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A Functional Role for Circulating Mouse L-Selectin in Regulating Leukocyte/Endothelial Cell Interactions In Vivo

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L-selectin mediates the initial capture and subsequent rolling of leukocytes along inflamed vascular endothelium and mediates lymphocyte migration to peripheral lymphoid tissues. Leukocyte activation induces rapid endoproteolytic cleavage of L-selectin from the cell surface, generating soluble L-selectin (sL-selectin). Because human sL-selectin retains ligand-binding activity in vitro, mouse sL-selectin and its in vivo relevance were characterized. Comparable with humans, sL-selectin was present in adult C57BL/6 mouse sera at ∼1.7 μg/ml. Similar levels of sL-selectin were present in sera from multiple mouse strains, despite their pronounced differences in cell surface L-selectin expression levels. Adhesion molecule-deficient mice prone to spontaneous chronic inflammation and mice suffering from leukemia/lymphoma had 2.5- and 20-fold increased serum sL-selectin levels, respectively. By contrast, serum sL-selectin levels were reduced by 70% in Rag-deficient mice lacking mature lymphocytes. The majority of serum sL-selectin had a molecular mass of 65–75 kDa, consistent with its lymphocyte origin. Slow turnover may explain the relatively high levels of sL-selectin in vivo. The t½ of sL-selectin, assessed by transferring sera from wild-type mice into L-selectin-deficient mice and monitoring serum sL-selectin levels by ELISA, was >20 h, and it remained detectable for longer than 1 wk. Short-term in vivo lymphocyte migration assays demonstrated that near physiologic levels (∼0.9 μg/ml) of sL-selectin decreased lymphocyte migration to peripheral lymph nodes by >30%, with dose-dependent inhibition occurring with increasing sL-selectin concentrations. These results suggest that sL-selectin influences lymphocyte migration in vivo and that the increased sL-selectin levels present in certain pathologic conditions may adversely affect leukocyte migration. The Journal of Immunology, 2002, 169: 2034–2043.
peptides derived from the lectin domain of L-selectin can inhibit leukocyte rolling and migration in vivo (29–32).

The physiological significance of L-selectin endoproteolytic release from the cell surface is not understood. Initially, it was proposed that L-selectin release was required for leukocytes to detach from the endothelial surface before entry into tissues (24). Alternatively, rapid L-selectin endoproteolytic release was proposed to modulate a leukocyte’s ability to migrate and enter sites of inflammation (25). However, more recent in vitro and in vivo studies using synthetic hydroxamic acid-based inhibitors of receptor endoproteolysis have generated conflicting results regarding the role of L-selectin endoproteolytic release in leukocyte migration (33–36). Nonetheless, the homeostatic regulation of sL-selectin levels may also influence leukocyte migration. To better assess the importance of sL-selectin in vivo, murine sL-selectin levels were measured, and the effect of sL-selectin on lymphocyte migration was assessed using L-selectin−/− mice. Mouse sL-selectin was readily detected in serum in which normal circulating levels of sL-selectin were observed to influence the in vivo migration of lymphocytes. In addition, sL-selectin was found to have a relatively long 1/2 in vivo. Thus, L-selectin endoproteolytic release contributes to a functionally relevant pool of serum sL-selectin that may regulate homeostatic leukocyte migration.

Materials and Methods

Animals

C57BL/6 (B6), DBA-1, BALB/c, CBA, MRL, ICAM-1-deficient (ICAM-1−/−) (37), CD18-hypomorphic (CD18<sup>hypo</sup>) (38), P-selectin-deficient (P-selectin−/−) (39), E<sub>μ</sub>-myc transgenic (40), and Rag1-deficient (Rag−/−) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). L-selectin−/− (10) and β<sub>2</sub> integrin-deficient (β<sub>2</sub> integrin−/−) (41) mice were generated as described and backcrossed onto the C57BL/6 background for ≥10 generations. E-selectin-deficient (E-selectin−/−) (42) and E-selectin/P-selectin-deficient (E/P-selectin−/−) (43) mice were housed at the University of Alabama. The E/P-selectin−/− mice were maintained on antibiotic water due to their increased susceptibility to mucocutaneous infection.

