Polymorphonuclear leukocytes L-selectin expression decreases as they age in circulation

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Polymorphonuclear leukocytes L-selectin expression decreases as they age in circulation. Am. J. Physiol. 272 (Heart Circ. Physiol. 41): H401–H408, 1997.—We recently reported that L-selectin expression increases on circulating polymorphonuclear leukocytes (PMN) during active bone marrow release, which suggests that older cells in the circulation have lower levels of L-selectin than those recently released from the bone marrow. The present study was designed to test the hypothesis that L-selectin expression decreases on PMN as they age in the circulation.

The purpose of the present study was to determine whether the lower expression on circulating PMN is due to a continuous shedding of L-selectin as PMN age in the circulation.

METHODS

In Vitro Experiments

Blood from female New Zealand White rabbits was used for in vitro studies. All studies were approved by the Animal Experimentation Committee of the University of British Columbia and St. Paul's Hospital. The blood was collected from the central ear artery in rabbits in tubes (Vacutainer, Becton-Dickinson, Rutherford, NJ) containing potassium EDTA, or acid-citrate-dextrose (ACD) or in heparin 100 U/ml. Blood was immediately transferred to a shaking water bath and incubated for 24 h at 37°C. PMN L-selectin and CD18 expression were determined at baseline, 1, 4, 8, and 24 h.

The expression of CD18 [monoclonal antibody (MAb) 60.3, kindly donated by Dr. J. M. Harlan] and L-selectin (MAb DREG 200, kind donation of Dr. E. C. Butcher) was determined on rabbit PMN by immunofluorescent flow cytometric analysis. The cells were prepared for flow cytometry analysis with a commercially available kit (Coulter Clone, Coulter, Hialeah, FL) and processed at 37°C until fixation. Briefly, 100 μl of EDTA-heparinized-ACD blood were incubated with 900 μl phosphate-buffered saline (PBS) buffer and 0.4 μg of the primary MAb (20 μg/ml) for 10 min at 37°C. The specimens were washed with PBS, and the supernatant was removed and incubated for 10 min in 1:20 dilution (0.1 μg) of fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Sigma). For each blood sample, a negative control was

with relaxed sequence specificity (6, 15, 25). Stimulation of PMN with activating agents and phorbol esters results in immediate receptor shedding (15, 23, 25). Several investigators (6, 15) have suggested that the shedding of L-selectin could provide a means for the de-adhesion of PMN, allowing them to return to the circulation when the signal for firm adhesion is insufficient or alternatively reduces PMN adherence to endothelium as the cells migrate into subendothelial tissues. L-selectin can be found in the supernatant of cultured lymphocytes (23) and can be demonstrated free in the plasma (22), suggesting that L-selectin may be constitutively lost from circulating leukocytes. This constitutive shedding of L-selectin has been demonstrated in lymphocytes and can be reexpressed by de novo synthesis. In contrast to lymphocytes, circulating PMN have reduced ability to resynthesize L-selectin (23).

We recently showed (28) that the mature PMN in the postmitotic pool in the bone marrow express high levels of L-selectin with a lower and more variable expression on circulating PMN. This led us to hypothesize that PMN in the circulation shed their L-selectin as they age. The purpose of the present study was to determine whether the lower expression on circulating PMN is due to a continuous shedding of L-selectin as PMN age in the circulation.
performed by using nonimmune mouse FITC-conjugated to IgG2a in the same concentration as the primary antibody (Becton-Dickinson). The leukocytes were fixed with 1% paraformaldehyde and stored at 4°C. Flow cytometry was performed on the specimens (model Profile EPIC II, Coulter), and analysis gates for the PMN were established by using the distinctive forward and side scatter profiles. A total of 3,000 gated cells were evaluated per specimen, and results are presented as relative mean fluorescence intensity.

**In Vivo Experiments**

**Animals.** Twenty-two female New Zealand White rabbits were used in this part of the study. Five (4 ± 0.3 kg, means ± SD) were used as blood donors and fifteen (2.6 ± 0.2 kg) as recipients. All these studies were approved by the Animal Experimentation Committee of the University of British Columbia.

