E-selectin is an activation-dependent, endothelial cell-restricted adhesion molecule that is internalized and degraded rapidly once expressed on the cell surface. Tyrosine-containing structural motifs play an important role in the internalization of a number of integral proteins, and the membrane-proximal E-selectin cytoplasmic tyrosine residue (Tyr582) conforms to the endocytosis motif proposed previously. To determine the endocytosis motif in E-selectin, we selectively introduced truncation, substitution, and deletion mutations to the cytoplasmic tail of E-selectin. We analyzed the internalization kinetics of surface-expressed wild-type and mutant E-selectin constructs in transiently transfected Chinese hamster ovary cells using 125I-labeled E-selectin monoclonal antibody (125I-P6E2) in an acid elution assay. Interestingly, truncation immediately membrane proximal to Tyr582 (ΔDGS construct) did not alter internalization kinetics significantly (ΔDGS versus wild-type, mean surface half-life = 42 versus 45 min, respectively). Thus, it appears that the tyrosine residues are not required for internalization of E-selectin. Additional analyses indicated that Ser381 was necessary but alone was insufficient for surface E-selectin endocytosis. Thus, we conclude that there exists a novel non-tyrosine-containing endocytosis signal in the cytoplasmic tail which involves Ser381 and residues membrane-proximal to it.

E-selectin (CD62E, ELAM-1) is a type I integral membrane protein restricted to activated endothelial cells with a defined time course of surface expression. It plays an important role in mediating leukocyte rolling (1–4), the first step of a cascade of leukocyte-endothelial cell adhesive interactions which leads to recruitment of neutrophils, eosinophils, basophils, monocytes, and subsets of memory T lymphocytes to sites of tissue inflammation. After stimulation with tumor necrosis factor-α, interleukin-1, or lipopolysaccharide, E-selectin expression in vivo typically peaks at 4–6 h and returns to baseline by 24 h (5). Under certain conditions, the expression of E-selectin is prolonged (6–8). The specific mechanisms involved in regulating E-selectin surface expression have not been elucidated fully.

Human vascular cell adhesion molecule-1 (VCAM-1), another type I integral membrane protein expressed on activated endothelium, is shed from the cell surface (9, 10) and does not undergo rapid internalization (11). This shedding process probably occurs as a result of metalloprotease cleavage of surface VCAM-1 at the juxtanuclear region of the extracellular domain (12). In contrast, most of the surface-expressed E-selectin undergoes efficient endocytosis and is degraded rapidly in the lysosomal compartment (11, 13–15). It is shed only slowly and in minute quantities in vitro (9, 10, 16). E-selectin internalization can be partially inhibited by the nonspecific protein kinase inhibitor staurosporine but not by inhibitors of protein kinase C, cAMP-dependent protein kinase A, or protein tyrosine kinase (11). The signal motif, presumably located within the cytoplasmic domain, mediating E-selectin endocytosis has not been determined.

Tyrosine-containing cytoplasmic motifs have been reported to mediate rapid endocytosis of a number of integral proteins. The NPXY motif in the low density lipoprotein receptor is a well defined example (17). A mutation of that tyrosine residue results in significant impairment of cellular low density lipoprotein cholesterol uptake and the phenotype of familial hypercholesterolemia in patients (18). These tyrosine-containing motifs have been postulated to assume a tight reverse turn structural conformation (19–22) that is recognized by cytoplasmic adaptor complexes that in turn mediate clathrin-coated pit localization and subsequent efficient endocytosis (23, 24). Although one study questioned the requirement for the proposed reverse turn structural conformation of the tyrosine-containing motifs (25), the medium chain subunits (μ1, also known as AP47, and μ2, also known as AP50) of clathrin-associated adaptor protein complexes (AP1 and AP2) have been shown to interact with specific tyrosine-containing motifs using yeast two-hybrid systems (26, 27). Phosphorylation of the critical tyrosine residue may not be a requisite event before internalization (27, 28). There are also endocytosis motifs that do not involve tyrosine, such as the di-leucine (29–31) and di-lysine (32) sequences. Depending on the particular molecule, phosphorylation of specific cytoplasmic serine residues membrane-proximal to the di-leucine motifs may (33, 34) or may not (31) be required for efficient endocytosis. Because the medium chains of either of the AP complexes failed to interact with a di-leucine motif studied (26), other subunits of the AP complexes or novel homologs might recognize and interact specifically with this motif. Alternatively, there are diverse molecular endocytic mechanisms by which a variety of signal motifs are recognized and processed (35, 36).

