0022-1554/96/$3.30
The Journal of Histochemistry and Cytochemistry

Vol. 44, No. 8, pp. 835–844, 1996
Printed in U.S.A.

Original Article

Quantitation of L-Selectin Distribution on Human Leukocyte Microvilli by Immunogold Labeling and Electron Microscopy[1]

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Received for publication October 6, 1995 and in revised form February 20, 1996; accepted March 26, 1996 (5A3789).

L-Selectin is a leukocyte cell adhesion receptor that contributes to neutrophil (PMN) rolling on activated endothelium at sites of inflammation and mediates lymphocyte attachment to high endothelial venules in peripheral lymph nodes. Localization of this receptor to the tips of PMN and lymphocyte microvilli has been demonstrated. However, its distribution on these cells has not been quantified, and its localization on other leukocytes and the morphometry of microvilli on different leukocyte subpopulations have not been previously examined. In this study, PMN and mononuclear leukocytes were isolated from anticoagulated blood by dextran sedimentation and density centrifugation, fixed in 2% paraformaldehyde and 0.05% glutaraldehyde, immunogold-labeled for L-selectin, and embedded in Epon resin. The distribution of L-selectin was determined by counting gold particles on the plasma membrane of sectioned cells, and the surface microstructure of these cells was surveyed on two-dimensional transmission electron micrographs. On average, 78% of PMN, 72% of monocyte, and 71% of lymphocyte L-selectin was observed on the microvilli, with more variance on lymphocytes than the other cell types. Typical PMN and monocyte sections had 26 microvilli, whereas typical lymphocyte sections had 23. Quantitation of the distribution of L-selectin and leukocyte surface topology offers a foundation from which to study the requirement of microvilli or microvillus-localized L-selectin for leukocyte tethering and rolling in model systems that mimic microvascular environments. (*J Histochem Cytochem 44:835–844, 1996*)

KEY WORDS: Human; L-Selectin; Neutrophil; Monocyte; Lymphocyte; Basophil; Eosinophil; Microvilli; Cell adhesion; Immunocytochemistry.

Introduction

Transendothelial migration of leukocytes to sites of injury or lymphocyte recirculation is central to an inflammatory or immune response. This is achieved through sequential interactions between leukocyte and endothelial cell adhesion molecules belonging to the selectin, integrin, and immunoglobulin gene families, and is controlled by cytokines and chemoattractants secreted from activated endothelial cells, connective tissue cells, and invading microorganisms (Carlos and Harlan, 1994; Rosen and Bertozzi, 1994; Springer, 1994). Leukocyte tethering and rolling precede extravasation and are mediated primarily by the selectins (Smith, 1993). Lymphocyte rolling, however, is also mediated by the α4β7 (Berlin et al., 1995) and α4β1 (Alon et al., 1995) integrins. Transient selectin-mediated binding, in combination with shear forces in the microvasculature, causes leukocytes to roll on vascular endothelium at a significantly reduced velocity, enabling them to survey the local environment and vessel wall for inflammatory signals (Bargatze and Butcher, 1993; Von Andrian et al., 1991; Kishimoto et al., 1989). If activated, leukocytes rapidly shed L-selectin (Mobley and Dailey, 1992; Spertini et al., 1991; Kishimoto et al., 1989), and upregulate β2 integrins (Borregaard et al., 1994; Mobley and Dailey, 1992; Dustin and Springer, 1989; Kishimoto et al., 1989; Miller et al., 1987), and subsequent interactions between leukocyte integrins and endothelial cell ligands contribute to firm adhesion, spreading, and extravasation (Carlos and Harlan, 1994; Hogg et al., 1992; Sánchez-Madrid and Corbi, 1992).

L-Selectin is constitutively expressed on all classes of leukocytes except a substantial population of memory T-cells, and binds transiently in a calcium-dependent manner (Geng et al., 1990) to sulfated (Imai et al., 1991,1993), sialylated (Foxall et al., 1992; Phillips et al., 1990; True et al., 1990) Lewis x-type carbohydrate determinants on peripheral lymph node high endothelial venules

[1] Supported by National Institutes of Health Grants HLB31610, DK10486, and CA31799.
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(Kikuta and Rosen, 1994; Rosen and Bertozzi, 1994) and on activated endothelial cells (Hallmann et al., 1991; Smith et al., 1991). The surface expression of L-selectin has been estimated by flow cytometry to occur at 65,000 sites per neutrophil (Simon et al., 1992) and at 50,000–100,000 sites per lymphocyte (Spertini et al., 1992), and localization to the tips of PMN and lymphocyte microvilli has been established by frozen thin section (Picker et al., 1991) and scanning electron microscopy (Erlandsen et al., 1993; Hasslen et al., 1995). Microvilli are pointed projections of the plasma membrane, and localization of L-selectin to these structures may facilitate leukocyte tethering under conditions of flow by concentrating the receptor to increase its avidity for endothelial ligand(s) and by making it more accessible to these ligands. Other leukocyte adhesion molecules involved in rolling, i.e., the $\alpha 4\beta 7$ integrin (Berlin and Bargatze, 1995) and the P-selectin glycoprotein ligand, PSGL-1 (Moore et al., 1995), are localized to the tips of microvilli.

In this study, the distribution of L-selectin was determined by counting gold particles on the plasma membrane of immunogold-labeled PMN, monocytes, and lymphocytes sectioned from Epon blocks, and the surface microstructure of these cells was surveyed on two-dimensional transmission electron micrographs. The distribution of L-selectin between the microvilli and the planar cell surface, the length of the plasma membrane and microvilli, the number of microvilli per section, and the contribution of microvilli to the total length of the plasma membrane were determined for leukocytes isolated from anticoagulated blood by dextran sedimentation and density centrifugation. Cells were prepared in this manner because this is typically the way in which leukocytes are isolated for study in laminar flow assays. This quantitation and comparison provides a baseline from which to study the requirement of microvilli and the localization of L-selectin to microvilli for leukocyte tethering and rolling in flow chambers that mimic microvascular environments (Lawrence et al., 1994; Lawrence and Springer, 1991).

