Biophysical Journal Volume 71 November 1996 2902–2907

Sialyl Lewis^x/E-Selectin-Mediated Rolling in a Cell-Free System

Debra K. Brunk, Douglas J. Goetz, and Daniel A. Hammer
School of Chemical Engineering, Cornell University, Ithaca, New York 14853 USA

ABSTRACT Selectins mediate transient adhesion of neutrophils to stimulated endothelial cells at sites of inflammation by binding counter-receptors that present carbohydrates such as sialyl Lewis^x. We have developed a cell-free adhesion assay using sialyl Lewis^x-coated microspheres and E-selectin-IgG chimera-coated substrates to investigate the premise that rolling primarily results from functional properties of selectin-carbohydrate bonds, whereas cellular morphology and signaling act as secondary effects. Sialyl Lewis^x-coated microspheres attach to and roll over E-selectin-IgG chimera-coated substrates between the physiological wall shear stresses of 0.7 and 2 dynes/cm^2. Rolling velocities vary with time and depend on E-selectin-IgG chimera site density and wall shear stress. Our results show that sialyl Lewis^x is a minimal functional recognition element required for rolling on E-selectin under flow.

INTRODUCTION

Adhesion of neutrophils to endothelial cells is a critical step in the inflammatory response. Transient adhesion, or rolling, of neutrophils over stimulated endothelial cells is a prerequisite to firm attachment and transendothelial migration (von Andrian et al., 1991; Lawrence and Springer, 1991). The selectin family of adhesion molecules (L-, P-, and E-selectin) are thought to mediate rolling by binding carbohydrate-presenting counter-receptors. L-selectin is constitutively expressed on the tips of neutrophil microvilli (Erlandsen et al., 1993; von Andrian et al., 1995), E-selectin is expressed by endothelial cells stimulated for 2–4 h with cytokines such as interleukin-1β (Bevilacqua et al., 1989), and P-selectin is rapidly upregulated from Weibel-Palade bodies within endothelium stimulated with thrombin or histamine (Hattori et al., 1989). Each selectin contains an amino-terminal, calcium-binding, lectin-like domain, an epidermal growth factor-like domain, a variable number of short consensus repeats, a transmembrane domain, and a cytoplasmic tail. Sialyl Lewis^x (sLe^x) is a sialylated, fucosylated carbohydrate that is widely distributed on both glycoprotein and glycolipid components of the neutrophil (Fukuda et al., 1984; Symington et al., 1985), is constitutively expressed by human umbilical vein endothelial cells (Majuri et al., 1994), and can bind all three selectins under static conditions (Polley et al., 1991; Foxall et al., 1992; Berg et al., 1992).

Although static assays may confirm putative receptor-ligand pairs, they do not provide information on how sLe^x interacts with the selectins under dynamic conditions (i.e., can these molecules mediate rolling?). An additional confounding aspect of rolling is the role of cellular features such as the clustering of receptors on microvilli, microvilli and cell deformability, and intracellular signaling. Lawrence and Springer (1993) observed that rolling velocities of formaldehyde-fixed neutrophils on E-selectin substrates were comparable to velocities measured for untreated neutrophils, which suggests that intracellular signaling is probably not necessary for rolling to occur. It would be interesting to know if sLe^x/selectin interactions have the requisite properties of recognition to mediate rolling in the absence of cellular features. Indeed, Pierres and co-workers have suggested that the initial formation of bonds may be a passive process, which could be studied using inert substrates as carriers for ligands (Pierres et al., 1994). Therefore, we developed a cell-free system to determine whether binding between sLe^x and E-selectin can mediate rolling under flow. Such a clean system will allow us to identify receptor-ligand pairs that mediate rolling and to better understand the functional properties of receptor recognition required for dynamic adhesion.

