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**CHIMERIC SELECTINS AS SIMULTANEOUS BLOCKING AGENTS
FOR COMPONENT SELECTIN FUNCTION**

RELATED APPLICATIONS

This application is a continuation-in-part of Tedder, U.S. Patent Application Serial No. 07/983,606, filed November 30, 1992, which is a continuation under 37 CFR 1.62 of Serial Nos. 07/730,503, filed July 8, 1991, and 07/313,109, filed February 21, 1989, both now abandoned. This application also is a continuation-in-part of Tedder, U.S. Patent Application Serial No. 07/700,773, filed May 15, 1991; of Tedder, U.S. Patent Application Serial No. 07/737,092, filed July 29, 1991; of Tedder et al., U.S. Patent Application Serial No. 07/770,608, filed October 3, 1991; and of Tedder et al., U.S. Patent Application Serial No. 07/862,483, filed April 2, 1992, the whole of which are hereby incorporated by reference herein.

FIELD OF THE INVENTION

This invention relates to the selectin family of receptor adhesion molecules and particularly to agents interfering with selectin function.

GOVERNMENT RIGHTS

Part of the work leading to this invention was made with United States Government funds. Therefore, the U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

The ability of leukocytes to leave the circulation and to migrate into tissues is a critical feature of the immune response. Normally, the infiltrating leukocytes phagocytize invading organisms or dead or damaged cells. However, in pathologic inflammation, infiltrating leukocytes can cause



serious and sometimes deadly damage. Leukocyte-mediated inflammation is implicated in a number of human clinical manifestations, including the adult respiratory distress syndrome, multi-organ failure and reperfusion injury.

5 The selectin family of receptor adhesion molecules mediates the initial interactions of leukocytes with endothelium (Springer, *Nature* 346:425-434 (1990); Lasky, *Science* 258:964-969 (1992)). L-selectin (also known as LAM-1) is expressed on the surface of most classes of leukocytes
10 (Griffin et al., *J. Immunol.* 145:576-584 (1990); Tedder et al., *J. Immunol.* 144:532-540 (1990)) and mediates the binding of lymphocytes to high endothelial venules (HEV) of lymph nodes and to activated endothelium. P-selectin (also called PADGEM, CD62 or GMP-140) is expressed by activated platelets
15 and endothelial cells (Johnston et al., *Cell* 56:1033 (1989); Hsu-Lin et al., *J. Biol. Chem.* 259:9121 (1984)) and mediates adhesion between myeloid cells and activated endothelium or activated platelets (Gallatin et al, *Nature* 304:30-34 (1983); Spertini et al, *J. Immunol.* 147:2565-2573 (1991); Larsen et
20 al, *Cell* 59:305-312 (1989); Geng et al, *Nature* 343:757-760 (1990)). Another member of the family E-selectin (also known as ELAM-1) is expressed by activated endothelial cells (Bevilacqua et al., *Science* 243:1160 (1989); Bevilacqua et
25 al., *Proc. Nat'l Acad. Sci. USA* 84:9238 (1987)) and partially mediates the binding of neutrophils to endothelium at sites of inflammation (Bevilacqua et al., *Proc. Nat'l Acad. Sci. USA* 84:9238 (1987)). All selectins are derived from evolutionarily related genes (Collins et al., *J. Biol. Chem.* 266:2466-2478 (1991); Johnston et al., *J. Biol. Chem.* 34:21381-21385 (1990); Ord et al., *J. Biol. Chem.* 265:7760-
30 7767 (1990); Watson et al., *J. Exp. Med.* 172:263-272 (1990)), and are characterized by an NH₂-terminal, Ca⁺-dependent lectin domain, an epidermal growth factor (EGF)-like domain followed by multiple short consensus repeat (SCR) domains,
35 a transmembrane region, and a cytoplasmic tail. Although the lectin domains are critical for the binding of specific



carbohydrate ligands (Springer, Nature 346:425-434 (1990); Lasky, Science 258:964-969 (1992)), the role of the conserved EGF-like domains is unknown.

5 It has been proposed that the treatment of a patient suffering from pathologic inflammation with an antagonist to adhesion receptor function can result in the reduction of leukocyte migration to a level manageable by the target endothelial cells and the subsequent dramatic recovery of the patient. Local administration of therapeutic agents can
10 block competitively the adhesive interactions between leukocytes and the endothelium adjacent to an inflamed region. Therapeutic agents can also be administered on a systemic level for the treatment of a patient suffering from disseminated inflammation (Harlan and Liu, eds., Adhesion: Its Role in Inflammatory Disease, W. H. Freeman (in press)).
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SUMMARY OF THE INVENTION

We report here that the EGF-like domains of P-selectin and of E-selectin can participate directly in cell adhesion, having ligand binding sites distinct from those of their
20 respective lectin domains. Cell adhesion mediated at least by the P and E-selectins may be complex, involving interactions between the lectin domain and a carbohydrate ligand, and separately, between the EGF-like domain and one or more proteins or other ligands. Our discovery of a
25 difference in at least one of the ligands recognized by the lectin and EGF domains of a given selectin has permitted the preparation of chimeric polypeptides that combine within one molecule the ability to target two or more different selectin ligands and the use of these agents in therapy or in the
30 preparation of additional classes of antagonists to selectin function.

Thus, the invention generally features chimeric peptides or polypeptides that combine ligand binding portions from within the lectin and EGF domains of two different selectins.
35 The peptides or polypeptides can be composed solely of the



indicated portions of lectin or EGF domains or they can include portions of any of the remaining domains (SCR, transmembrane or cytoplasmic), or the entire extracellular portion, of a generic selectin molecule. The peptides or polypeptides also can be joined to a carrier protein (e.g., a soluble portion of an immunoglobulin molecule) to increase the serum half-life of the therapeutic agent. In another aspect, the invention features nucleic acid encoding the chimeric polypeptides.

The chimeric polypeptides can be used as therapeutic agents to antagonize selectin function. They are also useful for screening for agents that are simultaneously antagonists of the function of lectin and EGF domains of different selectins.

As used herein, the term "polypeptide" is intended to include shorter molecules, or "peptides." The term "essentially purified" refers to a polypeptide sequence that has been separated or isolated from the environment in which it was prepared or in which it naturally occurs.

BRIEF DESCRIPTION OF THE DRAWINGS

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, taken in conjunction with the accompanying drawings in which:

Fig. 1 shows the structure and expression of L-selectin/P-selectin chimeric proteins (Lectin, lectin domain; EGF, epidermal growth factor-like domain; SCR, short consensus repeat; TM, transmembrane domain; C, cytoplasmic domain);

Fig. 2a shows binding of HL-60 cells to COS cells transfected with natural and chimeric selectins (Original magnification, 200x);

Fig. 2b shows mapping of the domain(s) of L2P responsible for adhesion of HL-60 cells (bars represent the



means $\pm$ SD, and are representative of at least six experiments);

Fig. 3a shows neuraminidase sensitivity for adhesion mediated by the lectin and EGF domains of P-selectin;

Fig. 3b shows protease sensitivity and Ca^{2+} requirement for adhesion mediated by the lectin and EGF domains of P-selectin;

Fig. 3c shows an analysis of the binding of the chimera L2P3L;

Figs. 4a, 4b, and 4c show the cDNA nucleotide sequence (SEQ ID NO: 1) encoding L-selectin and also show the amino acid sequence of L-selectin (SEQ ID NO: 2);

Fig. 5 shows the restriction map and the exon-intron organization of the *lyam-1* gene;

Figs. 6a, 6b, and 6c show the nucleotide sequence of exons II through X of the *lyam-1* gene; and

Fig. 7 shows the homology among the lectin and EGF domains of L-, P- and E-selectin.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

We have examined the role of the lectin and EGF-like domains of the L-, P-, and E-selectins in cell adhesion and have determined that the EGF-like domains of P- and E-selectin play a direct role in ligand recognition and leukocyte adhesion mediated by the respective selectin. This discovery has permitted the preparation of chimeric polypeptides that combine within one therapeutic agent the ability to target two or more different selectin ligands and the use of these agents in therapy or in the preparation of additional classes of antagonists to selectin function.

Determination of the binding specificity of selectin lectin and EGF domains

The functional activities of the lectin and EGF-like domains of L-, P-, and E-selectin in the adhesive events described above were examined using a panel of



L-selectin/P-selectin and L-selectin/E-selectin chimeric molecules. Referring to Fig. 1, these hybrid polypeptides were created by exchange of cDNA encoding the lectin, EGF, or both lectin and EGF domains of the desired selectin and expression of the chimeric nucleic acid, as described in the Experimental Procedures section. Many other methods familiar to those of skill in the art can be employed to prepare similar products.

The chimeras conserve the overall polypeptide backbone structure of the selectins, and resulted in minimal changes in the junctions between domains. The functional characterization of these chimeric selectins therefore offered a powerful approach to determining the molecular basis of cell adhesion mediated by these molecules and to preparing diagnostic and therapeutic agents incorporating properties of individual selectins into the same polypeptide.

Expression of the chimeric proteins in COS cells was verified and quantitated using panels of P-, E- and L-selectin domain-specific monoclonal antibodies (mAb) in direct adhesion assays. The chimeric cDNA were subcloned into the pMT-2 vector and used to transiently transfect COS cells as described under Experimental Procedures. Twenty-four hours before analysis, 2.5×10^4 transfected COS cells were replated into 96 well plates. Expression of domain-specific epitopes by chimeric selectins was assessed using mAb reactive with specific domains and a surface immunofluorescence assay as described (Luscinskas et al, J. Immunol. 149:2163-2171 (1992)).