Materials and Methods

Leukocyte Isolation and Preembedding Immunogold Labeling. Approval for obtaining human blood by vein puncture was obtained from the Human Subjects Committee at the University of California, San Francisco. Human leukocytes were isolated from venous blood drawn from consenting donors into syringes containing 0.14 ml acid–citrate–dextrose (ACD) per ml blood by dextran sedimentation and Ficoll–Hypaque density centrifugation as previously described (Miller et al., 1987). Neutrophils were isolated from the polymorphonuclear leukocyte band and monocytes and lymphocytes were isolated from the mononuclear leukocyte band. The isolated leukocytes were washed in Hank's buffered saline solution (HBSS; Gibco Laboratories, Grand Island, NY) supplemented with 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.3, and were allowed to recover in suspension for 5 min at 22°C. The cells were then fixed in 2% paraformaldehyde, 0.05% glutaraldehyde, 100 mM sodium phosphate, pH 7.4, for 30 min and were labeled with either control IgG_1 (X63, 1:100 dilution of tissue culture supernatant) or anti-L-selectin (DREG-200) (Kishimoto et al., 1990) (~10 μg/ml) monoclonal antibody (MAb), or with a polyclonal rabbit anti-β-2 microglobulin (A072; Dakopatts, Glostrup, Denmark) (~225 μg/ml) for 60 min. A polyclonal rabbit anti-mouse bridging antibody (Zymed Laboratories; South San Francisco, CA) at 10 μg/ml was added to all samples except the β-2 microglobulin for 60 min. To control for nonspecific binding of the rabbit anti-mouse bridging antibody and the polyclonal rabbit anti-β-2 microglobulin, cells that did not receive primary antibody were incubated with normal rabbit serum diluted 1:4 (~250 μg/ml antibody, assuming an initial concentration of 1 mg/ml). Protein A–gold 5 nm (Laboratory of Cell Biology; Utrecht, The Netherlands) diluted 1:25 in 0.1% BSA–PBS, pH 8.0, was added for 60 min. The immunogold-labeled cells were re-fixed in 1.5% glutaraldehyde in 0.067 M sodium cacodylate, pH 7.4, with 1% sucrose for 45 min. Some samples were further treated with diaminobenzidine and H_2O_2 to stain cytoplasmic granules for peroxidase (Bainton and Farquhar, 1970). All samples were postfixed in 2% OsO_4 in veronal acetate buffer, stained with aqueous 1% uranyl acetate, dehydrated in ethanol, and embedded in Epon (Bainton et al., 1971).

Morphometry and L-Selectin Distribution. Measurements of the plasma membrane and microvilli were obtained by tracing electron micrographs of sectioned leukocytes on a digital drawing tablet and interpreting the traces using morphometry software (Bioquant System IV; R & M Biometrics, Nashville, TN). To compare morphometric determinations among cell types, this study was limited to equatorial sections. Therefore, only micrographs that included sections of the Golgi complex or multiple lobes of the nucleus were chosen for analysis. Data were collected from 100 micrographs of whole PMN sections from four donors (25 each), and 50 micrographs of whole monocyte of lymphocyte sections from donors (25 each); A total of 2551 PMN microvilli, 1298 monocyte microvilli, and 1165 lymphocyte microvilli were measured. Cross-sectional area and plasma membrane length measurements were obtained simultaneously by tracing the perimeter of the sectioned cell. The diameter of the cells was determined by averaging the length and width of the sections. Length measurements were obtained by measuring the longest axis that passed through the center of the cell and width measurements were obtained by measuring the longest axis perpendicular to the length axis. The measured axes spanned the sections between flat regions of the plasma membrane and did not include microvilli.

Microvilli were defined as pointed projections of the plasma membrane extending at least 50 nm from what would otherwise be a smooth contour of the cell surface (Bongrand and Bell, 1984). Because of the two-dimensional nature of transmission electron micrographs, it was not possible to distinguish short, broad microvilli from folds or ruffles in the plasma membrane. Given this limitation, it is possible that plasma membrane extensions exceeding 150–200 nm in width at the base may in fact be folds or ruffles rather than true microvilli. Microvilli were measured along the central axis from the midpoint of the tip to what would otherwise be the surface of the cell if there were no microvilli.

The intraobserver error associated with the morphometric determinations in this study was determined by randomly selecting 20 micrographs and measuring the plasma membrane circumference, cross-sectional area, and diameter of the sections, and counting the microvilli on two separate occasions. To determine the intraobserver error associated with measurements of microvillous length, 232 microvilli from 10 randomly selected micrographs were measured on two separate occasions. Linear regression analysis of these determinations resulted in r values of 0.967, 0.999, 0.962, 0.991, and 0.978 for the plasma membrane circumference, cross-sectional area, diameter, number of microvilli per section, and microvillous length, respectively ($p<0.0001$).

The distribution of L-selectin was determined by counting gold particles on the plasma membrane of immunogold-labeled cells and distributing the counts into microvillous or planar cell surface categories. Because fixation decreased the number of functional epitopes and the reagents were multivalent, the number of gold particles counted did not quantitatively define the number of L-selectin molecules present. It did, however, provide a means of indexing the distribution of the receptor between the microvillous and nonmicrovillous compartments of the plasma membrane. Fifty micrographs of whole cell sections from two donors (25 each) were analyzed for each cell type.