MATERIALS AND METHODS

Microspheres

NeutrAvidin (Pierce, Rockford, IL) was covalently attached to carboxylated latex microspheres (Polysciences, Warrington, PA) (Kuo and Lauffenburger, 1993). Avidin-coated microspheres were then treated with 0.2 μg/ml biotin (Sigma, St. Louis, MO), fluorescein isothiocyanate (FITC) biotin (Molecular Probes, Eugene, OR) or biotinylated-(sLe^x)$_4$ (Syntesome, Munich, Germany) for 45 min. Microspheres were prepared for flow cytometry (Goetz et al., 1996) using a monoclonal antibody (MAb) against sLe^x (KM93, IgM, Kamiya Biomedical Co., Thousand Oaks, CA) and a species-matched secondary FITC-labeled MAb (Pharmingen, San Diego, CA) in 50 mM MES (pH 5.5, containing 1% bovine serum albumin, 1 mM $CaCl_2$, 1 mM $MgCl_2$). Conversion of fluorescence peaks to site densities was done using Quantum 26 calibration microspheres (Flow Cytometry Standards Corporation, San Juan, Puerto Rico) and has been previously described (Kuo and Lauffenburger, 1993).

Received for publication 7 June 1996 and in final form 20 August 1996.

Address reprint requests to Dr. Daniel A. Hammer, 392 Towne Bldg., Department of Chemical Engineering, University of Pennsylvania, Philadelphia, PA 19104. Tel: 215-573-6761; Fax: 215-573-2093; E-mail: hammer@seas.upenn.edu. Dr. Goetz's current address is Vascular Research Division, Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115.

0006-3495/96/11/2902/06 $2.00

E-selectin substrates

The E-selectin-IgG chimera (E-selectin-IgG) used in this study was a gift from Dr. Brian Brandley (Rush Medical Center, Chicago, IL). The chimera consisted of the lectin, epidermal growth factor, and two short consensus repeat domains for human E-selectin (Bevilacqua et al., 1989) linked to the Fc region of IgG_1 (Watson et al., 1990). E-selectin-IgG was incubated on silanated glass microscope slides (Sigma) in Flexiperm wells (Heraeus Instruments, South Plainfield, NJ) at 0.4 and 1.6 μg/ml for 2 h. Slides were washed two times with phosphate-buffered saline (PBS), then incubated in modified PBS (containing 1% bovine serum albumin, 1 mM $CaCl_2$, 1 mM $MgCl_2$) for 1 h to block nonspecific adhesion. 20 μg/ml anti-E-selectin (68–5H11, IgG_1, Pharmingen) was added to the wells for 30 min, followed by 30 μg/ml Fc-specific FITC anti-mouse IgG (Sigma) for 30 min. A negative control was prepared using the same method, replacing the E-selectin-IgG with human IgG_1. Fluorescence in counts/s was measured with a Photoscan (Nikon, Garden City, NJ) attached to a Nikon Diaphot inverted microscope and then converted to site densities using a calibration curve constructed from known concentrations of fluorescent IgG. Site densities obtained in this manner will overestimate the number of E-selectin molecules oriented in the correct position for binding ligand.

Flow chamber

The tapered channel design is based on the theory of Hele-Shaw flow between two parallel plates (Usami et al., 1993). The plates were separated by 175 μm Duralastic sheeting (Allied Biomedical, Goose Creek, SC). The chamber was assembled on the stage of a Nikon Diaphot inverted phase contrast microscope connected to a video camera (Dage-MTI, Inc., Michigan City, IN) and a S-VHS recorder (JVC, Elmwood Park, NJ). Buffer and bead suspensions were drawn through the chamber by an infusion/withdrawal syringe pump (Harvard Apparatus, South Natick, MA).

Adhesion experiments

E-selectin-IgG-coated slides were placed in the well of the flow chamber, the chamber was assembled in PBS then placed on the microscope stage. For a channel height of 175 μm, the perfusion buffer or microsphere suspension flow rate must be 128 μl/min to obtain a range in wall shear stress from 0 dynes/cm^2 to 4 dynes/cm^2 down the length of the channel. The chamber was perfused with PBS (+1 mM $CaCl_2$, 1 mM $MgCl_2$) for 15 min, then the microsphere suspension (5×10^5/ml) was introduced. Data was collected by stepping down the chamber from inlet to outlet in 0.5 cm steps, allowing ~1 min between steps. Microsphere interaction with the surface was recorded at a total magnification of 200× for future analysis. All experiments were done at 23°C.

Antibody blocking

Antibody blocking and antibody control experiments were carried out as previously described (Goetz et al., 1996). Briefly, E-selectin-IgG-coated slides were incubated for 45 min in 20 μg/ml anti-E-selectin (BBA2, IgG_2, R&D Systems, Minneapolis, MN), anti-vascular cell adhesion molecule-1 (VCAM-1) (51–10C9, IgG_1, Pharmingen), or anti-P-selectin (AK4, IgG_1, Pharmingen), then inserted in the chamber as usual. Perfusion buffer and microsphere suspension each contained 1 μg/ml of the desired antibody.