Referring again to Fig. 1, the individual constructs, containing the indicated domains of P-selectin and L-selectin, reacts only with anti-L-selectin and anti-P-selectin monoclonal antibodies recognizing the specific, indicated domains. These results demonstrate that the chimeric cDNA encoded the expected proteins and that each protein was expressed and readily detected on the surface of COS cells. Note that the LAM1-1 mAb fails to recognize



either the L2P or P2L proteins, and therefore defines an epitope composed of residues in both the lectin and EGF domains. Values given are means of absolute optical density (OD) units from which OD units obtained with a control non-binding mAb were subtracted. Experimental values less than the control values are represented as zero. The values presented are typical of those obtained in three experiments. The standard deviations for these fluorescence measurements were less than 15%.

Characterization of the adhesion reactions mediated by the selectin lectin and EGF domains

The HL-60 myelomonocytic cell line expresses ligands for P-selectin (Larsen et al, Cell 59:305-312 (1989); Geng et al, Nature 343:757-760 (1990)) and E-selectin (Bevilacqua et al., Science 243:1160 (1989); Bevilacqua et al., Proc. Nat'l Acad. Sci. USA 84:9238 (1987)) and was therefore used to assess P-selectin and E-selectin function. Referring to Fig. 2a, HL-60 cells bound at high levels to COS cells expressing P-selectin, but did not bind to COS cells expressing L-selectin. HL-60 cells also bound to COS cells expressing the chimeric protein P2L, in which the lectin domain from P-selectin was substituted for the lectin domain of L-selectin. Therefore, the lectin domain of P-selectin alone, when attached to the EGF-like and other domains from L-selectin, was sufficient to mediate high levels of HL-60 cell adhesion. However, the lectin domain of P-selectin alone was not solely responsible for all HL-60 binding, because HL-60 cells also bound to COS cells expressing L2P, in which the P-selectin lectin domain was replaced with that of L-selectin.

The domain(s) within L2P responsible for HL-60 adhesion were mapped using additional chimeric selectins. Referring to Fig. 2b, HL-60 cells bound to COS cells expressing L2P3L, which contains only the EGF domain from P-selectin, but did not bind to COS cells expressing L3P, which lacks both the



lectin and EGF-like domains of P-selectin but contains the P-selectin SCR domains. Furthermore, binding of HL-60 cells to P3L, which contains both the lectin and EGF domains from P-selectin, was approximately equivalent to that of native P-selectin and significantly ($P < 0.01$; Student's T test) higher than binding of cells to P2L, even though expression of P2L and P3L were approximately equal. These data indicate that both the lectin and EGF-like domains of P-selectin contain ligand binding sites capable of independently mediating HL-60 cell adhesion.

For study of ligand binding sites in E-selectin, four L-selectin/E-selectin chimeric molecules were constructed. L2E has the lectin of L-selectin and the remainder of E-selectin; E2L is the precise converse. L5E has the amino terminal 88 amino acids of L-selectin (i.e., 74% of the lectin domain) and E5L is the precise converse. Binding of HL-60 cells to COS cells expressing these chimeras was assessed as before. The constructs containing only a portion of a specific lectin domain exhibited zero binding, while the binding to chimeras containing lectin and EGF domains from different selectins was reduced by about a half. Therefore, the overall pattern of binding was similar to the results with L-selectin/P-selectin chimeras.

Determination of the influence of EGF-domains on the adhesive activity or specificity of the lectin domains

Adhesion mediated by P-selectin involves sialic acid-bearing carbohydrates, possibly including the sLe^x and/or related structures, as well as protein determinants (Larsen et al, Cell 63:467-474 (1990); Zhou et al, J. Cell Biol. 115:557-564 (1991); Moore et al, J. Cell. Biol. 118:445-456 (1992); Moore et al, J. Cell Biol. 112:491-499 (1991); Polley et al, Proc. Natl. Acad. Sci. USA 88:6224-6228 (1991); Larsen et al, J. Biol. Chem. 267:11104-11110 (1992)). Therefore, the effects of neuraminidase and protease



treatment of HL-60 cells on binding to COS cells expressing either P-selectin, P3L, or P2L was examined.

Removal of sialic acid residues from HL-60 cells significantly ($P < 0.01$) but incompletely reduced adhesion of HL-60 cells to COS cells expressing native P-selectin or P3L (Fig. 3A). In contrast, removal of sialic acid residues from HL-60 cells nearly completely eliminated adhesion of HL-60 cells to P2L (Fig. 3A). In addition, binding to the E-selectin/L-selectin construct E2L was partially inhibited by pretreatment of the HL-60 cells with CSLEX1 mAb directed against sLex. Similarly, pretreatment of COS cells with mAb directed against the lectin domain of P-selectin significantly ($P < 0.005$), but only partially, inhibited the adhesion of HL-60 cells to P-selectin or P3L, but abolished adhesion to P2L (Fig. 3A).

Treatment of HL-60 cells with either chymotrypsin or papain eliminated $>90\%$ of binding to COS cells expressing P-selectin, P3L, or P2L (Fig. 3B). In addition, 2.5 mM EGTA abolished adhesion (Fig. 3B). These results confirm the importance of the lectin domain in adhesion, and offer independent evidence that the EGF domain of P-selectin also plays a direct role in adhesion, recognizing a protein ligand.

A similar analysis was performed on L2P3L to characterize adhesion mediated by the EGF-like domain of P-selectin. Adhesion of HL-60 cells to COS cells expressing L2P3L was also reduced, but not eliminated, by neuraminidase treatment of the HL-60 cells (Fig. 3C). MAb directed against the L-selectin lectin domain present within L2P3L also significantly ($P < 0.01$) inhibited adhesion. These results suggest that recognition of specific carbohydrate structures on HL-60 cells by the lectin domain of L-selectin, which alone was not sufficient for cell adhesion, contributes to leukocyte adhesion in this system when this interaction is supported by the adhesive activity mediated by the EGF domain of P-selectin. Consistent with this hypothesis, rare



(~1 plate) rosettes of HL-60 cells were occasionally observed on COS cells transfected with L-selectin (data not shown), and a soluble L-selectin/IgG fusion protein has a measurable affinity for purified, immobilized sLe^x (Foxall et al, J. Cell Biol. 117:895-902 (1992)). Adhesion of HL-60 cells to L2P3L was eliminated by EGTA, indicating that adhesion mediated by the EGF-like domain is also Ca²⁺-dependent, and was significantly ($P < 0.01$) reduced by protease treatment (Fig. 3C).

Determination of the influence of EGF domains on the adhesive activity or specificity of the lectin domains

In order to determine if the EGF-like domains of selectins have any influence on the adhesive activity or specificity of the lectin domain, stable transfectants of the 300.19 murine pre-B cell line expressing L-selectin, P-selectin, L2P or L2P3L were generated. These cells were tested for binding to lymph node HEV using the standard *in vitro* frozen section assay (Stamper Jr. et al, J. Exp. Med. 144:828-833 (1976)). Transfectants expressing P-selectin did not bind to HEV, whereas transfectants expressing L-selectin, L2P or L2P3L bound to HEV equivalently and at high levels (Table I).



Table I. Adhesion of cell lines to lymph node HEV is mediated by the lectin domain of L-selectin.

	<u>CDNA</u>	<u>LAM1-3</u> <u>mAb</u>	<u>expt 1</u>	<u>expt 2</u>	<u>expt 3</u>
5	none	-	0.047 $\pm$ 0.02	0.12 $\pm$ 0.025	<0.01
		+	<0.01	ND	ND
	L-selectin	-	3.43 $\pm$ 0.42	6.85 $\pm$ 0.45	2.44 $\pm$ 0.51
		+	0.05 $\pm$ 0.2	ND	<0.01
	L2P	-	3.71 $\pm$ 0.26	6.64 $\pm$ 0.43	4.40 $\pm$ 0.3
		+	0.074 $\pm$ 0.03	ND	<0.01
	L2P3L	-	5.71 $\pm$ 0.33	6.85 $\pm$ 0.18	3.94 $\pm$ 0.27
		+	0.08 $\pm$ 0.03	ND	<0.01
	P-selectin	-	<0.01	<0.01	ND



5 The lectin domain of L-selectin alone was therefore
capable of mediating binding to HEV when attached to the
EGF-like and other domains of P-selectin. The L2P and L2P3L
chimeric selectins can therefore bind to different types of
cells which express ligand(s) for either L- or P-selectin,
reflecting the specificities of both of the parent selectins
from which they are constructed. This acquisition of novel
adhesive properties as a result of the exchange of lectin or
EGF-like domains between selectins reinforces our hypothesis
10 that the EGF-like domain of P-selectin can mediate cell
adhesion. In addition, these data directly demonstrate the
functional independence of the lectin and EGF-like domains,
and therefore argue against a role for the EGF-like domain
in determining the carbohydrate specificity of the lectin
15 domain.

Structure of the L-selectin gene

As disclosed in U.S. Patent Application Ser. No.
07/983,606, cDNA encoding L-selectin has been cloned and the
sequence determined, as shown in Figs. 4a, 4b and 4c (SEQ ID
20 NO: 1). The cDNA encodes a protein of 372 amino acids (SEQ
ID NO: 2).

25 The structure of the *lyam-1* gene, which encodes the LAM-
1 protein, was determined by isolating overlapping genomic
DNA clones that hybridized with a LAM-1 cDNA probe. The
lyam-1 gene spans greater than 30 kb of DNA and is composed
of at least 10 exons. The 5' end of the LAM-1 mRNA was
mapped by primer extension analysis, revealing a single
initiation region for transcription. Exons II through X
contain translated sequences; exon II (SEQ ID NO: 3) encodes
30 the translation initiation codon, residue 14 shown in Fig. 2;
exon III (SEQ ID NO: 4) encodes the leader peptide domain,
residues 15-41; exon IV (SEQ ID NO: 5) encodes the lectin-
like domain, residues 42-170; exon V (SEQ ID NO: 6) encodes
the epidermal growth factor-like domain, residues 171-206;
35 exons VI (SEQ ID NO: 7) and VII (SEQ ID NO: 8) encode the



short consensus repeat unit domains, residues 207-269 and 270-331; exon VIII (SEQ ID NO: 9) encodes the transmembrane region, residues 332-373; exon IX (SEQ ID NO: 10) encodes seven amino acids containing a potential phosphorylation site, residues 374-380; and exon X (SEQ ID NO: 11) encodes the five remaining amino acids of the cytoplasmic tail and the long 3' untranslated region.