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1 The abbreviations used are: VCAM-1, vascular cell adhesion molecule-1; AP, adaptor protein; PCR, polymerase chain reaction; WT, wild-type; CHO, Chinese hamster ovary; mAb, monoclonal antibody; PBS, phosphate-buffered saline; HUVEC, human umbilical vascular endothelial cells; PMN, polymorphonuclear leukocyte.
Endocytosis Signals in the Cytoplasmic Domain of E-selectin

**A**

| Species       | Sequence (32 amino acid residues) | COOH-terminal sequence |
|---------------|-----------------------------------|------------------------|
| Human         | RKCLRK.AKKFPASSCQS.LESDSGYQKPSYI   | -cooh                  |
| Dog           | LKLRLRKKAK joven PQCQVYKPSYI       | -cooh                  |
| Mouse         | MYTMKKAK joven PQCQVYKPSYI         | -cooh                  |
| Rat           | KEPKKAK joven PQCQVYKPSYI          | -cooh                  |
| Pig           | LKLRLRKKAK joven PQCQVYKPSYI       | -cooh                  |
| Bovine        | LKLRLRKKAK joven PQCQVYKPSYI       | -cooh                  |
| Rabbit        | LKLRLRKKAK joven PQCQVYKPSYI       | -cooh                  |
| **Consensus** | • • • • • • • • • • • • • • • • • • | • • • • • • • • • • • • |

**B**

Human: Human VCAM-1 cDNA was kindly provided by M. Bevilacqua, Amgen, Thousand Oaks, CA (5). The putative cytoplasmic domain of E-selectin contains two tyrosine residues (Tyr582 and Tyr587). One of these, the membrane-proximal residue at position 582 of the mature protein, in the context of its neighboring residues, was predicted to fit a generic structural motif for efficient endocytosis (39). Additionally, the cytoplasmic domain of E-selectin contains a potential endocytosis motif similar to the di-leucine motif at its COOH terminus as well as that similar to the di-lysine motif, described above, in the juxtamembrane region. Surface-expressed E-selectin is phosphorylated at serine residues but not tyrosine residues (14). However, the phosphorylation event was reported to be unrelated to the endocytosis of E-selectin (14). Interestingly, the cytoplasmic domain of human VCAM-1 has a single tyrosine residue that is conserved (Fig. 1B), but, as mentioned above, surface-expressed VCAM-1 is not internalized rapidly. The aim of our study was to define further the endocytosis motif in E-selectin. We show here that neither Tyr582 nor Tyr587 is required for efficient endocytosis of surface E-selectin. Instead, Ser281 and residues membrane-proximal to it are required.

**EXPERIMENTAL PROCEDURES**

**PCR-assisted Site-directed Mutagenesis**—Human E-selectin cDNA was a gift from M. Bevilacqua, Agen, Thousand Oaks, CA (5). The full-length coding region along with 360 base pairs of the 3′-untranslated region of the cDNA was subcloned into the mammalian cell expression vector pDIX (kindly provided by K. Kaushansky, University of Washington, Seattle) (40). To generate the various cytoplasmic mutants constructs (Fig. 2), we took advantage of the unique restriction sites, DraIII, located 120 base pairs 5′ to the transmembrane domain, and NotI, located at the 3′-end of the subcloned cDNA. For construction of the substitution mutants, sequential PCRs were carried out as described previously (41). In the first PCR, a sense primer corresponding to the DraIII site and an antisense internal mutagenic oligonucleotide were used as the primer pair. In the second PCR, the respective sense

**FIG. 1.** Alignments of known E-selectin (panel A) and transmembrane forms of VCAM-1 (panel B) cytoplasmic domain amino acid sequences among different species. Spaces (dots) are inserted for optimal alignment. Consensus residues are boldfaced. COOH-terminal residues are as indicated.

**FIG. 2.** Deduced cytoplasmic domain amino acid sequences of E-selectin constructs. Potential internalization motifs found previously in other integral membrane proteins identified in the cytoplasmic domain of WT E-selectin, including di-lysine (KKFV), tyrosine-containing (DGY, YQKP, KPSY, or YQKPSY), and di-leucine (IL) motifs, are underlined. Boldface denote substitution mutations. Dashes denote internal deletion of amino acid residues at corresponding positions. For positional reference, some of the COOH-terminal residues are numbered.