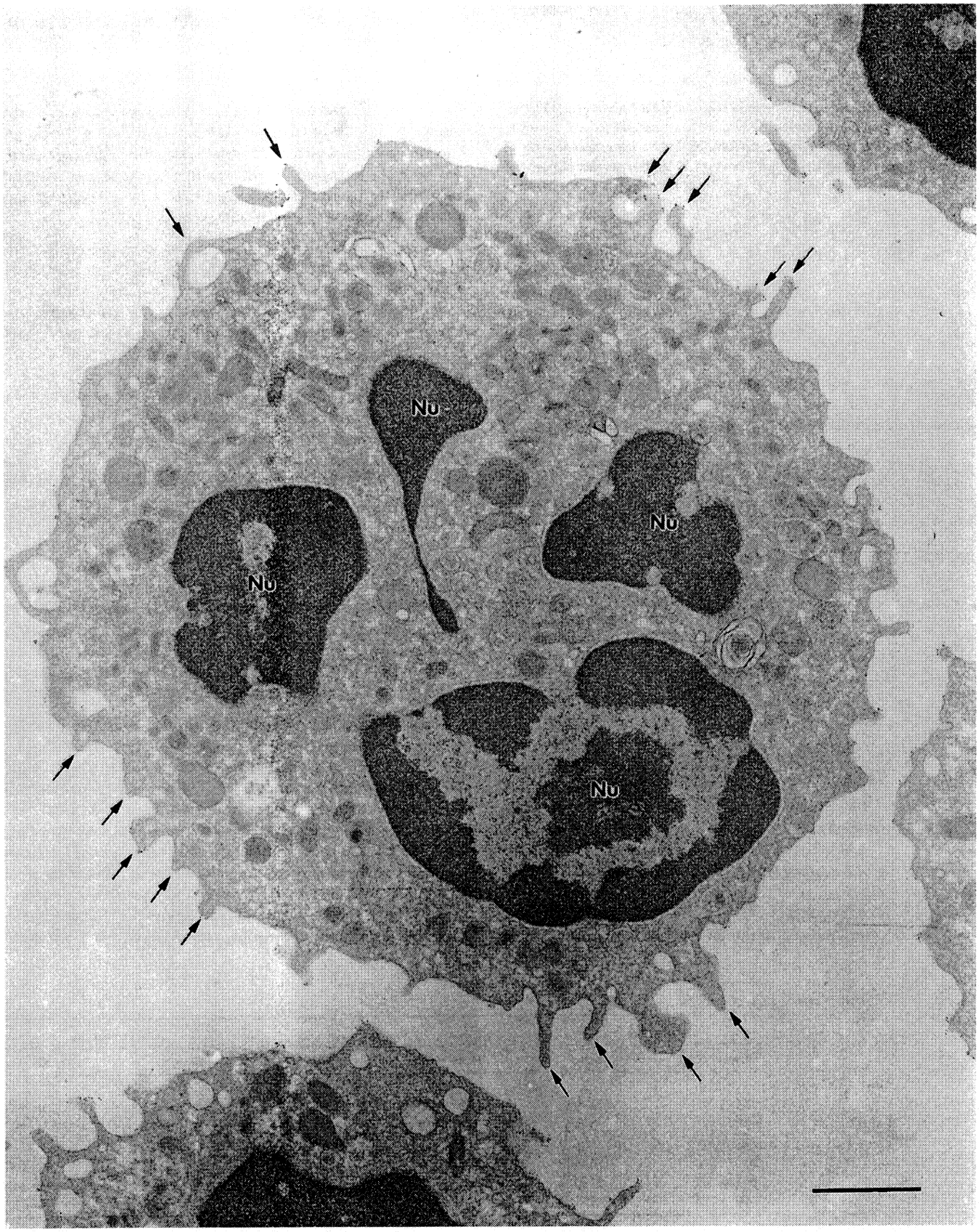

Figure 1. Transmission electron micrograph of a human PMN immunogold-labeled with the DREG 200 anti-L-selectin antibody. Note the many microvilli (arrows) of varied length, width, and shape, and irregular spacing on the surface of this cell. This particular section shows 31 microvilli and has a plasma membrane circumference of 42 μm. Although barely visible at this magnification, gold particles labeling L-selectin can be seen at the tips of most microvilli (black dots). A higher-magnification view of the plasma membrane with clearly visible gold particles is shown in Figure 2. Nu, nucleus. Original magnification × 17,900. Bar = 1 μm.

The intraobserver error associated with the distribution of gold particles was determined by randomly selecting 20 micrographs and counting the gold particles on two separate occasions. Linear regression analysis of the data generated r values of 0.995 and 0.999 for the percent of particles localized to microvilli and the total number of particles per section, respectively ($p<0.0001$).

Statistics. Values cited are the arithmetic mean ± one standard deviation (SD). One-way analysis of variance was used to compare the means of L-selectin distribution and morphometric parameters among PMN, monocytes, and lymphocytes for normally distributed data with equal variance. In cases where the data were not normally distributed or variance among samples was not equal, a Kruskal–Wallis one-way analysis of variance on ranks was used to compare medians. The statistical significance of differences between means was evaluated using a Student–Newman–Keuls all pairwise multiple comparison procedure at a significance level of 0.05. The statistical significance of differences between medians was evaluated using Dunn's method for all pairwise multiple comparisons at a significance level of 0.05. Correlations between the number of gold particles per microvillus and microvillous length and correlations between the proportion of L-selectin localized to the microvilli and the number of microvilli or gold particles per section were calculated using a Pearson product moment correlation. Data for the correlation between the number of particles per microvillus and microvillous length were obtained from 25 micrographs of each cell type from one donor; 758 PMN, 682 monocyte, and 461 lymphocyte microvilli were evaluated. Data for the correlations between the distribution of L-selectin and the number of microvilli or gold particles per section were obtained from 50 micrographs of each cell type from two donors (25 each). Statistical tests were performed using SigmaStat statistical software (Jandel Scientific; San Rafael, CA).

Results

Quantitation of L-Selectin Distribution

All classes of leukocytes were labeled with the anti-L-selectin MAb, although eosinophils were only lightly labeled and some lymphocytes were not labeled at all. Gold particles were most frequently observed in groups both at the tips of microvilli and along the planar cell surface. However, occasionally single particles were encountered on both compartments of the plasma membrane. The proportion of L-selectin localized to the microvilli of PMN, monocytes, and lymphocytes was determined by counting gold particles on the plasma membrane of sectioned cells and distributing the counts into microvillous or planar cell surface categories. Although particles were also observed on the tips of eosinophil and basophil microvilli, the distribution of L-selectin on these cell types was not quantified because of insufficient sampling.