Migration assays

Migration assays were performed as previously described (44), with slight modification. Single cell suspensions were prepared from the spleens of B6 mice. Erythrocytes were lysed in Tris-buffered 100 mM ammonium chloride solution. Splenocytes (5×10<sup>5</sup> to 10<sup>6</sup>) were incubated in 2 ml RPMI 1640 medium containing 0.7 μM calcine-AM (Molecular Probes, Eugene, OR) on ice for 30 min with gentle mixing every 5 min. Cells were then washed twice in 100 mM PBS, counted, and resuspended at 1×10<sup>6</sup> cells/ml in PBS. Cells in 300 μl (3×10<sup>5</sup> cells) were injected into the lateral tail vein of individual L-selectin−/− mice that had been injected i.p. with 4 h prior with 1 ml pooled sera from either B6, L-selectin−/−, or Eμ-myc transgenic mice. At 1 h following injection, recipient mice were bled (for blood and serum collection) and single cell suspensions of lymphoid tissues were prepared. One to five thousand calcine-labeled cells with the forward and side light scatter properties of mononuclear cells were analyzed by flow cytometry. The total number of calcine-labeled cells recovered from individual lymphoid tissues was determined by multiplying the total cell counts for individual tissues by the frequency of calcine-labeled cells within the tissue.

Septic shock model

B6 mice were injected i.p. with LPS (100,000 EU/g body weight; Escherichia coli serotype 0111:B4, Sigma-Aldrich), as previously described (45). Control mice were injected i.p. with an equal volume of 100 mM PBS. At various time points following injection, serum was collected and either used in ELISA to determine the concentration of sL-selectin or subjected to immunoprecipitation and Western blot analysis. In some experiments, blood was also collected to determine the surface expression level of L-selectin on leukocytes by FACS.

Statistical analysis

All data are shown as mean values ± SEM, unless indicated otherwise. The Student t-test was used to determine the level of significance in differences between population means.

Results

Quantitation of sL-selectin in serum by ELISA

To determine the amount of sL-selectin present within mouse serum, a quantitative sandwich ELISA was developed and optimized using the LAM1–102 mAb as a capture Ab and biotinylated LAM1–116 mAb as a detecting Ab. The LAM1–102 mAb identifies an epitope within the epidermal growth factor/shorthomucinquitous repeat regions of L-selectin, whereas the LAM1–116 mAb binds an epitope located within the lectin domain (5). Comparable results were obtained when the LAM1–101 mAb replaced the LAM1–102 mAb, which binds to a similar region of L-selectin (data not shown) (45). The presence of sL-selectin was readily detected in serum from normal adult B6 mice, with a mean
concentration of $1.7 \pm 0.1 \mu g/ml$ (Fig. 1A). By contrast, no reactivity was detected in serum from L-selectin $^{-/-}$ mice. Serum L-selectin was also identified in 2-wk-old B6 mice, although the mean concentration was lower ($1.46 \pm 0.07 \mu g/ml, n = 6$) than that of adult mice. As previously reported (26, 28), the concentration of sL-selectin within normal adult human serum was $1.6 \pm 0.1 \mu g/ml$ (Fig. 1A).

Sera from inbred strains of mice were analyzed to determine whether sL-selectin concentrations were consistent among mice. The DBA, CBA, and MRL strains of mice had comparable sL-selectin levels ($1.8 \pm 0.2, 1.8 \pm 0.1$, and $1.5 \pm 0.1 \mu g/ml$, respectively), while serum sL-selectin levels in BALB/c mice were ~30% higher ($2.3 \pm 0.1 \mu g/ml; p < 0.01$; Fig. 1A). Because the primary source of sL-selectin is endoproteolytic release from the surface of leukocytes, the expression level of cell surface L-selectin on circulating leukocytes from the above strains of mice was analyzed. Consistent with higher sL-selectin levels in BALB/c mice, leukocytes isolated from BALB/c mice showed markedly higher L-selectin expression levels relative to leukocytes from B6 mice (Fig. 1B). However, when direct comparisons between strains were made, CD4$^+$ T lymphocytes from DBA, BALB/c, CBA, and MRL mice all expressed significantly higher levels of cell surface L-selectin than CD4$^+$ T cells from B6 mice (by 1.3-, 1.7-, 2.1-, and 2.3-fold, respectively; $p < 0.001$; Fig. 1C). By contrast, human CD4$^+$ T cells and CD4$^+$ T cells from B6 mice expressed comparable levels of cell surface L-selectin (Fig. 1C). Thus, although a correlation between cell surface L-selectin expression and serum sL-selectin concentrations was not observed, sL-selectin was readily detected in serum from multiple strains of mice at concentrations similar to those found in humans.

