Identification of Novel Isoforms of Mouse L-selectin with Different Carboxyl-terminal Tails

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The leukocyte adhesion molecule L-selectin mediates the recruitment of lymphocytes to secondary lymphoid organs and is involved in the accumulation of neutrophils at sites of inflammation. In this study, we report the identification of novel isoforms of the mouse L-selectin gene, termed L-selectin-v1 and L-selectin-v2. Sequence analysis revealed that these isoforms are generated by alternative splicing: the L-selectin-v2 transcript includes a previously unknown exon of 100 bp located between the 7th and 8th exons of the mouse L-selectin gene, while the L-selectin-v1 transcript contains the first 49-bp sequence of this new exon. The insertion of each new sequence adds a downstream reading frame, giving rise to predicted proteins that differ in their carboxyl-terminal tails. These splice variants were found in cells that express conventional L-selectin, termed L-selectin-c, including B and T lymphocytes and granulocytes. Functionally, like L-selectin-c, both L-selectin-v1 and L-selectin-v2 expressed in cultured cells underwent phorbol ester-induced shedding, although L-selectin-v1 and L-selectin-v2 were shed to a greater and lesser degree, respectively, than L-selectin-c. Under flow conditions, both L-selectin-v1 and L-selectin-v2 mediated faster cell rolling than did L-selectin-c. In addition, ligation of L-selectin-c and L-selectin-v1, but not L-selectin-v2, induced p38 mitogen-activated protein kinase phosphorylation. These results suggest that alternative splicing is one mechanism for generating functional diversity in L-selectin.

Lymphocyte recirculation between the blood and lymphatic system is critical for the maintenance of immune surveillance. Lymphocytes in the blood extravasate into secondary lymphoid organs through specialized postcapillary venules called high endothelial venules, where they tether and roll via L-selectin (CD62L), a member of the selectin family of adhesion receptors expressed on most leukocytes (1, 2). L-selectin is also involved in leukocyte recruitment to both acute and chronic sites of inflammation (3). P-selectin (CD62P) and E-selectin (CD62E), the other two members of the selectin family, are expressed on the endothelial cells of inflamed venules and also mediate leukocyte recruitment to sites of inflammation.

The three selectins are type I membrane proteins with a C-type lectin domain at the amino terminus, an epidermal growth factor-like domain, several short consensus repeat domains, a single transmembrane domain, and a short cytoplasmic tail (4). The cytoplasmic tails of the three selectins are distinct, although highly conserved among species for any given selectin. The human and mouse L-selectin tails both consist of 17 amino acids, with only 4 residues different between them (5–7), suggesting that this domain has an important function. The L-selectin cytoplasmic tail has been reported to bind several proteins, including α-actinin, the ezrin/radixin/moesin (ERM) family of proteins, and calmodulin, and is implicated in the regulation of L-selectin function (8). For example, L-selectin-mediated tethering under flow is facilitated by the topographic distribution of L-selectin on the tips of microvilli, which is partly regulated by the cytoplasmic domain (9). L-selectin-mediated rolling is also regulated by the cytoskeletal anchoring of L-selectin via the cytoplasmic domain. Truncation of the 11 carboxyl-terminal residues of the human L-selectin tail disrupts association of L-selectin with α-actinin and abolishes rolling on inflamed venules in vivo without affecting its ligand recognition or microvilli localization (10, 11). This deletion mutant fails to associate with the cytoskeletal matrix in response to cross-linking by anti-L-selectin antibodies (Abs) or hyperthermia (12). In vitro flow assays demonstrated that this mutant can mediate tethering (13). However, the conversion of initial tethers to rolling is impaired by this truncation and is completely abolished by a further 4-residue truncation (13). These studies suggest that α-actinin acts as a link between L-selectin and the cortical actin cytoskeleton, which might be important for the transition from tethering to rolling. In addition, the L-selectin tail is implicated in the regulation of proteo-

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2 The abbreviations used are: ERM, ezrin/radixin/moesin; Ab, antibody; PMA, phorbol 12-myristate 13-acetate; mAb, monoclonal antibody; RT, reverse transcription; MAPK, mitogen-activated protein kinase.

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lytic cleavage of the ectodomain, called shedding, through its binding to calmodulin (14, 15).