Data analysis

To determine the particle flux, the number of transient interactions in a 0.03 mm^2 area were manually counted as a function of time. Transient attachment included all particles that interacted with the surface, i.e., rolling and stop/start interactions. Particles were considered firmly attached if they remained stationary for >10 s. p values are the results of two-tailed t-tests. Velocity measurements were obtained from recorded data using a SCION LG-3 frame grabber in a Power Macintosh 7100 running NIH Image, public domain image analysis software. Particle coordinates were obtained with 1 pixel accuracy (1 μm) by clicking the mouse at the center of a rolling microsphere. Average velocity was calculated by dividing the total displacement of a rolling particle by the observation time. Instantaneous velocity was determined by dividing the displacement of a rolling particle by the time between incremental captured frames (0.4 s for 2100 sites E-selectin-IgG/μm^2, 2 s for 3600 sites E-selectin-IgG/μm^2).

RESULTS

Development and characterization of cell-free system

An E-selectin-IgG chimera, consisting of the lectin, epidermal growth factor, and two short consensus repeat domains of human E-selectin linked to the Fc region of human IgG_1, was adsorbed to silanated glass slides. E-selectin-IgG solution concentration was varied and the adsorbed E-selectin-IgG site density was measured using a fluorescence method (see Materials and Methods) (Fig. 1 *A*).

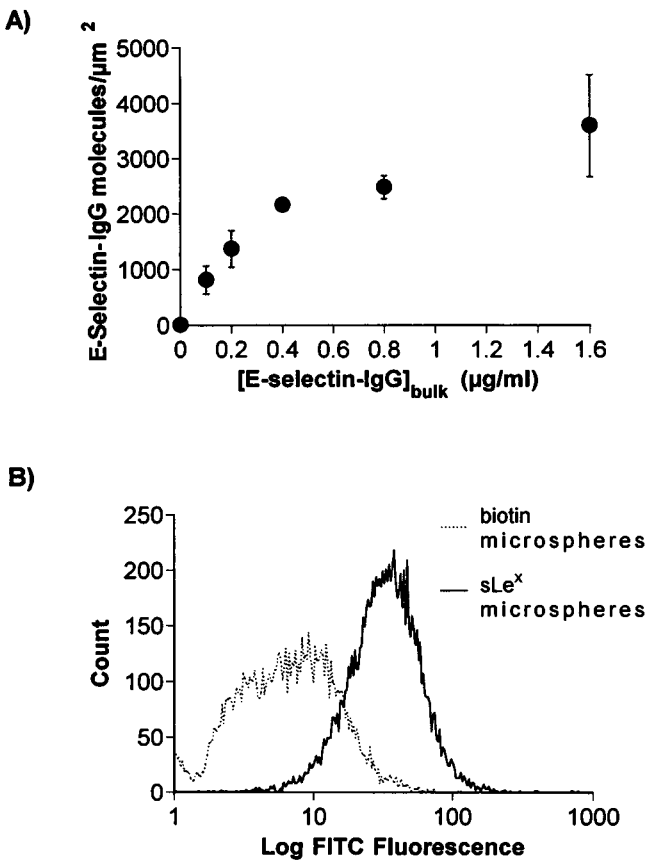

FIGURE 1 Characterization of cell-free system. (*A*) Site density of E-selectin-IgG adsorbed to glass slides versus bulk chimera concentration. Each point is the average ± standard deviation of three independent experiments, with 12 fluorescence measurements obtained per experiment. (*B*) Detection by flow cytometry of sLe^x on 10-μm diameter microspheres. sLe^x-microspheres show a large shift in mean log fluorescence compared to that for biotin-microspheres treated with the same MAbs (32 vs. 6). Peaks shown are representative of three separate measurements.