**Cell Preparation**

BrdU labeling of donor PMN. The DNA of rabbit leukocytes was labeled with 5′-bromo-2′-deoxyuridine (BrdU), a thymidine analogue, with the use of a previously described method (2). Five donor rabbits were given BrdU (Sigma) at a dose of 25 mg/kg daily for 7 days. The BrdU was infused through the marginal ear vein at a concentration of 5 mg/ml in normal saline over 15 min. Each day 1 ml of blood was collected from the central ear artery in tubes (Vacutainer, Becton-Dickinson) containing EDTA. Blood cell counts were determined on a model SS80 Coulter Counter (Coulter), and differential white cell counts were performed by counting 100 leukocytes on a randomly selected field on Wright's-stained blood smears. An additional 2-ml sample was collected in ACD for the preparation of leukocyte-rich plasma (LRP) using high-molecular-weight (100,000-200,000) dextran, final concentration 1.9% dextran wt/vol (Chemical Dynamics) to sediment residual red blood cells in the LRP was achieved by dilution with 11 ml of sterile water. After 18 s, 11 ml PBS was added, and the mixture was diluted in 20 ml PMN buffer. This specimen was divided into two equal aliquots, incubated in either denatured (control) or active chymotrypsin type IV, and infused into the recipients. The disappearance of PMN BrdU from the circulation was monitored over a 24-h period by obtaining blood specimens from the central ear artery at baseline (before infusion) at 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 min and then hourly for 6 h and at 24 h. The white blood cell counts were determined on a Coulter Counter (model SS80, Coulter), and differential white blood cell counts were determined on Wright's-stained blood smears. The BrdU-labeled PMN was determined with cytospins made from LRP (see above).

To determine the effect of removing L-selectin from the PMN surface, purified PMN from 75 ml of donor blood were diluted in 20 ml PMN buffer. This specimen was divided into two equal aliquots, incubated in either denatured (control) or active chymotrypsin type IV, and infused into the recipients. The disappearance of PMNBrdU from the circulation was monitored over a 24-h period. White blood cell counts and differential white blood cell counts were obtained, and cytospins were made from LRP as described earlier.

**L-selectin expression on transfused BrdU-labeled PMN.** Cytospins prepared from peripheral blood of recipients were stained for the presence of surface L-selectin and nuclear BrdU by using a double-alkaline phosphatase technique based on the APAAP technique (7) to determine the fraction of the BrdU-labeled PMN in each specimen (2).

**PMN purification.** The PMN were purified from donor rabbit blood by a previously described method (8). Briefly, the LRP obtained from 75 ml of donor blood was centrifuged and resuspended in 1 ml PMN buffer. Hypotonic lysis of the residual red blood cells in the LRP was achieved by dilution with 11 ml of sterile water. After 18 s, 11 ml 2% PBS (2% PBS is 2/ mM NaH2PO4, 132 mM KH2PO4, and 2.74 M NaCl) and 10 ml PMN buffer were added. The PMN were separated from the mononuclear cells by centrifugation in Histopaque (Sigma) with a density of 1.077 g/ml at 250 g for 13 min. The isolated PMN were 95–98% pure with a viability of 97%, as assessed by trypan blue exclusion.

**Removal of L-selectin by chymotrypsin treatment of PMN.** Selective removal of L-selectin from the PMN by chymotrypsin allowed us to study the behavior of a population of predominantly L-selectin-negative PMN in the circulation. L-selectin was cleaved from the surface of the PMN with chymotrypsin following the protocol of Julita et al. (13). Donor blood (75 ml) was obtained, and the PMN were purified and divided into two aliquots that were incubated in either denatured chymotrypsin (control) or chymotrypsin (type IV (Sigma) diluted 1 U/ml containing 3 × 106 cells/U incubated for 15 min at 37°C). The inability of heat-denatured chymotrypsin to cleave a substrate N-benzoyl-L-tyrosine ethyl ester was determined in preliminary experiments with a method described by Hummel (12). These studies showed that the ability of chymotrypsin to cleave the substrate was abolished by heating the enzyme for 30 min at 60°C, and this denaturing time was used in all subsequent experiments. The efficiency of L-selectin removal from the PMN surface by chymotrypsin was evaluated by both immunocytochemistry using the APAAP method and by immunofluorescence flow cytometric analysis of the PMN before and after the chymotrypsin treatment (see above).

By heating the enzyme for 30 min at 60°C and this denaturating time was used in all subsequent experiments. The efficiency of L-selectin removal from the PMN surface by chymotrypsin was evaluated by both immunocytochemistry using the APAAP method and by immunofluorescence flow cytometric analysis of the PMN before and after the chymotrypsin treatment (see above).
slides in 2 N HCl at 37°C for 60 min. The 2 N HCl was neutralized by washing the slides three times with 0.1 M borate buffer, pH 8.5. This step was followed by the second APAAP procedure in which mouse anti-BrdU, 0.1 µg/ml (Boehringer-Mannheim, Mannheim, Germany) was used as the primary antibody.