Neutrophils. Sectioned PMN presented many microvilli of varied length, width, and frequency (Figure 1) and, on average, 78 ± 9% of the gold particles labeling L-selectin were counted on microvilli (Table 1), with most observed at the tips (Figure 2A). The number of gold particles per microvillus was independent of microvillous length (Figure 3A), which is consistent with the observation that the majority of the receptor is positioned at the tip. The partitioning of L-selectin to microvilli was independent of the number of microvilli or gold particles per section (Figures 3B and 3C), which demonstrates that differences in the number of microvilli or particles per section among samples do not distort the distribution analysis. The anti-L-selectin MAb did not label red blood cells or platelets, and the class-matched control (X63) did not label any blood cells (not shown).

To demonstrate random cell surface labeling in contrast to microvillous localization, leukocytes were also labeled with a polyclonal antibody against the invariant HLA light-chain β-2 microglobulin. This antibody labeled the entire plasma membrane circumference of all leukocytes and platelets and was not preferentially localized to microvilli (Figure 2B). The anti-β-2 microglobulin antibody did not label red blood cells, and the normal rabbit serum control did not label any cells.

Other Cell Types. L-Selectin was also preferentially localized to the tips of monocyte (Figure 4A) and lymphocyte microvilli (Figure 4B). On average, 72 ± 10% of the gold particles labeling L-selectin on monocytes and 71 ± 14% of the gold particles on lymphocytes were counted on the microvilli (Table 1). As with PMN, the number of gold particles per microvillus on both cell types was independent of microvillous length, and the percent of L-selectin positioned on microvilli was independent of the number of microvilli or gold particles per section (Figures 3A–3C). Sections of immunogold-labeled basophils (Figure 4C) and eosinophils (Figure 4D) also presented the majority of the gold particles on the tips of microvilli.

Table 1. *L-Selectin distribution*[a]

	Neutrophil		Monocyte		Lymphocyte	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Percent of gold particles per compartment						
Microvilli	78 ± 9	51–95	72 ± 10	53–89	71 ± 14	24–92
Planar cell surface	22 ± 9	5–49	28 ± 10	11–47	29 ± 14	8–76
Number of gold particles per compartment						
Plasma membrane	209 ± 76	60–448	120 ± 45	30–212	106 ± 56	41–296
Microvilli	163 ± 63	51–354	87 ± 35	19–159	75 ± 39	11–203
Planar cell surface	46 ± 25	8–103	33 ± 16	9–79	31 ± 24	4–127

[a] Neutrophils partitioned a greater proportion of L-selectin to the microvilli than monocytes or lymphocytes ($p<0.0001$), whereas the difference in distribution between monocytes and lymphocytes was not statistically significant ($p = 0.8443$). The difference in means between PMN and monocytes was 6 ± 4% (± 95% confidence interval), and the difference in means between PMN and lymphocytes was 7 ± 5% (± 95% confidence interval). A statistically significant difference in the distribution of L-selectin between donors was not detected at a significance level of 0.05 for the lymphocyte data ($p = 0.3187$). However, there were statistically significant differences in the distribution between donors for the PMN and monocyte data ($p<0.0001$, $p = 0.0108$, respectively). The mean difference for the PMN data was 11 ± 5% (± 95% confidence interval), and the mean difference for the monocyte data was 7 ± 5% (± 95% confidence interval).

