Dynamics of Neutrophil Rolling Over Stimulated Endothelium in Vitro

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ABSTRACT Prior to extravasation at sites of acute inflammation, neutrophils roll over activated endothelium. Neutrophil rolling is often characterized by the average rolling velocity. An additional dynamic feature of rolling that has been identified but not extensively studied is the fluctuation in the rolling velocity about the average. To analyze this characteristic further, we have measured the instantaneous velocity of bovine neutrophils interacting with lipopolysaccharide-stimulated bovine aortic endothelium at shear stresses of 1, 2, 3, and 4 dynes/cm^2. The average velocities are quantitatively similar to those reported for human neutrophils rolling over reconstituted P-selectin at a surface density of 400 sites/μm^2. At all shear stresses tested, the population average variance in the instantaneous velocity is at least 2 orders of magnitude higher than the theoretical variance generated from experimental error, indicating that the neutrophils translate with a nonconstant velocity. Possible sources of the variance are discussed. These include "macroscopic" sources such as topological heterogeneity in the endothelium and microscopic sources, such as inherent stochastic formation and breakage of the receptor-ligand bonds that mediate the rolling. Regardless of the ultimate source of the variance, these results justify the use of mathematical models that incorporate stochastic processes to describe bond formation and breakage between the neutrophil and the endothelium and hence are able to generate variable velocity trajectories.

INTRODUCTION

Transient, heterotypic adhesion between leukocytes and the endothelium is an essential part of many immune and inflammatory reactions (Stoolman, 1989; Osborn, 1990; Springer, 1990; Lasky, 1992; Bevilacqua, 1993). The leukocyte/endothelial cell adhesion receptors known to date are grouped into three major families: the integrin family, the immunoglobulin superfamily, and the selectin family (Springer, 1990). The selectin family of adhesion molecules consists of three members: L-selectin, P-selectin, and E-selectin (Lasky, 1992; Bevilacqua, 1993). L-selectin is expressed on the surface of leukocytes, including neutrophils and lymphocytes (McEver, 1991), while P-selectin and E-selectin are inducible endothelial cell adhesion molecules (McEver et al., 1989; Lasky, 1992; Bevilacqua, 1993). P-selectin expression can be induced by stimulation of endothelial cells with thrombin or histamine (McEver et al., 1989) as well as tumor necrosis factor α (TNF-α) (Weller et al., 1992). E-selectin is induced by exposure of endothelial cells to various cytokines such as TNF-α, interleukin 1, or lipopolysaccharide (Bevilacqua et al., 1989; Bevilacqua, 1993). E-selectin expression is transient, reaching a plateau around 4 h after stimulation. Although the majority of these investigations involve the use of human umbilical vein endothelial cells (HUVEC), it appears that other endothelial cell systems respond similarly to stimulation (Weller et al., 1992; Hahne et al., 1993).

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Typically neutrophils exhibit transient attachment or "roll" on stimulated endothelial cell surfaces before firm attachment to the endothelium. Both P-selectin and E-selectin have been shown to mediate neutrophil rolling on activated endothelial cell surfaces (Abbassi et al., 1993; Jones et al., 1993). In addition, Lawrence and Springer (1991) have shown that reconstituted P-selectin supports neutrophil rolling. Firm adhesion is mediated by secondary neutrophil/endothelial cell adhesion receptors including LFA-1/ICAM-1 (Springer, 1990; Smith et al., 1989). This receptor pair provides ample adhesion under static conditions (Smith et al., 1989), but fails to mediate the rolling of neutrophils under hydrodynamic flow (Lawrence et al., 1990).

Not enough is known about the chemical and mechanical differences between selectins and other endothelial cell adhesion molecules (i.e., ICAM-1) and their respective neutrophil ligands to understand why one pair of molecules supports rolling while the others do not. Although firm conclusions regarding differences between the molecules await direct measurement of the physical and chemical properties of individual molecules, others have tried to address this question indirectly by formulating mathematical models that contain mechanicochemical parameters for adhesion molecules (Hammer and Apte, 1992; Tözeren and Ley, 1992). These models, when applied to neutrophil rolling data, can delineate regions of parameter space where the observed dynamic behavior can be recreated. To guide the development of such models it becomes important to have a full understanding of the dynamic nature of neutrophil rolling.

Although the quantity most often used in the literature to characterize neutrophil rolling is the average velocity (Lawrence et al., 1990; Lawrence and Springer, 1991, Jones et al., 1993), it appears that the dynamics of neutrophil rolling are not completely described by this quantity. Mention has

been made of the stochastic nature of neutrophil rolling over endothelial cell surfaces in vivo (House and Lipowsky, 1991; Schmid-Schönbein et al., 1987). This motion has been referred to as saltation (Lipowsky et al., 1991). Lawrence and Springer (1991) reported neutrophil saltation over surfaces reconstituted with selectins, which suggests that saltation is not solely dependent on the topology or receptor distribution of the endothelial surface. However, the trajectories or instantaneous velocities of individual neutrophils have never been fully analyzed. Our study therefore focuses on detailed analyses and measurements of the rolling of individual bovine neutrophils over stimulated bovine aortic endothelial cells. We have measured the average velocity, the instantaneous velocity, and the variance of the instantaneous velocity for neutrophils rolling at four different shear stresses that, typically, are observed in postcapillary venules (Atherton and Born, 1972, 1973). Our data show that neutrophils do not roll with a constant velocity over stimulated endothelium and that the variance in velocity may be 2 orders of magnitude higher than the theoretical variance generated from measurement error. This finding implies that mathematical models must contain a stochastic component to fully capture the dynamic nature of neutrophil rolling.

