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L-selectin: mechanisms and physiological significance of ectodomain cleavage

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Abstract

L-selectin is a cell adhesion molecule consisting of a large, highly glycosylated, extracellular domain, a single spanning transmembrane domain and a small cytoplasmic tail. It is expressed on most leukocytes and is involved in their rolling on inflamed vascular endothelium prior to firm adhesion and transmigration. It is also required for the constitutive trafficking of lymphocytes through secondary lymphoid organs. Like most adhesion molecules, L-selectin function is regulated by a variety of mechanisms including gene transcription, post-translational modifications, association with the actin cytoskeleton, and topographic distribution. In addition, it is rapidly downregulated by proteolytic cleavage near the cell surface by ADAM-17 (TACE) and at least one other "sheddase". This process of "ectodomain shedding" results in the release of most of the extracellular portion of L-selectin from the cell surface while retaining the cytoplasmic, transmembrane, and eleven amino acids of the extracellular domain on the cell. This review will examine the mechanism(s) of L-selectin ectodomain shedding and discuss the physiological implications.

Keywords: L-selectin - ectodomain shedding - cell adhesion molecules - leukocytes - TACE

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Discovery of ectodomain shedding of L-selectin

In 1989, it was found that treatment of neutrophils with phorbol ester or chemotactic factors (Complement factor C5a or leukotriene B4 (LTB4)) resulted in the downregulation of L-selectin (Mel-14 antigen) surface expression within minutes and a corresponding inability of these neutrophils to migrate effectively into sites of inflammation [1, 2]. Accompanying the reduced surface expression was a corresponding increase in soluble L-selectin (sL-sel) in the conditioned media (Fig. 1). Since chemotactic factors have other effects unrelated to L-selectin shedding, neutrophils were treated with low doses of several different proteases in an attempt to specifically cleave L-selectin without stimulating neutrophil activation. While most of the proteases tested had little effect, chymotrypsin produced a specific decrease in surface expression of L-selectin while having no effect on the expression of four other surface antigens, most notably the 2 integrin Mac-1 (CD11b/CD18) [3]. Along with the decrease in L-selectin expression, there was a corresponding