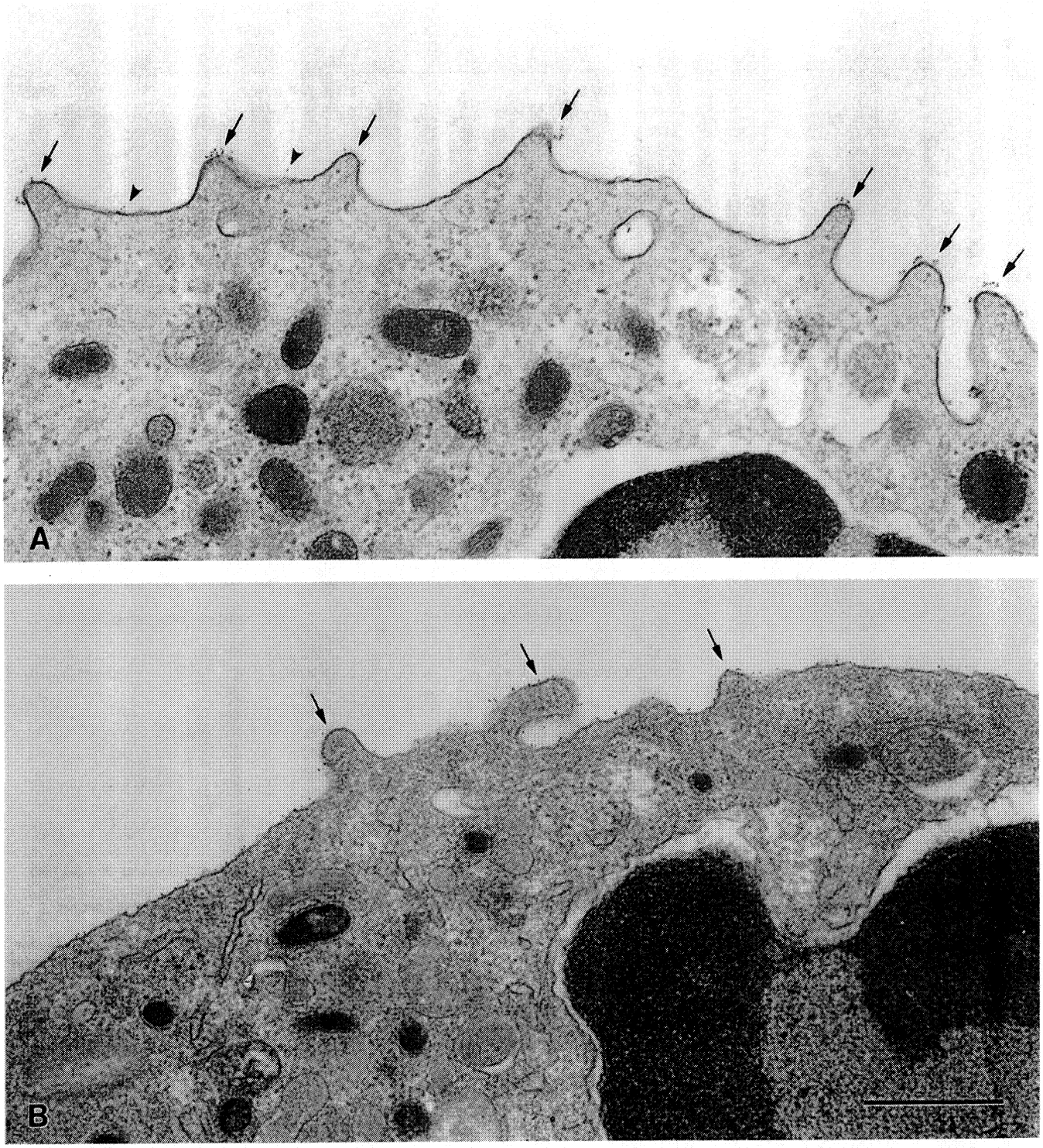

Figure 2. High-magnification of human PMN plasma membrane. **(A)** Microvillous positioning of L-selectin on microvilli. A typical segment of PMN plasma membrane showing seven microvilli with groups of gold particles labeling L-selectin localized at the tips (arrows). Note that the number of particles per microvillus is independent of microvillous length. Gold particles on the planar cell surface can also be seen (arrowheads). Of the 89 particles visible on this segment of plasma membrane, 80 (90%) can be seen on the microvilli. **(B)** Random distribution of β-2 microglobulin. β-2 microglobulin is labeled as a contiguous series of single gold particles without preferential partitioning to the microvilli or planar cell surface. Note that gold particles on microvilli are not concentrated at the tip (arrows). Original magnification × 42,000. Bar = 0.5 μm.

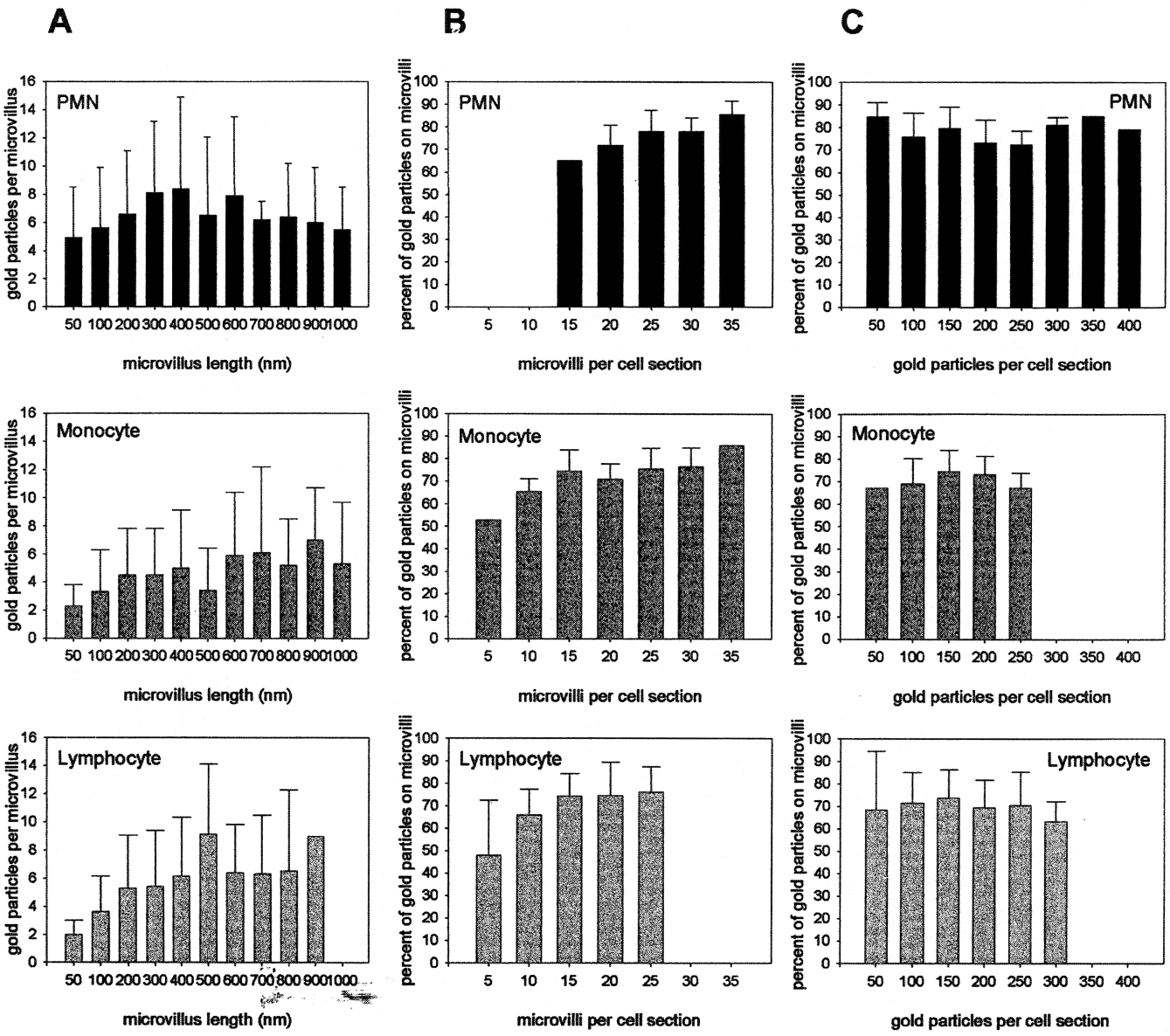

Figure 3. Correlations between the number of gold particles per microvillus and microvillous length, and between the distribution of L-selectin and the number of microvilli or gold particles per cell section. Data shown are the mean number of gold particles per microvillus or the mean percent of L-selectin localized to microvilli; error bars are 1 SD. **(A)** The average number of gold particles per microvillus was 6.6 ± 4.9, 4.3 ± 3.7, and 4.9 ± 3.7 for PMN, monocytes, and lymphocytes, respectively, and was independent of microvillous length (r = 0.038, 0.314, 0.339 for PMN, monocytes, and lymphocytes, respectively). Microvilli without gold particles were excluded from the analysis. Of all microvilli sampled, 17% of PMN, 23% of monocyte, and 29% of lymphocyte microvilli were not labeled. **(B)** The percent of L-selectin localized to microvilli was independent of the number of microvilli per section (r = 0.558, 0.586, 0.373 for PMN, monocytes, and lymphocytes, respectively). **(C)** The percent of L-selectin localized to microvilli was independent of the number of gold particles per section (r = −0.054, 0.105, −0.050 for PMN, monocytes, and lymphocytes, respectively).