MATERIALS AND METHODS

Preparation of endothelial cells

Bovine aortic endothelial cells (BAEC) were isolated from thoracic aortas of 18-month-old calves, essentially as described by Booyse et al. (1975). BAEC were grown in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO) and frozen in liquid nitrogen at passage 2 or 3. BAEC used in these experiments were recovered from liquid nitrogen and then passaged twice: once on tissue culture plastic and once on a glass slide precoated with 100 μg/ml collagen type I (Collagen Corporation, Palo Alto, CA). Cells were cultured for 48 h at standard culture conditions (37°C, 5% CO_2 in air, 100% humidity). Four hours before the experiments, lipopolysaccharide (LPS) (Sigma, St. Louis, MO) was added to the endothelial cell media at a final concentration of 1 μg/ml.

Preparation of neutrophils

Neutrophils were isolated from citrate-anticoagulated whole bovine blood using a modified version of Carlson and Kaneko (1973) as described by Gilbert et al. (1993). Recovered neutrophils were held on ice for <4 h until they were used in experiments. Immediately before use, the supernatant was removed and the neutrophils were resuspended in 20 ml DMEM supplemented with penicillin, streptomycin, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$, and 2.5 mM HEPES. The neutrophils were then placed in an incubator at 37°C, 5.0% CO_2, and 100% humidity for 30 min. An aliquot of the neutrophils was resuspended in supplemented DMEM (37°C) to a final concentration of 1.7×10^5 cells/ml. The suspension was allowed to cool to room temperature and poured into a glass syringe which was then placed on a syringe pump in preparation for the experiment.

Flow chamber assay

An adapted version of the radial flow chamber described by Cozens-Roberts et al. (1990) was used to measure the rolling velocity of the neutrophils (Fig. 1). Our chamber consists of a lower plate and an upper plate separated by a gasket (Silastic Sheeting, Dow Corning, Midland, MI). The top plate has an inlet for the cell suspension. The bottom plate has a trough where a coverslip can be deposited. For flow rates and chamber dimensions used in these experiments, an axisymmetric laminar flow field is created in the horizontal gap between the plates (Cozens-Roberts et al., 1990; Fowler and McKay, 1980). The shear stress at a radial position r on the bottom surface is given by $\tau(r) = 3q\mu/\pi r h^2$ where q is the volumetric flow rate, h is the thickness of the horizontal gap between the plates, and μ is the viscosity of the media (Fowler and McKay, 1980). For this particular application the flow rates used were 2.5 and 0.7 ml/min. The thickness of the horizontal gap was 0.20 mm, and the viscosity of the media was 0.01 p. The flow chamber was placed on top of an inverted phase-contrast microscope (Diaphot-TMD, Nikon Inc., Garden City, NJ) connected to a video camera (70S camera, Dage-MTI, Inc., Michigan City, IN). The events were recorded on a VCR (BR-S611U, JVC, Elmwood Park, NJ) connected to a monitor (PVM-91, Sony, Park Ridge, NJ) and in line with a frame code generator (SA-F911U, JVC, Elmwood Park, NJ). The resolution of the recording was 30 frames/s. In line with the camera was a reticle, which superimposed a grid of dimensions 250 μm × 250 μm onto the recorded image. Prints of the videoframes were made from a thermal printer (VideoGraphic Printer, UP-870 MD, Sony).

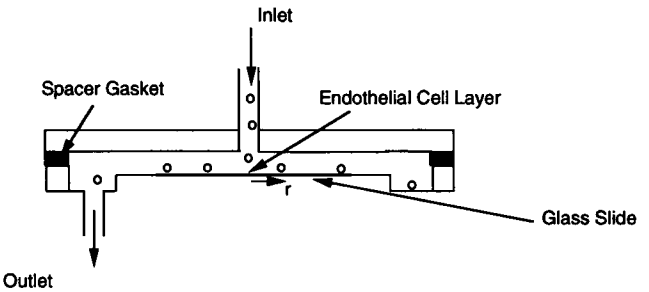

FIGURE 1 Radial flow chamber. The flow chamber consists of two pieces of Plexiglas separated by a Silastic gasket. The bottom piece has a hollow trough where the endothelial cell-coated coverslip is deposited. The top piece has an inlet for the neutrophil suspension. The flow chamber is assembled on top of a microscope stage and adhesion events are videotaped. The shear stress at the bottom surface of the chamber is inversely proportional to the radial position. Thus, several shear stresses can be realized at a single flow rate.

The experiments were performed at room temperature (23°C) as follows. The glass slide covered with the LPS-treated BAEC monolayer was placed in the bottom piece of the flow chamber. The flow chamber was assembled and the endothelial cell layer was washed with LPS-free supplemented DMEM (23°C) for 10 min. After the wash, the flow of the neutrophil suspension was initiated. Neutrophil trajectories were videotaped at radial positions corresponding to shear stresses of 4, 3, 2, and 1 dynes/cm^2.