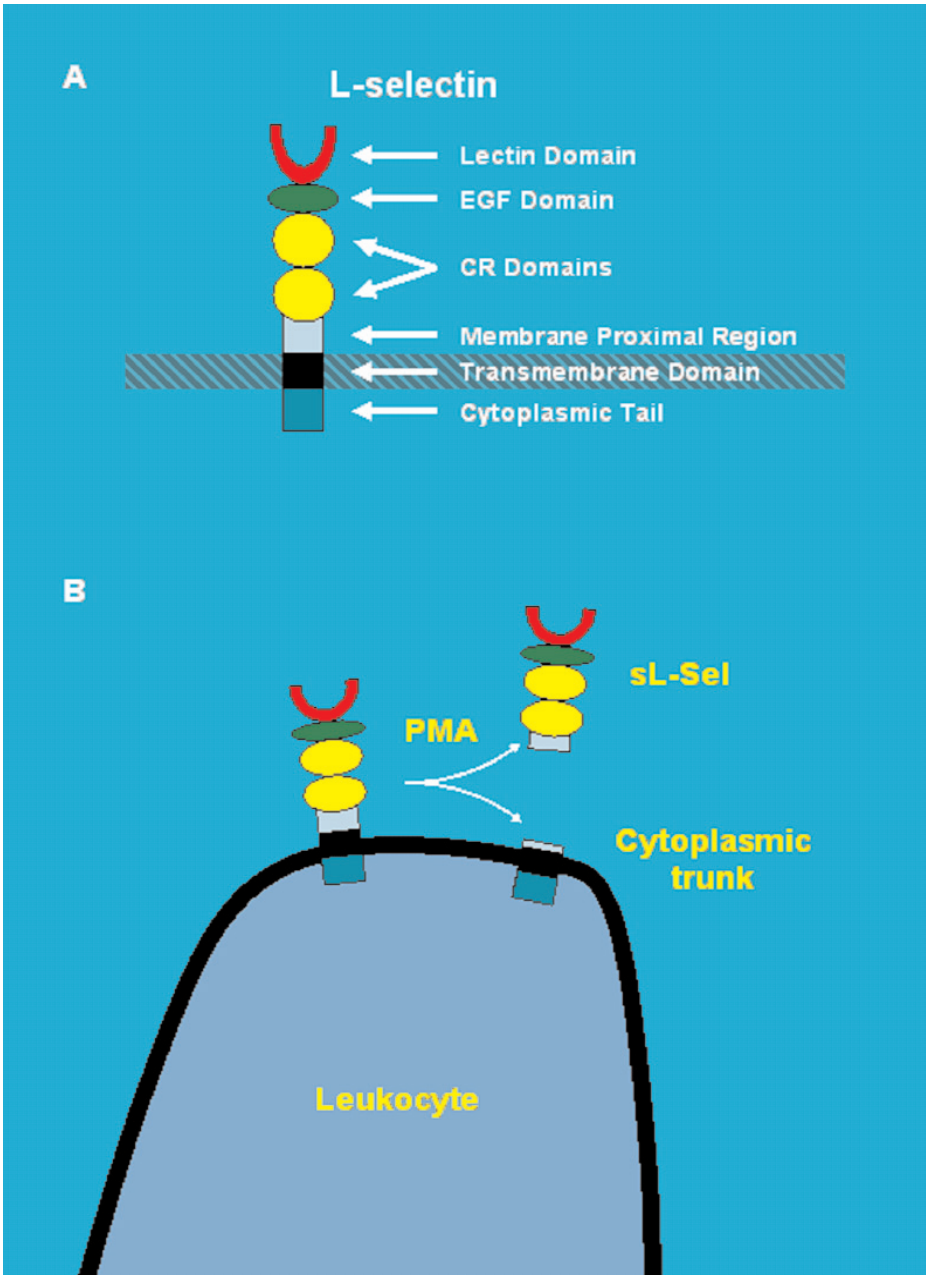

Fig. 1 Structure of L-selectin and the shedding of L-selectin from microvilli of leukocytes. A) Structure of Human L-selectin, composed of an N-terminal lectin domain, an epidermal growth factor (EGF) domain, two consensus repeats (CR) with homology to complement regulatory proteins, a transmembrane domain, an a cytoplasmic tail. B) L-selectin shedding from leukocytes following PMA treatement. L-selectin is cleaved in the membrane proximal region.

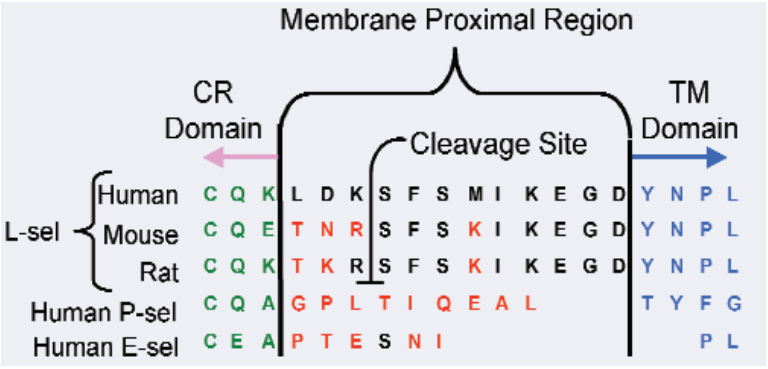

Fig. 2 Structure of L-selectin and the shedding of L-selectin from microvilli of leukocytes. A) Structure of Human L-selectin, composed of an N-terminal lectin domain, an epidermal growth factor (EGF) domain, two consensus repeats (CR) with homology to complement regulatory proteins, a transmembrane domain, an a cytoplasmic tail. B) L-selectin shedding from leukocytes following PMA treatement. L-selectin is cleaved in the membrane proximal region.

inability of the lymphocytes to bind to high endothelial venules in peripheral lymph nodes using an *ex vivo* model and neutrophils to home to an inflamed peritoneum using an *in vivo* model. The results of these studies indicated that L-selectin could be cleaved from the surface of neutrophils and this alters the ability of these cells to interact with high endothelial venules and migrate into inflamed tissues.