A synthetic, multivalent sLe^x-biotin conjugate was attached to avidin-coated polystyrene latex microspheres of 10 μm diameter. Presence of sLe^x on the microsphere surface was verified using flow cytometry (see Materials and Methods). sLe^x-microspheres have a mean log fluorescence of 32, compared to 6 for biotin-microspheres treated with the same MAbs (Fig. 1 *B*). Concentrations ranging from 0.1 μg/ml to 16 μg/ml biotinylated-sLe^x showed an equivalent shift; therefore, the shift in mean fluorescence corresponds to a saturating site density of $\sim 10^5$ sLe^x/particle (1300 $sLe^x/\mu m^2$).

Microspheres coated with sLe^x interact specifically with E-selectin substrates

sLe^x microspheres interact with E-selectin-IgG-coated substrates (Fig. 2). Nearly all adhesion is transient, with very few microspheres adhering firmly to the E-selectin-IgG surface. The interaction between sLe^x and E-selectin-IgG is specific, because replacing biotin-sLe^x with biotin-FITC or replacing the E-selectin-IgG with human IgG_1 eliminates microsphere interaction with the surface. Addition of BBA2, a monoclonal antibody that blocks the interaction between sLe^x and an E-selectin-IgG chimera under static conditions (Pigott et al., 1991), significantly reduces adhesion relative to isotype-matched control antibodies against P-selectin and VCAM-1. Adhesion is cation dependent, a hallmark of most selectin interactions (Vestweber, 1993; Carlos and Harlan, 1994), because adhesion does not occur in the presence of the cation chelator EDTA.

Rolling velocity varies with shear stress and E-selectin-IgG site density

To compare the adhesion dynamics of our cell-free system with those of cellular systems, we studied microsphere rolling as a function of shear stress and E-selectin-IgG density. In our analysis, we considered sLe^x-microspheres to be rolling on the E-selectin-IgG surface if their average velocity was <10% of the free stream velocity of a noninteracting particle near the surface (Goldman et al., 1967; Goetz et al., 1994). Fig. 3 *A* shows the effect of shear stress and E-selectin-IgG surface concentration on rolling velocity. As shear stress increases, the average rolling velocity increases at each concentration of E-selectin-IgG, whereas at a constant shear stress, the average rolling velocity decreases with increasing E-selectin-IgG concentration. For instance, at a shear stress of 1.5 dynes/cm^2, an increase in E-selectin-IgG surface density from 2100 to 3600 sites/μm^2 leads to a sevenfold decrease in the average rolling velocity.

Rolling velocity is a function of time

The velocity for a typical sLe^x-coated microsphere varies considerably with time at all shear stresses and E-selectin-IgG surface densities. Fig. 3 *B* shows rolling velocity versus time for two representative microspheres at a wall shear stress of 1.7 dynes/cm^2 for two chimera site densities. Variability in microsphere motion can be quantified using the variance of the instantaneous velocity (Goetz et al., 1994). The variance (σ^2) in motion increases with decreasing E-selectin-IgG site density (σ^2 = 515 $\mu m^2/s^2$ at 2100