### sL-selectin isoforms in serum

Two major isoforms of sL-selectin are present in human (26) and rat serum (27). By contrast, mouse sL-selectin migrated as a single broad band with an apparent molecular mass of 65–75 kDa (Fig. 2A). Similar proteins were not immunoprecipitated from L-selectin $^{-/-}$ mouse serum. To correlate Western blot and ELISA sensitivities, sL-selectin was immunoprecipitated from 1–100 $\mu l$ B6 mouse serum, which was calculated to contain between 2 and 180 ng sL-selectin, as determined by ELISA. By this analysis, between 2 and 9 ng sL-selectin was detectable by Western blot analysis (Fig. 2B).

Because the relatively broad size distribution of mouse sL-selectin could be due to the presence of multiple protein isoforms, serum from Rag $^{-/-}$ mice was analyzed. Rag $^{-/-}$ mice lack mature B and T lymphocytes, but have myeloid, granulocyte, and NK lineage cells (46). sL-selectin from Rag $^{-/-}$ mice migrated as a broad band with a higher average molecular mass than sL-selectin from B6 mice (Fig. 2C). In addition, Rag $^{-/-}$ mouse sL-selectin levels were markedly reduced compared with wild-type mice (Fig. 3). Serum sL-selectin from Eµ-myc transgenic mice was similar in size to that found in B6 mice (Fig. 2C). Because the Eµ-myc transgenic mice used for this analysis had developed significant spontaneous B lymphocyte leukemia/lymphomas (40), their serum sL-selectin was likely to predominantly represent lymphocyte-derived material. Thus, as with humans, sL-selectin present in normal mouse serum is composed of at least two major isoforms, one predominantly generated by granulocytes and a smaller lymphocyte-derived species.

### sL-selectin levels in adhesion molecule-deficient mice

A relationship between circulating leukocyte numbers and sL-selectin levels was assessed using serum from adhesion molecule-deficient mice. A general feature of most adhesion molecule-deficient mice is increased numbers of circulating leukocytes, primarily neutrophils, presumably reflecting their reduced ability to exit the bloodstream and enter tissues. Specifically, increases in cell number of up to 1.6-fold in $\beta_2$ integrin $^{-/-}$ mice (13), 2.4-fold in P-selectin $^{-/-}$ mice (39), 2.6-fold in CD18$^{b99}$ mice (38), 2.9- to 4.1-fold in ICAM-1 $^{-/-}$ mice (3, 37), and 7.1-fold in E/P-selectin $^{-/-}$ mice (43) have been reported. Increased numbers of circulating leukocytes have not been reported for E-selectin $^{-/-}$ mice (42). Nonetheless, serum from B6, $\beta_2$ integrin $^{-/-}$, CD18$^{b99}$, E-selectin $^{-/-}$, and P-selectin $^{-/-}$ mice all contained sL-selectin at comparable levels (Fig. 3). By contrast, serum sL-selectin concentrations in ICAM-1 $^{-/-}$ mice were 20% higher (2.1 ± 0.1 $\mu g/ml; p < 0.01$), while concentrations in E/P-selectin $^{-/-}$ mice were 240% higher (4.1 ± 0.2 $\mu g/ml; p < 0.001$) than in B6 mice.

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**FIGURE 1.** Cell surface L-selectin and serum sL-selectin levels in human and in different mouse strains. A, Serum was collected from normal adult human donors and from 2- to 4-mo-old B6, DBA, BALB/c, CBA, MRL, and L-selectin $^{-/-}$ (L$^{-/-}$) mice. Levels of sL-selectin were determined by ELISA, as described in Materials and Methods. Individual symbols represent mean values obtained in duplicate determinations for each sample. Horizontal bars represent mean concentrations of sL-selectin for each group. *, The difference between means of B6 and BALB/c mice was significant, $p < 0.01$. B, Cell surface L-selectin expression on circulating leukocytes from B6 (thin line) and BALB/c (heavy line) mice. Leukocytes in whole blood were stained with FITC-conjugated LAM1–116 mAb. Negative control staining (dashed line) was obtained using FITC-conjugated isotype-matched control mAb. L-selectin expression levels by all cells with the size characteristics of lymphocytes were determined by flow cytometric analysis. C, Cell surface L-selectin expression on circulating CD4$^+$ T lymphocytes from human and different mouse strains. Whole blood was stained simultaneously with FITC-conjugated LAM1–116 mAb and PE-conjugated anti-CD4 mAb and analyzed by flow cytometry. The mean linear fluorescence channel number for CD4$^+$ cells staining positive for L-selectin was determined. Gates for assessing positive L-selectin staining were established using directly conjugated isotype-matched control mAbs, with $<5\%$ of cells in control samples falling within this gate. Values represent the mean (±SEM) of results for 4 humans and 3–17 mice in each group. *, Significantly increased compared with B6, $p < 0.001$. **
Reduced by nearly 70% compared with wild-type mice (Fig. 3). Therefore, factors in addition to increased numbers of circulating leukocytes are likely to influence serum sL-selectin levels. Rather, the total number of leukocytes throughout the body and their activation status may have a greater influence on serum sL-selectin concentrations.