Similar to neutrophils, L-selectin is also cleaved from the surface of lymphocytes in response to a variety of stimuli. Jung and Dailey treated lymphocytes with phorbol ester and observed that L-selectin expression decreases on the cell surface and soluble L-selectin (sL-sel) can be immunoprecipitated from the conditioned media [4]. The sL-sel was found to be 12 KDa smaller than that bound form on the surface. These results appear to parallel those for neutrophils with two distinctions. First, while almost complete shedding of L-selectin on neutrophils occurs within the first five minutes, similar decreases in surface expression in lymphocytes take over 30 min [3, 4]. Second, since the extracellular domain of L-selectin on neutrophils is more highly glycosylated [5, 6], it results in a larger molecular weight complex being released into the supernatant.

Enzymatic cleavage of L-selectin shedding by TNF- α converting enzyme (TACE)

Once it was clearly established that L-selectin was shed from the surface of neutrophils and lymphocytes, it took several years before there was any significant advancement in our understanding of the enzyme(s) involved with this process. It was known that certain exogenous proteases, such as chymotrypsin, stromelysin, and collagenase could cleave L-selectin, but these did not appear to be the physiological mediator for a number of reasons, as originally outlined by Ager and coworkers [7]. First, these proteases are generally regulated transcriptionally following treatment with phorbol ester and no known mechanism existed to explain their rapid (< 5 min.) activation which would be required if they were the L-selectin sheddase [7]. Second, the activation-induced L-selectin sheddase operated only on the stimulated cells and not on adjacent, non-stimulated, cells [7]. This is referred to as operating in the cis, not in the trans, position and implies that a membrane associated factor or protease is involved. Third, the protease inhibitor profile did not appear to match any known protease [7]. In particular, TIMP-1, a natural inhibitor of all known matrix metalloproteinases, the most likely class of proteases involved with this process, had no effect on L-selectin shedding. These results indicated that the sheddase was not one of the typical proteases associated with the extracellular degradation of proteins.

Starting in 1995, there were several important papers which began to shed light on the nature of the sheddase. First, the exact cleavage site of human L-selectin was determined to be between Lys^{321} and Ser^{322} following phorbol ester treatment of COS cells transfected with the cDNA for L-selectin (Fig. 2) [8]. This was quickly followed by three groups modifying the cleavage site to determine the sequence specificity of the sheddase [9–11]. They all reported a very relaxed sequence specificity. The length of the membrane proximal region appeared to be much more important than the specific amino acid sequence. Altering any of

the amino acids in the membrane proximal region had little effect on shedding except if proline was inserted, suggesting that primary structure was of little importance. Deletion of several amino acids in this region essentially eliminated shedding, at least in response to PMA [12]. Stoddart and coworkers transfected L1-2 cells, a pre-B-cell line, with an L-selectin mutant which contained a 9 amino acid deletion (321.9) [9]. This mutant was resistant to cleavage in response to PMA, as predicted by the previous studies. However, it was still shed following cross-linking of L-selectin using antibodies, suggesting that more than one L-selectin shedding mechanism may exist. They went further and demonstrated that cross-linking induced shedding was resistant to staurosporine, a PKC inhibitor, while PMA induced shedding was inhibited. These results suggest that more than one mechanism exists for L-selectin shedding.

In 1997, two groups independently isolated and cloned a member of the A Disintegrin and Metalloprotease (ADAM) family, designated ADAM17 [13, 14]. This protease shed the TNF-α precursor from the surface to produce the active cytokine and is also referred to as TNF- α converting enzyme (TACE). While a number of ADAMs have been identified in mammalian tissues, this was first to have a known function. Peschon, *et al.*, [15] created mice with a targeted mutation in TACE which deletes the Zn^{2+} binding domain and inactivates the protease. While these mice were not viable, cells from the homozygous ($tace^{\Delta Zn/\Delta Zn}$) embryos failed to efficiently release TNF- α. They examined the shedding of a number of proteins and found that PMA-induced shedding of L-selectin from thymocytes of these mice was dramatically reduced. This study clearly demonstrated TACE is responsible for L-selectin shedding at least in mouse thymocytes following PMA stimulation.

