd was reduced at wall shear stresses below 0.5 dyn/cm² (Fig. 2 A). Based on these data, low shear inhibition of L-selectin-dependent adhesion does not appear to be exclusively a property of T lymphocytes or of T lymphocyte regulation of L-selectin avidity (Spertini et al., 1991). Similar to the pattern of adhesion observed with

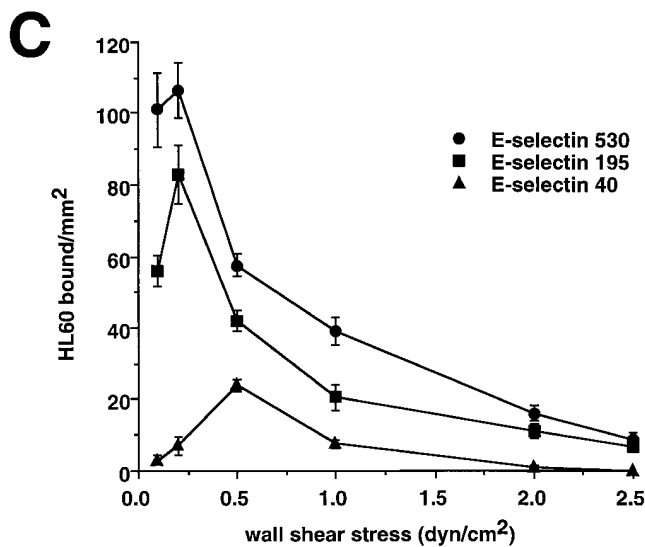
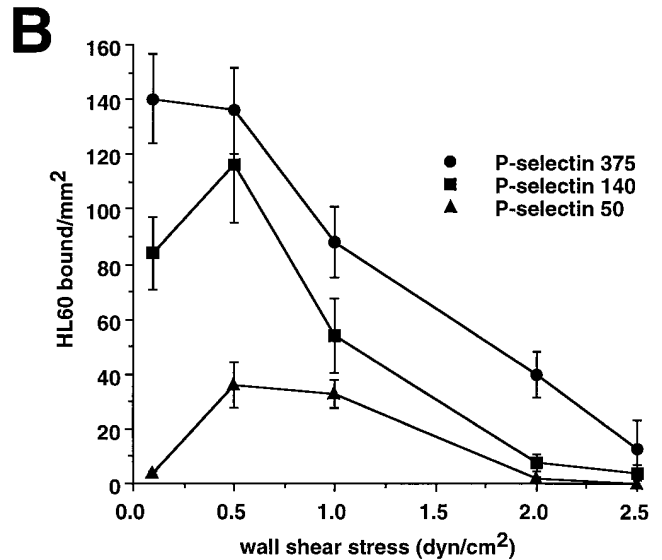
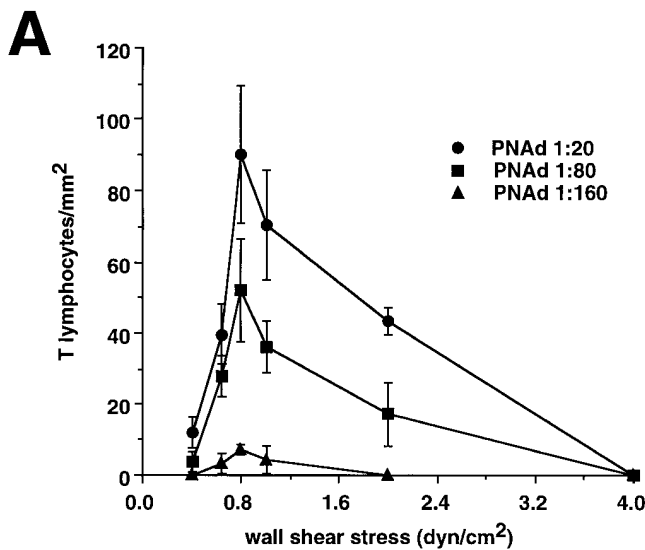


Figure 1. Biphasic pattern of leukocyte adhesion through L-, P-, and E-selectin under flow conditions. (A) T lymphocytes were perfused over surface immobilized PNAd at wall shear stresses ranging from 0.4 to 4.0 dyn/cm². Time of flow was adjusted to control for cell flux through the flow chamber (see Materials and Methods). PNAd was adsorbed at the indicated dilutions (see Materials and Methods). Adhesions were defined as cells remaining attached to the substrate for a minimum of 0.4 s (12 frames of videotape). Data points represent the means (\pm SEM) of three independent determinations. (B) HL-60 cells were perfused over immobilized purified P-selectin at the indicated wall shear stresses and P-selectin site densities (sites/ μ m²). (C) HL-60 cells were perfused over immobilized purified E-selectin at the indicated wall shear stresses and E-selectin site densities (sites/ μ m²). Data points represent means (\pm SEM) of rolling HL-60 cells averaged over 12–20 fields of view.

L-selectin transfectants rolling on PNAd, K562 cells cotransfected with PSGL-1 cDNA and a fucosyltransferase enzyme (FucT-VII) cDNA failed to form sustained rolling adhesions on P-selectin at wall shear stresses of 0.2 dyn/cm² or below (Fig. 2 B). PSGL-1 transfectants thus had a shear threshold similar to that of HL-60 cells interacting with P-selectin. The observation of fluid shear enhancing adhesion through PSGL-1 with P-selectin as well as that between L-selectin and its ligand in PNAd (MECA-79 antigen) suggests that a unique property of the selectin bond is responsible for the requirement of a threshold level of shear force to sustain rolling interactions.

Leukocyte Adhesion through Selectins Can Be Induced and Reversed by Varying Shear Stress

Jurkat T lymphocytes have previously been shown to use L-selectin to roll on PNAd at venular wall shear stresses (Lawrence et al., 1995). Adhesion of Jurkat T lymphocytes could be induced and reversed by a sequence of increasing and decreasing wall shear stress. Jurkat T cells that were rolling on PNAd (1:20 dilution) at 1.0 dyn/cm² wall shear

stress were individually tracked as flow was first lowered to 0.1 dyn/cm² wall shear stress and then raised back to the initial flowrate (Fig. 3 A). Lowering shear resulted in a highly significant reduction of Jurkat T lymphocyte rolling within 1 s, suggesting that the change in binding to PNAd can be altered on very short time scales relative to many intracellular signaling processes. Jurkat T lymphocyte rolling could be reestablished within a fraction of a second by an increase in wall shear stress, suggesting that L-selectin binding avidity was not altered by the change in flowrate (Fig. 3 A). Similarly, rolling of HL-60 cells on P-selectin could be dynamically modulated by first lowering wall shear stress to 0.1 dyn/cm² and then raising it back to 1.0 dyn/cm² (Fig. 3 B). Identical patterns were observed for HL-60 cell rolling on E-selectin after the same experimental manipulation (Fig. 3 C), showing that the adhesive modulation by shear was reversible.