Alternative splicing is widely recognized as a ubiquitous and important mechanism for generating protein diversity (16). Numerous cell-surface adhesion molecules have been found to undergo alternative splicing that may change their adhesion and/or signaling properties. These include the immunoglobulin superfamily members ICAM-1 (17), VCAM-1 (18), and PECAM-1 (CD31) (19) and the link protein family member CD44 (20). Among the selectin family members, human P-selectin is reported to have two variant forms, one predicting a molecule containing eight short consensus repeat domains instead of the typical nine and the other predicting a soluble form (21, 22). Rat E-selectin has two mRNA species, which differ in the presence of short consensus repeat domain 5 (23). To date, no splice variants have been reported for L-selectin from any species.

In this study, we report two novel isoforms of mouse L-selectin, termed L-selectin-v1 and L-selectin-v2, which are generated by alternative splicing. These isoforms have new sequence insertions that alter the amino acid sequences of the cytoplasmic tail: the 11-amino acid carboxyl-terminal sequence of conventional L-selectin (termed L-selectin-c) is replaced by a new 24-amino acid sequence in L-selectin-v1, and the 8-amino acid carboxyl-terminal sequence of L-selectin-v1 is further replaced with a new 10-amino acid sequence in L-selectin-v2. We found that, like L-selectin-c, both L-selectin-v1 and L-selectin-v2 underwent phorbol 12-myristate 13-acetate (PMA)-induced shedding as did L-selectin-c, although the three isoforms showed differences in their shedding efficiency. In addition, both L-selectin-v1 and L-selectin-v2 mediated faster cell rolling than did L-selectin-c under flow conditions. Furthermore, we found that ligation of L-selectin-c and L-selectin-v1, but not L-selectin-v2, induced p38 mitogen-activated protein kinase (MAPK) phosphorylation. These splice variants should prove useful in clarifying the function of the L-selectin cytoplasmic tail.

**EXPERIMENTAL PROCEDURES**

**Reagents**—The monoclonal antibody (mAb) MEL-14, which recognizes an epitope on the Fc portion of human IgG1, and it was a kind gift from Wyeth Research (Cambridge, MA). It was noted that this mAb recognizes an epitope in the extracellular domain of mouse P-selectin (24). Two hinge-proximal amino acids at positions 234 and 237 within the IgG1 Fc portion are critical for Fc receptor binding.

**Cells**—A mouse pre-B cell line L1.2 was kindly provided by InVitrogen. To examine L-selectin expression on transfected L1.2 cells, the cells were stained with phycoerythrin-labeled anti-L-selectin mAb MEL-14 (BD Biosciences) for 30 min on ice, washed, and analyzed using a FACSCalibur (BD Biosciences).

**Immunoelectron Microscopy**—L1.2 transfectants (5 × 10^6 cells) in phosphate-buffered saline containing 1% fetal calf serum and 0.05% NaN₃ were incubated with MEL-14 at 4 °C for 50 min. After washing with phosphate-buffered saline, the cells were incubated with affinity-purified biotinylated rabbit anti-rat IgG (mouse absorbed; Vector Laboratories) at 4 °C for 50 min. The cells were then reacted with streptavidin-conjugated 15-nm colloidal gold particles (EY Laboratories). The labeled
cells were spun at 400 × g, fixed with 1% glutaraldehyde in 0.1 M phosphate buffer at 4 °C for 1 h, washed in phosphate-buffered saline, and postfixed with 1% OsO4. Finally, the pellets of fixed samples were dehydrated in a graded ethanol series and embedded in Quetol 812 epoxy resin (Nisshin EM). Ultrathin sections were stained with 2% uranyl acetate and Reynold’s lead citrate and examined using a transmission electron microscope (JEM-1230; JEOL). Control staining was performed in the absence of primary Abs.

**PMA-induced Shedding of L-selectin**—To induce L-selectin shedding, 1 × 10^6 cells were incubated in 0.1 ml of medium containing 1 μg/ml PMA at 37 °C for 1 h. The cells were washed with phosphate-buffered saline containing 1% bovine serum albumin, 0.1% NaN₃, and 5 mM EDTA and analyzed for L-selectin surface expression by flow cytometry.