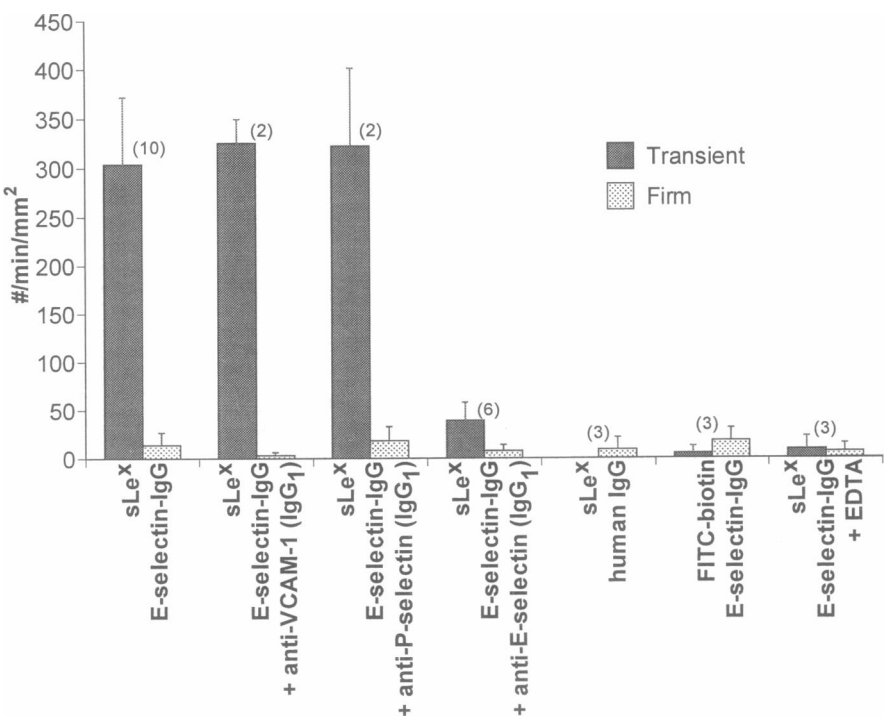

FIGURE 2 sLe^x-coated beads interact specifically with E-selectin-IgG surfaces. The number of interacting particles per minute per area are shown for different microsphere/surface treatments, including: sLe^x/E-selectin-IgG, sLe^x/E-selectin-IgG + anti-VCAM-1 (51–10C9, mouse IgG_1), sLe^x/E-selectin-IgG + anti-P-selectin (AK4, mouse IgG_1), sLe^x/E-selectin-IgG + anti-E-selectin (BBA2, mouse IgG_1), sLe^x/human IgG_1, biotin-FITC/E-selectin-IgG and sLe^x/E-selectin-IgG + EDTA at a wall shear stress of 0.7 dyn/cm^2, and 2100 molecules/μm^2 E-selectin-IgG or human IgG_1 on the substrate. Transient attachment is significantly reduced by adding a MAb to E-selectin, adding EDTA, replacing sLe^x with FITC-biotin, or replacing E-selectin-IgG with human IgG_1 ($p < 10^{-7}$). The data is the mean ± standard deviation of the indicated number of independent experiments, with the flux measured at two or three different times during one experiment.

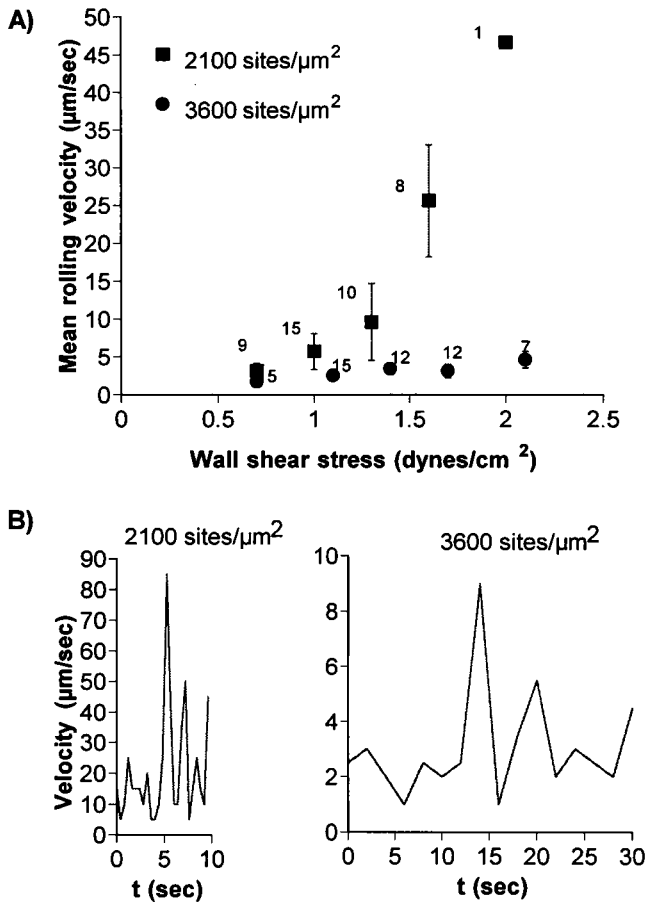

FIGURE 3 Measurements of sLe^x rolling velocity. (*A*) Average rolling velocity as a function of wall shear stress for two E-selectin-IgG site densities. Each point is the population average of the indicated number of particle mean rolling velocities for one experiment. The error bars represent the standard deviation of the mean rolling velocity. Velocity measurements were obtained only for consistently rolling particles, i.e., skips or jumps in the motion where velocities approached free stream levels were not included. The data are representative of three independent experiments. Average velocities for particles not experiencing molecular interactions range from 90 μm/s at a shear stress of 0.7 dynes/cm^2 to 700 μm/s at a shear stress of 2 dynes/cm^2. (*B*) Velocity versus time at a wall shear stress of 1.7 dynes/cm^2 for characteristic particles at 2100 and 3600 sites/μm^2 E-selectin-IgG. Note the range in the ordinate scales.

sites E-selectin-IgG/μm^2; σ^2 = 4 μm^2/s^2 for 3600 sites E-selectin-IgG/μm^2).