Other L-selectin sheddases

While there is substantial evidence demonstrating that TACE is the protease responsible for the shedding of L-selectin from the surface of thymocytes following activation with PMA, this does not eliminate the possibility that other proteases also play a role under a variety of conditions. As discussed above, shedding due to cross linking of L-selectin by antibodies does not have the same structural requirements suggesting that other proteases may be involved. While mutating L-selectin by eliminating 9 amino acids prevented its cleavage due to PMA, it had little effect on cleavage due to cross-linking using an antibody to the extracellular domain (DREG 200) [9]. In addition, proline substitution at the P2' or P3' (2 or 3 amino acids downstream from the cutsite) completely blocks phorbol ester induced cleavage but had no effect on basal shedding [16]. Interestingly, deletion of the epidermal growth factor (EGF) domain also eliminates PMA-induced shedding, suggesting that the extracellular domains of L-selectin and TACE may interact. Walcheck and associates recently extended these studies to look at L-selectin shedding in fibroblasts deficient in TACE and reconstituted with TACE retroviral transfection [17]. While TACE-reconstituted fibroblasts shed approximately three times more soluble L-selectin than fibroblasts from TACE-deficient animals, there was still significant shedding from these TACE-deficient fibroblasts. Of this, there appears to be a small fraction (19%) that is not inhibited by a protease inhibitor (KD-IX-73-4, 50 μg/ml), suggesting that there may be more than one additional sheddase. While this provides evidence that other proteases may play a role, it supports the hypothesis that TACE is the major L-selectin sheddase following PMA stimulation.

The importance of TACE in L-selectin shedding under physiological conditions remains unclear. While TACE appears to be essential for the shedding of tumor necrosis factor (TNF- α), it has also been implicated in the cell surface shedding of over thirty other proteins (Table 1) and more are being added to this list. While most of these studies suggest TACE is required, they do not necessarily show that TACE is the actual sheddase. TACE's role as a sheddase is also surprising since it is predominantly localized in the perinuclear compartments, similar to TNF- α [18]. In the original report implicating L-selectin cleavage as a TACE-dependent mechanism [15], TACE cleaves a peptide corresponding to the TNF- α cut site approximately 2250 times more efficiently than it cut a peptide corresponding to the L-selectin cut site. Mohan and coworkers did side-by-side comparisons of peptides corresponding to the cleavage site of a number of

Table 1 Proteins in which evidence exists to indicate that TACE is either the sheddase or required for shedding

Protein	Abreviation	Ref.
Amphiregulin	AR	[53]
Amyloid-beta precursor protein	APP	[54]
AXL Receptor Tyrosine Kinase	AXLr	[55]
CD30	CD30	[56]
CD40	CD40	[57]
CD117 (cKIT)	CD117	[58]
Cellular prion protein	PrP	[59]
Epiregulin		[60]
Fractalkine (CX3CL1)	CX3CL1	[61]
glycoprotein GP of Ebola virus	EBOV GP	[62]
Glycoprotein IB α	GP IB α	[63]
Glycoprotein V	GP V	[64]
Growth hormone receptor	GHR	[65]
Heparin binding epidermal growth factor	proHB-EGF	[60]
Human Epidermal Growth Factor Receptor 4	HER4	[66]
Human Meprin		[67]
IL-6 Receptor α	IL-6r α	[68]
Interleukin-1 receptor 2	IL-1 R2	[55]
interleukin-15 receptor α	IL-15R α	[69]
Low Density Lipoprotein Receptor	LDLr	[55]
L-selectin	L-sel	[15]
Macrophage Colony Stimulating Factor Recept	MCSF-R	[70]
Mucin 1	MUC1	[71]
Neuregulins		[72]
Neurogenic locus notch homolog protein	Notch	[73]
P75 neurotrophin receptor	p75NTR	[74]
Sortilin-related receptor	SorLA	[55]
Transforming Growth Factor α	TGF α	[15]
TNF-related activation-induced cytokine	TRANCE	[19]
Transforming Tyrosine Kinase Protein	TrkA	[75]
Tumor Necrosis Factor α	TNF α	[13]
Tumor Necrosis Factor Receptor I	TNFR-I	[15]
Tumor Necrosis Factor Receptor II	TNFR-II	[76]
Vascular Cell Adhesion Molecule 1	VCAM-1	[77]

hypothesized TACE substrates and found that only the TNF- α substrate is processed under their experimental conditions [19]. TACE exhibited little or no processing of any of the other peptides examined. It is possible that TACE, rather than cleaving L-selectin directly, is required to activate other proteases in a cascade-like fashion.