L-, P-, and E-Selectin-mediated Rolling Is Transient at Wall Shear Stresses below a Defined Threshold

Individual Jurkat T lymphocytes or HL-60 cells were

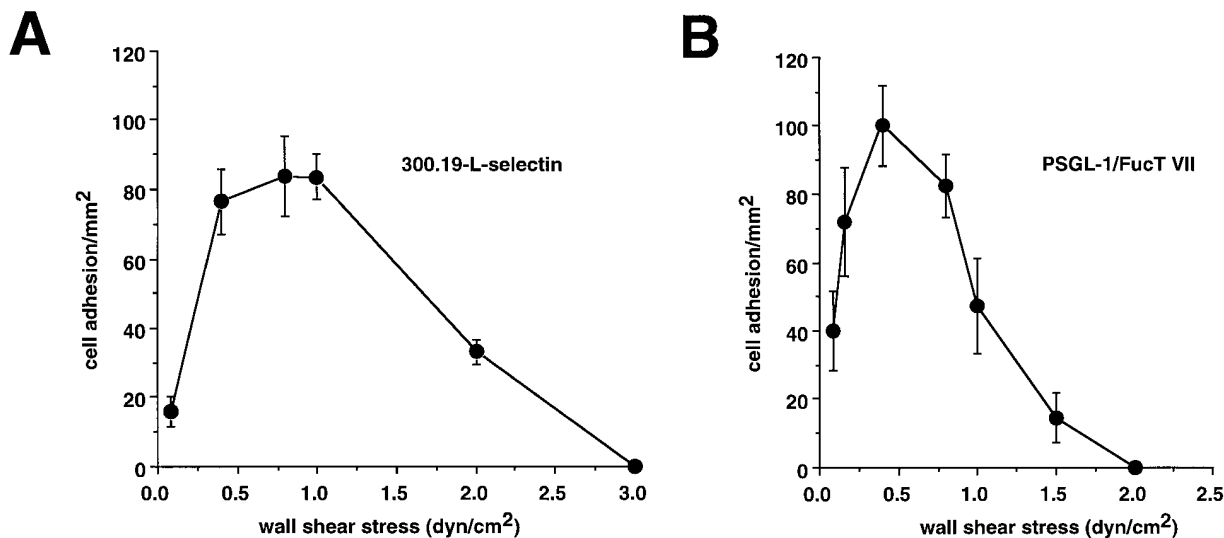


Figure 2. Attachment in flow of L-selectin transfectants to PNA (*A*) and PSGL-1/FucT VII transfectants to P-selectin (*B*). 300.19 cells transfected with L-selectin were perfused over isolated PNA (1:40) immobilized on plastic at wall shear stresses ranging from 0.1 to 4.0 dyn/cm². K562 cells transfected with PSGL-1 and FucT VII were perfused over isolated P-selectin (140 sites/μm²) at wall shear stresses ranging from 0.1 to 4.0 dyn/cm². Transfectants forming rolling adhesions for 0.4 s or longer were counted as adherent. Binding of both transfected cell lines to their respective substrates was 100% EDTA dependent. Figure represents the mean of two independent experiments for each cell type. Each point represents the average number (±SEM) of cells bound in a minimum of 15 fields of view.

tracked by videomicroscopy to measure the duration of selectin-dependent adhesions at and below the critical shear threshold required for rolling. By measuring the velocities of cells at closely spaced time intervals, the difference between cells transiently bound or steadily rolling could be determined by comparing their relative translational velocities. At sufficiently low wall shear stresses and sufficiently low site densities of selectin or selectin ligand, a flowing leukocyte will pause momentarily as a consequence of the formation of adhesive bonds (Kaplanski et al., 1993). After the momentary pause or capture event, the leukocyte either resumes its motion at the V_{crit} if it detaches, or alternatively, begins rolling at a velocity lower than the V_{crit} .

At a wall shear stress of 0.6 dyn/cm², Jurkat T lymphocytes formed rolling adhesions on PNA, often lasting several seconds, whose velocities varied between 20 and 50 μm/s (Fig. 4 *A*). In contrast, at 0.16 dyn/cm² wall shear stress (Fig. 4 *C*), L-selectin-dependent interactions did not convert to sustained rolling adhesions. Instead, interactions were observed to last only a fraction of a second. At an intermediate wall shear stress of 0.4 dyn/cm², adhesion (Fig. 4 *B*) consisted of both sustained rolling adhesions as well as momentary arrests as the lymphocytes rolled for a fraction of a cell diameter and released.

Tracking of HL-60 cells flowing over P- or E-selectin also demonstrated distinct patterns of adhesion when observed at either 0.5 or 0.1 dyn/cm² wall shear stress at site densities of 200/μm² or less (Fig. 5). HL-60 rolling on E- or P-selectin was sustained at a wall shear stress of 0.5 dyn/cm² (Fig. 5, *A* and *C*), while at 0.1 dyn/cm² wall shear stress, HL-60 cell rolling on both P- and E-selectin was intermittent and of brief duration, with interactions lasting between 0.5 and 1 s (Fig. 5, *B* and *D*). HL-60 cell detachment from P- or E-selectin was followed by a rapid increase of

the HL-60 cell's velocity to the measured V_{crit} (indicative of a noninteracting cell).

An inverted flow chamber assay was used to further distinguish rolling adhesions from recently detached HL-60 cells (see Materials and Methods, Fig. 6). In this procedure, reattachment of detached HL-60 cells from the top wall of the flow chamber is prevented by sedimentation of the unbound HL-60 cells away from the wall of the flow chamber because of the influence of gravity (sedimentation rates were approximately 1 μm/s). Once the HL-60 cell has fallen beyond the reach of selectin ligands present on microvilli, reattachment becomes improbable. This aids in distinguishing recently detached from reattached HL-60 cells.

HL-60 cells were perfused over a P- or E-selectin-coated substrate for ~2 min at 1.0 dyn/cm² wall shear stress to establish a baseline population of rolling cells for analysis of adhesion after inversion of the flow chamber. HL-60 cells were able to maintain stable rolling interactions on E-selectin at 1.0 dyn/cm² wall shear stress for several min after inversion of the chamber (Fig. 6 *A*). Lowering wall shear stress to 0.1 dyn/cm² resulted in detachment of rolling HL-60 cells from the E-selectin substrate within 15 s (Fig. 6 *B*). HL-60 cells maintained rolling adhesions on P-selectin at 1.0 dyn/cm² wall shear stress after inversion of the flow chamber for several min (Fig. 6 *C*). Lowering flow to 0.1 dyn/cm² wall shear stress resulted in the detachment of HL-60 cells from P-selectin within 15 s (Fig. 6 *D*). In Fig. 6 *B* and 5 *D*, virtually all the rolling HL-60 cells have detached because of the reduction in wall shear stress.