DISCUSSION

Using our cell-free system, we have achieved adhesion dynamics similar to those seen in leukocyte/endothelial experiments. The average rolling velocity of sLe^x-coated microspheres decreases with decreasing shear stress and increasing E-selectin-IgG site density. This is the same qualitative trend observed for neutrophil rolling as a function of E-selectin substrate density (Lawrence and Springer, 1993). We observed attachment and rolling only at shear stresses $\leq$2 dynes/cm^2, even at the highest E-selectin-IgG site density. This shear stress threshold dependence for rolling is in agreement with in vitro measurements of neutrophil rolling over interleukin-1β-stimulated endothelium (Lawrence et al., 1990). In addition, Alon and co-workers observed attachment only at wall shear stresses below 1.8 dynes/cm^2 for Chinese hamster ovary (CHO) cells expressing E-selectin interacting with sLe^x-glycolipid substrates (Alon et al., 1995a). Furthermore, at an E-selectin-IgG surface density of 3600 sites/μm^2, the average rolling velocity observed at all shear stresses is $\leq$5 μm/s, similar to rolling velocities reported for bovine neutrophils rolling over lipopolysaccharide-stimulated endothelium (Goetz et al., 1994). Microsphere rolling velocity varies with time, and the variance in velocity increases with decreasing E-selectin-IgG substrate density. Similar fluctuations in rolling velocity have been observed in neutrophil/endothelial cell (Goetz et al., 1994; Kaplanski et al., 1993) and neutrophil/E-selectin (Patel et al., 1995) attachment assays. As predicted by adhesive dynamic simulations of receptor-coated hard spheres binding to ligand-coated surfaces under flow (Hammer and Apte, 1992) and as confirmed here, rolling velocity varies with time and this fluctuation is controlled at the molecular level. Transient rolling theory suggests that velocity fluctuations are caused by a small number of bonds in the sphere-substrate contact region; therefore, we expect these fluctuations to be amplified at high shear stresses or low E-selectin-IgG densities, where very small numbers of bonds can form between sLe^x and E-selectin-IgG. Observations of rolling in cellular systems suggest that rolling velocity depends on shear stress, molecule site density, and time. Our experiments demonstrate that these parameters have a similar effect on microsphere dynamics in our cell-free system, indicating that we have captured the essential physical features of rolling with an artificial system.

Recent work by Alon and co-workers demonstrated that E-selectin expressed on CHO cells mediated rolling on sLe^x substrates (Alon et al., 1995a); however, this interaction required large sLe^x substrate densities (13,000 sites/μm^2) and their experiments were nonphysiological because E-selectin was in the mobile phase. In contrast, we observed rolling at E-selectin densities between 1300 and 3600 sites/μm^2, with sLe^x on microspheres at 1300 sites/μm^2 in the mobile phase. Quantitative comparisons between this work and that of Alon and co-workers is difficult as there is a lack of knowledge about the expression and clustering of E-selectin on the CHO cell surface.

Cell adhesion molecules important in transient attachment (L-selectin, P-selectin glycoprotein ligand-1 (PSGL-1), very late antigen-4 (VLA-4) are concentrated at the tips of microvilli (von Andrian et al., 1995; Moore et al., 1995; Berlin et al., 1995). If the cytoskeletal domain of these molecules is removed, the molecules do not cluster on microvilli and the extent of cell attachment under flow is substantially reduced (von Andrian et al., 1995; Kansas et al., 1993). It has also been suggested that the positioning of selectin counter-receptors on microvilli generates a lever

arm of several micrometers, which shields the selectin bond from shear stress preventing rapid dissociation (Alon et al., 1995b). Our results show that hard spheres coated with sLe^x at a modest density of 1300 sites/μm^2 roll over E-selectin-IgG-coated substrates, suggesting that binding between E-selectin and its ligand controls rolling, and that cell surface topology or counter-receptor presentation serve as secondary effects, perhaps by facilitating initial capture between the cell and substrate at high shear stresses. Our work is the first to show that molecular recognition between sLe^x and E-selectin is sufficient to mediate rolling in the absence of all confounding cellular features such as morphology, deformability, or signaling.