The pLAM-1 cDNA was labeled with ^{32}P and used as a probe to isolate hybridizing DNAs from a human leukocyte genomic DNA library. Approximately 1×10^6 plaques were screened, and 13 plaques that hybridized with the cDNA probe were identified and isolated. Seven of these clones were found to contain inserts with unique restriction enzyme maps representing overlapping genomic fragments spanning at least 30 kb. These inserts, LAMG-17, -19, -20, -28, -35, -37, and -47, were further digested and subcloned into plasmids. Detailed restriction maps of these subclones were made and compared to those of intact inserts to determine their correct locations (Figs. 4A and 4B).

The correctness of the restriction map was verified with Southern blot analysis. DNA isolated from two B cell lines, BL and BJAB, and one T cell line, HSB-2, was digested to completion with *Bam* HI, *Bal* II, or *Pvu* II, size-fractionated, and transferred onto nitrocellulose. This filter was probed with the LAM-1 cDNA clone, pLAM-1. All genomic fragments derived from endonuclease digested DNA hybridized with cDNA probe to generate hybridizing bands of the appropriate size.

The pLAM-1 cDNA clone encodes an 85-bp 5' untranslated region. An oligonucleotide homologous with the 5' sequence of the pLAM-1 cDNA was used as a probe for primer extension analysis. This oligonucleotide was hybridized with poly (A⁺) RNA isolated from the human B cell line RAJI, the LAM-1 negative human B cell line Namalwa, the mouse pre-B cell line A20, and yeast tRNA as a control. Complementary DNA was synthesized by extending the primer with reverse transcriptase. The major primer extension product obtained



using the human LAM-1 positive B cell line RNA was extended 126 nucleotides beyond the translation initiation site. There was a single cluster of transcription initiation sites for the *lyam-1* gene apparent in the reaction with RAJI RNA that was not found with the LAM-1 negative Namalwa RNA. Several primer extension products of size similar to those of the human B cell line RNA were obtained with mouse B cell RNA. Therefore, murine B cells may express an RNA species that cross-hybridizes with the oligonucleotide probe used. No primer extension products were obtained in the yeast tRNA control reactions.

The relationship of the primer extension results to the cloned LAM-1 cDNAs and the most 5' exon of *lyam-1* isolated was used to determine the nucleotide sequence of the exon that encodes the translation initiation AUG codon. This exon ends immediately after the site that encodes the translation initiation codon (Fig. 5) and overlaps precisely with the pLAM-1 cDNA sequence. The length of the cDNA clone obtained by Bowen et al., J. Cell Biol. 109:421-427 (1989) agrees precisely with the primer extension results except for two nucleotides. However, 15 nucleotides before the 5' end of the cDNA the sequence diverges from the genomic sequence at a site homologous with the 3' splice acceptor site consensus sequence. Therefore, it is most likely that this 15-bp region is derived from the exon upstream of this potential splice site. Thus, the primer extension results indicate that exon I would most likely be composed of 15 or fewer base pairs.

A 15-bp oligonucleotide homologous with the 5' nucleotides present in the cDNA clone of Bowen et al., supra but not encoded by exon II, was used to probe the 10 kb of cloned DNAs 5' of exon II; however, specific hybridization was not detected by Southern blot analysis. Under the conditions necessary for hybridization of this oligonucleotide, significant cross-hybridization occurred



with λ -DNA, making it difficult to use this oligonucleotide to isolate the first exon from a λ -based genomic library.

5 These results suggest that the exon which encodes the translation initiation site is the second exon of the *lyam-1* gene (Fig. 4C). Consistent with this, the 900 bp upstream of exon II did not contain any apparent "TATA" or "CCAAT" sequences frequently found in promoter regions of eukaryotic genes (Fig. 5). Therefore, it is likely that the transcription initiation region and exon I are further than 10 kb upstream from exon II of the *lyam-1* gene. S1 nuclease protection analysis was carried out using the 5' region of exon II as a labeled probe for hybridization with poly (A⁺) RNA from RAJI, Namalwa, and A20 cells. Two mRNA species were protected in the RAJI mRNA, while no S1 protection was provided by the other RNAs. The length of these fragments was consistent with differential splicing at the two potential CAG/N splice sites located within the potential splice acceptor site in exon II. It is therefore likely that the transcription initiation region has not been identified.

20 The majority of the exons were localized by comparison of the restriction enzyme maps of the genomic clones and the pLAM-1 cDNA. In cases where this method did not provide definitive results, subcloned DNA fragments were digested with selected restriction enzymes, electrophoresed through agarose gels, and transferred to nitrocellulose. Fragments containing exons were identified by Southern blot analysis using labeled cDNA or oligonucleotide probes. The exon that encodes the 3' untranslated region of the LAM-1 cDNA was not contained within the 30 kb of isolated DNA fragments. Therefore, a labeled 0.9-kb *Dra* I fragment containing most of the 3' untranslated region of the pLAM-1 cDNA was used as a probe to identify a homologous 3.2-kb fragment generated by complete *Eco* RI digestion of genomic DNA. *Eco* RI-digested genomic DNA fragments of this size were used to make a partial λ -gt11 genomic library from which the 3.2-kb *Eco* RI



fragment was cloned. This 3.2-kb fragment did not overlap with the previously isolated genomic DNAs.

The exact boundaries of the exons were determined by nucleotide sequence analysis. From this analysis, nine exons were identified which make up the entire pLAM-1 cDNA. Exon II encodes the translation initiation codon, and exon III encodes the leader domain of the LAM-1 protein (Fig. 5). Each of the lectin-like, epidermal growth factor-like, transmembrane, and short consensus repeat domains was encoded by a separate exon. The smallest exon, IX, is 19 bp in length and may encode a carboxyl-terminal phosphorylation cassette. The last 5 amino acids of the LAM-1 protein and the 3' untranslated region which includes the poly(A) attachment site, AATAAA, are encoded by exon X as shown in Fig. 5. The nine exons which encode pLAM-1 were split inside codons in all cases, except the junction between exons II and III. In each instance, the consensus sequences of 5' donor splice sites and 3' acceptor splice sites were adhered to. Nucleotide sequence polymorphisms within the coding region were observed between the genomic clones containing exon V that encoded SCR I and the pLAM-1 clone at cDNA nucleotide positions 741 and 747 (A to G), leading to a coding change from Asn to Ser in both cases, and at position 816 (A to G) changing the Glu to a Gly.

Experimental Procedures Construction of Chimeric Selectin cDNAs

Four new restriction endonuclease recognition sites were introduced separately into the pLAM-1 cDNA by polymerase chain reaction (PCR)-based site-directed mutagenesis as follows. Oligonucleotides surrounding and including each new restriction sequence were synthesized in both the sense and antisense directions. PCR was carried out using the new restriction site sense oligonucleotide and an antisense oligonucleotide anchor located in the plasmid near the 3' end of the cDNA. In a separate reaction, the new restriction site antisense oligonucleotide plus a sense oligonucleotide



anchor from the plasmid 5' end were used to amplify the 5' end of the LAM-1 cDNA. The individual PCR products from these two reactions were gel purified, subcloned into the pSP65 vector (Promega Biotec, Madison, WI), digested with the appropriate restriction enzymes, and ligated together. The nucleotide changes were as follows: (a) GA³⁶¹ATCC to GGATCC in the lectin domain, creating a BamHI site, corresponding to amino acid number 53 in the mature protein (Fig. 2); (b) A⁵⁸³TGCAG to CTGCAG in the EGF domain, creating a PstI site, corresponding to amino acid number 127; (c) GAGG⁸⁸¹C⁸⁸²C to GAGCTC in the first SCR domain, creating a SacI site, corresponding to amino acid number 164; and (d) GTCAAA¹⁰²³ to GTCAAC in the second SCR domain, creating a HincII site, corresponding to amino acid number 279. The fidelity of the site-directed mutagenesis was confirmed by restriction mapping and sequence analysis of the cDNAs. Each of these sites is naturally present in the PADGEM cDNA (Larsen et al., Cell 59:305-312 (1984)).

Domain Mapping of Binding Sites

The chimeric selectin cDNAs were subcloned into the pMT-2 expression vector (Kaufman et al, J. Mol. Cell. Biol. 9:946-958 (1989)), and COS cells at ~50% confluency were transiently transfected with 3 µg of the indicated cDNA by the DEAE dextran method. One day after transfection, the COS cells were replated at ~50% confluency onto 35 mm Petri dishes (assay plates) (Becton-Dickinson, Lincoln Park, New Jersey). The following day, HL-60 cells were washed twice in cold RPMI 1640 media (Gibco, Grand Island, NY), resuspended in RPMI 1640 at a final concentration of 3.3 x 10⁶ cells ml, and 0.6 ml (2 x 10⁶ cells) were added to assay plates which had been washed twice with cold RPMI 1640 media. The plates were gently rocked for 20 min at 4°C, washed five times, and the number of HL-60 cells bound to individual COS cells were counted on a minimum of 100 COS cells.