The slides were washed between antibody applications with 0.1% Tween 20 in TBS (pH 7.6) for 10 min. The alkaline phosphatase was then developed with Histomark Blue for 10 min in the dark. Slides were washed for 30 min in distilled water, mounted in an aqueous medium (Gelvatol), and analyzed on a Zeiss Universal Research light microscope (model IIR). The influence of the double-labeling procedure on the presence of surface L-selectin and nuclear BrdU expression was evaluated by comparing the number of positive PMN for each antigen after the double-staining procedure with paired slides stained for each single antigen using the APAAP method.

Evaluating PMN\textsuperscript{BrdU}. The slides were coded, and the PMN\textsuperscript{BrdU} (Fig. 1A) were evaluated in computer-generated randomly selected fields counting 100 PMN/slide. In double-stained slides, the PMN\textsuperscript{BrdU} were identified by a deep blue staining of the cell nucleus (Fig. 1B). Cells were categorized as BrdU positive, BrdU and L-selectin positive (double-labeled), L-selectin positive, or negative for both labels (Fig. 1B). If <10% of the PMN were BrdU labeled, 200 PMN were counted; if <5%, 500 PMN were counted, and if <1%, 1,000 PMN were counted. The number of PMN\textsuperscript{BrdU} evaluated in each slide was an average of 102 ± 19 with a range of 50–156. The number of PMN\textsuperscript{BrdU} present in the circulation of the recipients at each time point was expressed as a fraction of the total number of labeled PMN infused, corrected for the calculated blood volume (BV) of the rabbit (22a) in the following manner

\[
\text{Fraction PMN}_{\text{BrdU, recipient}} = \frac{\text{PMN}_{\text{BrdU, recipient}} \times \text{BV} \times \%\text{PMN}_{\text{BrdU}}}{\text{PMN}_{\text{BrdU, infused}}} \tag{1}
\]

where the fraction PMN\textsuperscript{BrdU, recipient} is the number of PMN\textsuperscript{BrdU} in the circulation as a fraction of the total number of PMN\textsuperscript{BrdU} infused, PMN\textsuperscript{circ} is the calculated number of PMN ($\times 10^6$) in the circulation (total white blood cell count times the fraction of leukocytes that are PMN), BV is the calculated blood volume, %PMN\textsuperscript{BrdU, recipient} is the fraction of PMN\textsuperscript{BrdU} in a cytospin of peripheral blood in the recipient, and PMN\textsuperscript{BrdU, infused} is the number ($\times 10^6$) of PMN\textsuperscript{BrdU} infused [PMN count/ml $\times$ vol of fluid infused (ml) $\times$ %PMN\textsuperscript{BrdU}].
The PMN<sup>BrdU</sup> were further stratified as either stained for BrdU alone or double stained for BrdU and L-selectin, and these fractions were calculated separately with the same equation.

**Calculation of half-life of PMN<sup>BrdU</sup>**. The time required to achieve the maximal number of PMN<sup>BrdU</sup> in the circulating blood of the recipients (t<sub>max</sub>) was applied to the rate of decay equation to calculate the half-life (t<sub>1/2</sub>) of PMN in the circulation:

\[
N_t = N_{\text{max}} e^{-kt}
\]

where \(k\) is the rate of loss of PMN<sup>BrdU</sup> from the circulation, \(t\) is time after \(t_{\text{max}}\), \(e\) is 2.71828, \(N_t\) is number of PMN<sup>BrdU</sup> in the circulation at time \(t\); \(N_{\text{max}}\) is number of PMN<sup>BrdU</sup> in the circulation at time \(t_{\text{max}}\).

Because the \(t_{1/2}\) can be estimated as the time at which \(N_t = N_{\text{max}}/2\), the rate-decay equation for \(t_{1/2}\) becomes \(t_{1/2} = \ln 2/k\). The constant, \(k\), was calculated with the restricted maximum likelihood method described by Feldman (10). The confidence interval (CI) was obtained by deriving the lower and upper bounds of the 95% CI for the slope, \(k\).