The cytoplasmic domain of E-selectin is relatively short (32 amino acid residues). Although E-selectin is related to L- and P-selectin structurally and phylogenetically, they share little homology within this domain. This is in contrast to the relative cross-species conservation of the cytoplasmic domain of E-selectin (Fig. 1A). Accordingly, the fate of E-selectin after surface expression is quite divergent from that of L- and P-selectin. Unlike surface-expressed E-selectin, L-selectin is shed rapidly after leukocyte activation (37). P-selectin, once expressed on the endothelial cell surface, is internalized and subsequently recycled to the cell surface or degraded in lysosomes (15, 38).

**Transfection**—Chinese hamster ovary (CHO) cells were plated onto three 12-well tissue culture plates (Costar, Cambridge, MA) and grown to 70–80% confluence. Each of the wells was inoculated with 500 µl of Opti-MEM (Life Technologies, Inc.) containing LipofectAMINE (Life Technologies, Inc.) at 8 µg/ml and an expression vector construct at 2 µg/ml for 4 h. These were further incubated overnight with the addition of 500 µl well of regular CHO medium consisting of 10% fetal calf serum and 2 µg/ml of 125I. For each internalization assay, 90 µl of 125I was added immediately downstream from the preactivation stop codon in the design of the respective antisense mutagenic oligonucleotides. Each of these oligonucleotides, along with the sense DraIII primer as the primer pair and the WT construct as the template, were used to generate a PCR product that was subcloned subsequently into the expression vector as before. Each of the mutant clones generated was sequenced to verify the region spanning from the DraIII site to the NotI site. The deduced cytoplasmic tail amino acid sequences for the various mutants constructs are summarized in Fig. 2.

**125I Labeling of CD62E mAb**—The CD62E mAb, P6E2 (CY1878), was a generous gift from L. Phillips and J. Paulson, Cytel Corporation, San Diego. For each internalization assay, 90 µg of P6E2 was iodinated with 1 MCl of 125I using IODO-BEADS (Pierce Chemical Co.). The reaction was terminated at 12 min of incubation by removing the iodination mixture from the IOODO-BEADS. Labelled P6E2 was separated from unincorporated 125I using a size-exclusion desalting column (Presto Column, Pierce). The final eluted volume was adjusted to 18 ml with PBS containing 1 mM MgCl2, 1 mM CaCl2, and 0.1% bovine serum albumin (PBS†).

**CD62E mAb Acid Elution/Internalization Assay**—We modified the procedure reported previously by von Amsuth et al. (13) to measure internal mutagenic oligonucleotide and the antisense primer corresponding to the NotI site were used as the primer pair. The wild-type (WT) E-selectin construct was used as the template in both of these reactions. In the final PCR, aliquots of melted gel slices containing PCR products from the above reactions were combined as the template; the sense DraIII and the antisense NotI primers were used as the primer pair. The final PCR product was then isolated and extracted from agarose gel, digested with DraIII and NotI restriction enzymes, and subcloned into the purified WT E-selectin expression vector previously digested with the same enzymes. Applying a similar strategy, we substituted the full-length cytoplasmic tail of human VCAM-1 for that of E-selectin using hybrid mutagenic oligonucleotides having one end complementary to E-selectin transmembrane domain and the other end to VCAM-1 cytoplasmic domain. Human VCAM-1 cDNA (kindly provided by L. Osborn, Biogene, Inc., Cambridge Center, MA) (42) was used as the template in the second PCR reaction described above. For construction of the premature cytoplasmic domain termination mutants, the NotI site sequence was added immediately downstream from the premature stop codon in the design of the respective antisense mutagenic oligonucleotides. Each of these oligonucleotides, along with the sense DraIII primer as the primer pair and the WT construct as the template, were used to generate a PCR product that was subcloned subsequently into the expression vector as before. Each of the mutant clones generated was sequenced to verify the region spanning from the DraIII site to the NotI site. The deduced cytoplasmic tail amino acid sequences for the various mutants constructs are summarized in Fig. 2.
the surface kinetics of expressed E-selectin. For a typical experiment, one 12-well CHO cell plate/time point was assayed at 0, 30, and 60 min. Each plate/time point included four groups (in triplicate wells) of CHO cells that had been transiently transfected with the following constructs: WT E-selectin (positive control), ΔCyto with near total cytoplasmic domain truncation of E-selectin (Fig. 2), vector alone without cDNA insert (background control), and the test construct.