Assay for leukocyte attachment to HEV

The cDNAs encoding native L-selectin, L2P and L2P3L were subcloned into the Bam HI site of the pZIPneoSV(X) vector (Cepko et al, Cell 37:1053-1062 (1984)), and were used to transfect the mouse pre-B cell line 300.19. Stable transfectants were selected in medium containing 0.5-1.0 mg/ml G418 (geneticin; Sigma), and cells expressing the proteins were selected by panning with the LAM1-3 mAb (Spertini et al, J. Immunol. 147:942-949 (1991)). To generate P-selectin transfectants, the P-selectin cDNA in the pMT-2 vector was cotransfected with the pSV2neo vector containing the neomycin resistance marker. Rat lymph nodes were obtained from freshly euthanized Lewis rats, snap frozen in isopentane/liquid nitrogen, and stored at -70°C in isopentane until use. For the HEV assay, 5×10^6 of each cell type were incubated on three 12 mm sections/ slide at 64 rpm for 25 min at ~4°C, the excess cells were gently removed, and the slides were placed vertically in ice-cold fixative (PBS/2.4% glutaraldehyde) overnight. The slides were then counterstained with Gill's hematoxylin, overlaid with glycerol gelatin, and cover slips were applied. Each slide was scored for the number of lymphocytes bound/HEV. Between 100-200 HEV were counted for each experiment. Data are presented as mean $\pm$ standard deviation of lymphocytes bound/HEV. ND, not determined.

Determination of neuraminidase sensitivity, protease sensitivity and Ca^{2+} requirements for adhesion

COS cells were transfected with the indicated cDNA and replated as described above. HL-60 cells were incubated in RPMI 1640 medium at 37°C for two hours with either 0.25 U/ml *Clostridium perfringens* neuraminidase (Type VI, Sigma, St. Louis, MO), 50 mg/ml chymotrypsin or papain (Sigma), washed twice, and the assay was performed as described in the Fig. 2 legend. Neuraminidase treatment of the HL-60 cells was effective in eliminating all reactivity with the CSLEX1



anti-sLe^x mAb (data not shown). In some groups, COS cells were preincubated with either 5 µg/ml G1 mAb (a gift of Dr. R.P. McEver) (Geng et al, Nature 343:757-760 (1990)), or ascites fluid containing the LAM1-3 mAb (Spertini et al, J. Immunol. 147:942-949 (1991)) diluted 1:200. To determine the effect of EGTA, both the HL-60 cells and the COS cells were washed in RPMI 1640 containing 2.5 mM EGTA, and this medium was also used for the assay. No detachment of COS cells occurred under these conditions. These results are representative of at least four experiments.

Use

The adhesion of leukocytes to the endothelial wall of blood vessels and their infiltration into the surrounding tissues contributes to inflammation of tissue at the cellular level. Normally, the infiltrating leukocytes phagocytize invading organisms or dead or damaged cells. However, in pathologic inflammation, infiltrating leukocytes can cause serious and sometimes deadly damage. Leukocyte-mediated inflammation is involved in a number of human clinical manifestations, including the adult respiratory distress syndrome, multi-organ failure and reperfusion injury.

Local administration of therapeutic agents antagonistic to the function of the selectins can block competitively the adhesive interactions between leukocytes and the endothelium adjacent to an inflamed region. On a systemic level, treatment of a patient in shock (e.g., from a serious injury) with an antagonist to selectin function can result in the reduction of leukocyte migration and adhesion and the subsequent dramatic recovery of the patient.

Chimeric peptides or polypeptides combining ligand binding portions from within the lectin and EGF domains of two different selectins can be used for therapeutic treatment to interfere in the binding of leukocytes at the site of inflammation. Referring to Fig. 7, the close homology among the lectin and EGF domains of L-, P- and E-selectin can be



seen, and the intron/exon organization of the respective genes encoding P- and E-selectin (Collins et al., J. Biol.Chem. 266:2466 (1991); Johnston et al., J. Biol. Chem.265:21381 (1990)) is the same as that of the *lyam-1* gene encoding L-selectin, disclosed herein. These chimeric agents will offer more efficient targeting than using a single function antagonist. Therapeutic agents of the invention can also be used to block platelet or platelet and mononuclear cell aggregation, thus preventing thrombus formation.

The therapeutic peptides or polypeptides can be composed solely of the indicated portions of lectin or EGF domains or they can include portions of any of the remaining domains (SCR, transmembrane or cytoplasmic), or the entire extracellular portion of a generic selectin molecule. The peptides or polypeptides also can be joined to a carrier protein (e.g., a soluble portion of an immunoglobulin molecule) to increase the serum half-life of the therapeutic agent. Immunoglobulin chimera are easily purified through standard immunochemistry procedures, including IgG-binding protein A-Sepharose chromatography. The chimera have the ability to form an immunoglobulin-like dimer with the concomitant higher avidity and serum half-life.

cDNA sequences encoding the chimeric polypeptides can be incorporated into replicable expression vectors and the vectors transfected into an appropriate host to express the encoded polypeptide. Stop codons can be incorporated into the DNA sequences or any other chain termination method can be used to prepare a soluble polypeptide. The expressed polypeptide can be purified by ordinary techniques well known to those of skill in the art, e.g., by affinity chromatographic methods.

Other agents can also be joined to the therapeutic polypeptides to form a useful product. For example, the ligand binding portions of the lectin and EGF domains of two different selectins can be combined with the toxic portion of a cytotoxin to produce a fusion protein. In addition, the



selectin chimeric polypeptides may be coupled to a chemotherapeutic drug or drugs which could simultaneously bind to cells expressing their respective ligands, to administer the drug to a site of tissue damage or inflammation to treat, e.g., acute inflammation or vasculitis. Such drugs may include, anti-inflammatory agents or agents that provide regional relief from inflammatory distress. Syndromes, diseases, and conditions that could be treated by these agents would include, but not be limited to, treating inflammation, microbial/parasitic infections, post-reperfusion injury, leukemia, lymphoma, vasculitis, inhibition of the metastatic spread of tumor cells, organ transplantation, or graft rejection. As is well known to those of skill in the art, the fusion proteins can be transcribed from a hybrid cDNA molecule, or the agent may be covalently bonded to the chimeric polypeptide by routine procedures.

One method of imaging the sites of inflammation in a patient involves detecting the expression of the L-selectin ligand on the inflamed endothelium. The method includes administering to a patient a pharmaceutical composition consisting of a detectable amount of a labeled ligand binding fragment of L-selectin, alone or joined to a carrier protein, including a ligand binding portion of a different selectin receptor, in a pharmaceutically acceptable carrier. Sufficient time is allowed for the labeled polypeptide to localize at the site of L-selectin ligand expression, unbound polypeptide is permitted to clear from healthy tissue in the patient, and signal generated by the label is detected and converted into an image of the site of inflammation. The amount of labeled ligand binding fragment of L-selectin preferably would be from 1 pg/kg to 10 μ g/kg although higher or lower doses are possible depending on the imaging agent label used and the sensitivity of the detection method.

Some of the labels which can be detected externally from within the body of a human patient include radionuclides,



radiopaque labels, and paramagnetic isotopes. A radionuclide for *in vivo* diagnosis should have a half-life long enough that it is still detectable at the time of maximum uptake, but short enough that after diagnosis unwanted radiation does not remain in the patient. Coupling of radionuclides to antibodies or proteins is well known in the art (see, e.g., Daddona et al., U.S. Pat. No. 5,026,537, the teachings of which are incorporated by reference herein) and is often accomplished either directly or indirectly using an intermediary linking group. Examples of radioisotopes that could be used for *in vivo* diagnosis are ⁹⁹Tc, ¹²³I, ¹³¹I, ¹¹¹In, ⁸⁷Ru, ⁶⁷Cu, ⁶⁷Ga, ⁶⁸Ga, ⁷²As, ⁸⁹Zr, and ²⁰¹Ti. Paramagnetic isotopes for purposes of *in vivo* diagnosis can also be used according to the methods of this invention. Examples of elements that are particularly useful for use in magnetic resonance energy techniques include ¹⁵⁷Gd, ⁵⁵Mn, ¹⁶²Dy, ⁵²Cr, and ⁵⁶Fe. For radiopaque imaging, the LAM-1 protein or ligand binding fragment is coupled to an agent that produces an opaque field at the site of inflammation upon X-ray imaging.

A ligand binding portion of the lectin or EGF-like domains of various selectins can be experimentally determined using this invention. In a typical procedure, fragments of the selectin cDNA can be fused with cDNA encoding a carrier protein, such as immunoglobulin heavy chain or another non-ligand binding portion of a different selectin. These chimeric proteins can be expressed on the cell surface, or as soluble molecules, and their ability to bind ligand assessed. Further segregated fragments of the lectin or EGF-like domain under study can be examined for their ability to bind ligands, as well as their ability to inhibit the binding of the parent selectin molecule to ligand.

Any of the peptides or polypeptides of the invention comprising lectin and EGF ligand binding portions from two different selectin molecules (including portions of any of the remaining domains (SCR, transmembrane or cytoplasmic) of a generic selectin molecule or including a carrier protein)



can be used to screen for antagonists of lectin or EGF function individually or for agents that can simultaneously antagonize the functions of the lectin and EGF domain of different selectins. This invention will allow the development of pharmacologic reagents composed of peptides, carbohydrate moieties, RNAs or other small molecules which may mimic the ligand of the lectin or EGF domains of the selectins or may mimic the ligand-binding epitopes of the respective selectin domains and thus be useful therapeutic agents.

In another therapeutic method, chimeric selectin polypeptides can be used in combination therapy with any other selectin or any other cell surface molecule, or soluble fragments thereof, involved in adhesion of leukocytes to endothelial surfaces. In addition, antagonists to any of the above receptors or receptor portions (or mAb reactive to the receptors or receptor portions) can be used in the above combinations or as independent antagonist combinations for treatment of a patient. Examples include ICAM-1, VCAM-1, VLA-4, CD18, CD11a, CD11b and CD31, and the mAb reactive with them.