Quantitation of Leukocyte Microvilli

Typical PMN sections had 26 ± 5 microvilli at a frequency of 1 per 1.4 μm plasma membrane. Monocyte sections had 26 ± 6 microvilli per section at a frequency of 1 per 1.7 μm plasma membrane, and lymphocyte sections had 23 ± 6 microvilli at a frequency of 1 per 1.4 μm plasma membrane (Table 2). Microvilli were shortest on PMN, where the average length measured 290 ± 170 nm and 95% were less than 530 nm. Monocyte microvilli were longest and most varied in length and measured 390 ± 280 nm, with 95% less than 780 nm. The average lymphocyte microvillus measured 340 ± 190 nm, with 95% less than 600 nm. The distribution of microvillous length on these cells is shown in Figure 5.

To estimate the percent of the plasma membrane circumference occupied by the microvilli, the difference between the measured

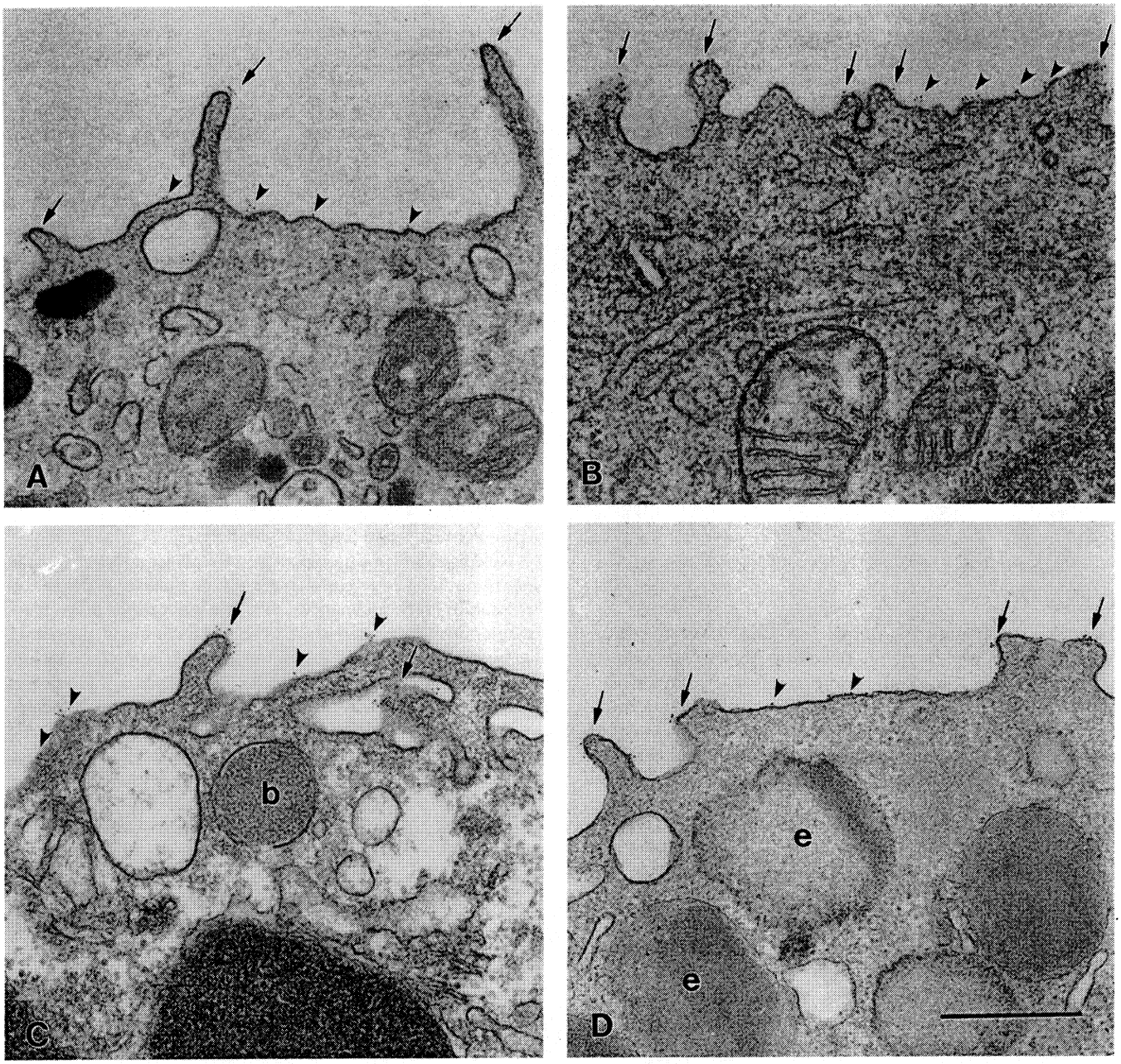

Figure 4. Human leukocytes immunogold-labeled for L-selectin. **(A)** Monocyte, reacted for peroxidase; **(B)** lymphocyte; **(C)** basophil; **(D)** eosinophil. Gold particles can be seen primarily at the tips of microvilli (arrows), but also on the sides and on the planar cell surface (arrowheads). Monocyte microvilli are longer than microvilli on the other cells in **B, C,** and **D,** and in Figures 2A and 2B. In this study, only three eosinophil and three basophil whole cell sections were quantified. Counting gold particles on these sections showed that 76 ± 8% of eosinophil and 75 ± 3% of basophil gold particles were localized to the microvilli. Although this sample size is clearly too small to reach definitive conclusions regarding the quantitative distribution of L-selectin on these cell types, they suggest that the receptor is preferentially positioned on microvilli. A distinctive basophil granule can be seen at b in **C,** and eosinophil granules with crystalline content can be seen at e in **D.** Original magnification × 42,000. Bar = 0.5 μm.