**Statistical Analysis**

Statistical analysis was performed with SYSTAT Version 5.1 software (Systat, Evanston, II.). To evaluate the difference in the behavior of chymotrypsin-treated or control PMN<sup>BrdU</sup>, the disappearance rate of labeled PMN in each recipient was estimated as the relationship between the fraction of PMN<sup>BrdU</sup> and time. A log transformation of the fraction of PMN<sup>BrdU</sup> in the circulation was done and fits a monoexponential line. This family of lines of each group were then compared with the restricted maximum likelihood method (10). The estimates were then compared with a \(\chi^2\) statistic, and differences in slopes, intercepts, and lines were considered significant when \(P\) was <0.05. The estimated slope was then used to calculate the half-life for each group (3). A one-way analysis of variance was used to evaluate the changes in L-selectin expression on PMN in the circulation over time, and a two-way analysis of variance was used to compare the transfusion of whole blood with isolated PMN over time.

**RESULTS**

*In Vitro Experiments*

Figure 2 shows that the expression of L-selectin on PMN decreased to 14.6 ± 2.3% of the original baseline value when EDTA whole blood was incubated for 24 h at 37°C in a shaking water bath (Fig. 2K, \(P < 0.01\)). Figure 2 shows typical changes in L-selectin (Fig. 2, A–E) and CD18 (Fig. 2, F–J) in blood over 24 h. No significant change in CD18 was observed over the 24-h incubation period. This result was the same when
L-SELECTIN ON PMN IN CIRCULATION

Fig. 3. Effect of BrdU labeling and PMN purification of L-selectin expression. Expression of L-selectin (filled bars) and CD18 (open bars) on control (unlabeled), BrdU labeled (60 ± 2.3% labeled), and purified (BrdU labeled) PMN as measured by flow cytometry. Monoclonal antibodies DREG 200 (anti-L-selectin) and 60.3 (anti-CD18) were used. Values (n = 5) are MFI (means ± SE). No difference was apparent between different cell populations.

either heparin, EDTA, or ACD was used as anticoagulant (data not shown).

In Vivo Labeling of PMN With BrdU

The staining of PMN for nuclear BrdU and surface L-selectin is shown in Fig. 1, A and B. The expression of both L-selectin and BrdU was similar in paired single- (Fig. 1A) and double-stained slides (Fig. 1B) (data not shown). In donor blood (n = 5), 10.2 ± 4.5% of BrdU labeled PMN stained negative for L-selectin with immunocytochemistry, which is similar to values in animals before BrdU-labeling treatment. Donor animals after 7 days of BrdU treatment have ±80% of the circulating PMN labeled. BrdU-labeled donors show the same level of expression of CD18 and L-selectin by flow cytometry (Fig. 3).

Changes in PMN L-Selectin Expression

To determine the change in L-selectin expression with time in the circulation, PMN^BrdU were infused into normal recipients as whole blood. The fraction of L-selectin-negative PMN^BrdU increased with time spent in the circulation (Fig. 4, P < 0.001). The fraction of L-selectin-negative PMN^BrdU in the circulation increased rapidly during the first 30 min and then more slowly over the 24-h study period. Nearly all PMN still present in the circulation 24 h after the infusion were negative for L-selectin (Fig. 4). Similar results were obtained with purified PMN^BrdU (n = 5, data not shown).

Effect of L-Selectin Removal on Clearance of PMN^BrdU From Circulation

To determine whether L-selectin expression influenced clearance of PMN from the circulation, PMN^BrdU were stripped of their L-selectin by chymotrypsin in vitro before their infusion into the recipients (n = 5). The flow cytometric analysis (Fig. 5, A and F) shows that 78 ± 8.2% (n = 5) of the L-selectin present on PMN was removed by chymotrypsin treatment. This loss was confirmed by immunocytochemistry in which the expression of L-selectin on PMN was graded (G0–G4) as previously described (28). The results showed that the PMN that stained intensely positive for L-selectin (G4) decreased from 63 ± 6.8 to 4.6 ± 3% and the negative PMN (G0) increased from 10.2 ± 4.5 to 58 ± 5.1% with chymotrypsin treatment (n = 5). The viability of PMN, accessed by the trypan blue exclusion, was not changed by chymotrypsin treatment, and the treatment of PMN did not change the expression of CD18 (Fig. 5, E and F) measured by flow cytometry.