Plates were precultured on ice and washed twice with ice-cold PBS+. Five hundred μl of prechilled 125I-P6E2/well was added for a 15-min incubation on ice. Each well was then washed three times with 2 ml of cold PBS + to remove unbound 125I-P6E2. After the last wash, 1 ml/well of regular CHO medium prewarmed to 37 °C was added (at time 0) to the 30- and 60-min plates to initiate active endocytosis, and the plates were placed in 37 °C incubator for the designated duration. At the same time, 1 ml/well of ice-cold regular CHO medium was added to the time 0 plate, which had been kept on ice.

At the appropriate times, the supernatant medium in each well was collected and saved. Each well was then washed once with 1 ml and once with 2 ml of cold PBS+. The washes were saved. The saved supernatant medium and the washes from each respective well were combined and represented the supernatant fraction for that well. Subsequently, each well was incubated for 10 min with 1 ml of cold sodium citrate (0.1 M, pH 3.0) to dissociate surface-bound 125I-P6E2. Additional washes were completed with 1 ml/well sodium citrate followed by 2 ml/well PBS+. The acid-eluted and wash aliquots were combined and represented the surface fraction for that well. Finally, the cells in each well were lysed with 1 ml of 1 N NaOH for 30 min at room temperature. Each well was scraped and the content collected. Additional washes with 1 ml/well NaOH followed by 2 ml/well PBS were carried out. The lysis and wash aliquots were combined and represented the internalized fraction for that well. The total radioactivity in each fraction was counted and recorded. Thus, each well represented an independent transfection reaction, yielding three data points (supernatant, surface, and internalized fractions) at a given time point.

**Data Analysis for Surface-bound 125I-CD62E mAb Kinetics**—For each fraction at a time point, the radioactivity counts from the background control wells (CHO cells transiently transfected with vector alone without cDNA insert) were averaged. The radioactivity count from each of the other wells on the same plate was then adjusted by subtracting the mean background count. The mean background count of the surface fraction at any time point was typically <5% and frequently <1% of the surface fraction counts from the other wells. The adjusted count of each fraction from each well was then expressed as a percentage of the adjusted total count (supernatant, surface, and internalized fractions) of that well. The estimated surface half-lives of the various protein constructs were calculated from rate constants derived from curve fitting using the software KaleidaGraph (Synergy Software, Reading, PA), assuming first-order exponential decay characteristics. Analyses of variance were used to test differences of the surface fractions between relevant constructs at each time point.

**RESULTS**

**The Cytoplasmic Tail of E-selectin Contains an Internalization Signal**—In contrast to VCAM-1, E-selectin is internalized efficiently in tumor necrosis factor-activated human umbilical vascular endothelial cells (HUVEC), suggesting that the cytoplasmic tail of E-selectin contains an internalization signal (11, 13). To investigate this possibility further, we first conducted pilot experiments utilizing 125I-labeled human E-selectin mAb P6E2 in the acid elution assay described above, in previously cloned CHO cells stably transfected with the WT human E-selectin cDNA. Consistent with previous studies in HUVEC (13), our results indicated that the majority of the initially surface-bound 125I-P6E2 was internalized by 60 min (Fig. 3). Of note, recycling of surface E-selectin has not been reported, and, compared with that initially internalized, only a small fraction of the face-expressed E-selectin is shed in HUVEC (13, 16). Furthermore, over the time course studied, the amount of initially surface-bound 125I-P6E2 released into the supernatant medium at 4 °C was negligible (11). Thus, all subsequent experiments were performed on CHO cells transiently transfected with the various cytoplasmic domain constructs and focused on the kinetics of expressed surface E-selectin in the 1st h after labeling with the iodinated mAb.