Data analysis

The average velocity of the cells at a given shear stress was determined from the time taken for a cell to cross a 250-μm section of the field of view defined by the reticle. To determine the instantaneous velocities, the tapes were played back on a VCR connected to an image analysis workstation (TCL-IMAGE Software, Multihouse TSI, Amsterdam, Holland, running on a Mac IIx computer, Data Translation frame grabber). Every 2 s, determined by the time codes, an image was sent to the image analysis work station. We then utilized the image analysis software to determine the position of the neutrophil in the field of view to a resolution of 0.76 μm.

The theoretical variance of the instantaneous velocity measurement was determined using standard error analysis. The velocity is defined by $V = d/t$ where d is the distance the cell moved in time t (2 s). The theoretical measurement variance was then determined from the equation $\langle\sigma_v\rangle = (\delta d/t)^2 + (V\delta t/t)^2$ where δd is the theoretical error in the distance measurement (one-half the smallest resolution, 0.38 μm) and δt is the theoretical error in the time measurement (one-half the smallest time resolution, 1/60 s). In calculating the theoretical measurement variance, the average velocity at the given shear stress was used.

The experimental variance of the instantaneous velocities of cell j were determined from the equation $\langle \sigma_{vj} \rangle = \sum (V_i - V_j)^2/n - 1$, where the sum is taken from 1 to n where n is the number of instantaneous velocity measurements for cell j, V_j is the average instantaneous velocity for cell j, and V_i is the instantaneous velocity measurement corresponding to time i. Error bars are ±SEM.

RESULTS

Selection of neutrophils for analysis

Neutrophils exhibited various interactions with the endothelium as illustrated in Fig. 2, *A–D*: (*A*) neutrophils which entered the field of view with a velocity close to the hydrodynamic velocity and then decelerated to a velocity considerably less than the hydrodynamic velocity (The "hydrodynamic velocity" is the velocity we expect neutrophils to display if they experience only hydrodynamic forces); (*B*) neutrophils which came into the field of view at a velocity considerably less than the hydrodynamic velocity and then accelerated to a velocity close to the theoretical velocity; (*C*) neutrophils which translated at a velocity considerably less than the hydrodynamic velocitys then accelerated to a velocity close to the hydrodynamic velocity and then decelerated to a velocity considerably less than the hydrodynamic velocity; and (*D*) neutrophils which moved at considerably less than the hydrodynamic velocity for the entire field of view. Because we are only interested in the dynamics of neutrophil motion when the neutrophil is in continuous contact with the endothelium, only neutrophils from category D were considered for this analysis. This represented about 30% of the neutrophils that contacted the endothelium. Further, none of the neutrophils used in the subsequent analyses ever exhibited an instantaneous velocity (discretized to a resolution of ⅓oth of a second), which was >50% of the hydrodynamic velocity indicating that, at least to a resolution of ⅓oth s, the neutrophils used for the analysis were continuously in contact with the endothelium. In the following analyses, 8 neutrophils were analyzed at 2, 3, and 4 dynes/cm^2 and 4 neutrophils were analyzed at 1 dyne/cm^2. These numbers of observations were sufficient to make statistical comparisons.

Videoprints of neutrophil rolling

A video record of neutrophils rolling over a stimulated endothelial cell layer at a shear stress of 2 dynes/cm^2 is shown in