Leukocyte Rolling in Venules In Vivo Is Reduced at Very Low Flow Velocities

To determine whether selectin-mediated leukocyte adhe-

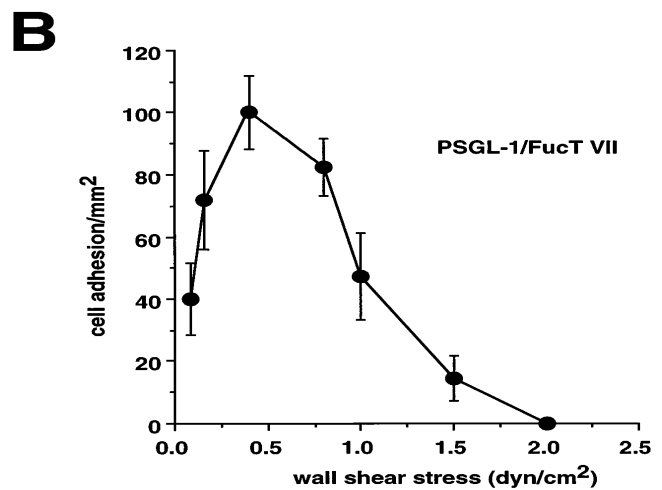
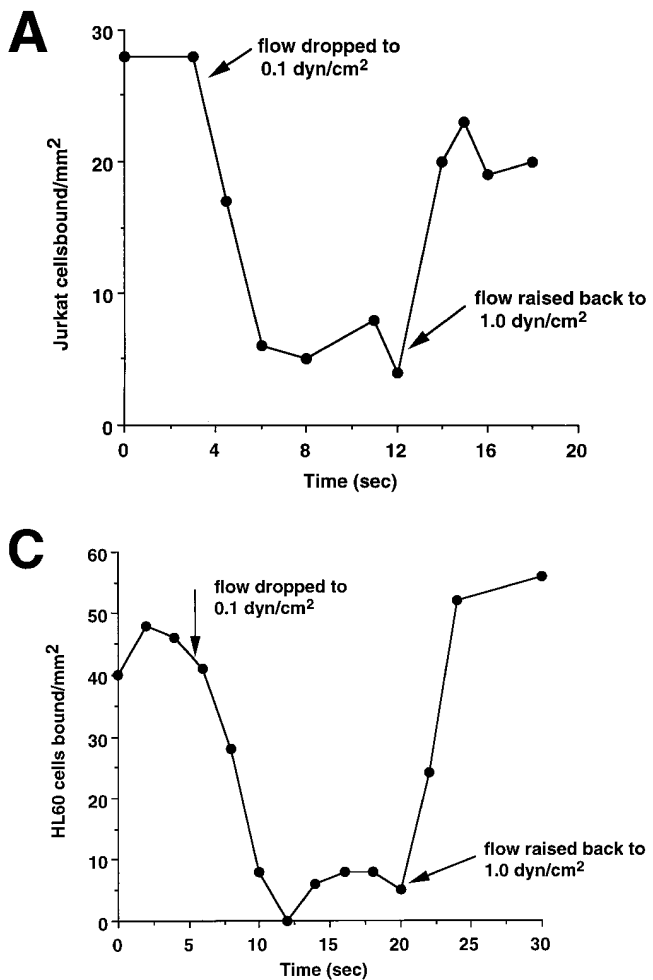


Figure 3. Changes in shear dynamically regulate selectin mediated rolling. (A) Jurkat T lymphoma cells (which express L-selectin [Lawrence et al., 1995]) were perfused over immobilized PNAd (1:40 dilution) at 1.0 dyn/cm² wall shear stress before the flow was reduced to 0.1 dyn/cm² using an electronically controlled syringe pump. Individual Jurkat T lymphocytes were tracked frame by frame on the video monitor. The criteria for adhesion was a rolling velocity of less than the Vcrit (see Materials and Methods) of a noninteracting lymphocyte flowing at 0.1 dyn/cm². Flow was raised back to 1.0 dyn/cm² and the numbers of interacting Jurkat cells counted as a function of time. Data shown is representative of four experiments. For B and C, the assay procedure was identical, except that HL-60 cells were used in place of Jurkat cells to assess the dynamic adhesion of leukocytes with (B) P-selectin (140 sites/ μ m²), and (C) E-selectin (195 sites/ μ m²).

sion in postcapillary venules is inhibited at the lower range of in vivo shear rates, a range of venule sizes corresponding to a range of wall shear rates was examined by intravital microscopy. In venules of the acutely exteriorized mouse cremaster muscle, leukocyte rolling flux fraction decreased at low centerline blood flow velocities (<1 mm/s) (Fig. 7 A). Rolling leukocyte flux fraction was normalized for systemic leukocyte counts and represents the percentage of leukocytes that interact with the microvessel wall. During the first 2 h after exteriorization, this rolling is known to be mediated by both P- and L-selectin (Ley and Tedder, 1995; Kunkel et al., 1996). Interestingly, the same general pattern of rolling leukocyte flux is seen in L-selectin-deficient mice (Fig. 7 B). Leukocyte rolling in L-selectin-deficient mice is entirely P-selectin dependent under these experimental conditions because a blocking P-selectin antibody completely blocks any residual rolling (Ley et al., 1995). As reported earlier, rolling in L-selectin-deficient mice is reduced compared to wild-type controls (Ley et al., 1995). The comparatively large standard errors seen in both data sets are caused by variations of other parameters influencing leukocyte rolling, particularly the time elapsed after exteriorization and the microvessel diameter (Ley et al., 1995).

Discussion

Using an in vitro flow chamber assay, we have observed

that fluid shear forces promote leukocyte adhesion mediated by P-, E-, or L-selectin. Under flow conditions, cell adhesion to surfaces or other cells varies inversely with the magnitude of applied shear forces. Cell detachment or failure to adhere may be due to either mechanical disruption of existing molecular bonds or the limited time allowed for bonds to form between membranes and surfaces (Lotz et al., 1989; Tempelman and Hammer, 1994). In this report, we show that levels of fluid shear forces above a defined threshold enhance leukocyte adhesion mediated by all three selectins. This finding extends recent observations made for L-selectin (Finger et al., 1996) to both P- and E-selectin-mediated leukocyte adhesion and establishes an upper limit for selectin bond lifetimes. Intravital microscopic observations of leukocyte/vessel wall interactions in wild-type and L-selectin-deficient mice also support a requirement for fluid shear for optimal P- and L-selectin-dependent leukocyte adhesion in postcapillary venules.

A recent report of fluid shear-enhancing L-selectin-mediated rolling used both PNAd and adherent neutrophils as substrates to present an L-selectin ligand to flowing leukocytes. It was concluded that L-selectin was unique among the three selectins in this regard based on the failure of fluid shear to enhance leukocyte binding to E-selectin and VCAM-1 (Finger et al., 1996). Several factors have been proposed to explain how fluid shear may promote adhesion, namely, that L-selectin bond lifetimes are relatively