We first generated a near total cytoplasmic domain truncation construct, ΔCyto (Fig. 2). At 60 min, the surface fraction of the WT E-selectin decreased substantially to about one-third of the initial value (mean = 36.5%, S.D. = 5.1%, n = 69). With most of the putative cytoplasmic domain truncated except for one amino acid residue, the internalization of ΔCyto was significantly (p < 0.0001) impaired compared with WT (mean surface fraction at 60 min = 53.0%, S.D. = 4.1%, n = 42, Fig. 4A). Consequently, the surface half-life of ΔCyto was nearly twice that of the WT (t_{1/2-WT} = 45 min versus t_{1/2-ΔCyto} = 79 min). The mean internalized fraction of the WT increased to 29.4% (S.D. = 4.3%) at 30 min and plateaued at 60 min (mean = 28.6%, S.D. = 4.1%), whereas that of the ΔCyto increased to a mean of 18.2% (S.D. = 2.6%) at 30 min and plateaued at 60 min (mean = 17.8%, S.D. = 2.0%) (Fig. 4B). Although reduced compared with WT, a significant fraction of initial ΔCyto was still internalized. Presumably, the internalization of ΔCyto occurred as a result of the normally constitutive membrane bulk flow in CHO cells. To define this observation further in CHO cells, the putative cytoplasmic domain of WT E-selectin was replaced with that of human VCAM-1 (ΔVCAM, Fig. 2). Because VCAM-1 internalization is reduced in cytokine-activated HUVEC compared with E-selectin (13), we expected that ΔVCAM would have an internalization kinetics similar to that of ΔCyto. Indeed, this was the case (Fig. 4). Thus, although elements other than the cytoplasmic domain might also contribute to the endocytosis of E-selectin (43, 44), these results demonstrate that there is a determinant within the cytoplasmic domain which promotes endocytosis of surface-expressed E-selectin in CHO cells.

**Tyrosine Residues Are Not Required for the Efficient Internalization of E-selectin**—The tyrosine-based endocytosis motif has been suggested to consist of 4–6 amino acid residues with the critical tyrosine residue located as the 3rd or 6th residue in the signal (45). These include the proposed general motif of XXT (46), where X represents an amino acid residue with a bulky aliphatic or hydrophobic side chain and the critical tyrosine residue within a motif NPYX (17) and others (45). Inspecting the E-selectin cytoplasmic domain reveals several potential tyrosine-based endocytosis motifs: DGSY, YQKP, KPSY, and YQKPSY. Interestingly, the cytoplasmic domain of human VCAM-1 also contains two potential endocytosis motifs, KGSY and YSLV (Fig. 1B) but failed to promote E-selectin endocytosis (ΔVCAM,
Endocytosis Signals in the Cytoplasmic Domain of E-selectin

that Tyr582 was a critical residue that conferred the function of

motoring E-selectin endocytosis and to test the previous proposal

cytoplasmic region containing the two tyrosine residues in pro-

late to predicted biologic function. To investigate the role of the

quences in the cytoplasmic domain does not necessarily trans-

Fig. 4). Clearly, the mere presence of tyrosine motif-like se-

sequences in the cytoplasmic domain does not necessarily trans-

Fig. 4. Cytoplasmic domain of E-selectin contains an internal-

Summary of 125I-P6E2 surface kinetics

Table I

Table I

| Construct   | Characteristics | Mean $t_{1/2}$ (min) |
|-------------|-----------------|----------------------|
| ΔCyto       |                 | 79                   |
| ΔVCAM + SDGS|                 | 95                   |
| ΔΔKF        |                 | 83                   |
| ΔLES        |                 | 80                   |
| ΔSDG        |                 | 57                   |
| SΔA         |                 | 54                   |
| SYΔGA       |                 | 57                   |
| ΔΔGS        |                 | 42                   |
| ΔΔGSY       |                 | 35                   |
| ΔΔGSA       |                 | 31                   |
| DΔA         |                 | 49                   |
| ΔΔASY       |                 | 42                   |
| YΔAF        |                 | 47                   |
| YΔA         |                 | 49                   |
| ΔPAS        |                 | 49                   |
| WT          |                 | 45                   |

Fig. 5. Tyrosine residues are not required for efficient inter-


efficient E-selectin internalization (39), we designed several

Fig. 5). To confirm this observa-

DISCUSSION

The exact mechanism effecting E-selectin endocytosis has

been elucidated. Studies have shown that it is a tempera-
The roughly 20-amino acid residue juxtamembrane region of the E-selectin cytoplasmic domain is highly conserved among species (Fig. 1). The functional role of this region remains obscure, although it contains a conserved di-lysine sequence similar to a previously presumed ER retention signal, KXXK. When overexpressed, this motif acted as a non-tyrosine-based endocytosis signal (32). The truncation construct ΔLES contains this region but failed to support internalization, having kinetics identical to that of ΔCyto (Fig. 5). Therefore, the juxtamembrane cytoplasmic segment containing di-lysine residues is alone not sufficient for efficient E-selectin internalization.