The therapeutic agents may be administered orally, topically, or parenterally, (e.g., intranasally, subcutaneously, intramuscularly, intravenously, or intra-arterially) by routine methods in pharmaceutically acceptable inert carrier substances. Optimal dosage and modes of administration can readily be determined by conventional protocols.

The *lyam-1* gene itself or portions thereof can be used to construct chimeric selectins. The gene can also be used in genetic therapy. Individuals having a genetic defect in the *lyam-1* gene would be unable to produce a fully active L-selectin leukocyte "homing" receptor and thus would be unable to mobilize sufficient leukocytes to a site of inflammation. Individuals suspected of having a congenital defect in the *lyam-1* gene could be screened for this genetic disorder using



the sequence and structural information described. Treatment of affected individuals would then be possible using the *lyam-1* gene or fragments thereof.

5 The normal regulation of the *lyam-1* gene, as evidenced by the appearance and disappearance of L-selectin on the surface of a specific leukocyte sub-population can be monitored to test the effects of drugs or specific therapies that would alter gene expression.

10 While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection
15 granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Tedder, Thomas F.
Kansas, Geoffrey S.

(ii) TITLE OF INVENTION: CHIMERIC SELECTINS AS SIMULTANEOUS
BLOCKING AGENTS FOR COMPONENT SELECTIN FUNCTION

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Weingarten, Schurgin, Gagnebin & Hayes
(B) STREET: Ten Post Office Square
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/008,459
(B) FILING DATE: 25-JAN-1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/983,606
(B) FILING DATE: 30-NOV-1992

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/862,483
(B) FILING DATE: 02-APR-1992

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/770,608
(B) FILING DATE: 03-OCT-1991

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/737,092
(B) FILING DATE: 29-JUL-1991

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/730,503
(B) FILING DATE: 08-JUL-1991

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/700,773
(B) FILING DATE: 15-MAY-1991

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/313,109
(B) FILING DATE: 21-FEB-1989

(viii) ATTORNEY/AGENT INFORMATION:

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(C) REFERENCE/DOCKET NUMBER: DFCI-318XX



(ix) TELECOMMUNICATION INFORMATION:
 (A) TELEPHONE: (617) 542-2290
 (B) TELEFAX: (617) 451-0313
 (C) TELEX: 940675

5 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2330 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

15

(A) NAME/KEY: CDS
 (B) LOCATION: 53..1210

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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	Gly Cys Arg Arg Thr Arg Glu Gly Pro Ser Lys Ala Met Ile Phe Pro	
	5 10 15	
25	TGG AAA TGT CAG AGC ACC CAG AGG GAC TTA TGG AAC ATC TTC AAG TTG	151
	Trp Lys Cys Gln Ser Thr Gln Arg Asp Leu Trp Asn Ile Phe Lys Leu	
	20 25 30	
	TGG GGG TGG ACA ATG CTC TGT TGT GAT TTC CTG GCA CAT CAT GGA ACC	199
	Trp Gly Trp Thr Met Leu Cys Cys Asp Phe Leu Ala His His Gly Thr	
	35 40 45	
30	GAC TGC TGG ACT TAC CAT TAT TCT GAA AAA CCC ATG AAC TGG CAA AGG	247
	Asp Cys Trp Thr Tyr His Tyr Ser Glu Lys Pro Met Asn Trp Gln Arg	
	50 55 60 65	
	GCT AGA AGA TTC TGC CGA GAC AAT TAC ACA GAT TTA GTT GCC ATA CAA	295
	Ala Arg Arg Phe Cys Arg Asp Asn Tyr Thr Asp Leu Val Ala Ile Gln	
35	70 75 80	
	AAC AAG GCG GAA ATT GAG TAT CTG GAG AAG ACT CTG CCT TTC AGT CGT	343
	Asn Lys Ala Glu Ile Glu Tyr Leu Glu Lys Thr Leu Pro Phe Ser Arg	
	85 90 95	
40	TCT TAC TAC TGG ATA GGA ATC CGG AAG ATA GGA GGA ATA TGG ACG TGG	391
	Ser Tyr Tyr Trp Ile Gly Ile Arg Lys Ile Gly Gly Ile Trp Thr Trp	
	100 105 110	
	GTG GGA ACC AAC AAA TCT CTC ACT GAA GAA GCA GAG AAC TGG GGA GAT	439
	Val Gly Thr Asn Lys Ser Leu Thr Glu Glu Ala Glu Asn Trp Gly Asp	
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This Sheet Has Been Replaced



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	Gly Glu Pro Asn Asn Lys Lys Asn Lys Glu Asp Cys Val Glu Ile Tyr	
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5	ATC AAG AGA AAC AAA GAT GCA GGC AAA TGG AAC GAT GAC GCC TGC CAC	535
	Ile Lys Arg Asn Lys Asp Ala Gly Lys Trp Asn Asp Asp Ala Cys His	
	150 155 160	
	AAA CTA AAG GCA GCC CTC TGT TAC ACA GCT TCT TGC CAG CCC TGG TCA	583
	Lys Leu Lys Ala Ala Leu Cys Tyr Thr Ala Ser Cys Gln Pro Trp Ser	
	165 170 175	
10	TGC AGT GGC CAT GGA GAA TGT GTA GAA ATC ATC AAT AAT TAC ACC TGC	631
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	180 185 190	
15	AAC TGT GAT GTG GGG TAC TAT GGG CCC CAG TGT CAG TTT GTG ATT CAG	679
	Asn Cys Asp Val Gly Tyr Tyr Gly Pro Gln Cys Gln Phe Val Ile Gln	
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	TGT GAG CCT TTG GAG GCC CCA GAG CTG GGT ACC ATG GAC TGT ACT CAC	727
	Cys Glu Pro Leu Glu Ala Pro Glu Leu Gly Thr Met Asp Cys Thr His	
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20	CCT TTG GGA AAC TTC AAC TTC AAC TCA CAG TGT GCC TTC AGC TGC TCT	775
	Pro Leu Gly Asn Phe Asn Phe Asn Ser Gln Cys Ala Phe Ser Cys Ser	
	230 235 240	
	GAA GGA ACA AAC TTA ACT GGG ATT GAA GAA ACC ACC TGT GAA CCA TTT	823
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25	GGA AAC TGG TCA TCT CCA GAA CCA ACC TGT CAA GTG ATT CAG TGT GAG	871
	Gly Asn Trp Ser Ser Pro Glu Pro Thr Cys Gln Val Ile Gln Cys Glu	
	260 265 270	
30	CCT CTA TCA GCA CCA GAT TTG GGG ATC ATG AAC TGT AGC CAT CCC CTG	919
	Pro Leu Ser Ala Pro Asp Leu Gly Ile Met Asn Cys Ser His Pro Leu	
	275 280 285	
	GCC AGC TTC AGC TTT ACC TCT GCA TGT ACC TTC ATC TGC TCA GAA GGA	967
	Ala Ser Phe Ser Phe Thr Ser Ala Cys Thr Phe Ile Cys Ser Glu Gly	
	290 295 300 305	
35	ACT GAG TTA ATT GGG AAG AAG AAA ACC ATT TGT GAA TCA TCT GGA ATC	1015
	Thr Glu Leu Ile Gly Lys Lys Lys Thr Ile Cys Glu Ser Ser Gly Ile	
	310 315 320	
	TGG TCA AAT CCT AGT CCA ATA TGT CAA AAA TTG GAC AAA AGT TTC TCA	1063
	Trp Ser Asn Pro Ser Pro Ile Cys Gln Lys Leu Asp Lys Ser Phe Ser	
	325 330 335	
40	ATG ATT AAG GAG GGT GAT TAT AAC CCC CTC TTC ATT CCA GTG GCA GTC	1111
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	340 345 350	
45	ATG GTT ACT GCA TTC TCT GGG TTG GCA TTT ATC ATT TGG CTG GCA AGG	1159
	Met Val Thr Ala Phe Ser Gly Leu Ala Phe Ile Ile Trp Leu Ala Arg	
	355 360 365	
	AGA TTA AAA AAA GGC AAG AAA TCC AAG AGA AGT ATG AAT GAC CCA TAT	1207
	Arg Leu Lys Lys Gly Lys Lys Ser Lys Arg Ser Met Asn Asp Pro Tyr	
	370 375 380 385	

This Sheet Has Been Replaced



5 TAAATCGCCC TTGGTGAAAG AAAATTCTTG GAATACTAAA AATCATGAGA TCCTTTAAAT 1267
 CCTTCCATGA AACGTTTTGT GTGGTGGCAC CTCCTACGTC AAACATGAAG TGTGTTTCCT 1327
 TCAGTGCATC TGGGAAGATT TCTACCTGAC CAACAGTTCC TTCAGCTTCC ATTCACCCCC 1387
 TCATTTATCC CTCAACCCCC AGCCCACAGG TGTTTATACA GCTCAGCTTT TTGTCTTTTC 1447
 TGAGGAGAAA CAAATAAGAC CATAAAGGGA AAGGATTCAT GTGGAATATA AAGATGGCTG 1507
 ACTTTGCTCT TTCTTGACTC TTGTTTTAG TTTCAATTCA GTGCTGTACT TGATGACAGA 1567
 CACTTCTAAA TGAAGTGCAA ATTTGATACA TATGTGAATA TGGACTCAGT TTTCTTGCAG 1627
 ATCAAATTTT GCGTCGTCTT CTGTATACGT GGAGGTACAC TCTATGAAGT CAAAAGTCTA 1687
 CGCTCTCCTT TCTTTCTAAC TCCAGTGAAG TAATGGGGTC CTGCTCAAGT TGAAAGAGTC 1747
 10 CTATTTGCAC TGTAGCCTCG CCGTCTGTGA ATTGGACCAT CCTATTAAAC TGGCTTCAGC 1807
 CTCCCCACCT TCTTCAGCCA CCTCTCTTTT TCAGTTGGCT GACTTCCACA CCTAGCATCT 1867
 CATGAGTGCC AAGCAAAAGG AGAGAAGAGA GAAATAGCCT GCGCTGTTTT TTAGTTTGGG 1927
 GGTTTTGCTG TTTCCTTTTA TGAGACCCAT TCCTATTTCT TATAGTCAAT GTTCTTTTAA 1987
 TCACGATATT ATTAGTAAGA AAACATCACT GAAATGCTAG CTGCAACTGA CATCTCTTTG 2047
 15 ATGTCATATG GAAGAGTTAA AACAGGTGGA GAAATTCCTT GATTACAAAT GAAATGCTCT 2107
 CCTTTCCCCT GCCCCAGAC CTTTTATCCA CTTACCTAGA TTCTACATAT TCTTTAAATT 2167
 TCATCTCAGG CCTCCCTCAA CCCCACCACT TCTTTTATAA CTAGTCCTTT ACTAATCCAA 2227
 CCCATGATGA GCTCCTCTTC CTGGCTTCTT ACTGAAAGGT TACCCTGTAA CATGCAATTT 2287
 TGCATTTGAA TAAAGCCTGC TTTTAAAGTG TAAAAAGAA TTC 2330