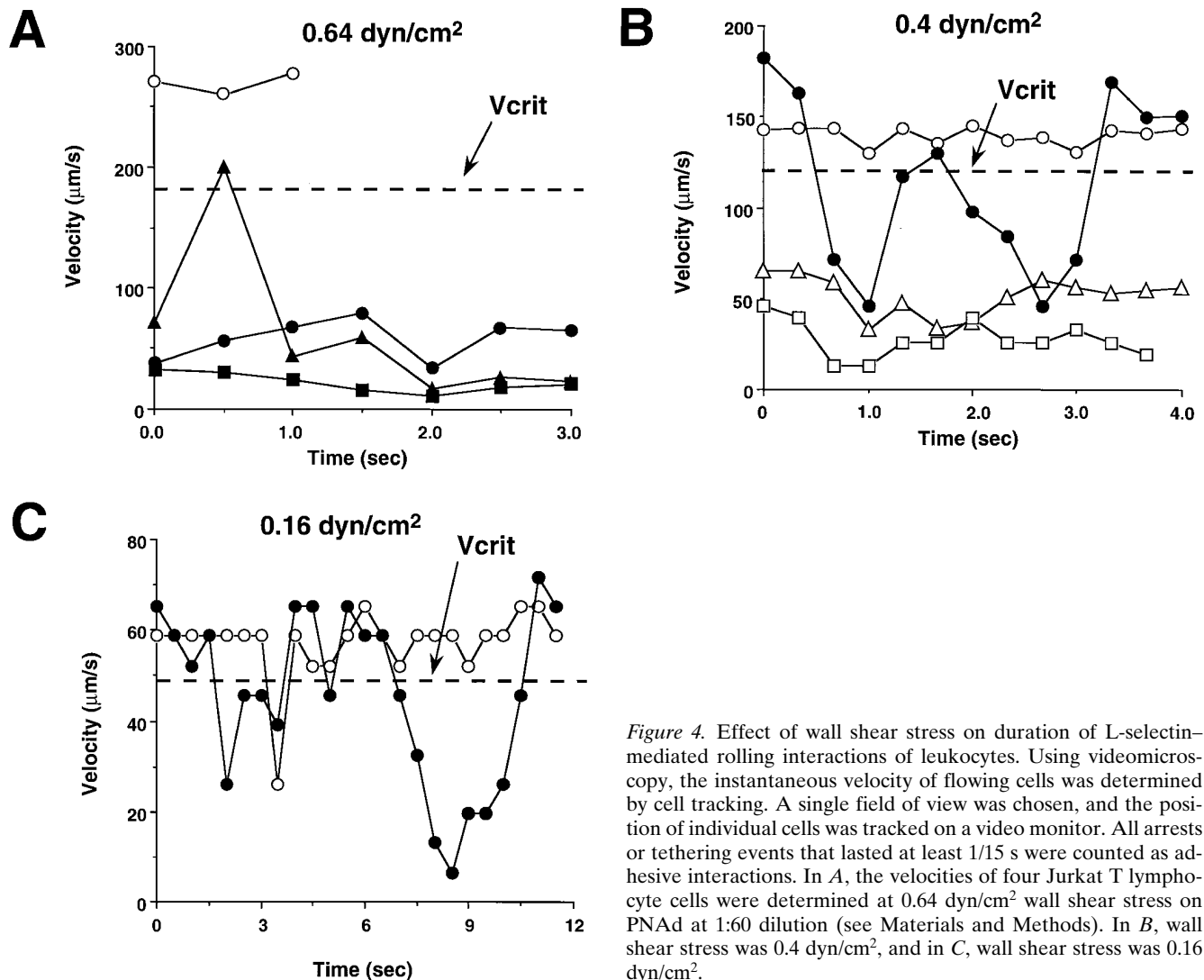


Figure 4. Effect of wall shear stress on duration of L-selectin-mediated rolling interactions of leukocytes. Using videomicroscopy, the instantaneous velocity of flowing cells was determined by cell tracking. A single field of view was chosen, and the position of individual cells was tracked on a video monitor. All arrests or tethering events that lasted at least 1/15 s were counted as adhesive interactions. In *A*, the velocities of four Jurkat T lymphocyte cells were determined at 0.64 dyn/cm^2 wall shear stress on PNAd at 1:60 dilution (see Materials and Methods). In *B*, wall shear stress was 0.4 dyn/cm^2 , and in *C*, wall shear stress was 0.16 dyn/cm^2 .

brief and that L-selectin may be shed while the leukocyte is rolling (Finger et al., 1996; Walcheck et al., 1996). However, in this report, L-selectin and PSGL-1 transfectants were observed to display a similar pattern of fluid shear enhancement of adhesion, suggesting that shedding of L-selectin may not be the principal mechanism for the reduction in rolling as wall shear stress is lowered. It was also proposed that the minimum shear needed to promote L-selectin-dependent rolling depended in part on the magnitude of the L-selectin bond lifetime (Finger et al., 1996). Since E- and P-selectin bond lifetimes may be greater than those of L-selectin (Kaplanski et al., 1993; Alon et al., 1995a; Zhao et al., 1995) (M.B. Lawrence, unpublished observations), we postulated that the shear threshold that promoted adhesion might be lower for the vascular selectins. Consistent with this hypothesis, HL-60 cells readily detached from both P- and E-selectin substrates once wall shear stress was lowered below 0.25 dyn/cm^2 , though the time required for release was longer than that required for L-selectin. E- and P-selectin mediate slower leukocyte rolling than L-selectin in vivo (Jung et al., 1996; Kunkel and Ley, 1996), consistent with the hypothesis that E- and P-selectin bonds may have longer lifetimes than

those involving L-selectin. HL-60 cell adhesion to P-selectin expressed in CHO cells has been observed to plateau at low wall shear stresses (Moore et al., 1995), although the earlier study did not examine wall shear stresses as low as those used in this report. The differences between adhesion mediated by L-, P-, and E-selectin appear quantitative and not qualitative, as previously hypothesized.

Enhancement of leukocyte rolling at wall shear stresses above 0.5 dyn/cm^2 does not appear to be unique to L-selectin-expressing T lymphocytes, as shown by L-selectin transfectants and Jurkat T cells rolling on surface immobilized PNAd. Similarly, transfection of PSGL-1 into K562 cells along with the appropriate fucosyltransferase confers binding of K562 cells to P-selectin under flow conditions, and rolling of the PSGL-1 transfectants on P-selectin was also enhanced relative to static conditions by raising wall shear stress above a threshold level, demonstrating that adhesion mediated by either L- or P-selectin is promoted by fluid shear forces. Taken together, these data suggest that a requirement for threshold levels of shear force is characteristic of selectin-mediated adhesion and is not dependent on the cell type expressing the selectin or ligand. The reversibility of selectin-mediated leukocyte rolling in