The internalization signal for E-selectin appears to be complex but novel. Our results indicate that Ser581 is necessary, but additional residue(s) NH2-terminal to this residue, other than aspartate579 and perhaps glycine580, also contribute(s) to the internalization process. Because surface E-selectin is phosphorylated at serine but not tyrosine residues (14), Ser581 may be phosphorylated and potentially serve as a constitutive endocytosis signal. This is supported by the fact that staurosporine inhibits E-selectin internalization and is an inhibitor of serine kinases (48). Furthermore, the result of applying a neural network-based prediction algorithm, PHDacc, developed at EMBL, Heidelberg, which assess solvent accessibility of amino acid residues (49), to the cytoplasmic sequences of E-selectin suggests that Ser581, among the six serine residues and the two tyrosine residues, is the one most likely exposed.

There are precedents in which cytoplasmic protein–protein-binding interactions are based on the recognition of a phosphoserine and its neighboring sequence motifs (50). Similar associations of serine phosphorylation with non-tyrosine motif-related membrane protein trafficking have also been reported in several studies. For the polymeric immunoglobulin receptor, phosphorylation of a specific cytoplasmic serine residue is required for efficient receptor transcytosis; an alanine mutant is transcytosed only slowly (51). Additionally, an alanine mutation introduced at another cytoplasmic serine residue impairs both internalization and transcytosis (52). Because E-selectin is not known to be transcytosed, it likely utilizes an endocytosis mechanism distinct from that of the polymeric immunoglobulin receptor. Alternatively, the difference might be accounted for by cell-specific mechanisms. For CD4, its down-regulation from the cell surface requires the phosphorylation of three cytoplasmic serine residues (34). However, unlike those in E-selectin, the phosphorylated serine residues in CD4 may be part of the di-leucine endocytosis motif operating in the cytoplasmic domain (33, 53). Finally, specific cytoplasmic serine phosphorylation of some surface receptors has been shown to be associated with ubiquitination of neighboring lysine residues that in turn signal for endocytosis (54, 55).

Recent studies suggested that the cytoplasmic domain of E-selectin was not required for PMN adhesion under static conditions (56), although its association with cytoskeletal proteins upon adhesion did occur (57). Under conditions of flow, PMNs rolled on surface-immobilized soluble E-selectin in a density-dependent manner (4). To confirm our suspicion that localization to clathrin-coated pits, definitive proof of the interaction of these motifs with adaptor-associated proteins is lacking (26). Ile-Leu and Leu-Ile motifs are also considered members of the di-leucine motifs that confer similar functions (33). Interestingly, E-selectin also has an Ile-Leu sequence at its COOH terminus. In fact, most of the E-selectin molecules cloned to date have isoleucine and leucine at their COOH termini (Fig. 1). Our results, however, indicate that this motif is not necessary for efficient E-selectin internalization (∆DGS, Fig. 5).

The membrane-proximal tyrosine residue in E-selectin has been proposed to be part of a structural motif that fits a generic model for efficient endocytosis (39). However, the mere presence of any tyrosine residue, or even a tyrosine motif-like sequence, in the cytoplasmic domain of an integral membrane protein does not necessarily indicate either the utilization of clathrin-dependent endocytotic machinery or the critical involvement of the tyrosine residue in the internalization pathway. VCAM-1, for example, contains a tyrosine in the cytoplasmic domain but is not efficiently internalized (11) (ΔVCAM, Fig. 4). Our results demonstrate that neither the two internalization motifs nor the DGSY, KPSY, or YQKPSY sequence is necessary for the efficient endocytosis of E-selectin. Recently, di-leucine motifs in several integral membrane proteins have been identified as non-tyrosine-based internalization as well as lysosomal targeting signals (29, 30, 33, 47). Although evidence suggests that these motifs probably mediate
the cytoplasmic domain of E-selectin was not essential for PMN rolling under shear, we selected CHO clones stably transfected with WT or ΔCyto construct and compared the rolling velocities of PMNs under defined shear stresses in a flow chamber. Using methodologies similar to that described (3), we did not observe an impairment in PMN rolling over stable ΔCyto clones at all shear stresses applied.2

In conclusion, we have identified a critical E-selectin cytoplasmic domain serine residue that is necessary for efficient endocytosis. Other unidentified determinants NH2-terminal to this residue are also required. Contrary to prior prediction, cytoplasmic tyrosine residues appear not involved. Because this residue are also required. Contrary to prior prediction, cytoplasmic tyrosine residues appear not involved. Because E-selectin is one of the important molecules in leukocyte recruitment in inflammation, additional studies to identify precise biochemical and structural basis of its internalization may provide insights into the regulation of the inflammatory response.

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