**Cell Adhesion Assays under Flow Conditions**—Cell adhesion assays under flow conditions were performed according to the method of Nandi et al. (26) with slight modifications. rPSGL-Ig or control human IgG (Sigma) (10 μg/ml) was immobilized on the inside walls of glass capillaries (inner diameter, 0.69 mm; Drummond Scientific) at 4 °C overnight. The capillaries were then blocked with 3% bovine serum albumin for 1 h at room temperature. The capillaries were mounted on the stage of an inverted microscope (Diaphot 300; Nikon) with a ×4 objective. At this magnification, all the cells rolling at a fixed position of a capillary could be monitored. L1.2 cells expressing each L-selectin isoform were resuspended at 1 × 10^6 cells/ml in Hanks’ balanced salt solution containing either CaCl₂ or EDTA and infused into the capillaries at a shear force of 1 dyn/cm². The rate of flow was controlled by a PHD 2000 syringe pump (Harvard Apparatus). Three minutes after the start of infusion, cell images were recorded with a cell-viewing system (SRM-100; Nikon) and video recorder (BR-S600; Victor), and the number of rolling cells passing through a fixed plane (at three quarters of the capillary tube from the entrance) perpendicular to the capillary axis was counted. The results are expressed as the number of rolling cells/min. The cells that rolled stably along the wall of the glass capillary tube for at least 3 s were considered to be rolling cells in this assay. The rolling velocity of individual cells was determined from video recordings by measuring the time required for a cell to travel a fixed distance (0.5 mm) for 200 cells.

**Stimulation of Cells**—L1.2 transfectants were washed with RPMI 1640 containing 10 mM HEPES (RPMI-HEPES) and serum-starved in RPMI-HEPES for 3 h at a concentration of 1 × 10^7 cells/ml. Serum-starved cells were stimulated with 20 μg/ml fucoidin (Sigma) at 37 °C for various time periods. At the end of the stimulation, the cells were washed with RPMI-HEPES, lysed in SDS sample buffer, sonicated, boiled for 5 min, and cleared by centrifugation. The lysates were resolved by SDS-PAGE, and the proteins were transferred to an Immobilon-P membrane (Millipore). Membranes were probed with Abs against p38 MAPK or phospho-p38 MAPK (Cell Signaling) followed by peroxidase-conjugated anti-rabbit IgG (Cell Signaling).

**Statistical Analysis**—Data are presented as the mean ± S.D. (Figs. 2 and 4) or S.E. (Fig. 5). Statistical analyses were performed using the two-tailed unpaired Student’s t-test.

**RESULTS**

**Identification of Novel L-selectin Isoforms**—RT-PCR was performed from RNA isolated from mouse bone marrow cells

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**Figure 1.** Schematic representation of the gene and three isoforms of mouse L-selectin. A, genomic organization of the mouse L-selectin gene and the three mRNA transcripts. B, nucleotide sequences of the new exon v1 + v2 and surrounding regions. C, amino acid sequences of the intracellular regions of the three isoforms. The carboxyl-terminal 11-amino acid sequence in L-selectin-c (underlined) is replaced with new sequences in L-selectin-v1 and L-selectin-v2 (bold letters). The sequences unique to L-selectin-v1 and L-selectin-v2 are also underlined.
Novel Mouse L-selectin Isoforms

Expression of the Three L-selectin mRNA Species in Mouse Tissues and Cells—The mRNA expression of the three L-selectin isoforms was examined by quantitative real-time RT-PCR. The primers and probes are shown in Fig. 2A and Table 1. The 3′-primers for L-selectin-c and L-selectin-v1 were designed to span the unique junctions. The 3′-primer for L-selectin-v2 was located in exon v2. The specificity of the primers was verified using the plasmid for each isoform as a template. As expected, L-selectin-c expression was found in lymphoid organs, including lymph nodes and spleen (Fig. 2B). The L-selectin-v1 and L-selectin-v2 transcripts were expressed in the same tissues where L-selectin-c was expressed (Fig. 2B). The expression of mRNA for the three isoforms was also confirmed in various cell types, including B and T lymphocytes and granulocytes (Fig. 2C). In addition, the amount of L-selectin-v1 and L-selectin-v2 mRNA relative to L-selectin-c mRNA was determined using the plasmid for each isoform as a standard. In B and T lymphocytes, L-selectin-v1 mRNA expression was <2% of that of L-selectin-c, whereas L-selectin-v2 mRNA expression was ∼4–5% (Fig. 2D). In activated T cells, such as Th1 and Th2 cells, and granulocytes, the mRNA expression of