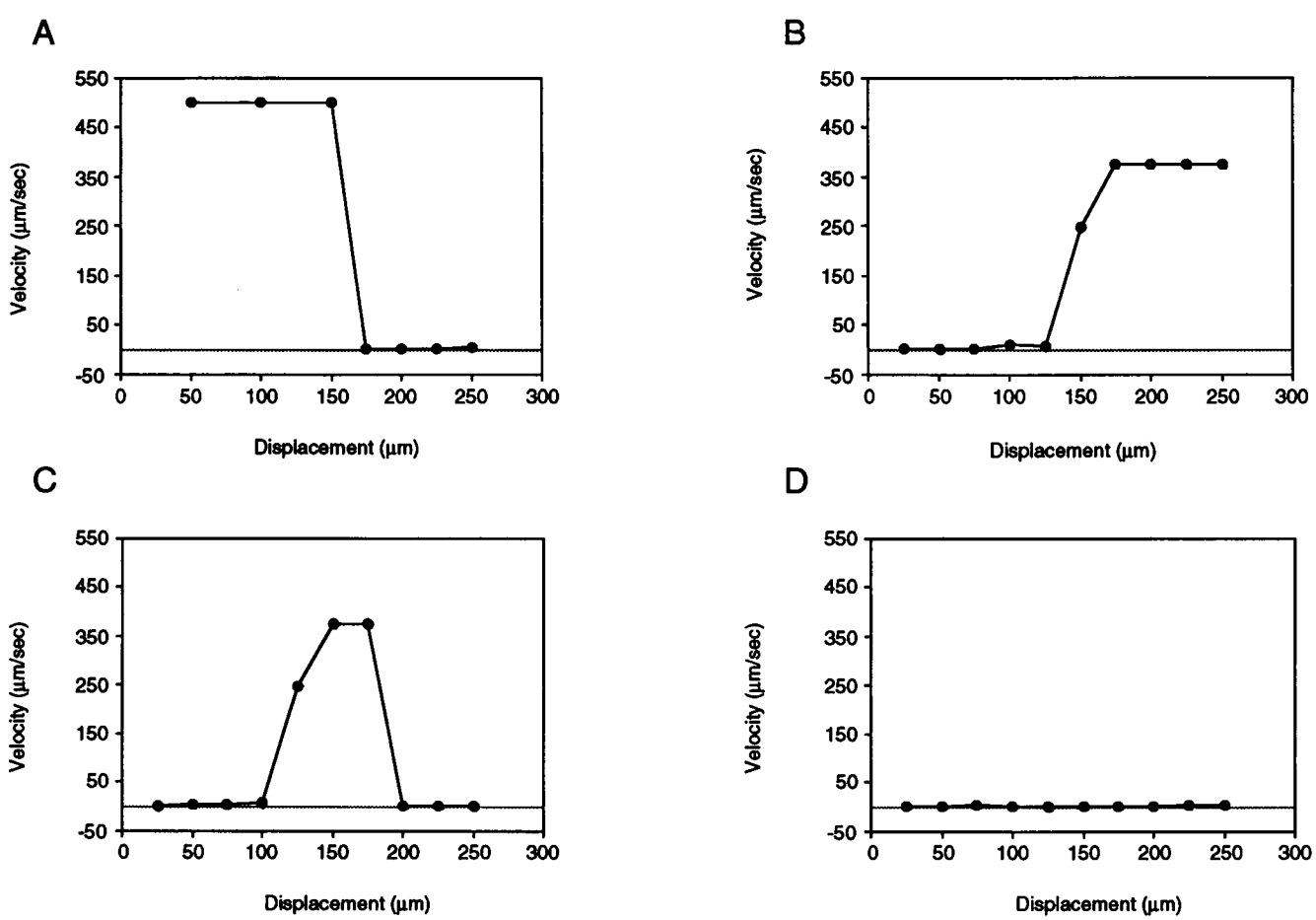

FIGURE 2 (*A–D*) Four neutrophil trajectories observed in the flow chamber assay. Trajectories were generated by discretizing the 250-µm field of view into 25-µm segments using the image superimposed on the tape by the reticle. The velocities exhibited by the neutrophils over the 10 25-µm segments were then determined and plotted as a function of position in the field of view. *A–C* are trajectories of neutrophils not used in the analysis. Neutrophils used in the analysis exhibited trajectories as shown in *D*. Shear Stress = 2 dynes/cm^2.

Fig. 3, *A–D*. As shown by the time codes at the top of each image, 10 s elapsed between each image. The entire grid covers an area of 250 × 250 μm^2. Each smaller square in the grid is 25 × 25 μm^2 and can be referenced alphanumerically by the letter and the number corresponding to the row and column of the grid. By following the trajectories of the cells through the sequence of videoimages, it becomes apparent that the cells do not roll with a constant velocity. For example, consider the two cells in column 4. In Fig. 3 *A*, the cell indicated by the *black arrow* (black cell) is slightly ahead of the cell indicated by the *white arrow* (white cell). However, 10 s later (Fig. 3 *B*) the black cell is >25 μm ahead of the white cell indicating that, in the recent past, the black cell had a higher velocity than the white cell. Fig. 3 *C* shows the two cells at approximately the same lateral position indicating that the white cell recently had a higher velocity than the black cell. In the final frame, Fig. 3 *D*, the black cell is ahead of the white cell indicating that the black cell recently had a higher velocity than the white cell. These observations indicate that the velocities of the two cells are not constant. Because both cells were observed simultaneously in a single field of view, fluctuations in their velocities cannot be a result of fluctuations in the fluid velocity in the flow chamber. Later we will show formally that the instantaneous rolling velocities are not constant.

Average velocity versus shear stress

To determine the average rolling velocity of the neutrophils we measured the time required for several cells to transverse the 250 × 250 μm^2 grid superimposed on the videotaped image. Fig. 4 shows how the average velocity varies with shear stress. The rolling velocity appears to increase with increasing shear stress up to about 3 dynes/cm^2. (Analysis of variance indicated that the average velocity was a function of shear stress (*F* test, $p < 0.0001$)). The average velocities

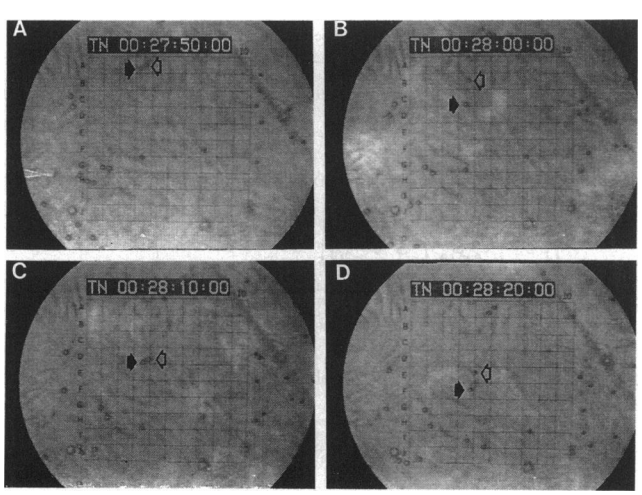

FIGURE 3 (*A–D*) Video images of neutrophils rolling at 2 dynes/cm^2. A sequence of video images taken from the videorecord of an experiment. Between each image 10 s have elapsed. By following the trajectory of the cells, it becomes apparent that each cell is moving with a nonuniform velocity. The discussion in the text refers to the two highlighted cells.