and the theoretical length of the plasma membrane was compared to the measured length. The theoretical length approximated the circumference of a cell without microvilli and was calculated as the circumference of an ellipse based on the length and width of the section. Using this calculation, we estimated that microvilli occupied 30–40% of the plasma membrane circumference of PMN, monocytes, and lymphocytes (Table 2). Using the length and width determinations to calculate section diameter, we obtained values of 8 ± 1 μm for PMN and monocyte diameter and 6 ± 1 μm for lymphocyte diameter (Table 2). These values are in good agree-

Table 2. *Morphometric determinations of sectioned PMN, monocytes, and lymphocytes*[a,b]

	PMN		Monocyte		Lymphocyte	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
MV per section	26 ± 5	14–39	26 ± 6	9–36	23 ± 6	7–39
Microvillous length (nm)	290 ± 170	50–1900	390 ± 280	50–3420	340 ± 190	50–1500
Percent of PM occupied by MV	32 ± 8	14–50	40 ± 8	21–60	37 ± 9	19–50
PM circumference (μm)	35 ± 4	28–47	44 ± 6	34–60	32 ± 5	20–45
Cross-sectional area (μm^2)	47 ± 6	31–62	57 ± 6	40–70	34 ± 5	18–67
Diameter (μm)	8 ± 1	6–9	8 ± 1	7–10	6 ± 1	5–8

[a] Morphometric determinations were obtained from 100 PMN sections from four donors (25 each) and 50 monocyte and 50 lymphocyte sections from two donors (25 each). Analysis of variance of mean values for PMN cross-sectional area and diameter did not detect significant differences among donors ($p = 0.4777$ and 0.1938, respectively). Variations among donors were detected for PMN plasma membrane circumference, the number of microvilli per section, and microvillous length ($p<0.0001$ for all tests). The greatest difference in PMN plasma membrane circumference was 7 μm, the greatest difference in the number of microvilli per section was 8, and the greatest difference in microvillous length was 100 nm. Comparison of the mean number of microvilli per monocyte section by Student's *t*-test did not detect significant differences between donors ($p = 0.241$). Statistically significant differences between donors were detected, however, for the cross-sectional area (4 μm^2, $p = 0.0249$), diameter (0.3 μm, $p = 0.0454$), plasma membrane circumference (6 μm, $p = 0.0078$) and microvillous length (80 nm, $p<0.0001$). No differences were detected in mean values for lymphocyte cross-sectional area and diameter between donors ($p = 0.263$ and 0.367, respectively) by Student's *t*-test. However, differences in mean values between donors were detected for plasma membrane circumference (6 μm, $p<0.0001$) and the number of microvilli per section (10 microvilli, $p<0.0001$), and the median values for microvillous length (140 nm, $p<0.0001$).

[b] MV, microvilli; PM, plasma membrane.

ment with those obtained by Schmid–Schönbein and co-workers (1980).

Discussion

This is the first attempt to quantitate the distribution of L-selectin on the plasma membrane of circulating human leukocytes by preembedding immunogold labeling, and to determine the number and length of microvilli on two-dimensional electron micrographs of sectioned PMN, monocytes, and lymphocytes. Gold particles labeling L-selectin were typically observed in groups both at the tips of microvilli and on the planar cell surface, and the number of particles counted on a microvillus was independent of microvillous length. On average, 78% of PMN, 72% of monocyte and 71% of lymphocyte L-selectin partitioned to the microvilli. It is noteworthy that by the same techniques used here, ~90% of human L-selectin transfected into murine pre-B-cells was observed on microvilli (Pavalko et al., 1995; Von Andrian et al., 1995). The more complete partitioning to microvilli in transfection experiments could be due to intrinsic differences between human leukocytes and murine lymphoblast cell lines. Localization of L-selectin to microvilli on rabbit PMN has also been observed (Burns and Doerschuk, 1994) which, combined with the observations of murine lymphoblasts, demonstrates that partitioning of L-selectin to leukocyte microvilli is not unique to humans.

Although the data presented here quantitatively extend the observations of others that L-selectin is concentrated on the tips of PMN (Erlandsen et al., 1993; Picker et al., 1991) and lymphocyte microvilli (Hasslen et al., 1995), they also demonstrate that L-selectin is localized to the tips of monocyte, basophil, and eosinophil microvilli. Although a significant portion of L-selectin is localized to microvilli, it should be noted that ~30% of this receptor is not localized to the microvilli. On the lymphocyte, for example, L-selectin on the planar cell surface ranges from 8% to 76%. This distribution is independent of the labeling intensity of the cells or the number of microvilli per section, and is much broader than that observed on PMN (5–49%), or monocytes (11–47%). This broad variability in planar surface labeling may reflect the presence of a heterogeneous population of lymphocytes.

Gold particles labeling L-selectin on the planar surface may mark sites of budding or receding microvilli, or auxiliary stores of the receptor. In PMN, for example, L-selectin is not stored in cytoplasmic granules (Borregaard et al., 1994) and is not found on granule membranes (Picker et al., 1991), even though it is progressively lost from the surface during intravascular life by shedding (Van Eeden et al., 1994) and is rapidly cleaved after leukocyte activation (Mobeley and Dailey, 1992; Spertini et al., 1991; Kishimoto et al., 1989). Alternatively, planar surface L-selectin may be a receptor that is not associated with the cytoskeleton (see below). Although a difference in the carbohydrate binding ability of L-selectin on the microvilli or planar cell surface is unlikely, electron microscopic studies using labeled carbohydrate have not been conducted to address this possibility.

L-selectin is anchored to the cytoskeleton through interactions between its cytoplasmic tail and α-actinin (Pavalko et al., 1995). Truncation of the tail has no effect on the microvillous positioning (Pavalko et al., 1995) or carbohydrate binding activity (Kansas et al., 1993) of the receptor. However, murine pre-B-cells (300.19) transfected with truncated L-selectin do not roll in ex vivo adhesion assays (Kansas et al., 1993). Moreover, cytochalasin B treatment of 300.19 cells transfected with wild-type L-selectin blocks rolling in ex vivo adhesion assays but does not affect carbohydrate binding (Kansas et al., 1993). Transfection of L1-2 cells, another murine pre-B-cell line, with wild-type L-selectin or an L-selectin–CD44 chimera that does not localize to microvilli showed that microvillous localization significantly enhanced the ability of these cells to adhere to a physiological ligand in flow (Von Andrian et al., 1995). Combined, these data suggest that microvillous positioning of L-selectin is necessary but not sufficient for leukocyte rolling, and that the cytoplasmic tail regulates leukocyte binding to endothelium in flow, independent of ligand recognition.