20 (2) INFORMATION FOR SEQ ID NO:2:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 385 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
 25 (ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Cys Arg Arg Thr Arg Glu Gly Pro Ser Lys Ala Met Ile Phe
 1 5 10 15
 30 Pro Trp Lys Cys Gln Ser Thr Gln Arg Asp Leu Trp Asn Ile Phe Lys
 20 25 30
 Leu Trp Gly Trp Thr Met Leu Cys Cys Asp Phe Leu Ala His His Gly
 35 40 45
 Thr Asp Cys Trp Thr Tyr His Tyr Ser Glu Lys Pro Met Asn Trp Gln
 50 55 60
 35 Arg Ala Arg Arg Phe Cys Arg Asp Asn Tyr Thr Asp Leu Val Ala Ile
 65 70 75 80



	Gln	Asn	Lys	Ala	Glu	Ile	Glu	Tyr	Leu	Glu	Lys	Thr	Leu	Pro	Phe	Ser	
					85					90					95		
	Arg	Ser	Tyr	Tyr	Trp	Ile	Gly	Ile	Arg	Lys	Ile	Gly	Gly	Ile	Trp	Thr	
				100					105					110			
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		130					135					140					
10	Tyr	Ile	Lys	Arg	Asn	Lys	Asp	Ala	Gly	Lys	Trp	Asn	Asp	Asp	Ala	Cys	
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	His	Lys	Leu	Lys	Ala	Ala	Leu	Cys	Tyr	Thr	Ala	Ser	Cys	Gln	Pro	Trp	
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15	Cys	Asn	Cys	Asp	Val	Gly	Tyr	Tyr	Gly	Pro	Gln	Cys	Gln	Phe	Val	Ile	
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20	His	Pro	Leu	Gly	Asn	Phe	Asn	Phe	Asn	Ser	Gln	Cys	Ala	Phe	Ser	Cys	
	225					230					235					240	
	Ser	Glu	Gly	Thr	Asn	Leu	Thr	Gly	Ile	Glu	Glu	Thr	Thr	Cys	Glu	Pro	
					245					250					255		
	Phe	Gly	Asn	Trp	Ser	Ser	Pro	Glu	Pro	Thr	Cys	Gln	Val	Ile	Gln	Cys	
				260					265					270			
25	Glu	Pro	Leu	Ser	Ala	Pro	Asp	Leu	Gly	Ile	Met	Asn	Cys	Ser	His	Pro	
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		290					295					300					
30	Gly	Thr	Glu	Leu	Ile	Gly	Lys	Lys	Lys	Thr	Ile	Cys	Glu	Ser	Ser	Gly	
	305					310					315					320	
	Ile	Trp	Ser	Asn	Pro	Ser	Pro	Ile	Cys	Gln	Lys	Leu	Asp	Lys	Ser	Phe	
				325						330					335		
	Ser	Met	Ile	Lys	Glu	Gly	Asp	Tyr	Asn	Pro	Leu	Phe	Ile	Pro	Val	Ala	
				340					345					350			
35	Val	Met	Val	Thr	Ala	Phe	Ser	Gly	Leu	Ala	Phe	Ile	Ile	Trp	Leu	Ala	
			355					360					365				
	Arg	Arg	Leu	Lys	Lys	Gly	Lys	Lys	Ser	Lys	Arg	Ser	Met	Asn	Asp	Pro	
		370					375					380					
40	Tyr																
	385																



(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1192 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGAGAGCTGT TATAAAGATT AAACAATATA ATAAATATGG CGCGTGAGCT TCAGAGTTTT	60
TGTTGTTGTT ATTATTATTT TCCTAAAAAT GCAAATCTGA TTTGCATTG ACTCATTGAC	120
TCACATCAGT GGGTCTTCCT TTTTATTGTC CTTTCATCATA TGGGTCCTAA TTTACATGC	180
AGTCTTATAA AACCATCTCA TTTTATAGTC CAAGAATATT AAAGGTACTT GTAGGCTCCC	240
AAACCTACAC GGTGAAAAGC TAGAGAGCAT GGGCTCTCTT CAGGGGTAA CTTCAGGAAG	300
TGCCACTAAC AAGGACGTCC ACTAGGTGGT GAGCAAGGAA AGACGGAGGT GAAGGAACCG	360
AAACGAGTCA AGTCCACTGC TTAGCTCTAC TGAAGTTTTG CAAACATCAT AAATATGTCT	420
GAAATGCAGT TTTGATTTGT AGTATTTGCA ATTTCCAAGG GCCATTTACC ACAGGTAGCC	480
AAGAGTTAGT TTAGCATTTA TGAAAAAGAT AGGGGAGGGT GGTGGTTAAG AAGGAGGTGG	540
AGGAGAGAGT GAAGGAGGAA GAGGAGAACA AGAACCAAAC AAAACAAGA ACAAGAACAA	600
GTAGAAGAAG AGGAGCAGGG AGGAAAAGA AGAGGAAGAA GAACAGCAAC AACAATGAGT	660
GAAGGAGGAG GAGGGTAAGG AAAGATGCAT AGGAGAATGG AAGGAAGGAT AGAAAGGAGG	720
GAAGGAAGAG AGAATCTAGT CACATTACTT TCTGATCAGC AGTTCATTTT TGTCTCAGTG	780
GGAGGCAATA GAGGCCAGTC TAGGAAAGGG GTGGGGAAAG AGGAAAGAGA AGTGCAGGAG	840
GAAGGGGAGG CCCAAGGGGA GGAGGAGGAG GATGTGAGAC TGGGTTAGAG AAATGAAAGA	900
AAGCAAGGCT TTCTGTTGAC ATTCAGTGCA GTCTACCTGC AGCACAGCAC ACTCCCTTTG	960
GGCAAGGACC TGAGACCCTT GTGCTAAGTC AAGAGGCTCA ATGGGCTGCA GAAGAACTAG	1020
AGAAGGACCA AGCAAAGCCA TGGTGAGCCT TTCAGCCTAA AAGACGTTTA GATGCTCAGA	1080
TAGAACTCT TGGGGTTGTA GAGGCAGGTG GCAAGGATAG GAATCACCCC ATTTCAATTC	1140
TGGTTTTAAA TAATATAGAA ACTAAACATT TTCTCAGACC CTCAAAAAAA GT	1192

This Sheet Has Been Replaced



(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 363 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CACTGAGACT AAGCGTAAAA TAAATAGAAC AAACAAACTG TGCATCAGTT CTGATGTAAA 60
TTTGAAGTAA TTTTCATCTA TGTCTGAGAA ACCTGTTACC TCAGACAGGG TTAGTAGACA 120
TATGTGTTTT ATTCTGATTA TTAAGAAAGT TGTAAGCACC ACCTCAAAGG CTATAAATGT 180
GTGGTTTAAAG GGTATACATC TAAATATAAT TTTGTATTTC ATTTGCAGAT ATTTCCATGG 240
AAATGTCAGA GCACCCAGAG GGACTTATGG AACATCTTCA AGTTGTGGGG GTGGACAATG 300
CTCTGTTGTG GTATGTTATG ATATTTATAT ATCACTAAGT CTATTTTACT TATATTCATT 360
TTT 363

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 531 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGGAGTAGT GCTAGGTTCT TTTTAGCTGT AACATTATGT AAGTCTGCAT AGGTCACACT 60
GATGTCTTGC AGATTTCCCTG GCACATCATG GAACCGACTG CTGGACTTAC CATTATTCTG 120
AAAAACCCAT GAACTGGCAA AGGGCTAGAA GATTCTGCCG AGACAATTAC ACAGATTTAG 180
TTGCCATACA AAACAAGGCG GAAATTGAGT ATCTGGAGAA GACTCTGCCT TTCAGTCGTT 240
CTTACTACTG GATAGGAATC CGGAAGATAG GAGGAATATG GACGTGGGTG GGAACCAACA 300
AATCTCTCAC TGAAGAAGCA GAGAACTGGG GAGATGGTGA GCCCAACAAC AAGAAGAACA 360
AGGAGGACTG CGTGGAGATC TATATCAAGA GAAACAAAGA TGCAGGCAAA TGGAACGATG 420
ACGCCTGCCA CAAACTAAAG GCAGCCCTCT GTTACACAGG TAGGGAGTGA CAAGACGGCT 480
ATGCTGCCTC AGACTCAGGA AGGGCCACGG TTAAGAGAAT ACTCAGATTT A 531