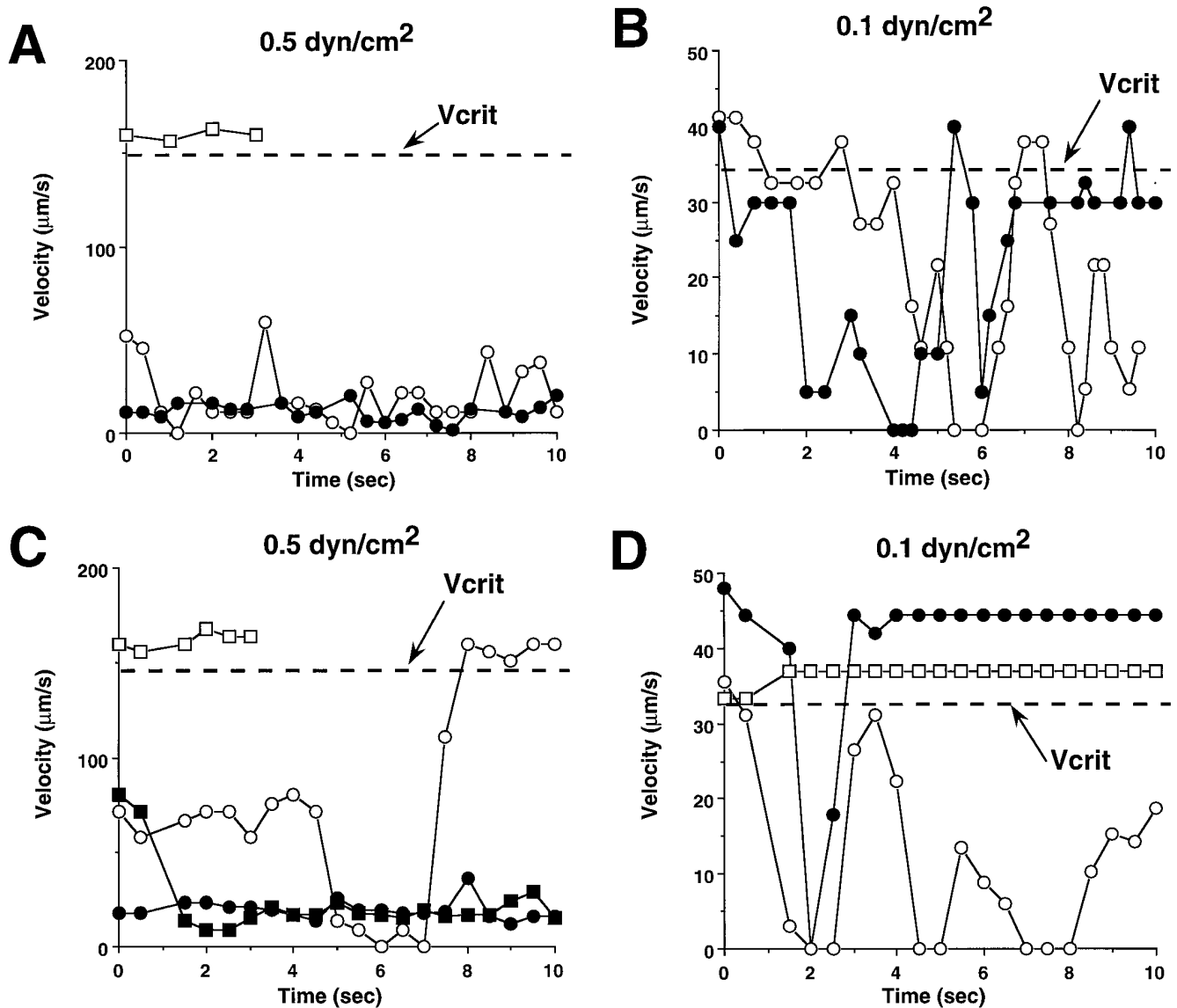


Figure 5. Effect of wall shear stress on duration of P- and E-selectin-mediated rolling interactions of leukocytes. HL-60 cells were perfused over P- or E-selectin-coated substrates at the indicated wall shear stresses. For P-selectin substrates (140 sites/ μm^2 , **A** and **B**), wall shear stress was 0.5 and 0.1 dyn/cm², respectively. For E-selectin substrates (195 sites/ μm^2 , **C** and **D**), wall shear stress was 0.5 and 0.1 dyn/cm², respectively.

response to changes in wall shear stress over the time interval of seconds indicates that cell signaling processes are not likely to be responsible for the changes in leukocyte rolling on E-selectin, P-selectin, or PNAd.

Promotion of selectin-mediated leukocyte adhesion by fluid shear may be explained in part by the induction of a torque as the passing leukocyte attempts to pivot around a bond cluster. Single cell tracking of leukocytes flowing over either PNAd, E-selectin, or P-selectin indicated that the frequency of cell-substrate interactions (manifested by transient arrests) did not change appreciably over the range of fluid shear stresses where attachment and rolling were minimal, suggesting that less frequent contact with the substrate was not responsible for the drop in rolling stability at wall shear stresses below 0.25 dyn/cm². Increasing selectin site density could partially overcome the low

shear inhibition of adhesion on all three substrates, suggesting that part of the observed phenomenon could be attributed to the time elapsed between formation of successive bonds.

After a reduction in fluid shear forces below a threshold level, HL-60 cells will detach from E- or P-selectin under the influence of gravity acting normal to the substrate, presumably because of dissociation of existing bonds and failure to form additional bonds. However, if the resultant moment generated by the fluid shear forces is large enough, additional bonds are apparently able to form as the cell experiences a torque into the wall of the flow chamber during the lifetime of existing bonds. The inverted flow chamber assay demonstrates both the importance of continuous bond formation and a concurrent need for shear-induced torque for sustaining and stabilizing selectin-mediated ad-