**sL-selectin turnover in vivo**

The relatively high levels of sL-selectin found in both human and mouse serum suggest that L-selectin is either continuously cleaved from the surface of leukocytes and/or that sL-selectin is relatively stable in vivo. To test this, 1 ml pooled serum from ICAM-1−/− mice was adoptively transferred into L-selectin−/− mice and serum sL-selectin levels were monitored by ELISA. Serum sL-selectin was detected as early as 15 min after i.p. injection, reached peak concentration at ~4 h, and declined to half-maximal levels at ~25 h (Fig. 4A). Detectable levels of sL-selectin remained in the serum for greater than 1 wk. In another set of experiments, 0.5 ml B6 serum was similarly transferred into an L-selectin−/− mouse with serum samples analyzed by both ELISA and Western blotting to directly visualize the transferred sL-selectin in the serum. Consistent with the kinetics of the above results, sL-selectin concentrations of 79, 345, and 142 ng/ml were found at 0.5, 5, and 20 h following transfer, respectively. Western blot analysis of the same samples demonstrated intact sL-selectin at 0.5 h, the highest concentration at 5 h, and a decreasing amount at 20 h (Fig. 4B). Therefore, sL-selectin appears to accumulate and remain stable in vivo with an estimated t_{1/2} of >20 h.

**FIGURE 3.** Serum sL-selectin levels in adhesion molecule-deficient mice. Serum sL-selectin concentrations for B6, CD18 hypo , ICAM−/−, E-selectin−/−, P-selectin−/−, and Rag−/− mice were determined by ELISA. Individual symbols represent the mean values obtained in duplicate determinations for each sample. Horizontal bars represent mean sL-selectin concentrations for each mouse line. *, Differences between means for B6 and test mice were significant, p < 0.01; **, p < 0.001.

**FIGURE 4.** Half-life of sL-selectin in vivo. A, L-selectin−/− mice were injected i.p. with 1 ml pooled ICAM-1−/− mouse serum that contained 3.30 (Exp. 1) and 2.95 (Exp. 2) μg/ml sL-selectin, as determined by ELISA. Small aliquots of blood (25–50 μl) were collected at the indicated time points following injection, and the resulting serum samples were measured for levels of sL-selectin. Individual symbols represent the mean values obtained in duplicate determinations for each sample. The dashed line indicates the time when the concentration of injected sL-selectin had decreased to half of maximum. B, An L-selectin−/− mouse was injected with 0.5 ml B6 mouse serum, containing 1.83 μg/ml sL-selectin, as above. Serum samples (50 μl) collected at the indicated time points were immunoprecipitated, followed by Western blot analysis, as described in Fig. 2.
Septic shock effects on sL-selectin levels