(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AAAATTTTAG CCATATGATT TTTATGCTAT GAATTTACCA AATAAACCTT TCCTGATTAT 60
TTAAATCATC TCAGACAAAA GGTTATCTAT GTCTAAAGAA ATGACTTTGA GTACTAAAT 120
GTAATCACAT TAAATATTT TTTTCTGAC CTCCTTAAAG CTTCTTGCCA GCCCTGGTCA 180
TGCAGTGGCC ATGGAGAATG TGTAGAAATC ATCAATAATT ACACCTGCAA CTGTGATGTG 240
GGGTACTATG GGCCCCAGTG TCAGTTTGGT AAGTCTCTTT CTTTCTTTG CTTCTTCTTA 300
GGTAAAGTCA CAGGAATCAT TATAGCTTAT CATGAAGCTG GTTGAACAA AATGATACTA 360
GCCACTCTGA GAAATGGGAA GTTTTGATCA GAAAGCTCTG CTTTCACAAT ATTGTTACCT 420
TTCCGTAAAG ATTTTCATAAG TCAGCATGAA GTTTCGATTG ACTTCTCAAC AAGTCTTTTT 480
GAGTACCACA AGAAGCACAG TGTGTTGGATA AAGCTGTCAG GGTACAATA AGGAATTAGC 540
ATGGTAGATT CCCGCTCTCA AGAAGCTCAC GATCTAATGA GCTTGTTAGA TTAATTAGAA 600
CTCTAAGGTC TGGAAGAAAC TATGCCATTT ATCATTAGGA GGCTGAGTTA CCCAGAAAGT 660
ATCTTGCTTT TTCCTTCTAG TAGTTCCTTT CTTTCTTGCA GTTCTCCACA CTTAACACAT 720
GTGCTCTGTA GCACACTGAC TTTGCTGGTG GCCTTCTCTC TCATTTTGCA CATGGCCAAA 780
AAACATGTCA TCTTTAAGAC ATTGTTCAAA GACAGTTTCT TCTAGGAAGC TT 832

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 712 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCTGATGTG ATAGTTATTT CCCGACTAAG CTGGTCATTC CCAGTTACAC CTATTTGGCT 60
TTAAGGATTC TCACTACAGA TAATACTGAA GATAATAATA TGAAGACTAG CTAATGTTTA 120
CTTAGAATTT CTGATGAGTC AGGCTTTGTT CTAACGTCCT TGACTTATGC TAATTGAATT 180



ACATTTAGTT TCCATATCAA TTTGATAAAG ATAACACAAT TTCATTATTC CTCTTATATA 240
 GATGAAGAAA CTGAAGTTGG AGGGTTCAAG TAACCTTGTT TAAAGGCACA TGGTTATCAA 300
 GTGGCAGGGC TAGGATTCAA ATCCAGGCGT CAGTTCCTCT TAACTCTTCC CCATACTGTT 360
 TCTTTCCCTA TTGAAGTGAT TCAGTGTGAG CCTTTGGAGG CCCCAGAGCT GGGTACCATG 420
 GACTGTACTC ACCCTTTGGG AACTTCAGC TTCAGCTCAC AGTGTGCCTT CAGCTGCTCT 480
 GAAGGAACAA ACTTAACTGG GATTGAAGAA ACCACCTGTG GACCATTGG AACTGGTCA 540
 TCTCCAGAAC CAACCTGTCA AGGTGAGTAA CTTCAGACTA GAGGTTTTGT CATGCAATCC 600
 TGGGCTTACA GTCAGAACAT TCAGTAGAAG TTTGCTGAGA AGTCAAACCTT AGGATCCTAA 660
 TTTAACCTAA CTTTTGTTTA ACCTACTGTG ATGTTTCTCA AAGGACTTAT TC 712

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 451 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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 TGGAAGCAAT ATCACAAGTA ATGTAGTCAG GCAGCTTTGG CTAAAAATCC AAAGCTCAAG 180
 GGAGGGTCTC TACTCAGAAA TACTGTTTTG TCTTTTTTTT TTTTCTTTT TCATTGAAGT 240
 GATTCAGTGT GAGCCTCTAT CAGCACCAGA TTTGGGGATC ATGAACTGTA GCCATCCCCT 300
 GGCCAGCTTC AGCTTTACCT CTGCATGTAC CTTTCATCTGC TCAGAAGGAA CTGAGTTAAT 360
 TGGGAAGAAG AAAACCATTG GTGAATCATC TGGAACTGG TCAAATCCTA GTCCAATATG 420
 TCAAAGTGAG TAAGTTTGTC CTGGAAGTGA A 451



(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 544 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TATCAGAACT AAGAAAGCTT GGGCTGCAGG TCGACTCTAG GTGCATTTTC AGGAACTCTA 60
TGAACCACAA ATCTGGGCAT TGAGATTCTG TAGGCATTAG ACTAGCAAGG CTGGTCAGTC 120
TTTGCTATG CTGTAGACTC ATCAGGGGCC TTCCCATGCC AGTTTCCTCA TCTGTCAAAT 180
GGCATCATTT GGGCTACTAC TGGGAGATGT AAGGAGGAAA AAAGTCAAAT ATCATGAGAT 240
AGACTAAGGA AATAATGCTG GTGGTCTCAT GCTATGTGCC TTACTGATTT CTCTTTCAGA 300
ATTGGACAAA AGTTTCTCAA TGATTAAGGA GGGTGATTAT AACCCCTCT TCATTCCAGT 360
GGCAGTCATG GTTACTGCAT TCTCTGGGT GGCATTTATC ATTTGGCTGG CAAGGAGATT 420
AAAAAAGGT ATGTGAGTTT AACTTCACAT GAAAAGAACA CAACTTTAAA GTGAAAAAGA 480
AAAAAAAAG AAACCCACAG GAAATTAAAT GTGATAGATT CAACACAAGC AGGATGCCAA 540
GCTT 544

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 524 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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TTGGGTGGGA TAAACACTTG AATGACATAT TGGGTCTTGC CACCAGGCAA TTTAGCAATT 120
CTGTCTTCTT GAGTAGCAG GAGATGGAAT GGAACCTCAG GAGGCATCTG CATCAACATG 180
TCTGTTCTGT ATTAGTGTCT ACCACTGTTT ATTAAGCCAG TTCCTCAAAT CTCCTTTGAC 240
ACAGATAGGG TCCACCTAAC AAATACCTAA TATACTTCAA AAGACAGTTT TGAGAGTGGG 300
AGTCTTCCTT CTCCCTTACT TGAAAACTT TAAATTGTCT AATTTTGTCT AATGCCTTTT 360

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TCTCTATTTT CTATTTTCAGG CAAGAAATCC AAGAGAAGGT AAGTTTTATT AGTGGCGAGG 420
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CATACCCATA GTAGTTATTA CTGGGTCTAT CAATGACAGA TAGG 524

(2) INFORMATION FOR SEQ ID NO:11:

5

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1696 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

15

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TGCTGCTACT AGGGTTTGCT TACATTTGTT CATCTTCAGA GGTTCCTAAA GCCTGGCCTC 180
TTGTCTGAGA TTTCCAGCTG AAAGCATTTT CTGCTCCTC TTCTCATCTC TAATGAATAT 240
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TTCATGTGGA ATATAAGAT GGCTGACTTT GCTCTTTCTT GACTCTTGTT TTCAGTTTCA 660
25 ATTCAGTGCT GTACTTGATG ACAGACACTT CTAAATGAAG TGCAAATTTG ATACATATGT 720
GAATATGGAC TCAGTTTTCT TGCAGATCAA ATTTGCGGTC GTCTTCTGTA TACGTGGAGG 780
TACACTCTAT GAAGTCAAAA GTCTACGCTC TCCTTTCTTT CTAAGTCCAG TGAAGTAATG 840
GGGTCTGCT CAAGTTGAAA GAGTCCTATT TGCAGTGTAG CCTCGCCGTC TGTGAATTGG 900
ACCATCCTAT TTAAGTGGCT TCAGCCTCCC CACCTTCTTC AGCCACCTCT CTTTTTCAGT 960
30 TGGCTGACTT CCACACCTAG CATCTCATGA GTGCCAAGCA AAAGGAGAGA AGAGAGAAAT 1020
AGCCTGCGCT GTTTTTTAGT TTGGGGGTTT TGCTGTTTCC TTTTATGAGA CCCATTCCCTA 1080
TTTCTTATAG TCAATGTTTC TTTTATCAG ATATTATTAG TAAGAAAACA TCACTGAAAT 1140
GCTAGCTGCA ACTGACATCT CTTTGATGTC ATATGGAAGA GTTAAACAG GTGGAGAAAT 1200
TCCTTGATTC ACAATGAAAT GCTCTCCTTT CCCCTGCCCC CAGACCTTTT ATCCACTTAC 1260
35 CTAGATTCTA CATATTCTTT AAATTCATC TCAGGCCTCC CTCAACCCCA CCACTTCTTT 1320



5

TATAACTAGT CCTTTACTAA TCCAACCCAT GATGAGCTCC TCTTCCTGGC TTCTTACTGA	1380
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TAGTTGCCT AGTTTGTTAT TTTGAAAATT GATCATATGT TTTGTTTTCT CCCCAGTGAG	1500
TTACATGCTC CTTCAGGGCA GAGTTTGTGT CAGATCCCTG GAGTATCTAG TGCATTACTT	1560
GAACTCAAT AAATGAATGT TCAAATAAAT CAGAAAGAGC ATACAGTGCA CTGCTGATAT	1620
AAGTTTCAGC ATCCCTCTTT CTCTATGGCA TCTGATGACC TGGGTCAGAT ATCACCTAAT	1680
GTCAACAGCT GAATTC	1696

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CLAIMS

What is claimed is:

1. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
P-selectin and
a ligand binding portion found within the lectin domain
of L-selectin.
2. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
E-selectin and
a ligand binding portion found within the lectin domain
of L-selectin.
3. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
P-selectin and
a ligand binding portion found within the lectin domain
of E-selectin.
4. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
E-selectin and
a ligand binding portion found within the lectin domain
of P-selectin.
5. The polypeptide of claim 1 wherein
the ligand binding portion found within the EGF domain
of P-selectin is the entire EGF domain of P-selectin and
wherein
the ligand binding portion found within the lectin
domain of L-selectin is the entire lectin domain of L-
selectin.