There are also questions about why all these proteins and possibly many others would be shed by the same protease. Mohen and associates had suggested that the presence of additional factors may play a role in alternate substrate presentation [19]. Cui *et al.*, identified ARTS-1 (aminopeptidase regulator of TNFR1 shedding-1) as an additional factor regulating the shedding of TNF receptor 1 (TNFR1), Interleukin 6 receptor (IL-6 R), and Type II IL-1 Decoy [20–22]. Whether this protein or others are involved with L-selectin shedding is currently not known. There is solid evidence that TACE is required for efficient L-selectin shedding. However, there may be other sheddase and/or additional factors involved with L-selectin shedding that have yet to be fully examined. In addition, the sheddase/cofactors used may be dependent on the type of activation.

Agents inducing L-selectin shedding

Two agents which elicit L-selectin shedding on both neutrophils and lymphocytes are phorbol esters and antibodies which crosslink adjacent L-selectin molecules on the surface of leukocytes. Others, such as synthetic sulfonated glycoproteins containing multivalent ligands presumably operate via the same mechanism as the crosslinking antibodies [23]. In addition, there are a number of other agents which induce L-selectin shedding, including LPS, both hypertonic and hypotonic shock, and chemotactic factors [2, 24, 25]. There are others that induce shedding of L-selectin without general cell activation which provide important information about this process. For example, Diaz-González and associates have investigated the nonsteroidal anti-inflammatory drugs' (NSAIDs) ability to shed L-selectin without affecting neutrophil viability, activation, or expression levels of other surface molecules [26–28]. Their initial report demonstrated in an *in vitro* system that NSAIDs prevented neutrophil attachment to endothelial cells due to shedding of L-selectin [26]. In addition, certain diphenylamine-based NSAIDs' were more effective at inducing shedding than other NSAID, especially if the diphenylamine group contained a carboxylic acid [28]. They hypothesize that this may have significant implications and may explain some of the differences observed clinically between various NSAIDs. These compounds also decreased neutrophil ATP concentrations that correlated with their ability to induce shedding. NSAID-induced down-regulation of L-selectin occurs by a TACE dependent mechanism, based on studies using a TACE-deficient murine monocytic cell line [28].

Bennett *et. al.* reported that thiol-oxidizing or -blocking reagents promoted L-selectin shedding while reducing agents inhibited it [29]. From these results, they hypothesized that regulatory molecules capable of forming and rearranging disulfide bonds, such as protein disulfide isomerase (PDI), could regulate this process. While this hypothesis has not been fully explored, PDI is expressed on the cell surface of neutrophils and altering its function using inhibitors and antibodies resulted in L-selectin shedding. This is supported by evidence that other domains of the L-selectin molecule, including the EGF domain [16], are involved in regulating shedding.

Role of the cytoplasmatic tail in L-selectin shedding

As discussed above, various regions of the L-selectin molecule appear to be important in its shedding. The cytoplasmic tail has received considerable attention. Zhao and associates have simply truncated the cytoplasmic tail and found that PMA-induced shedding was reduced from 88% to 44% [16]. Kishimoto and associates examined three different antibodies to the fragment of the L-selectin retained by the cell following shedding (Fig. 3) [30, 31]. JK924 recognized the remaining approximately 10 amino acid fragment of the extracellular portion, JK564 recognized the entire cytoplasmic domain, and CA21 recognized the C-terminal 8 amino acids of the cytoplasmic tail. They found that a 17 KDa protein coprecipitated with L-selectin when they used either JK924 or CA21, but not