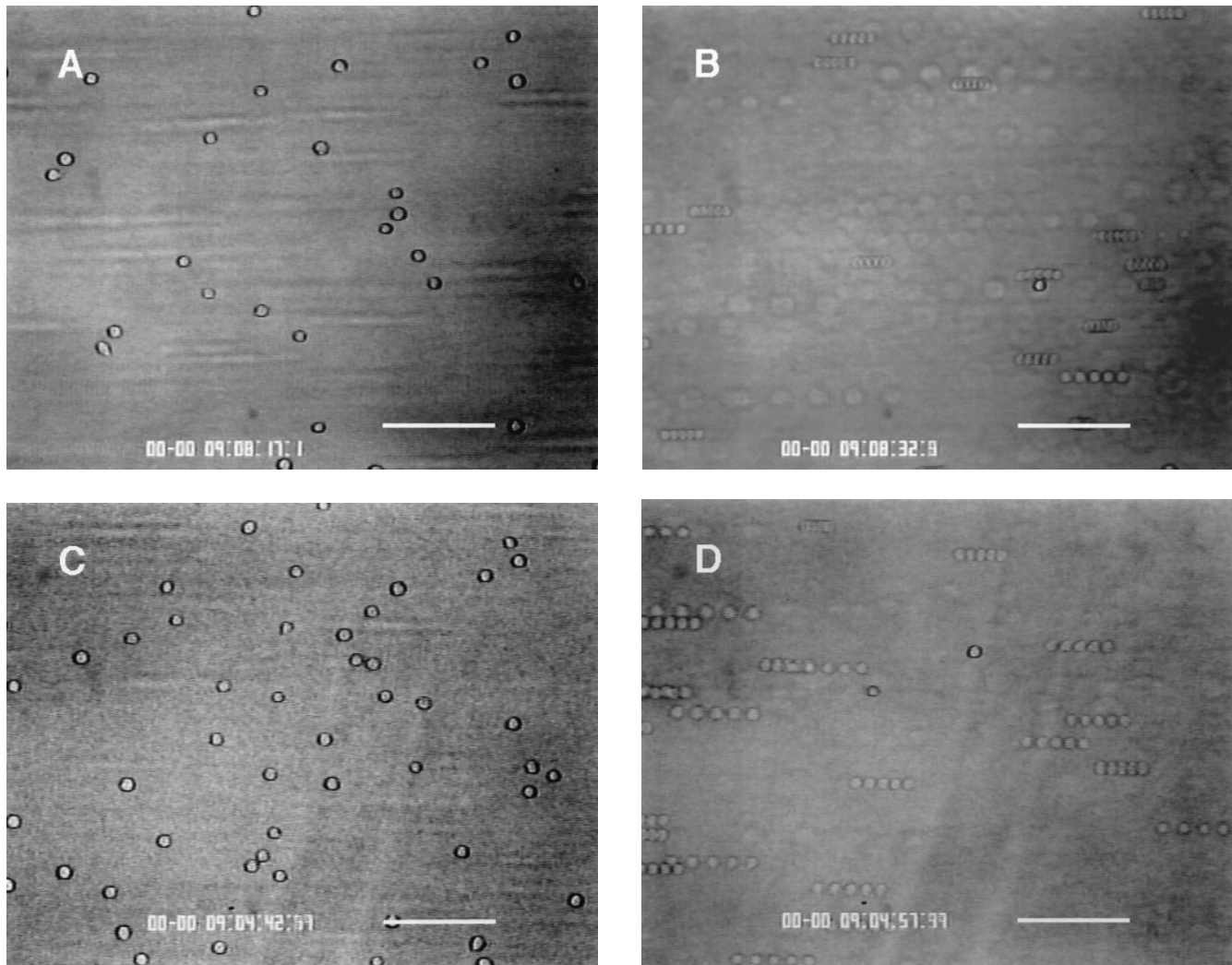


Figure 6. Sequence of photomicrographs of HL-60 cells rolling on either P- or E-selectin before and after flowrate was lowered. A suspension of HL-60 cells was perfused over the selectin-coated substrate at 1.0 dyn/cm² wall shear stress with the flow chamber in the normal orientation before being inverted along the axis of flow (see Materials and Methods). Images in each panel consist of five superimposed video frames (0.1 s apart) to show the multiple images of fast-moving nonadherent HL-60 cells compared to slow-rolling, adherent HL-60 cells. (A) HL-60 cells are rolling on P-selectin (140 sites/μm²) at 1.0 dyn/cm² wall shear stress 30 s after the chamber was inverted. (B) 15 s after wall shear stress was lowered to 0.1 dyn/cm², showing that all but one HL-60 cell have released from the surface. (C) HL-60 cells are rolling on E-selectin (195 sites/μm²) at 1.0 dyn/cm² wall shear stress 40 s after inversion of flow chamber. (D) 14 s after wall shear stress was lowered to 0.1 dyn/cm², showing that all but one HL-60 cell have released from the surface. In B and D, HL-60 cells released or detached from the substrate after the drop in wall shear stress are free to fall away from the wall of the flow chamber under the influence of gravity sedimentation and therefore are prevented from reattaching. Bar, 100 μm.

hesion. Under the low Reynolds number flow conditions found in vivo and present in the in vitro flow system used in this study ($Re \ll 1$), inertial effects are negligible. Consequently, a cell impinging on the wall of the chamber will undergo deformation solely because of the magnitude of the fluid shear forces. Fluid shear may therefore stabilize leukocyte rolling by deforming the cell slightly after the first bond cluster forms, thereby increasing the time and cell/substratum contact area to favor further bond formation.

Intravital microscopy observations of both wild-type and L-selectin-deficient mice also suggest that a minimum level of blood flow is required for optimal binding of leukocytes through selectins in vivo. After surgical trauma in

both wild-type and L-selectin knockout mice, leukocyte rolling flux was reduced at blood centerline velocities below 1.5 mm/s. Similar in vivo data were obtained previously in high endothelial venules of Peyer's patch high endothelial venules (Finger et al., 1996) and showed a reduction of L-selectin-mediated neutrophil rolling at very low shear rates. In the present study, we extend these findings to leukocyte rolling in postcapillary venules. Furthermore, the reduction of leukocyte rolling flux fraction at low flow velocities seen in L-selectin-deficient mice provides direct evidence that P-selectin-mediated leukocyte rolling in vivo is also reduced by low flow. P- and E-selectin site densities in vivo have not yet been determined, but rolling velocities in vivo correlate with leukocyte rolling velocities in vitro

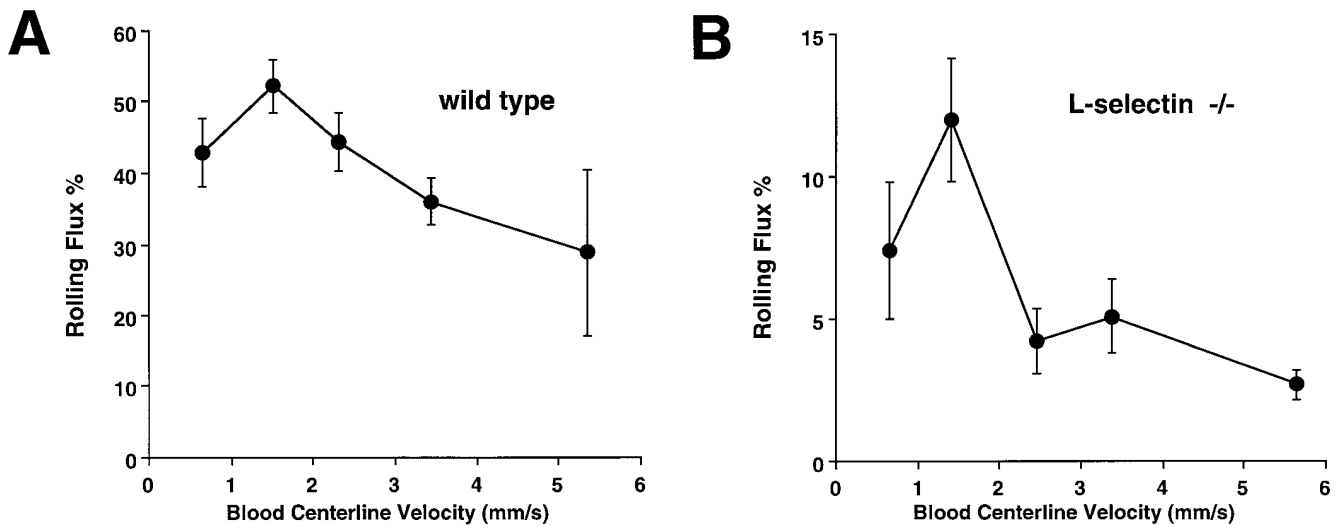


Figure 7. Dependence of in vivo leukocyte rolling flux fraction on vessel centerline blood velocity. (A) Leukocyte rolling in 151 venules of the cremaster muscle of 12 wild-type mice after surgical exteriorization at $t = 0$ min. Mean \pm SEM of leukocyte rolling flux fraction grouped in velocity classes spanning 1 mm/s (5–16 venules per class). (B) Leukocyte rolling flux in 54 venules of five L-selectin-deficient mice after surgical exteriorization. Rolling under these conditions is entirely P-selectin dependent as shown by antibody-blocking studies (Ley et al., 1995). Mean \pm SEM, class width 1 mm/s, highest class all venules with velocities >4 mm/s.

at site densities of <100 sites/ μm^2 , suggesting that the low shear inhibition of HL-60 cell adhesion observed at these concentrations of selectin may be physiologically significant. Based on the data obtained in vitro, it appears reasonable to predict that E-selectin-mediated rolling in vivo will also show a requirement for a minimum level of flow.