Increased cleavage of L-selectin from the surface of activated leukocytes may be responsible for the increased levels of serum sL-selectin observed in patients suffering from septic shock (28, 47). To examine this issue and its time course in mice, septic shock was induced by injecting mice i.p. with a sublethal dose of LPS. All mice quickly demonstrated obvious symptoms of shock that included ruffled fur, shivering, lethargy, and watery eyes. Serum and/or blood samples were collected following LPS injection, and serum and leukocyte cell surface L-selectin levels were determined. Following LPS injection, cell surface L-selectin expression was reduced on both neutrophils and lymphocytes (Fig. 5A). Specifically, L-selectin expression on neutrophils decreased by 65% and 78% at 1.5 and 4 h, respectively, following LPS injection. L-selectin expression on circulating lymphocytes was less affected and reached a maximal decrease of 30% at 1.5 h following LPS injection. Despite increased release of L-selectin from the surface of leukocytes following LPS injection, serum sL-selectin levels remained comparable with those of PBS-injected control mice at all time points examined (Fig. 5A). However, sL-selectin levels did increase by 1.5- and 1.7-fold in the serum from both control and LPS-injected mice, respectively, at 24 h following injection (p < 0.05). This was most likely due to local trauma from repeated blood sample collection. To address this, mice were injected with either LPS or PBS as above and serum samples were collected from different sites at 0 and 24 h following injection. No change in the level of sL-selectin was observed in either group of animals (Fig. 5B). To control for any effects of collecting the preinjection blood sample, 10 littermate mice were separated into two groups, in which one group was injected with PBS, and the other with LPS. Serum was only collected 24 h following injection. There was no significant difference in sL-selectin levels between the two groups of mice (Fig. 5B). One possibility of why an increase in sL-selectin was not found following LPS injection could be increased degradation as a result of systemic inflammation. To examine this, sL-selectin in serum from mice injected with either LPS or PBS as above was immunoprecipitated and subjected to Western blot analysis. There were no differences in the amount or size of sL-selectin, and other immunoreactive fragments were not detected in blots from LPS-treated mice compared with controls (Fig. 5C). Thus, loss of L-selectin from the surface of circulating leukocytes in a murine model of acute inflammation did not result in significantly increased levels of serum sL-selectin.

Effects of sL-selectin on leukocyte migration

L-selectin mediates lymphocyte migration to PLN, while L-selectin and β2 integrin synergistically mediate migration to mesenteric lymph nodes (MLN) and PP (10, 13, 48). To determine whether sL-selectin influences lymphocyte migration to peripheral lymphoid tissues, in vivo migration studies were performed using lymphocytes adoptively transferred into L-selectin−/− mice in the presence or absence of sL-selectin. Calcein-labeled splenocytes from B6 mice were injected into the tail veins of individual L-selectin−/− mice that had been injected i.p. ~4 h prior with 1 ml serum from either B6 or L-selectin−/− mice. This time point was chosen so that migration would be occurring when serum levels of sL-selectin were maximal (Fig. 4). Following 1 h of migration,

![FIGURE 5. Serum sL-selectin levels during LPS-induced septic shock. B6 mice were injected i.p. with either LPS (100,000 EU/g body weight) or PBS as control. A. Blood and serum were collected at the indicated time points following injection. Leukocytes were labeled for L-selectin expression using FITC-conjugated LAM1–116 mAb. L-selectin expression levels on leukocytes having the light scatter properties of either neutrophils (left panel) or lymphocytes (center panel) were determined by FACS. Columns represent the mean fluorescence intensity expressed as a percentage of preinjection control staining (100%, dashed line) from two independent experiments. Individual symbols represent the results obtained for each experiment. Serum was analyzed for sL-selectin concentration by ELISA (right panel). Values represent the mean results (±SEM) obtained from three mice in each group. B. Serum was collected at 0 and 24 h following PBS or LPS injection, using a different anatomical site for each time point, and levels of sL-selectin were determined by ELISA (left panel). Values represent the mean results (±SEM) obtained from three mice in each group. In addition, mice were injected with PBS or LPS as above and serum was harvested at only the 24-h time point for analysis by ELISA (right panel). Values represent the mean results (±SEM) obtained from five mice in each group. C. Serum samples (20 μl) collected at the 24-h time point from the PBS- and LPS-injected mice described in B were immunoprecipitated, followed by Western blot analysis, as described in Fig. 2.](http://www.jimmunol.org/)

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serum and peripheral lymphoid tissues were collected. Mean sL-selectin levels were 0.9 ± 0.1 μg/ml (n = 4 independent experiments) in L-selectin$^{-/-}$ mice injected with B6 mouse serum, whereas sL-selectin was not detected in mice injected with L-selectin$^{-/-}$ mouse serum. Similar numbers of injected splenocytes migrated into the spleen, MLN, and PP of mice injected with either B6 or L-selectin$^{-/-}$ mouse serum (Fig. 6A). By contrast, the migration of calcein-labeled splenocytes into the PLN of mice injected with B6 mouse serum was reduced by 38% ($p < 0.05$) compared with that of mice injected with L-selectin$^{-/-}$ mouse serum (Fig. 6A).