The clearance studies showed that the fraction of PMN^BrdU present in the circulation of recipients directly after infusion was the same in both the control (48 ± 12.6%) and the chymotrypsin-treated (46 ± 9.6%) groups. The chymotrypsin-treated PMN^BrdU disappeared more rapidly from the circulation than control cells during the first hour after infusion but were present in the circulation to the same levels as the nontreated cells by 3 h. The rate of disappearance of PMN^BrdU in the chymotrypsin and control groups between 3 and 24 h was similar (Fig. 6) with a similar overall t1/2 (296 vs. 296 min) (Table 1). In both the chymotrypsin-treated and control groups, L-selectin-negative PMN appeared to be cleared from the circulation faster than L-selectin negative PMN (Table 1). The t1/2 of chymotrypsin-treated L-selectin-negative PMN were not different from the t1/2 of the whole population of PMN (Table 1), suggesting that a lack of L-selectin expression does not result in faster clearance of PMN from the circulation. The more rapid clearance of L-selectin-positive PMN may be due to the continuous shift of L-selectin positive-to-negative PMN in the circulation rather than faster clearance of L-selectin-positive PMN.

DISCUSSION

This study shows that PMN L-selectin expression decreases as cells age both in vitro and in vivo. The
Fig. 5. Effect of chymotrypsin (1 U/ml containing $3 \times 10^6$ PMN incubated for 15 min) on the expression of L-selectin (A and C) and CD18 (D and F) as measured with flow cytometry. In panels, the events left of cursor represent nonspecific labeling. A and D: baseline values obtained in whole blood. B and E: baseline values in purified PMN. C and F: after purified PMN were treated with chymotrypsin. Note significant loss of L-selectin from PMN with chymotrypsin treatment but no changes in expression of CD18. Y-axis represents fluorescent events, and x-axis is MFI plotted on a log-scale. MFI decreased from 10.8 to 2.4 in B to C and was 8.1 and 8.9 in E and F, respectively, in this example.

L-SELECTIN ON PMN IN CIRCULATION

A decrease in L-selectin expression on PMN in the circulation could be explained by either the loss of L-selectin from the cell surface with time or preferential removal of L-selectin-positive PMN from the circulation. Our data favor the conclusion that L-selectin is progressively lost from the surface of PMN as they age in the circulation.

The thymidine analogue, BrdU, was used to label PMN in donor animals and follow them after transfusion into serum-compatible recipients. This labeling technique is similar to previous reports by Maloney and Patt (19) who showed that [H3]thymidine was a convenient label for cells such as PMN that divide rapidly in the bone marrow. Our method, which is fully described elsewhere, eliminates the need for in vitro purification and labeling of PMN with isotopes (8) and allows activation-sensitive surface molecules, such as L-selectin, to be studied with minimal manipulation of the cells.

The interaction of L-selectin with endothelial cell surface ligands results in rolling and margination of PMN in postcapillary venules. This rolling phenomenon occurs on normal unactivated endothelium (18) but is enhanced on activated endothelium (24). The fraction of PMN rolling increases from <20% on nonactivated endothelium to >30% on cytokine-activated endothelium with a marked decrease in rolling velocity (18, 24). The concept that ligand binding initiates receptor shedding provides a mechanism for the return of PMN into the circulation when conditions for firm adhesion and migration are not present. This may be

**Table 1. Half-life of PMN treated with and without chymotrypsin**

<table>
<thead>
<tr>
<th>Cell Populations</th>
<th>$t_{1/2}$ (min)</th>
<th>95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>270</td>
<td>248 - 296</td>
</tr>
<tr>
<td>Control PMN</td>
<td>262</td>
<td>320 - 184</td>
</tr>
<tr>
<td>L-selectin +</td>
<td>178</td>
<td>311 - 134</td>
</tr>
<tr>
<td>L-selectin -</td>
<td>219</td>
<td>458 - 135</td>
</tr>
<tr>
<td>Chymotrypsin-treated PMN</td>
<td>296</td>
<td>570 - 199</td>
</tr>
<tr>
<td>L-selectin +</td>
<td>153</td>
<td>238 - 118</td>
</tr>
<tr>
<td>L-selectin -</td>
<td>302</td>
<td>722 - 180</td>
</tr>
</tbody>
</table>

*Half-life ($t_{1/2}$) of the L-selectin-negative polymorphonuclear leukocytes (PMN) was significantly longer than the L-selectin-positive PMN in the group that was infused with chymotrypsin-treated PMN. All other differences between half-lives were not significant.*
relevant to recent studies from our laboratory showing that only a small percentage of PMN delivered to a local area of inflammation actually migrates out of the vascular space into the inflamed tissue (9). Furthermore, the high levels of circulating L-selectin, demonstrated in plasma by Schleiffenbaum et al. (22), suggests that leukocytes continuously shed L-selectin within the intravascular space. Our results show that the expression of L-selectin on PMN decreases with time spent in the circulation in normal unstimulated animals. This finding is consistent with the shedding of L-selectin by the PMN as either a part of the normal margination process or as they come in contact with mildly activated vascular beds present in the gums, upper respiratory, gastrointestinal, and urinary tract.