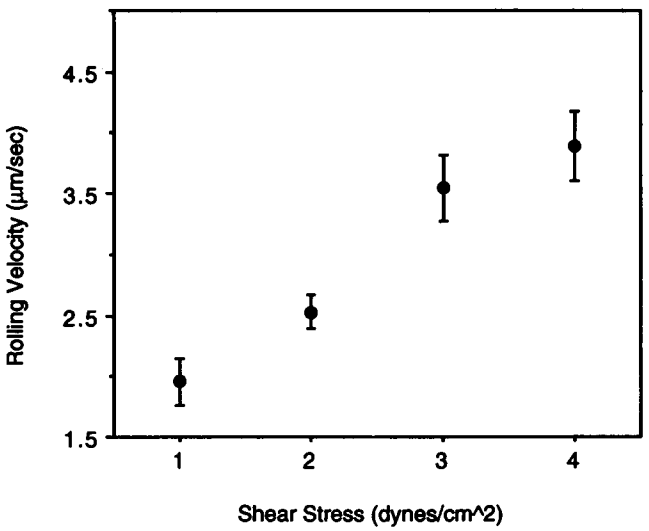

FIGURE 4 Average rolling velocity versus shear stress. Between 1 and 3 dynes/cm^2, the rolling velocity increases with increasing shear stress. The rolling velocities at the different shear stresses are significantly different ($p < 0.05$). The rolling velocities at 3 and 4 dynes/cm^2 are not significantly different ($p < 0.4$).

at the three lower shear stresses are significantly different from one another (*t* test, $p < 0.05$) whereas the average velocity at 4 dynes/cm^2 is not significantly different from that seen at 3 dynes/cm^2 (*t* test, $p = 0.4$).

From the work of Goldman et al. (1967), it is possible to predict the velocity at which a solid sphere having the same radius as the neutrophil would be moving if it were 500 Å from the wall and in low Reynolds number linear shear flow (500 Å being the approximate maximal separation distance for two selectin adhesion receptors to interact (Springer, 1990)). Table 1 shows this prediction, as well as the average velocities reported in Fig. 3 as a function of shear stress. As shown, the neutrophils roll between 1.0 and 0.5% of the velocity predicted by Goldman and co-workers (1967). The translational velocities we measured for bovine neutrophils over LPS-stimulated BAEC are quantitatively similar to those reported by Lawrence and Springer (1991) for human neutrophils rolling over a substrate consisting of reconstituted P-selectin at a surface density of 400 sites/μm^2.

Instantaneous velocities

To further characterize the motion of the neutrophils, we measured the instantaneous velocities for several neutrophils at the four different shear stresses. This was done by importing an image from the videotape to an image analysis workstation. The position of the neutrophils in the image was then determined up to a theoretical resolution of 0.78 μm. This process was repeated many times for frames 2 s apart. Distance versus time plots were then converted to velocity versus time using finite difference. Typical results for a sample of neutrophils are given in Figs. 5 and 6. Fig. 5 shows neutrophils translating at a wall shear stress of 2 dynes/cm^2; Fig. 6 shows neutrophils translating at a wall shear stress of 3 dynes/cm^2. Data from 1 and 4 dynes/cm^2 are omitted for conciseness. At both 2 and 3 dynes/cm^2 the instantaneous

TABLE 1 Comparison of rolling velocities on various substrates with hard sphere motion in couette flow

Shear Stress	Substrate	Rolling velocity	Hydrodynamic velocity*	% of theory	Source
dynes/cm^2		μm/s	μm/s		
1	Stimulated endothelial cells	2.0	200	1.0	This paper
2		2.5	400	0.63	
3		3.6	600	0.60	
4		3.9	800	0.49	
1	P-selectin, 400 sites/μm^2	1.8	200	0.90	Lawrence and Springer, 1991
2		3.4	400	0.85	
3		3.9	600	0.65	
4		4.8	800	0.60	
1	P-selectin, 200 sites/μm^2	4.0	200	2.0	Lawrence and Springer, 1991
2		5.8	400	1.5	
3		6.4	600	1.1	
4		7.4	800	0.93	
1	P-selectin, 50 sites/μm^2	6.2	200	3.1	Lawrence and Springer, 1991
2		10.1	400	2.5	
3		13.0	600	2.2	
4		15.2	800	1.9	

* The hydrodynamic velocities for unencumbered cells were determined from Goldman et al. (1967) using a separation distance of 500 Å. The rolling velocity of neutrophils over surfaces coated with different densities of reconstituted P-selectin were taken from Lawrence and Springer (1991).