To further evaluate the effect of sL-selectin on lymphocyte migration, Eμ-myC transgenic mouse serum was used to assess the effect that elevated sL-selectin levels would have on lymphocyte migration. Serum from Eμ-myC transgenic mice had sL-selectin concentrations of 2.8 ± 0.4 μg/ml before the onset of detectable disease, whereas mice with pronounced disease had mean sL-selectin levels of 28.0 ± 4.7 μg/ml ($p < 0.05$; Fig. 6B). Serum from Eμ-myC transgenic mice containing increasing concentrations of sL-selectin was injected into L-selectin$^{-/-}$ mice, and 1-h lymphocyte migration assays were performed. Consistent with the above results, increasing levels of serum sL-selectin resulted in further inhibition of lymphocyte migration into PLN of L-selectin$^{-/-}$ mice, but did not have a consistent effect on lymphocyte migration into MLN (Fig. 6C). Therefore, lymphocyte migration into PLN is influenced by the presence of physiologically relevant sL-selectin levels, with increased levels resulting in more profound inhibition.

**Discussion**

Although the physiological significance of L-selectin endoproteolytic release from the cell surface is unknown, these studies suggest that sL-selectin is a homeostatic regulator of mouse leukocyte migration. Importantly, sL-selectin was present in serum at high levels in both humans and mice (1.6 and 1.7 μg/ml, respectively; Fig. 1A). Although mice with different genetic backgrounds varied markedly in their levels of cell surface L-selectin expression (Fig. 1, B and C), similar levels of sL-selectin were present in the sera of four of the five inbred strains examined (Fig. 1A). Similarly, serum sL-selectin levels were within a normal range for the majority of adhesion molecule-deficient mice, despite their significantly increased numbers of circulating neutrophils (Fig. 3). This may reflect the finding that the majority of serum sL-selectin derives from mature lymphocytes, and circulating lymphocytes far outnumber circulating myelomonocytic cells in mice. Specifically, serum sL-selectin levels in Rag$^{-/-}$ mice lacking mature lymphocytes were ~70% below the levels found in normal mice (Fig. 3). Furthermore, the majority of serum sL-selectin in normal mice displayed a molecular mass comparable with lymphocyte-derived receptor (Fig. 2C). In part, this may also explain why in vivo neutrophil activation during septic shock, with resultant L-selectin endoproteolytic release from the cell surface, did not significantly increase the serum pool of sL-selectin (Fig. 5). Moreover, the relatively high levels of serum sL-selectin in humans and mice may reflect the slow turnover of sL-selectin because serum sL-selectin had an in vivo $t_{1/2}$ of $>20$ h in mice (Fig. 4). The relatively high serum levels and stability of sL-selectin further imply that sL-selectin influences leukocyte migration in vivo (26).