Hydrodynamic interactions of red blood cells appear to play an important role in facilitating the margination of leukocytes to the vessel wall where interactions with the endothelial cell adhesive receptors becomes possible (Schmid-Schonbein et al., 1980). Since margination appears to depend in part on the extent of erythrocyte deformation induced by fluid shear stresses, lower wall shear stresses may reduce the frequency of leukocyte contact with the vessel wall. Additionally, in vitro flow chamber assays have shown recently that erythrocytes augment lymphocyte adhesive interactions with the vascular endothelium (Melder et al., 1995). Consequently, a minimum shear requirement for rolling observed for selectin-mediated adhesion may depend in part on blood cell hydrodynamic interactions as well as the molecular properties of selectins.

Leukocyte extravasation depends on a coordinated sequence of signals consisting of expression and activation of specific adhesion mechanisms. A number of points in this sequence of events are possible regulatory steps during an inflammatory response, from margination of leukocytes by hydrodynamic interactions with erythrocytes to steps governed by adhesive bond formation such as rolling and transendothelial migration. Our observations suggest that leukocyte rolling in vivo requires a threshold level of hydrodynamic shear in addition to other hydrodynamic mechanisms that control margination.

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References

- Alon, R., D.A. Hammer, and T.A. Springer. 1995a. Lifetime of the P-selectin-carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature (Lond.)* 374:539–542.
- Alon, R., P.D. Kassner, M.W. Carr, E.B. Finger, M.E. Hemler, and T.A. Springer. 1995b. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J. Cell Biol.* 128:1243–1253.
- Arbones, M.L., D.C. Ord, K. Ley, H. Ratch, C. Curry-Maynard, G. Otten, D.J. Capon, and T.F. Tedder. 1994. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. *Immunity* 1:247–260.
- Asako, H., I. Kurose, R. Wolf, S. Defrees, Z.L. Zheng, M.L. Phillips, J.C. Paulson, and D.N. Granger. 1994. Role of H1-receptors and P-selectin in histamine-induced leukocyte rolling and adhesion in postcapillary venules. *J. Clin. Invest.* 93:1508–1515.
- Bargatze, R.F., S. Kurk, E.C. Butcher, and M.A. Jutila. 1994. Neutrophils roll on adherent neutrophils bound to cytokine-induced endothelial cells via L-selectin on the rolling cells. *J. Exp. Med.* 180:1785–1792.
- Berg, E.L., M.K. Robinson, R.A. Warnock, and E.C. Butcher. 1991. The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor. *J. Cell Biol.* 114:343–349.
- Berlin, C., R.F. Bargatze, J.J. Campbell, U.H. von Andrian, M.C. Szabo, S.R. Hasslen, R.D. Nelson, E.L. Berg, S.L. Erlandsen, and E.C. Butcher. 1995. α_4 integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell* 80:413–422.
- 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* 84:9238–9242.
- Finger, E.B., K.D. Puri, R. Alon, M.B. Lawrence, U.H. von Andrian, and T.A. Springer. 1996. Adhesion through L-selectin requires a threshold hydrodynamic shear. *Nature (Lond.)* 379:266–269.
- Fuortes, M., W.W. Jin, and C. Nathan. 1994. β_2 integrin-dependent tyrosine phosphorylation of paxillin in human neutrophils treated with tumor necrosis factor. *J. Cell Biol.* 127:1477–1483.
- Geng, J.-G., M.P. Bevilacqua, K.L. Moore, T.M. McIntyre, S.M. Prescott, J.M. Kim, G.A. Bliss, G.A. Zimmerman, and R.P. McEver. 1990. Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature (Lond.)* 343:757–760.
- Goldman, A.J., R.G. Cox, and H. Brenner. 1967. Slow viscous motion of a