These experiments suggest that sLe^x is a minimal functional E-selectin ligand required for rolling. The underlying scaffold on which sLe^x is presented may contribute to the efficiency of attachment and perhaps modulate rolling. As an adhesion ligand, sLe^x is analogous to the fibronectin tri-peptide, arg-gly-asp (RGD), which can bind integrin receptors and mediate cell attachment, but does not elicit precisely the same binding response as fibronectin itself (Pierschbacher and Ruoslahti, 1980). As fibronectin does not bind all integrins, it is not yet clear if sLe^x is a minimal functional ligand for rolling on all selectins.

There are several technological applications of our findings. First, a cell-free assay can be used to identify unambiguously other receptor-ligand pairs that mediate rolling. Second, identification of a minimal functional element for rolling can be used to develop pharmaceuticals that interrupt selectin-ligand interactions in an intelligent and systematic way. Third, because sLe^x is a simple molecule and because the structure of E-selectin is known (Graves et al., 1994), it should be possible to develop mimetic, nonbiological adhesion molecules with full functional activity as selectin ligands.

We thank B. Brandley for the gift of the E-selectin-IgG chimera.

Supported by National Institutes of Health grant HL 18208, and grants from Pfizer and the Department of Defense.

REFERENCES

Alon, R., T. Feizi, C.-T. Yuen, R. C. Fuhlbrigge, and T. A. Springer. 1995a. Glycolipid ligands for selectins support leukocyte tethering and rolling under physiologic flow conditions. *J. Immunol.* 154:5356–5366.

Alon, R., D. A. Hammer, and T. A. Springer. 1995b. Lifetime of the P-selectin-carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature.* 374:539–542.

Berg, E. L., J. Magnani, R. A. Warnock, M. K. Robinson, and E. C. Butcher. 1992. Comparison of L-selectin and E-selectin ligand specificities: the L-selectin can bind the E-selectin ligands. *Biochem. Biophys. Res. Commun.* 184:1048–1055.

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. $\alpha 4$ integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell.* 80:413–422.

Bevilacqua, M. P., S. Stengelin, M. A. Gimbrone, Jr., and B. Seed. 1989. Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science.* 243:1160–1165.

Carlos, T. M., and J. M. Harlan. 1994. Leukocyte-endothelial adhesion molecules. *Blood.* 84:2068–2101.

Erlandsen, S. L., S. R. Hasslen, and R. D. Nelson. 1993. Detection and spatial distribution of the β_2-integrin (Mac-1) and L-selectin (LECAM-1) adherence receptors on human neutrophils by high-resolution field emission SEM. *J. Histochem. Cytochem.* 41:327–333.

Foxall, C., S. R. Watson, D. Dowbenko, C. Fennie, L. A. Lasky, M. Kiso, A. Hasegawa, D. Asa, and B. K. Brandley. 1992. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl $Lewis^x$ oligosaccharide. *J. Cell Biol.* 117:895–902.

Fukuda, M., E. Spooncer, J. E. Oates, A. Dell, and J. C. Klod. 1984. Structure of sialylated fucosyl lactosaminoglycans isolated from human granulocytes. *J. Biol. Chem.* 259:10925–10935.

Goetz, D. J., B. K. Brandley, and D. A. Hammer. 1996. An E-selectin-IgG chimera supports sialylated moiety dependent adhesion of colon carcinoma cells under fluid flow. *Ann. Biomed. Eng.* 24:87–98.

Goetz, D. J., M. E. El-Sabban, B. U. Pauli, and D. A. Hammer. 1994. Dynamics of neutrophil rolling over stimulated endothelium in vitro. *Biophys. J.* 66:2202–2209.

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.* 22:653–659.

Graves, B. J., R. L. Crowther, C. Chandran, J. M. Rumberger, S. Li, K.-S. Huan, D. H. Presky, P. C. Familletti, B. A. Wolitzky, and D. K. Burns. 1994. Insight into E-selectin/ligand interaction from the crystal structure and mutagenesis of the lec/EGF domains. *Nature.* 367:532–538.