Normal human serum sL-selectin concentrations typically range between ~0.5 and 1.9 μg/ml in multiple studies using different assay systems (26, 28, 45, 47, 49–52). By contrast, sL-selectin is present in normal rat serum at ≤1 ng/ml (27). Although it is difficult to understand the basis for the molecular difference between humans and rats, the mouse serum sL-selectin levels quantified in this study were similar, if not identical with those of humans. This was true when sL-selectin levels were quantified both by ELISA (Fig. 1A) and by Western blot analysis (Fig. 2A). In the current study, mouse serum sL-selectin levels were quantified using a previously described human serum standard that contained a known concentration of sL-selectin (26, 28). Others have used recombinant human L-selectin as a standard for measuring human sL-selectin levels (45). Therefore, it is likely that humans and mice have similar, high levels of serum sL-selectin that are likely to influence leukocyte migration.
In the present study, the \( t_{1/2} \) of sL-selectin was determined in L-selectin \(^-\) mice, and thus in the absence of any endogenous sL-selectin. In most cases, \( t_{1/2} \) of plasma proteins are determined in the presence of endogenous serum protein that is at steady state. Therefore, the injected sL-selectin may initially have had a faster clearance rate than would occur in a mouse containing SL-selectin due to the availability of unbound endothelial- and leukocyte-expressed L-selectin ligands. This may result in the \( \sim 25\text{-}h \) \( t_{1/2} \) for sL-selectin being an underestimate of the actual turnover rate of SL-selectin in wild-type animals. However, as with all circulating proteins, multiple factors, including size, charge, catabolism, and association with other plasma proteins, predominantly determine their turnover rate. Thus, it is still possible to compare the \( t_{1/2} \) of sL-selectin with that of other plasma proteins. Small molecular mass proteins such as IL-2 and bikunin have reported \( t_{1/2} \) of 4 and 10 min, respectively, due to rapid kidney clearance (53, 54). Because sL-selectin is a relatively high molecular mass protein, it would not be expected to be removed to any significant amount by filtration in the kidney. However, very large serum proteins can also be rapidly removed from the circulation. For example, hyaluronic acid is very efficiently taken up and degraded by sinusoidal endothelial cells in the liver, resulting in a circulating \( t_{1/2} \) of only 2–5 min (reviewed in Ref. 55). Acute-phase reactants such as serum amyloid A proteins and C-reactive protein, which largely circulate in association with plasma lipoproteins, have \( t_{1/2} \) of between 30 min and 4 h (56, 57). Proteins in the coagulation or fibrinolytic pathways can have short (e.g., factor VIIa, \(~2.5\text{ h}\)), intermediate (e.g., factor VIII, 12–14 h), or longer \( t_{1/2} \) (e.g., factor IX, plasminogen, prothrombin, 24–48 h) (58–61). Much longer \( t_{1/2} \) are reported for serum albumin (4–5 days) (62) and IgG (4–8 days) (63). It is possible that the \( t_{1/2} \) of sL-selectin may be shortened under inflammatory conditions as a result of binding to newly expressed vascular L-selectin ligands (64). This could be a contributing factor to why increased levels of sL-selectin were not found in the serum of mice during septic shock (Fig. 5). Consistent with this idea, decreased levels of SL-selectin correlate with progression to acute respiratory distress syndrome among at-risk patients (65). Therefore, the circulating \( t_{1/2} \) of sL-selectin is regulated by multiple factors and may be altered during inflammation.

The relatively high serum levels of sL-selectin in mouse serum suggest that it may compete with cell surface L-selectin for ligand binding and thereby influence lymphocyte migration into PLN and leukocyte migration into sites of inflammation. Consistent with this, increasing serum L-selectin levels in L-selectin \(^-\) mice to near physiological levels by serum infusion significantly inhibited wild-type lymphocyte migration into PLN during in vivo migration assays (Fig. 6A). In addition, the infusion of serum SL-selectin from mice with leukemia/lymphoma further increased sL-selectin levels, which further inhibited the in vivo migration of lymphocytes into PLN (Fig. 6B and C). Significant inhibition of migration into other peripheral lymphoid tissues was not observed, most likely due to the presence of alternate adhesion pathways (13, 48).

In agreement with these results, a separate study has demonstrated that administration of recombinant human soluble L-selectin to mice at estimated systemic concentrations of 2.3 and 8 \( \mu \)g/ml diminishes trauma-induced leukocyte/endothelial cell interactions in cremaster venules in vivo, as assessed using intravital microscopy (31). In these experiments, the addition of human L-selectin to 8 \( \mu \)g/ml, for a total 5-fold increase in sL-selectin levels based on the current studies (Fig. 1), resulted in a \( \sim 50\% \) decrease in leukocyte rolling flux and increased leukocyte rolling velocities. Because alterations in leukocyte rolling flux and rolling velocities do not always predict alterations in leukocyte entry into tissues (18), the demonstration that sL-selectin levels also influenced leukocyte migration into tissues (Fig. 6) confirms that sL-selectin levels influence homeostatic leukocyte/endothelial interactions, leading to leukocyte rolling and entry into tissues. Similar in vivo results using recombinant soluble L-selectin-Ig chimeric protein also suggest that during inflammation, sL-selectin binding its vascular ligands has physiological effects (29). In further support of this, sL-selectin has been shown to bind the luminal surface of inflamed vascular endothelium in vivo (64). Consistent with these in vivo observations, purified human sL-selectin at concentrations of 8–15 \( \mu \)g/ml completely inhibits L-selectin-mediated lymphocyte binding to activated endothelial cells during in vitro binding assays, whereas physiological concentrations of sL-selectin inhibit binding by 15–20\% (26). Therefore, sL-selectin retains functional activity and may function to down-regulate low-grade inflammatory responses in vivo.