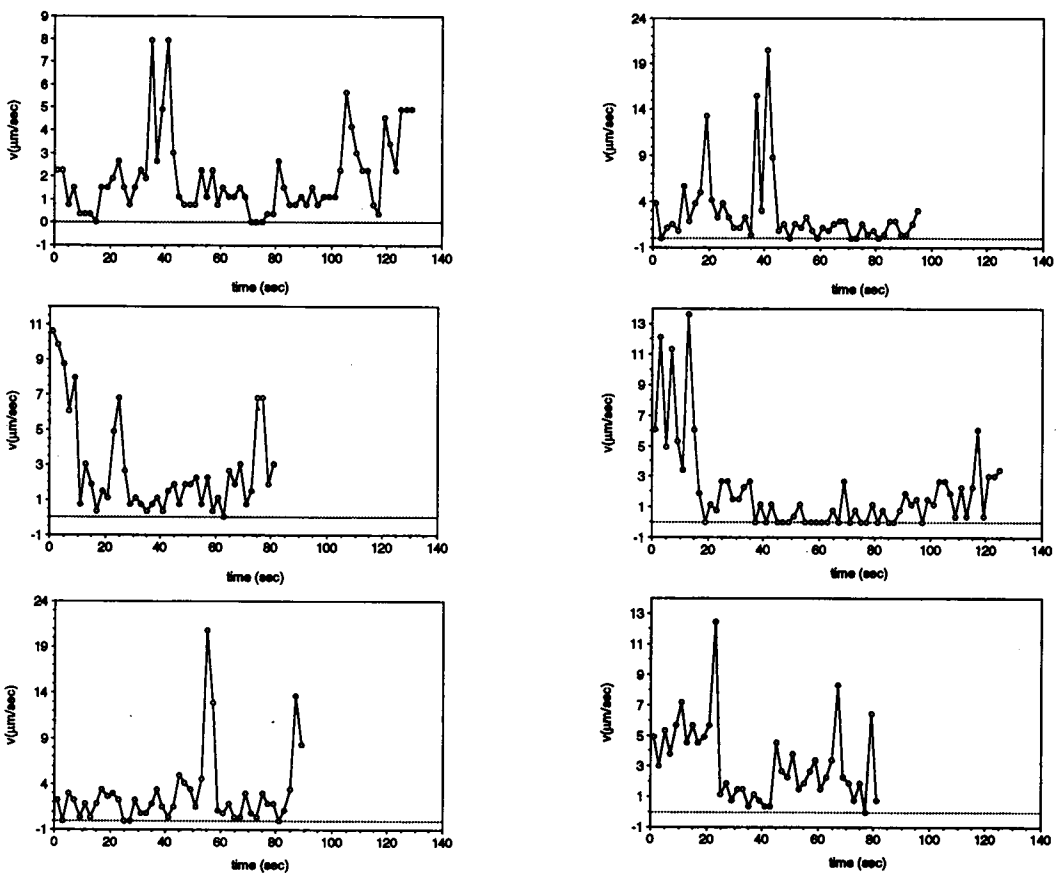

FIGURE 5 Velocity trajectories for individual neutrophils at 2 dynes/cm^2. In all cases the trajectory of the neutrophil was followed through one field of view (250 μm). The trajectories show that neutrophils exhibit a large variance in their instantaneous velocities.

velocity for a typical neutrophil varies considerably during its transit over the field of view. This was true for all neutrophils observed at all shear stresses. To quantify the variability in the neutrophil motion, we calculated the variance in the instantaneous velocity for each individual neutrophil as described under Materials and Methods. We then averaged these variances and plotted the average variance as a function of shear stress (Fig. 7). As expected the variance is nonzero