Using a 5′-primer located in the 5′-untranslated region and a 3′-primer located in the carboxyl end of the coding region of mouse L-selectin-c. Nucleotide sequence analysis of the cloned PCR fragments revealed the presence of two novel transcripts, termed L-selectin-v1 and L-selectin-v2 (Fig. 1, A and B). The L-selectin-v1 transcript has a 49-bp insertion, due to the inclusion of a new exon (exon v1) located between the 7th and 8th exons of the mouse L-selectin gene (nucleotides 11026–11074, GenBank™ accession number NC_000067, Gene ID 20343). Part of the L-selectin-v1 sequence is found in the GenBank™ Expressed Sequence Tag data base (BF714815). The L-selectin-v2 transcript includes an additional 51-bp sequence (exon v2) just after the 49-bp insert of the L-selectin-v1 transcript (nucleotides 11075–11125, NC_000067). These two sequences exist continuously without an intervening sequence in the mouse L-selectin gene. Thus, L-selectin-v2 is generated by the inclusion of the full cassette exon v1+v2 that was newly identified, while L-selectin-v1 is generated by the use of an alternative 5′-splice site within this cassette exon.

Insertion of the new sequences resulted in new downstream reading frames in L-selectin-v1 and L-selectin-v2 (Fig. 1C). Consequently, the 11-amino acid carboxy-terminal sequence of the cytoplasmic domain of L-selectin-c (residues 362–372) is replaced with a new 24-amino acid sequence in the carboxy end of L-selectin-v1 (residues 362–385). In L-selectin-v2, the 8-amino acid carboxy-terminal sequence of the new sequence in L-selectin-v1 (residues 378–385) is further replaced with a new 10-amino acid sequence (residues 378–387). In contrast to the hydrophilic nature of the L-selectin-c cytoplasmic domain, the L-selectin-v1 and L-selectin-v2 cytoplasmic domains contain many hydrophobic amino acids, especially in the portion of the new sequence shared by L-selectin-v1 and L-selectin-v2 and in the unique carboxy-terminal tail of L-selectin-v2. An in silico analysis of the nucleotide sequence of the human L-selectin gene failed to detect an equivalent of the exon v1+v2, and RT-PCR using RNA from human thymus failed to detect a human ortholog of L-selectin-v1 or L-selectin-v2.

Expression of the Three mRNA Transcripts of Mouse L-selectin. A, schematic representation of the primers and probes used for quantitative RT-PCR. Primers and probes are shown in arrows and bold lines, respectively. B, expression of the three mRNA species in various tissues. C, expression of the three mRNA species in various cell types. In B and C, the amount of each transcript was normalized to 18 S RNA and is shown relative to the expression in placenta. D, expression of L-selectin-v1 and L-selectin-v2 relative to L-selectin-c in various cell types. The amount of each isoform was calculated using plasmid cDNA as a standard. The amount of the L-selectin-v1 and L-selectin-v2 transcript is expressed as a percentage of that of L-selectin-c in each cell type. Results in B–D represent one of three independent experiments and are expressed as the mean ± S.D. of triplicate wells.

The nucleotide sequences reported here have been submitted to the DNA Data Bank of Japan with accession numbers AB353767 and AB353768 for the mouse L-selectin-v1 and L-selectin-v2, respectively.
Novel Mouse L-selectin Isoforms

TABLE 1
Sequences of primers and probes used in quantitative real-time RT-PCR

Sequences in exon 8 are underlined, sequences in exon v1 are underlined with a bold line, and sequences in exon v2 are underlined with a dotted line.

| Isoform             | Forward primer                  | Reverse primer                        | Probe                      |
|---------------------|---------------------------------|---------------------------------------|----------------------------|
| L-selectin-c        | 5′-cattcctgtacggctatgg-3′       | 5′-tcttgagttcttctgtcatcc-3′           | 5′-cattcctgtacggctatgg-3′ |
| L-selectin-v1       | 5′-cattcctgtacggctatgg-3′       | 5′-tcttgagttcttctgtcatcc-3′           | 5′-cattcctgtacggctatgg-3′ |
| L-selectin-v2       | 5′-ggtttaaaaagatgtcggaccag-3′   | 5′-ggttaaaaagatgtcggaccag-3′          | 5′-ggttaaaaagatgtcggaccag-3′ |

L-selectin-v1 and L-selectin-v2 was ~2–3% that of L-selectin-c. Thus, the relative mRNA expression of L-selectin-v1 is higher in activated T cells and granulocytes than in unstimulated lymphocytes, while the relative L-selectin-v2 mRNA expression is higher in unstimulated lymphocytes than in activated T cells and granulocytes.