The ligand-binding activity of the selectins is primarily localized to the lectin domain, although the epidermal growth factor-like and short consensus repeat domains also contribute (66, 67). A high degree of homology among the selectin lectin domains allows each to recognize similar heavily glycosylated mucin-like proteins, the prototype carbohydrate ligand being the tetrasaccharide sialyl Lewis\(^x\) (68). The dominant physiologic ligand for P-selectin, P-selectin glycoprotein ligand-1 (PSGL-1) (69–72), is expressed by most leukocytes and also serves as a ligand for L-selectin (66, 73, 74) and E-selectin (75, 76). The interaction of L-selectin with PSGL-1 accounts for the majority of leukocyte rolling on adherent leukocytes at sites of inflammation (77, 78). In addition to PSGL-1, L-selectin binds to inducible ligands expressed on activated endothelium (79–83) and to at least five ligands constitutively expressed by HEV within lymphoid tissues: glycosylation-dependent cell adhesion molecule 1 (84), CD34 (85), CD62L (86), L-selectin binds to inducible ligands expressed on activated endothelium (79–83) and to at least five ligands constitutively expressed by HEV within lymphoid tissues: glycosylation-dependent cell adhesion molecule 1 (84), CD34 (85), CD62L (86), podocalyxin-like protein (87), HEV-like vessels that express sL-selectin ligands are also present at sites of chronic inflammation, including inflamed islets (89–91), salivary glands (90), kidney (91), and rheumatoid synovium (92, 93). The interaction of L-selectin with these vascular ligands regulates lymphocyte migration into peripheral lymphoid tissues and mediates leukocyte/endothelial cell interactions at sites of inflammation. Thus, elevated levels of sL-selectin that occur in multiple disease conditions may interfere with L-selectin-mediated, as well as P- and E-selectin-mediated leukocyte migration, by competing for ligand binding.

Significantly increased levels of sL-selectin are found in the serum of patients suffering from chronic myeloid and lymphocytic leukemia (94–96), sepsis (28, 47), HIV infection (28), atopic dermatitis (50), severe psoriasis (97), and active systemic lupus erythematosus (45). High sL-selectin levels in cases of hematological malignancies or inflammatory disorders may explain why leukocytes in these patients circulate for longer periods of time as compared with normal individuals and are delayed in their entry into tissues. This may also be true in mice with lymphoid malignancies that demonstrated sL-selectin levels up to 20-fold higher than normal (Fig. 6B). Moreover, even the smaller increases in sL-selectin levels observed in E/P-selectin \(^-\) mice (2- to 3-fold) may have considerable functional effect on leukocyte migration. The effect of increased sL-selectin levels is likely to be even further magnified given that the finding that cell surface L-selectin levels are dramatically diminished in these mice, presumably due to increased rates of infection (43). In addition, while serum levels of sL-selectin were found to be normal in CD18\(^{\text{hyp}}\) mice, it is likely that levels in CD18-deficient mice are significantly increased because these animals suffer from chronic dermatitis and mucocutaneous infections.
(98). Thus, both sL-selectin levels and L-selectin cell surface density may be critical factors for directing leukocyte migration and recirculation.

Although circulating sL-selectin was found to influence lymphocyte migration to PLN (Fig. 6), it may not affect the migration of all lymphocyte subsets to the same degree. In fact, there is considerable heterogeneity in L-selectin expression by activated lymphocytes (99) and among lymphocyte subsets (44). It is likely that sL-selectin levels will more significantly modify the migratory patterns of leukocytes expressing lower levels of cell surface L-selectin. The finding that a 50% decrease in L-selectin expression results in a 70% decrease in leukocyte entry into PLNs suggests that L-selectin surface density is tightly regulated, with small differences markedly influencing leukocyte migration (44). Thus, increased levels of sL-selectin in vivo may adversely affect the migration of L-selectinlow leukocytes and provide an effective mechanism for preventing the entry of activated leukocytes with lower levels of sL-selectin expression into lymphoid tissues or sites of inflammation. Likewise, increased cell surface L-selectin expression by lymphocytes among the different inbred lines of mice examined (Fig. 1C) suggests that lymphocyte migration may be more efficient in these mice because sL-selectin levels were comparable between the different lines. Thus, these studies suggest an important role for sL-selectin in regulating lymphocyte entry into peripheral lymphoid tissues. Because there is considerable overlap in adhesion pathways mediating lymphocyte recirculation and inflammation-induced leukocyte migration, a role for sL-selectin during inflammation is also likely. In addition, measurement of sL-selectin may be a potentially useful parameter to monitor certain inflammatory or immune disorders in mice.

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References

CHARACTERIZATION OF MOUSE sL-SELECTIN