Quantitative descriptions of the leukocyte surface in terms of plasma membrane specializations and the distribution of adhesion molecules are required to establish references from which to study

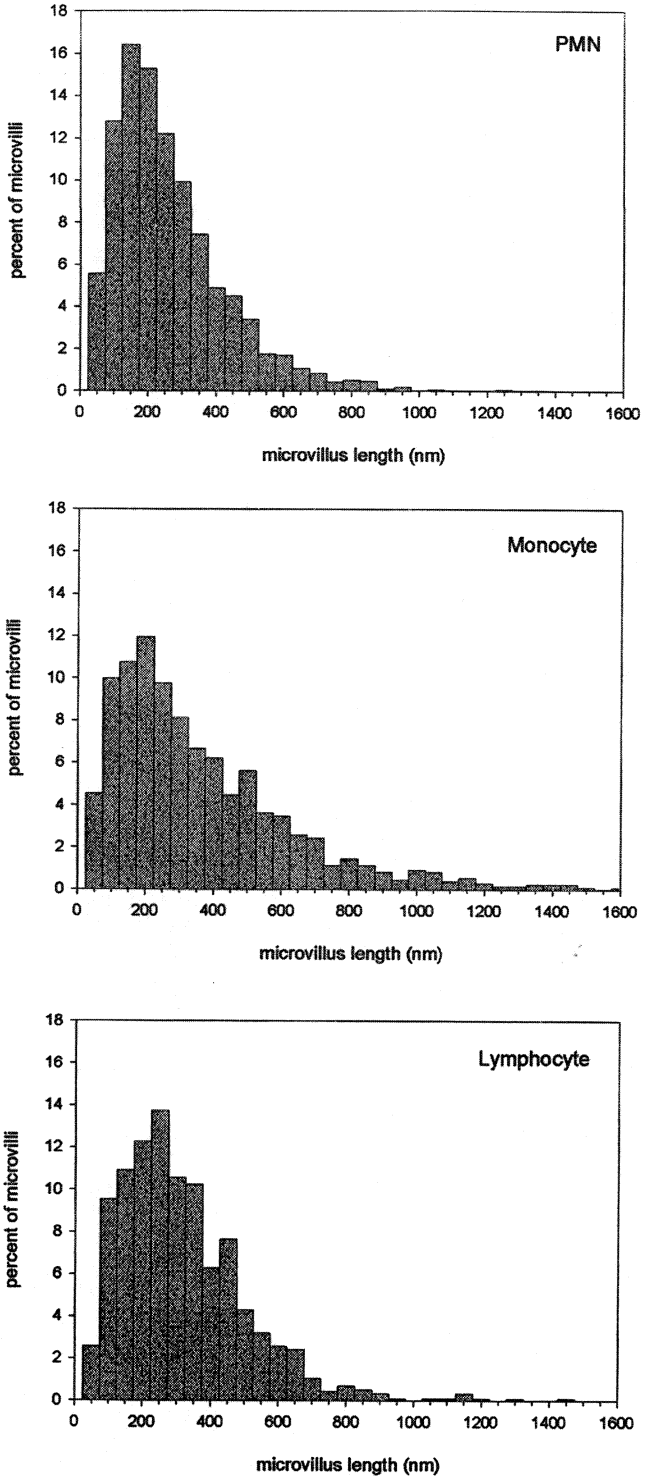

Figure 5. Distribution of microvillous length on PMN, monocyte, and lymphocyte cell sections. The percent of microvilli per length category in increments of 50 nm is shown. A total of 2551 PMN, 1298 monocyte, and 1165 lymphocyte microvilli were measured from 100 PMN micrographs from four donors (25 each) and 50 monocyte and lymphocyte micrographs from two donors (25 each).

the roles of microvilli and the positioning of adhesion molecules in transient intercellular adhesion, adhesion to extracellular matrix material, and the dynamics of leukocyte–endothelial interaction in the microvasculature. Functional studies of isolated leukocytes in laminar flow chambers (Moore et al., 1995; Lawrence and Springer, 1991) or exteriorized mesentery (Von Andrian et al., 1992,1993), for example, can be performed to dissect the contribution of leukocyte or endothelial cell adhesion molecules in leukocyte tethering and rolling and to identify receptor–ligand pairs involved in these interactions.

Quantitation of the surface microstructure also has application in the development of mathematical models constructed to describe leukocyte–endothelial cell interaction in flow. Methods that can simulate adhesion under conditions of viscous flow can be applied to explore the relationship between the molecular properties of leukocyte adhesion molecules and the attachment of leukocytes to endothelial surfaces. Hammer and Apte (1992), for example, developed a model to describe a range of leukocyte–endothelial cell adhesive events such as rolling, tumbling, transient adhesion, and firm attachment, using dimensional parameters including the length and spring constants of microvilli. The values for microvillous length (Bongrand and Bell, 1984) and the number of microvilli per cell (Knutton et al., 1975; Loor and Hägg, 1975), however, were based on determinations from other cell types, and the average length of PMN microvilli was assumed to be 500 nm. This is almost twice the measured value of 290 nm determined here for PMN microvilli.

The work presented in this report was conducted to establish a foundation from which to study the physiological contributions of microvilli and receptor clustering to leukocyte behavior in flow under a variety of conditions. We are now investigating the role of microvilli and the distribution of L-selectin on PMN tethering and rolling on lipid bilayers containing P- or E-selectin in flow (E. Finger, REB, DFB, TAS, submitted for publication).

Acknowledgments

The expert technical assistance of Ivy Hsieh and the editorial advice of David Geller are gratefully appreciated. We thank Dr Takashei Kishimoto for the DREG 200 antibody.

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