Distribution of L-selectin Isoforms on the Cell Surface—To examine the localization of L-selectin isoforms, L1.2 transfectants expressing L-selectin-c, L-selectin-v1, or L-selectin-v2 were prepared. The novel isoforms were expressed on the cell surface of these cells and were glycosylated with both N-linked and O-linked glycans, similarly to L-selectin-c (supplemental Fig. S1). L-selectin-c is known to be localized to the tips of microvilli. Immunogold staining with the anti-L-selectin mAb MEL-14 showed L-selectin-c was preferentially localized to microvilli (Fig. 3A). Similarly, L-selectin-v1 and L-selectin-v2 were also localized to microvilli (Fig. 3, B and C). No specific staining was observed in the control sample lacking the primary mAb MEL-14 (Fig. 3D). These results suggest that the carboxyterminal 11-amino acid sequence of L-selectin-c is not required for its localization to microvilli. Thus, the first 6-amino acid region of the cytoplasmic domain appears to be sufficient for the microvillar localization of L-selectin.

FIGURE 3. Localization of the three L-selectin isoforms on the cell surface. L1.2 cells stably expressing L-selectin-c (A and D), L-selectin-v1 (B), or L-selectin-v2 (C) were incubated with (A, B, and C) or without (D) the mAb MEL-14, followed by biotinylated anti-rat IgG and streptavidin-conjugated 15-nm colloidal gold particles. The three isoforms were all predominantly localized to microvilli.

Shedding of the Three L-selectin Isoforms—Because the cytoplasmic domain of L-selectin has been shown to be critical for L-selectin shedding, we next examined whether these isoforms differ in shedding efficiency. L1.2 cells stably expressing L-selectin-c, L-selectin-v1, or L-selectin-v2 were stimulated with PMA, and the remaining cell surface expression of L-selectin was examined by flow cytometry. As shown in Fig. 4A, the cell surface expression of L-selectin-c was decreased following PMA stimulation. The cell surface expression of L-selectin-c was decreased after PMA stimulation (Fig. 4A). Comparison of the geometric mean fluorescence intensity of the L-selectins showed that the PMA-induced decrease in the geometric mean fluorescence intensity of L-selectin-v1 was larger than that of L-selectin-c, while that of L-selectin-v2 was smaller (Fig. 4B). These results show that although all three isoforms are shed after PMA stimulation, they differ in their efficiency of shedding.

Rolling Behavior of L1.2 Cells Expressing the Three L-selectin Isoforms—We next investigated whether the novel isoforms...
could mediate cell rolling under flow conditions. When L1.2 cells expressing L-selectin-c, L-selectin-v1, or L-selectin-v2 were infused into capillary tubes coated with rPSGL-Ig under a shear stress of 1 dyn/cm², the cells rolled (Fig. 5A). This rolling was completely inhibited by the presence of EDTA (data not shown), verifying that it was calcium-dependent. The number of rolling cells was slightly decreased when L-selectin-v1-expressing cells were infused, compared with L-selectin-c-expressing cells, although the difference did not reach statistical significance (Fig. 5A). L-selectin-v2-expressing cells rolled in similar numbers as L-selectin-c-expressing cells and in slightly greater numbers than L-selectin-v1-expressing cells (Fig. 5A).

We next examined the rolling velocity of the cells expressing the three L-selectin isoforms. L1.2 cells expressing L-selectin-c or L-selectin-v1, or L-selectin-v2 were infused into capillary tubes coated with rPSGL-Ig under a shear stress of 1 dyn/cm², and the number of rolling cells was determined. Results are expressed as the mean ± S.E. of three independent experiments. B, average rolling velocity of L1.2 cells expressing each isoform. Results represent one of three independent experiments and are expressed as the mean ± S.E. of 200 cells for each isoform. C, cumulative histograms of the rolling velocities of L1.2 cells expressing each isoform. *, p < 0.05; ***, p < 0.001; n.s., not significant.

**DISCUSSION**

In this study, we identified novel isoforms of the mouse L-selectin gene, termed L-selectin-v1 and L-selectin-v2. These isoforms are generated by alternative splicing and differ from conventional L-selectin, L-selectin-c, only in their cytoplasmic tails. The mRNAs for these novel isoforms are present in the cells and tissues that express L-selectin-c. Functionally, both L-selectin-v1 and L-selectin-v2 underwent PMA-induced shedding, although L-selectin-v1 and L-selectin-v2 were shed to a greater and lesser degree, respectively, than L-selectin-c. In addition, both L-selectin-v1 and L-selectin-v2 mediated faster cell rolling compared with L-selectin-c under flow conditions. We also showed that ligation of L-selectin-v1, but not L-selectin-v2, induced p38 MAPK phosphorylation.