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. *J. Biol. Chem.* 264:7768–7771.

Kansas, G. S., K. Ley, J. 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. *Biophys. J.* 64:1922–1933.

Kuo, S. C., and D. A. Lauffenburger. 1993. Relationship between receptor/ligand binding affinity and adhesion strength. *Biophys. J.* 65:2191–2200.

Lawrence, M. B., C. W. Smith, S. G. Eskin, and L. V. McIntire. 1990. Effect of venous shear stress on CD18-mediated neutrophil adhesion. *Blood.* 75:227–237.

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.* 65:859–873.

Lawrence, M. B., and T. A. Springer. 1993. Neutrophils roll on E-selectin. *J. Immunol.* 151:6338–6346.

Majuri, M.-L., M. Pinola, R. Niemela, S. Tiisala, J. Natunen, O. Renkonen, and R. Renkonen. 1994. $\alpha 2,3$-sialyl and $\alpha 1,3$-fucosyltransferase-dependent synthesis of sialyl Lewis x, an essential oligosaccharide present on L-selectin counterreceptors, in cultured endothelial cells. *Eur. J. Immunol.* 24:3205–3210.

Moore, K. L., Patel, K. D., Bruehl, R. E., L. Fugang, D. A. Johnson, H. S. Lichenstein, R. D. Cummings, D. F. Baintain, and R. P. McEver. 1995. P-selectin glycoprotein ligand-1 mediates rolling of human neutrophils on P-selectin. *J. Cell Biol.* 128:661–671.

Patel, K. D., K. L. Moore, M. U. Nollert, and R. P. McEver. 1995. Neutrophils use both shared and distinct mechanisms to adhere to selectins under static and flow conditions. *J. Clin. Invest.* 96:1887–1896.

Pierres, A., O. Tissot, B. Malissen, and P. Bongrand. 1994. Dynamic adhesion of CD8-positive cells to antibody-coated surfaces: the initial step is independent of microfilaments and intracellular domains of cell binding molecules. *J. Cell Biol.* 125:945–953.

Pierschbacher, M. D., and E. Ruoslahti. 1980. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature.* 309:30–33.

Pigott, R., L. A. Needham, R. M. Edwards, C. Walker, and C. Power. 1991. Structural and functional studies of the endothelial activation antigen endothelial leukocyte adhesion molecule-1. *J. Immunol.* 147:130–135.

Polley, M. J., M. L. Phillips, E. Wayner, E. Nudelman, A. K. Singhal, S. I. Hakomori, and J. C. Paulson. 1991. CD62 and endothelial cell-leukocyte adhesion molecule 1 (ELAM-1) recognize the same carbohydrate ligand, sialyl-Lewis x. *Proc. Natl. Acad. Sci. USA* 88:6224–6228.

Symington, F. W., D. L. Hedges, and S. I. Hakomer. 1985. Glycolipid antigens of human polymorphonuclear neutrophils and the inducible HL-60 myeloid cell line. *J. Immunol.* 134:2498–2506.

Usami, S., H.-H. Chen, Y. Zhao, S. Shien, and R. Skalak. 1993. Design and construction of a linear shear stress flow chamber. *Ann. Biomed. Eng.* 21:77–83.

Vestweber, D. 1993. Adhesion in leukocyte homing and differentiation: the selectins and their ligands. *In* Current Topics in Microbiology and Immunology, Vol. 184: Adhesion in Leukocyte Homing and Differentiation. D. Dunon, C. R. Mackay, and B. A. Imhof, editors. Springer-Verlag, Berlin. 65–75.

von Andrian, U. H., J. D. Chambers, L. M. 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 the leukocyte β_2 integrins in vivo. *Proc. Natl. Acad. Sci. USA.* 88:7538–7542.

von Andrian, U. H., S. R. Hasslen, R. D. Nelson, S. L. Erlandsen, and E. C. Butcher. 1995. A central role for microvillous receptor presentation in leukocyte adhesion under flow. *Cell.* 82:989–999.

Watson, S. R., Y. Imai, C. Fennie, J. S. Geoffroy, S. D. Rosen, and L. A. Lasky. 1990. A homing receptor-IgG chimera as a probe for adhesive ligands of lymph node endothelial venules. *J. Cell Biol.* 110:2221–2229.