- sphere parallel to a plane wall. II. Couette flow. *Chem. Eng. Sci.* 22:653–660.
- Hammer, D.A., and S.M. Apte. 1992. Simulation of cell rolling and adhesion on surfaces in shear flow: general results and analysis of selectin-mediated neutrophil adhesion. *Biophys. J.* 63:35–57.
- Hattori, R., K.K. Hamilton, R.D. Fugate, R.P. McEver, and P.J. Sims. 1989. Stimulated secretion of endothelial von Willebrand factor is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. *J. Biol. Chem.* 264:7768–7771.
- Jones, D.A., L.V. McIntire, C.W. Smith, and L.J. Picker. 1994. A two-step adhesion cascade for T cell/endothelial cell interactions under flow conditions. *J. Clin. Invest.* 94:2443–2450.
- Jung, U., D.C. Bullard, T.F. Tedder, and K. Ley. 1996. Velocity differences between L-selectin and P-selectin dependent neutrophil rolling in venules of the mouse cremaster muscle *in vivo*. *Am. J. Physiol.* 271:H2740–H2747.
- Kansas, G.S. 1996. Selectins and their ligands: current concepts and controversies. *Blood.* 88:3259–3287.
- Kansas, G.S., K. Ley, M. Munro, and T.F. Tedder. 1993. Regulation of leukocyte rolling and adhesion to high endothelial venules through the cytoplasmic domain of L-selectin. *J. Exp. Med.* 177:833–838.
- Kaplanski, G., C. Farnarier, O. Tissot, A. Pierres, A.M. Benoliel, M.C. Alessi, S. Kaplanski, and P. Bongrand. 1993. Granulocyte-endothelium initial adhesion. Analysis of transient binding events mediated by E-selectin in a laminar shear flow. *Biophys. J.* 64:1922–1933.
- Kishimoto, T.K., M.A. Jutila, and E.C. Butcher. 1990. Identification of a human peripheral lymph node homing receptor: a rapidly-downregulated adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 87:2244–2248.
- Kubes, P., and S. Kanwar. 1994. Histamine induces leukocyte rolling in post-capillary venules: a P-selectin mediated event. *J. Immunol.* 152:3570–3577.
- Kunkel, E.J., and K. Ley. 1996. Distinct phenotype of E-selectin deficient mice: E-selectin is required for slow leukocyte rolling *in vivo*. *Circ. Res.* 79:1196–1204.
- Kunkel, E.J., U. Jung, D.C. Bullard, K.E. Norman, B.A. Wolitzky, D. Vestweber, A.L. Beaudet, and K. Ley. 1996. Absence of trauma-induced leukocyte rolling in mice deficient in both P-selectin and intercellular adhesion molecule-1 (ICAM-1). *J. Exp. Med.* 183:1–9.
- Larsen, E., A. Celi, G.E. Gilbert, B.C. Furie, J.K. Erban, R. Bonfanti, D.D. Wagner, and B. Furie. 1989. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. *Cell.* 59:305–312.
- Lawrence, M.B., D.F. Bainton, and T.A. Springer. 1994. Neutrophil tethering to and rolling on E-selectin are separable by a requirement for L-selectin. *Immunity.* 1:137–145.
- Lawrence, M.B., E.L. Berg, E.C. Butcher, and T.A. Springer. 1995. Rolling of lymphocytes and neutrophils on peripheral node addressin and subsequent arrest on ICAM-1 in shear flow. *Eur. J. Immunol.* 25:1025–1031.
- Ley, K. 1994. Histamine can induce leukocyte rolling in rat mesenteric venules. *Am. J. Physiol.* 267:H1017–H1023.
- Ley, K., and T.F. Tedder. 1995. Leukocyte interactions with vascular endothelium. New insights into selectin-mediated attachment and rolling. *J. Immunol.* 155:525–528.
- Ley, K., T.F. Tedder, and G.S. Kansas. 1993. L-selectin can mediate leukocyte rolling in untreated mesenteric venules *in vivo* independent of E- or P-selectin. *Blood.* 82:1632–1638.
- Ley, K., D.C. Bullard, M.L. Arbones, R. Bosse, D. Vestweber, T.F. Tedder, and A.L. Beaudet. 1995. Sequential contribution of L- and P-selectin to leukocyte rolling *in vivo*. *J. Exp. Med.* 181:669–675.
- Lobb, R.R., G. Chi-Rosso, D.R. Leone, M.D. Rosa, S. Bixler, B.M. Newman, S. Luhowskyj, C.D. Denjamin, I.G. Douglas, S.E. Goelz, et al. 1991. Expression and functional characterization of a soluble form of endothelial-leukocyte adhesion molecule 1. *J. Immunol.* 147:124–129.
- Lotz, M.M., C.A. Burdsal, H.P. Erickson, and D.R. McClay. 1989. Cell adhesion to fibronectin and tenascin: quantitative measurements of initial binding and subsequent strengthening response. *J. Cell Biol.* 109:1795–1805.
- Luscinskas, F.W., G.S. Kansas, H. Ding, P. Pizcueta, B.E. Schleiffenbaum, T.F. Tedder, and M.A. Gimbrone, Jr. 1994. Monocyte rolling, arrest and spreading on IL-4-activated vascular endothelium under flow is mediated via sequential action of L-selectin, β_1 -integrins, and β_2 -integrins. *J. Cell Biol.* 125:1417–1427.
- Ma, L., L. Raycroft, D. Asa, D.C. Anderson, and J.-G. Geng. 1994. A sialoglycoprotein from human leukocytes functions as a ligand for P-selectin. *J. Biol. Chem.* 269:27739–27746.
- Melder, R.J., L.L. Munn, S. Yamada, C. Ohkubo, and R.K. Jain. 1995. Selectin and integrin-mediated T-lymphocyte rolling and arrest on TNF- α -activated endothelium: augmentation by erythrocytes. *Biophys. J.* 69:2131–2138.
- Moore, K.L., K.D. Patel, R.E. Bruehl, L. Fugang, D.A. Johnson, H.S. Lichtenstein, R.D. Cummings, D.F. Bainton, and R.P. McEver. 1995. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *J. Cell Biol.* 128:661–671.
- Natsuka, S., K.M. Gersten, K. Zenita, R. Kannagi, and J.B. Lowe. 1994. Molecular cloning of a cDNA encoding a novel human leukocyte α -1,3-fucosyltransferase capable of synthesizing the sialyl Lewis^x determinant. *J. Biol. Chem.* 269:16789–16794.
- Norman, K.E., K.L. Moore, R.P. McEver, and K. Ley. 1995. Leukocyte rolling *in vivo* is mediated by P-selectin glycoprotein ligand-1. *Blood.* 86:4417–4421.
- 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 selectins ELAM-1 and GMP-140. *Cell.* 66:921–933.
- Sako, D., X.J. Chang, K.M. Barone, G. Vachino, H.M. White, G. Shaw, G.M. Veldman, K.M. Bean, T.J. Ahern, B. Furie, et al. 1993. Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell.* 75:1179–1186.
- Schmid-Schonbein, G.W., S. Usami, R. Skalak, and S. Chien. 1980. The interaction of leukocytes and erythrocytes in capillary and postcapillary vessels. *Microvasc. Res.* 19:45–70.
- Simon, S.I., A.R. Burns, A.D. Taylor, P.K. Gopalan, E.B. Lyman, L.A. Sklar, and C.W. Smith. 1995. L-selectin (CD62L) cross-linking signals neutrophil adhesive functions via the Mac-1 (CD11b/CD18) β_2 integrin. *J. Immunol.* 155:1502–1514.
- Smith, C.W., S.D. Marlin, R. Rothlein, C. Toman, and D.C. Anderson. 1989. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils *in vitro*. *J. Clin. Invest.* 83:2008–2017.
- Snapp, K.R., A.J. Wagers, R. Craig, L.M. Stoolman, and G.S. Kansas. 1997. P-Selectin glycoprotein ligand is essential for adhesion to P-selectin but not E-selectin in stably transfected hematopoietic cell lines. *Blood.* In press.
- Spertini, O., G.S. Kansas, J.M. Munro, J.D. Griffin, and T.F. Tedder. 1991. Regulation of leukocyte migration by activation of the leukocyte adhesion molecule-1 (LAM-1) selectin. *Nature (Lond.)* 349:691–694.
- Springer, T.A. 1995. Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration. *Annu. Rev. Physiol.* 57:827–872.
- Sriramarao, P., U.H. von Andrian, E.C. Butcher, M.A. Bourdon, and D.H. Broide. 1994. L-selectin and very late antigen-4 integrin promote eosinophil rolling at physiological shear rates *in vivo*. *J. Immunol.* 153:4238–4246.
- Tempelman, L.A., and D.A. Hammer. 1994. Receptor-mediated binding of IgE-sensitized rat basophilic leukemia cells to antigen-coated substrates under hydrodynamic flow. *Biophys. J.* 66:1231–1243.
- Tozeren, A., and K. Ley. 1992. How do selectins mediate leukocyte rolling in venules? *Biophys. J.* 63:700–709.
- von Andrian, U.H., J.D. Chambers, L.M. McEvoy, R.F. Bargatzke, K.-E. Arfors, and E.C. Butcher. 1991. Two-step model of leukocyte-endothelial cell interaction in inflammation. *Proc. Natl. Acad. Sci. USA.* 88:7538–7542.
- Waddell, T.K., L. Fiakow, C.K. Chan, T.K. Kishimoto, and G.P. Downey. 1994. Potentiation of the oxidative burst of human neutrophils: a signaling role for L-selectin. *J. Biol. Chem.* 269:18485–18491.
- Walcheck, B., J. Kahn, J.M. Fisher, B.B. Wang, R.S. Kisk, D.G. Payan, C. Feehan, R. Betageri, K. Karlak, A.F. Spatola, and T.K. Kishimoto. 1996. Neutrophil rolling altered by inhibition of L-selectin shedding *in vitro*. *Nature (Lond.)* 380:720–723.
- Zhao, Y., S. Chien, and R. Skalak. 1995. A stochastic model of leukocyte rolling. *Biophys. J.* 69:1309–1320.