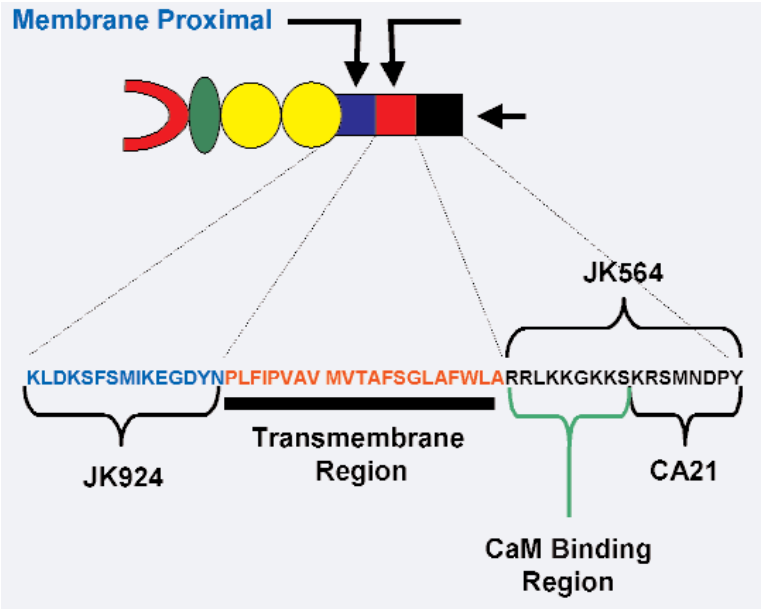

Fig. 3 Specificity of Anti-L-selectin Antibodies. The membrane proximal, transmembrane, and cytoplasmic domains of L-selectin are enlarged to show the specificity of three anti-L-selectin antibodies. Since both JK924 serum and CA21 MAb did not block the binding site, but JK564 serum did, Calmodulin must bind in the region depicted [31].

when they used JK564, which suggested that the unknown 17 KDa protein bound to the membrane proximal region of the cytoplasmic tail. Based on the unusual observation that this 17 KDa protein was not retained by PVDF membranes following immunotransfer, they suspected and later confirmed that it was calmodulin. They then blocked this interaction using calmodulin inhibitors and found a dramatic increase in L-selectin shedding in less than five minutes. Calmodulin is constitutively bound to the cytoplasmic tail of L-selectin and when it is removed, L-selectin is shed suggesting a general pathway for L-selectin shedding.

Ivetic *et al.* [32], recently created an affinity column using a peptide corresponding to the 17 amino acid cytoplasmic tail of L-selectin. They then applied either untreated or PMA-treated lymphocyte cell extracts and determined which proteins bound to the column. While ezrin was found adhering to the column following addition of either unstimulated or stimulated lymphocytes, moesin from only stimulated lymphocytes bound to the column. Erzin and moesin are members of the Ezrin-Radixin-Moesin (ERM) family of proteins which are believed to be important in the interaction between actin and the cell membrane in participate in the formation of microvilli. Whether this family is directly involved in L-selectin shedding is still being investigated.

Since it is well established that L-selectin is selectively localized on the tips of microvilli, two groups have independently investigated whether its topographic distribution plays a role in its shedding. One group transfected either wild type L-selectin, or chimeric molecules consisting of the ectodomain of L-selectin connected to the transmembrane and intracellar domains of CD44 or CD31 into murine L1-2 cells [33]. Unlike the wild type L-selectin, the L-selectin-CD44 and the L-selectin-CD31 are excluded from the microvilli and randomly distributed, respectively. All three are shed to similar degrees following PMA treatment suggesting that subcellular localization is not important for PMA-induced L-selectin shedding. As expected, the calmodulin inhibitor, trifluoperazine, was much more effective at inducing wild type L-selectin shedding than either of the other two constructs [33]. At high concentrations, trifluoperazine did induce shedding suggesting that at least two mechanisms were involved with calmodulin inhibitor-induced shedding.

Another group examined shedding in Jurkat cells following crosslinking of L-selectin using antibodies [34]. They found that crosslinking enhanced the percentage of the L-selectin in lipid rafts and this L-selectin was exclusively tyrosin phosphorylated. Using various inhibitors, they provided evidence to indicate that this process was independent of $p56^{lck}$ tyrosine kinase activity, but required other tyrosine kinases and the neutral sphingomyelinase. This suggests that the mechanisms behind shedding induced by different agents are different.