The rapid increase in L-selectin-negative PMN in the circulation within the first 30 min after the infusion of labeled PMN (Fig. 4) suggests a preferential margination of L-selectin-positive PMN that return to the circulation with less L-selectin. This finding is consistent with previous studies demonstrating preferential margination of L-selectin-positive PMN in the lungs of rabbits receiving lipopolysaccharide- or zymosan-activated plasma (11, 27) and extend these observations by showing that L-selectin-positive PMN preferentially marginate in normal unstimulated animals.

Spontaneous shedding of L-selectin has been demonstrated from lymphocytes in culture by Sperini et al. (23). The in vitro studies reported here extend these observations to PMN, in which incubation for 24 h resulted in >80% reduction in L-selectin expression in rabbits PMN (Fig. 2K). This supports the near total loss of L-selectin-positive PMN within 24 h in vivo where leukocyte-endothelial interaction may be responsible for the additional loss.

The fact that PMN L-selectin expression decreases over time in the circulation implies that the L-selectin-negative PMN represent an older population of cells. Removal of L-selectin by chymotrypsin did not lengthen the half-life of these PMN and rules out the alternative explanation that L-selectin-positive PMN were preferentially removed from the vascular space into the tissues. Furthermore, permanent removal of PMN expressing high levels of L-selectin from the circulation will result in a progressive decrease in L-selectin expression on PMN in the circulation over time. This explanation is unlikely because the majority of circulating PMN are L-selectin positive (5, 28).

The fact that PMN, in which L-selectin was removed from their surface, behave in a similar fashion to a population of PMN of the same age suggests that the lack of L-selectin on the PMN surface is not a signal for their permanent removal from the intravascular space. This is consistent with normal circulating leukocyte counts in L-selectin knockout mice (26).

Neither the purification procedure nor the chymotrypsin treatment activates the PMN significantly. Margination of chymotrypsin PMN could account for their rapid temporary disappearance from and reappearance in the circulation (Fig. 6). This suggests that chymotrypsin may have additional effects (e.g., cleaving P-selectin glycoprotein ligand) on the PMN to account for this temporary margination. However, the $t_{1/2}$ of chymotrypsin-treated PMN in the circulation was similar to control cells; therefore, the loss of L-selectin from the PMN with time in the circulation can be attributed to the normal aging process of PMN in the circulation and does not signal their permanent removal from the intravascular space.

We previously demonstrated that circulating PMN express variable levels of L-selectin in humans (28) and rabbits (14). One of these studies showed that mature PMN in the bone marrow express higher levels of L-selectin compared with PMN in the circulation (28). Stimuli that cause bone marrow release of PMN result in an increase in PMN expressing high levels of L-selectin. Because L-selectin is essential for the recruitment of PMN to an area of inflammation (30), this implies that PMN recently released from the bone marrow may preferentially marginate in inflammatory foci. Reduced levels of L-selectin on neonatal PMN (±50% of adult values) interfere with their ability to adhere to activated endothelium (1). This neonatal PMN deficit in L-selectin suggests there may be a critical receptor density for L-selectin-dependent function.

In summary, the data reported here show that PMN gradually lose their L-selectin as they age in vitro and in vivo. This suggests that the decreased levels of L-selectin on older circulating PMN result from both constitutive shedding of L-selectin and shedding when the PMN interact with endothelium in microvessels. We conclude that PMN with a reduced or absent L-selectin represent a subpopulation of older circulating PMN. Because L-selectin is essential for the recruitment of PMN across pulmonary and extrapulmonary vascular beds, we speculate that this population of older PMN may have a reduced ability to marginate and emigrate to areas of inflammation.

We gratefully acknowledge Dean English for help with the animal studies, Stuart Greene for photography, and Lorri Verbrugt for statistical analysis.

This work was supported by the Medical Research Council of Canada (MRC 4219), the British Columbia Lung Association, and the Medical Research Council of South Africa. S. F. Van Eeden was financially supported by the South African Medical Research Council.

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Received 24 January 1996; accepted in final form 21 June 1996.

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