Alternative splicing is a widespread mechanism for generating protein diversity. Although human P-selectin and rat E-selectin are known to have splice variants, no alternative splicing
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events have been reported for L-selectin from any species; thus, this is the first demonstration of alternative splicing events of the L-selectin gene. The L-selectin gene comprises 9 exons and locates on chromosome 1, region q23–25 and H2.2 in humans and mice, respectively. The splice variants we identified here contain the new exon v1 or v1 + v2 located between exons 7 and 8. As the cytoplasmic domain of L-selectin spans from exon 7 through exon 9, the insertion of the new sequences resulted in the alteration in the amino acid sequence of the cytoplasmic domain. The selectin splice variants identified previously either exist as a soluble form or differ in the extracellular domain (21–23). Thus, the L-selectin splice variants are unique among selectin variants in that they differ in the cytoplasmic domain. Because the cytoplasmic domain of L-selectin has been implicated in various functions, these variants should serve as valuable tools to elucidate the role of this domain.

Quantitative RT-PCR analyses showed that the mRNA species for these novel isoforms were present in the cells and tissues that express L-selectin-c, although the amount of each was <5% of the amount of L-selectin-c mRNA. It has been shown that alternative splicing events of numerous molecules show changes following cell activation (29). Thus, we examined the mRNA expression of the novel isoforms in activated T cells such as Th1 and Th2 cells. There was a tendency for the L-selectin-v1 mRNA to be up-regulated in Th1 cells compared with unstimulated lymphocytes and for the L-selectin-v2 mRNA to be down-regulated relative to the L-selectin-c mRNA. It would be interesting to find situations or diseases where the expression of these novel variants is altered.

Our data showed that all three isoforms were localized similarly to microvilli, suggesting that the carboxy-terminal 11-amino acid region of the cytoplasmic domain of L-selectin-c is not required for microvillar localization. This finding is in agreement with a previous report showing that a deletion mutant of human L-selectin lacking the carboxy-terminal 11 amino acids localizes normally to microvilli (11). Another study identified Arg<sup>357</sup> and Lys<sup>362</sup> of the human L-selectin cytoplasmic domain as critical residues for binding to ERM proteins and microvillar positioning (30). However, L-selectin-v2 was unable to induce p38 MAPK phosphorylation, suggesting that the unique cytoplasmic tail of L-selectin-c, as well as the unique tail of L-selectin-v1, is involved in the interaction with α-actinin and the regulation of cell rolling.

The L-selectin cytoplasmic domain is also involved in signal transduction. Previous studies showed that ligation of human L-selectin with Abs or sulfated polysaccharide ligands stimulates intracellular signaling, including MAPK phosphorylation. We showed that ligation of L-selectin-c and L-selectin-v1 also induced p38 MAPK phosphorylation. Interestingly, L-selectin-v2 was unable to induce p38 MAPK phosphorylation, suggesting that the unique cytoplasmic tail of L-selectin-c, as well as the unique tail of L-selectin-v1, is involved in the induction of p38 MAPK phosphorylation. In this regard, it is interesting to note that the unique cytoplasmic tails of L-selectin-c and L-selectin-v1 have serine residues that can be phosphorylated, Ser<sup>364</sup> and Ser<sup>381</sup>, respectively, as predicted by the NetPhos server (34), whereas L-selectin-v2 has no potential sites of phosphorylation in its cytoplasmic tail. Future studies are required to clarify the phosphorylation patterns of the three isoforms and to assess whether L-selectin phosphorylation is linked to downstream signaling events, including MAPK phosphorylation.

In conclusion, our study shows for the first time alternatively spliced events of the L-selectin gene. L-selectin mediates not only the homing of lymphocytes to secondary lymphoid organs but also the recruitment of leukocytes to inflamed tissues in various chronic inflammatory conditions, such as rheumatoid arthritis, multiple sclerosis, thyroiditis, and inflammatory bowel diseases, as well as to acutely inflamed sites. Thus, regulation of L-selectin function may be a way to regulate leukocyte infiltration at these sites. The three isoforms identified in this study should serve as tools to clarify the functions of L-selectin and the roles of its cytoplasmic region, as well as the regulation of L-selectin-mediated leukocyte trafficking.
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