Functional implications of L-selectin shedding

Once it was clear that L-selectin shedding occurred, investigators began trying to decipher its implications. Kishimoto and associates were the first to report on inhibitors which prevented this shedding. They found that a hydroxamic acid-based peptide inhibitor of matrix metalloprotease (KD-IX-73-4) inhibited the downregulation of L-selectin following either fMLP (formyl-methionylleucylpheylalanine) or phorbol ester but had no effect on neutrophil activation [35, 36]. This group went on to demonstrate that this inhibitor reduced neutrophil rolling velocities on immobilized peripheral lymph node vascular addressin substrates from human tonsils under hydrodynamic flow resulting in increased neutrophil accumulation. Allport and associates, using another hydroxamic acid-based peptide inhibitor, Ro 31-9790, reported that L-selectin shedding did not affect neutrophil rolling, adherence, or transmigration on TNF- α activated human vascular endothelial cells [37]. These studies were extended to an in vivo model of leukocyte rolling in mouse cremaster venules [38, 39]. KD-IX-73-3 did not alter rolling velocity of leukocytes after treatment (2.5 to 3 hours) with TNF- α. However, this inhibitor did decrease the rolling velocity in both untreated wild-type mice and TNF- α treated E-selectin-deficient mice. Additional studies using E- and P-selectin knockout mice and L-selectin conjugated to microbeads indicate that inhibition of L-selectin shedding prevent the "jerkiness" (variability in velocity over time) of leukocyte rolling. These studies, using synthetic inhibitors of L-selectin shedding, indicate that L-selectin shedding participates in regulating neutrophil rolling.

For leukocytes to firmly adhere and transmigrate into tissues, they must be activated. A number of groups have demonstrated that L-selectin can participate in this activation [40–44]. In one study, antibodies were used to crosslink L-selectin on neutrophils and several indicators of activation were monitored. This crosslinking altered the neutrophil's ability to deform and enhanced β_2 integrin activation. Shedding of L-selectin limits this activation and thus may limit inflammation [39].

A third mechanism by which L-selectin shedding may regulate inflammation has been proposed. Plasma of healthy humans and mice contains approximately 1.6 μg/ml of soluble L-selectin [45]. This is an extremely high level, especially considering that the lectin domain, by itself, has been shown to inhibit leukocyte rolling and migration in vivo [46–49]. The level of soluble L-selectin is very consistent between several mouse strains even though L-selectin levels on their leukocytes vary by 2.5 fold suggesting that soluble L-selectin levels are controlled [45]. At a concentration of 0.9 μg/ml, soluble L-selectin reduced lymphocyte migration to peripheral lymph nodes by over 30% indicating that soluble L-selectin, presumably derived from shedding, regulates normal lymphocyte trafficking and possibly the inflammatory response. Intravenous injection of LPS resulted in septis and shedding of L-selectin from both neutrophils and lymphocytes. However, soluble L-selectin was not elevated versus injection of vehicle. Even though this study failed to demonstrate soluble L-selectin levels were elevated in inflammatory conditions, there are clinical studies which suggest that soluble L-selectin levels are elevated during sepsis [50].

Transgenic mice expressing shedding-resistant L-selectin

Recently, two papers report the creation of transgenic mice with shedding-resistant L-selectin. In the first, the membrane proximal region of the gene was replaced with the analogous region of P-selectin producing the LΔP mouse [51]. Trangenic mice expressing either LΔP or wild type L-selectin under the human CD2 promoter were crossed with L-selectin knockout (L-sel$^{+/-}$) mice generating mice that expressed either shedding resistant (LΔP$^{T\text{-cell}}$) or wild type (WT$^{T\text{-cell}}$) L-selectin only on T-cells. This mutation appears to prevent both PMA-induced and basal shedding. While there was still a small amount of L-selectin in the plasma of LΔP$^{T\text{-cell}}$ mice, it was less than 5% of that of WT$^{T\text{-cell}}$ and actual wild type (C57BL/6) mice and may represent L-selectin contained within microparticles generated from evagination of the cellular membrane and not a cleavage product. The levels of L-selectin in the plasma of the WT$^{T\text{-cell}}$ were about 70% of the actual wild type mice suggesting that a majority of the shed protein is from lymphocytes. The cellularity and composition of