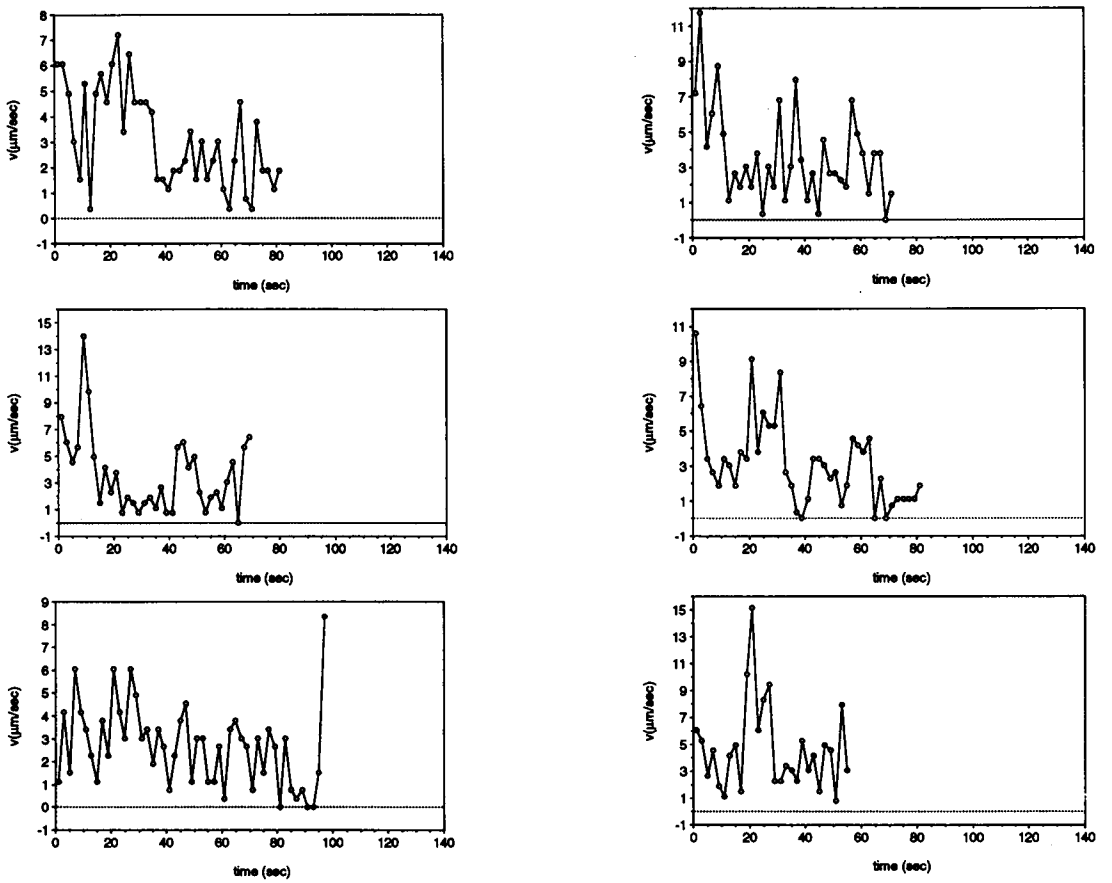

FIGURE 6 Velocity trajectories for individual neutrophils at 3 dynes/cm^2. As at 2 dynes/cm^2, the neutrophils exhibit a large variance in their instantaneous velocities. This was true for all neutrophils observed at all shear stresses.

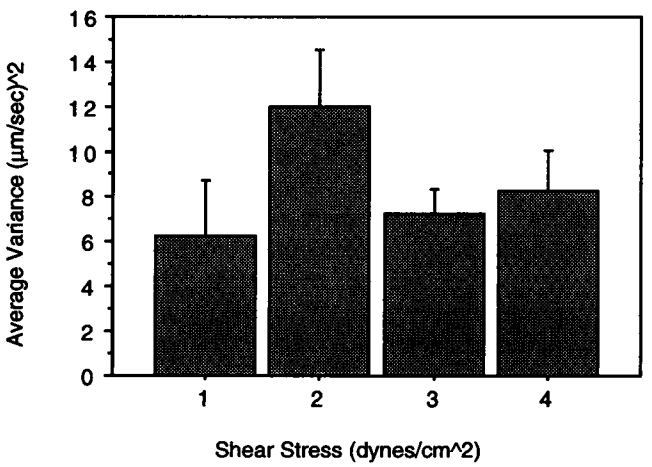

FIGURE 7 The variance in the instantaneous velocity over a 250 μm $\times$ 250 μm field of view was determined for several neutrophils at each shear stress. For each shear stress, these values were averaged. Table 2 list the results of a t test between the measured values and the theoretical variance that arises from experimental measurement error. The average variance was not dependent on shear stress ($p < 0.22$).

at all shear stresses. However, even for perfectly smooth motion, the variance will be nonzero because of inherent measurement error. Hence, it is important to determine if the variance is significantly different from the theoretical measurement variance that arises from the measurement error. The theoretical measurement variance was calculated as shown under Materials and Methods and is compared with the observed variance at the four different shear stresses (Table 2). As shown, the observed variance is >2 orders of magnitude higher than the theoretical variance and these differences appear to be significant (t test, $p < 0.01$ for shear stresses > 1 dyne/cm^2; $p < 0.08$ for shear stress = 1 dyne/cm^2).

Analysis of variance indicated that the shear stress had no effect on the variance (F test, $p < 0.22$). It seems reasonable

TABLE 2 Comparison of measured and theoretical variances

Shear stress	Measured variance	Theoretical variance*	Ratio: measured/ theoretical	t-test p value
dynes/cm^2	(μm/s)^2	(μm/s)^2		
1	6.27	0.036	170	0.08
2	12.01	0.036	330	<0.01
3	7.27	0.037	200	<0.01
4	8.25	0.037	220	<0.01

* The theoretical measurement variance was determined by standard error analysis as described under Materials and Methods. Because the error in measuring distance accounts for 95% of the theoretical measurement variance, the theoretical measurement variance varied very little from one shear stress to another (0.036–0.037 (μm/s)^2).

to question whether the variance relative to the mean (coefficient of variance) was dependent on shear stress. Hence, the coefficient of variance was determined for each cell, and the results were averaged (Fig. 8). The coefficient of variance was dependent on shear stress (F test, $p < 0.0001$). The coefficients of variance at 1 and 2 dynes/cm^2 were significantly different than the coefficients of variance at 3 and 4 dynes/cm^2 ($p < 0.005$; Scheffe's analysis (Woolson, 1987)).

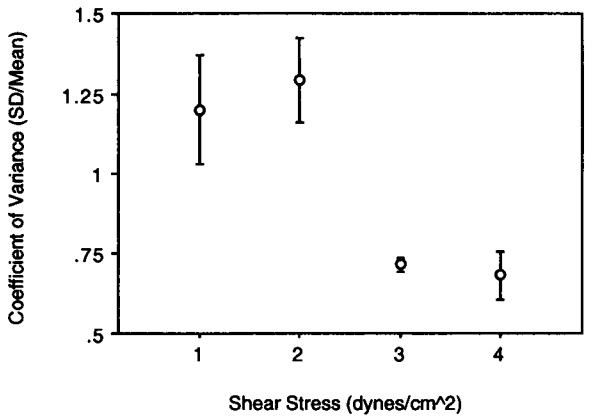

FIGURE 8 The coefficient of variance for several cells at each shear stress was determined. These results were averaged and plotted versus the shear stress. The coefficient of variance was dependent on shear stress ($p < 0.0001$). The coefficients of variance at the two lower shear stresses (1 and 2 dynes/cm^2) were significantly different from the coefficients of variance at the two higher shear stresses (3 and 4 dynes/cm^2) ($p < 0.005$).