secondary lymphoid organs were not altered in L PT-cell mice versus those expressing $WT^{T\text{-}cell}$-L-selectin. Two functional differences were noted between T-cells from the $WT^{T\text{-}cell}$ and the $L\Delta P^{T\text{-}cell}$ mice. First, T-cells from the $L\Delta P^{T\text{-}cell}$ mice transmigrated high endothelial venules (HEV) more slowly resulting in a consistently lower number of T-cells outside the HEV. Second, since T-cells normally shed their L-selectin following TCR engagement, once activated, they lose their ability to reenter the PLN. In the $L\Delta P^{T\text{-}cell}$ mice, L-selectin was retained on the surface of activated T-lymphocytes and these cells were still able to enter the lymph nodes. These results suggest that the main function of L-selectin shedding on T-cells is to prevent these cells from reentering the PLN once activated.

In the second paper, several knock-in mice were generated [52]. The first, denoted L, had seven amino acids deleted from the membrane proximal region and knocked into the L-selectin locus. This form of L-selectin was undetectable on leukocytes but was increased two fold in the plasma suggesting that it was readily cleaved. Another, denoted L(E), replaced 7 amino acids from wild type L-selectin with 7 amino acids from E-selectin in the membrane proximal domain and was overexpressed in blood cells (168%), spleen (214%), and PLN (178%) but significantly less was in the plasma versus wild type mice. To produce a mouse with approximately the same amount of L-selectin on various cell types as wild type mice, the L(E) mice were crossed with the LΔ mice producing the $L(E)^{same}$ mice which resulted in roughly equivalent levels of L-selectin on cells of the blood, spleen, and PLN. Lympophocytes from these mice did not shed L-selectin in response to either PMA or calmodulin inhibitors. Lymphocytes from wt, L(E), and $L(E)^{same}$ mice were labeled and injected into wild type mice. Lymphocyte migration into the PLN, mesenteric lymph node (MLN) and Peyer's patch (PP) were examined at one and 48 hours. At one hour, mice expressing the shedding resistant L-selectin had approximately a 33% decrease in labeled lymphocytes in these tissues and an increase in the blood. After 48 hrs, these differences disappeared. In in vitro experiments, L(E) and wild type lymphocytes had similar rolling properties over a wide range of shear rates, while $L(E)^{same}$ lympocytes rolled faster and had lower rolling capacity. While the ability of lymphocytes to shed L-selectin did slightly alter its function in unstimulated lymphocytes, as in the previous study, the major differences appear following lymphocyte activation. Following CD3-induced T-cell activation *in vitro*, there was a dramatic decrease in L-selectin surface expression on wild type but not $L(E)^{same}$ mice. However, by three days, cell surface levels of L-selectin were similar, implying that shedding controls L-selectin expression shortly following activation, but eventually transcription regulation controls this process. Using activated labeled T-cells, the authors found that T-cells from L-selectin shedding resistant mice were about four times more likely to enter the PLN than T-cells of wild type mice. Similar to the results in the previous paper [51], L-selectin ectodomain shedding alters the migration of activated lymphocytes.

In the knockout mice, all cells normally expressing wild type L-selectin express shedding resistant L-selectin, which allows one to examine the importance of L-selectin shedding on other cell types, most notably neutrophils [52]. On wild type neutrophils, most of the cell surface expression of L-selectin is lost following entry into an inflamed peritoneum, but L-selectin is retained on neutrophils from the $L(E)^{same}$ mice. Neutrophil interactions with inflamed vascular endothelium were not altered in $L(E)^{same}$ mice, but lack of shedding prevented the leukocytes, presumably mostly neutrophils, from migrating into the tissue following activation with keratinocyte-derived cytokine (KC).

Conclusion

L-selectin downregulation by ectodomain shedding has been the subject of intense study. While it appears that TACE is at least one of the protease needed for L-selectin shedding following PMA stimulation, it is unclear how important this sheddase is under more physiological conditions. Other sheddases and accessory molecules are probably involved. L-selectin shedding has a role in minimizing reentry of T-cells into PLN following activation and limits neutrophil adhesion at sites of inflammation. However, mice lacking the ability to shed L-selectin on neutrophils show reduced neutrophil migration to inflammatory chemokines, suggesting that L-selectin shedding is required for efficient transendothelial migration.

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