DISCUSSION

The experiments and associated analysis in this article demonstrate that neutrophils do not roll with a constant velocity, but rather translate with a velocity whose average has a variance >2 orders of magnitude higher than the theoretical measurement variance. There are several possible sources of this variance in the cell motion.

First, there might be an inherent stochasticity in binding between neutrophil ligand and endothelial cell counter receptor, which becomes pronounced when there are small numbers of bonds formed between the neutrophil and the endothelium. A second source of variance might be topological heterogeneity in the endothelial substrate, either in the variation of shear stress over the substrate or in the distribution of adhesion molecules over the surface. For example, Satcher et al. (1992) have shown that the surface shear stress on the endothelial cell surface may vary by as much as 34% as a result of surface convolutions. Transient changes in the microstructure of the neutrophil surface or heterogeneous distribution of adhesion molecules on the neutrophil surface may also be an important source of nonconstant velocity. All of these sources contribute to the variances reported in this paper, and it is impossible for us to quantify how much each source contributes to the variance. However, it is conceivable that the variance is due largely to inherent stochasticity in the level of binding itself. Lawrence and Springer (1991) measured the rolling of human neutrophils over reconstituted P-selectin in planar-supported lipid bilayers, and state that, "Individual cells rolled with a relatively, but not completely, uniform velocity." (Lawrence and Springer, 1991; page 863) They did not, however, quantify the variance. This result suggests that the topology of the endothelium alone is not the only source of the variable velocity observed in our system. Furthermore, Hammer and Apte (1992) have shown using computer simulations of neutrophil motion that nonzero variances are possible in the neutrophil system, without considering endothelial heterogeneities.

We suspect probabilistic binding is an inherent feature of cell attachment in general. Probabilistic binding will manifest itself in different ways depending on the cell system. In neutrophils, probabilistic binding would, of course, manifest itself in fluctuations in the rolling velocity. In a recent study, we showed that rat basophilic leukemia cell adhesion under flow, mediated by antigen-antibody binding, did not display rolling; rather these cells existed in one of two states: firmly adherent (zero velocity) or motion at the hydrodynamic velocity. In this system, the probabilistic nature of binding manifested itself in the spatial location of cell binding (Tempelman and Hammer, 1994). Thus, probabilistic binding may be a fundamental controlling feature of cell attachment.

The topology of the endothelial cell monolayer may have a regular repeating pattern, and this regularity may lead to periodicities in the instantaneous velocity. In addition, heterogeneities on the neutrophil surface may also give rise to periodicities in the velocity. Given the average rolling velocity of the neutrophils (2–4 μm/s) and an average length of an endothelial cell (30 μm), the time scale for translation over a particular endothelial cell is approximately 10 s. The time scale for one rotation of the neutrophil is given by $\tau = 2\pi R/v$ where R is the radius of the neutrophil and v is the translational velocity. This time scale is also on the order of 10 s. Hence, the sampling rate of 1 sample every 2 s, which was used in the measurements of the instantaneous velocities, should have been at a sufficient frequency to resolve periodicities arising from these two phenomena. No periodicities were observed by Fourier analysis of our data. However, we feel this subject deserves a more thorough investigation. For example, it has been shown that subjecting endothelial cells to a high shear stress causes them to align in the direction of flow (Davies et al., 1986). Hence, by subjecting the endothelium to high shear stresses before the experiment, an endothelium with a more regular topology may be achieved. This increased regularity may lead to a predominant frequency in the instantaneous velocity data.

It was of interest that while the variance was not dependent on the shear stress, the coefficient of variance was dependent on shear stress, and this dependency was biphasic. Although we are not quite sure how to interpret this result, one intriguing possibility is that at the higher shear stresses (3 and 4 dynes/cm^2) perhaps only one set of receptors mediate the rolling while at the lower shear stresses (1 dyne/cm^2 and 2 dynes/cm^2) two or more sets of receptors mediate the rolling.

Most previous neutrophil rolling data have been presented for the HUVEC system. The average velocities presented here are quantitatively similar to those reported by Lawrence and Springer (1991) where the adhesive surface was reconstituted P-selectin at a surface density of 400 sites/μm^2. Unfortunately, the expression of adhesion molecules on a stimulated BAEC substrate has not been completely characterized. It has been shown that stimulation of BAEC with TNF-α leads to a twofold increase in the expression of P-selectin (Weller et al., 1992); however, the surface density was not reported. Further characterization of the BAEC system, i.e., the concentration and type of selectin molecules on the surface of the stimulated cells, should ultimately give insight into the strength of the receptor ligand pairs relative to that seen in the HUVEC system.

In summary, we have measured bovine neutrophil rolling over LPS-stimulated BAEC in a flow chamber under shear stresses from 1 to 4 dynes/cm^2. Cells do not roll with a constant velocity, but rather with a measurable, nonzero variance. The existence of velocity fluctuations justifies the development of stochastic/probabilistic mathematical models, which account for this type of motion and therefore capture the true dynamic nature of neutrophil rolling.

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