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6. The polypeptide of claim 2 wherein
the ligand binding portion found within the EGF domain
of E-selectin is the entire EGF domain of E-selectin and
wherein
the ligand binding portion found within the lectin
domain of L-selectin is the entire lectin domain of L-
selectin.
7. The polypeptide of claim 3 wherein
the ligand binding portion found within the EGF domain
of P-selectin is the entire EGF domain of P-selectin and
wherein
the ligand binding portion found within the lectin
domain of E-selectin is the entire lectin domain of E-
selectin.
8. The polypeptide of claim 4 wherein
the ligand binding portion found within the EGF domain
of E-selectin is the entire EGF domain of E-selectin and
wherein
the ligand binding portion found within the lectin
domain of P-selectin is the entire lectin domain of P-
selectin.
9. The polypeptide of any one of claims 1-4 further
comprising a selectin SCR domain and a selectin transmembrane
domain.
10. The polypeptide of any one of claims 1-4 further
comprising a
carrier region/joined to a carrier protein.
11. The polypeptide of claim 10 wherein said carrier protein
comprises an immunoglobulin heavy chain constant region.

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12. The polypeptide of claim 10 wherein said carrier protein comprises non-ligand binding portions of a selectin molecule.
13. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of P-selectin and
a ligand binding portion found within the lectin domain of L-selectin.
14. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of E-selectin and
a ligand binding portion found within the lectin domain of L-selectin.
15. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of P-selectin and
a ligand binding portion found within the lectin domain of E-selectin.
16. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of E-selectin and
a ligand binding portion found within the lectin domain of P-selectin.
17. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising
the exon encoding the EGF domain of P-selectin and
the exon encoding the lectin domain of L-selectin.
18. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising
the exon encoding the EGF domain of E-selectin and
the exon encoding the lectin domain of L-selectin.

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19. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising the exon encoding the EGF domain of P-selectin and the exon encoding the lectin domain of E-selectin.

20. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising the exon encoding the EGF domain of E-selectin and the exon encoding the lectin domain of P-selectin.

21. A pharmaceutical composition for use in treating the polypeptide of any one of claims 1-4 in a pharmaceutically acceptable carrier substance.

22. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said condition is tissue damage or an autoimmune disorder.

23. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said condition is a post-reperfusion injury.

24. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said condition is an organ or tissue transplant recipient.

25. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said polypeptide is joined to a therapeutic agent.

26. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said polypeptide is joined to a carrier protein.

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27. The pharmaceutical composition of leukocyte-mobilizing claim 25 wherein said therapeutic agent comprises a chemotherapeutic drug or an anti-inflammatory agent.

28. The use of the polypeptide of any one of claims 1-4 to identify a ligand or ligands that bind to said polypeptide.

29. The use of the polypeptide of any one of claims 1-4 to screen for antagonists of selectin EGF domain function.

30. The use of the polypeptide of any one of claims 1-4 to screen for simultaneous antagonists of the function of lectin and EGF domains of different selectins.

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**CHIMERIC SELECTINS AS SIMULTANEOUS BLOCKING AGENTS
FOR COMPONENT SELECTIN FUNCTION**

ABSTRACT

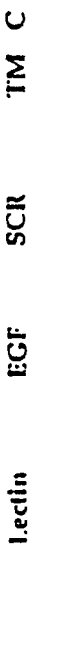
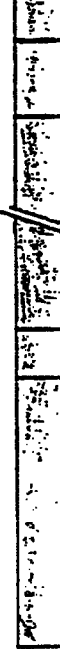
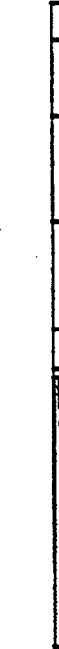
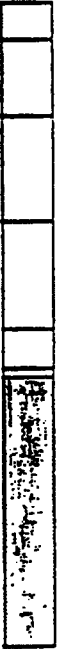
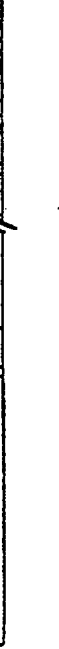
Chimeric peptides or polypeptides that combine ligand binding portions from within the lectin and EGF domains of two different selectins are disclosed. The peptides or polypeptides can be constructed solely of the indicated portions of lectin or EGF domains or they can include portions of any of the remaining domains (SCR, transmembrane or cytoplasmic), or the entire extracellular portion, of a generic selectin molecule. The peptides or polypeptides also can be joined to a carrier protein (e.g., a soluble portion of an immunoglobulin molecule) to increase the serum half-life of the therapeutic agent. The chimeric polypeptides can be used as therapeutic agents to antagonize selectin function. They are also useful for screening for agents that are simultaneously antagonists of the function of lectin and EGF domains of different selectins.

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Monoclonal antibody reactivity

DESIGNATION	Lectin	EGF	SCR	TM C	G1 (P Lectin)	LAM1-3 (L Lectin)	LAM1-1 (L Lectin/EGF)	LAM1-14 (L SCR)	S12 (P SCR)
L-selectin (L)					0	525	374	576	0
P-selectin (P)					1712	0	0	0	1059
P2L					839	0	68	419	0
P3L					714	0	0	350	0
L2P					19	1484	0	0	1093
L3P					0	1779	1503	0	1018
L2P3L					0	363	0	266	0

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Fig. 1

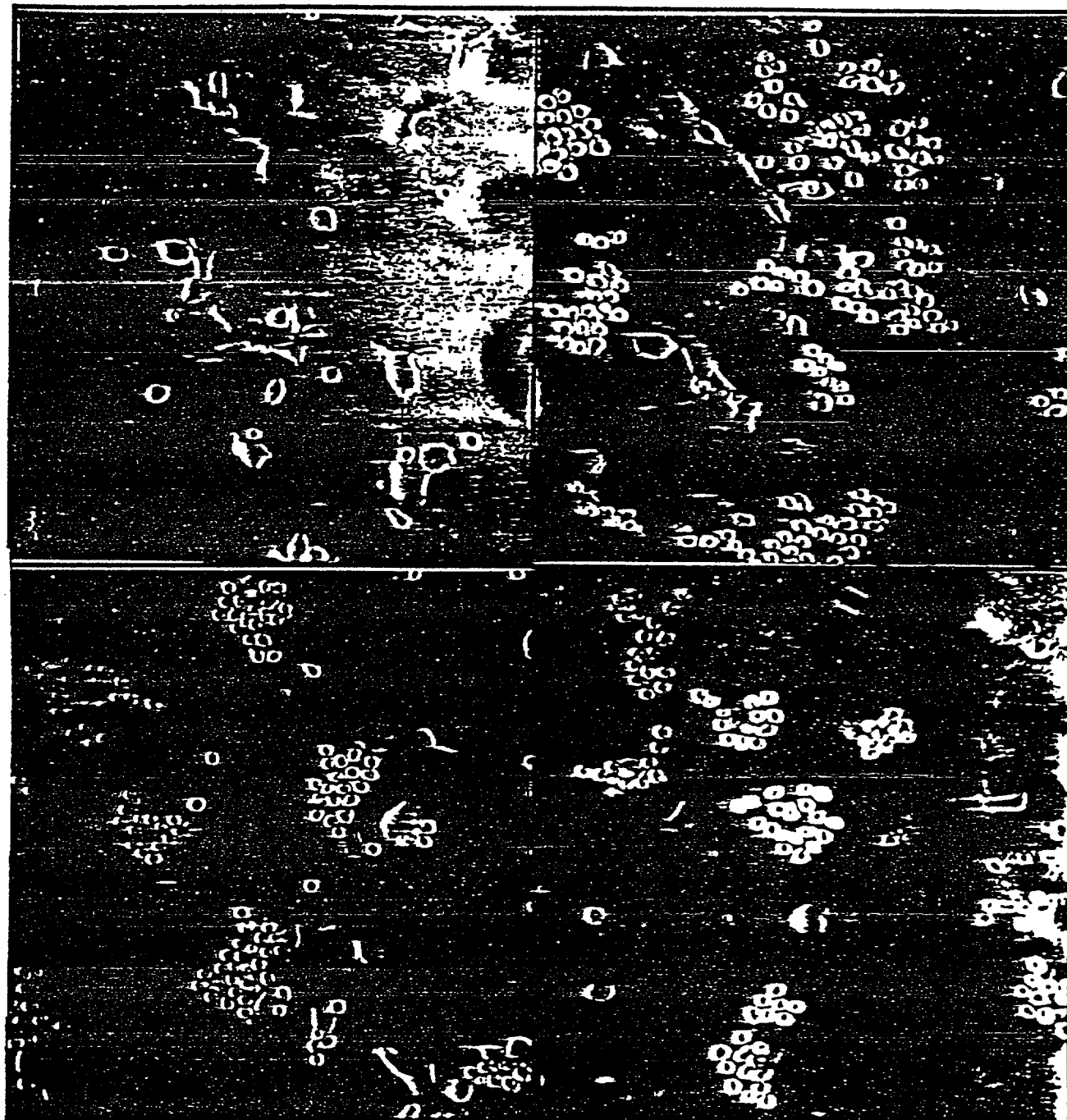
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Fig. 2A

P-selectin

L-selectin



L2P

P2L



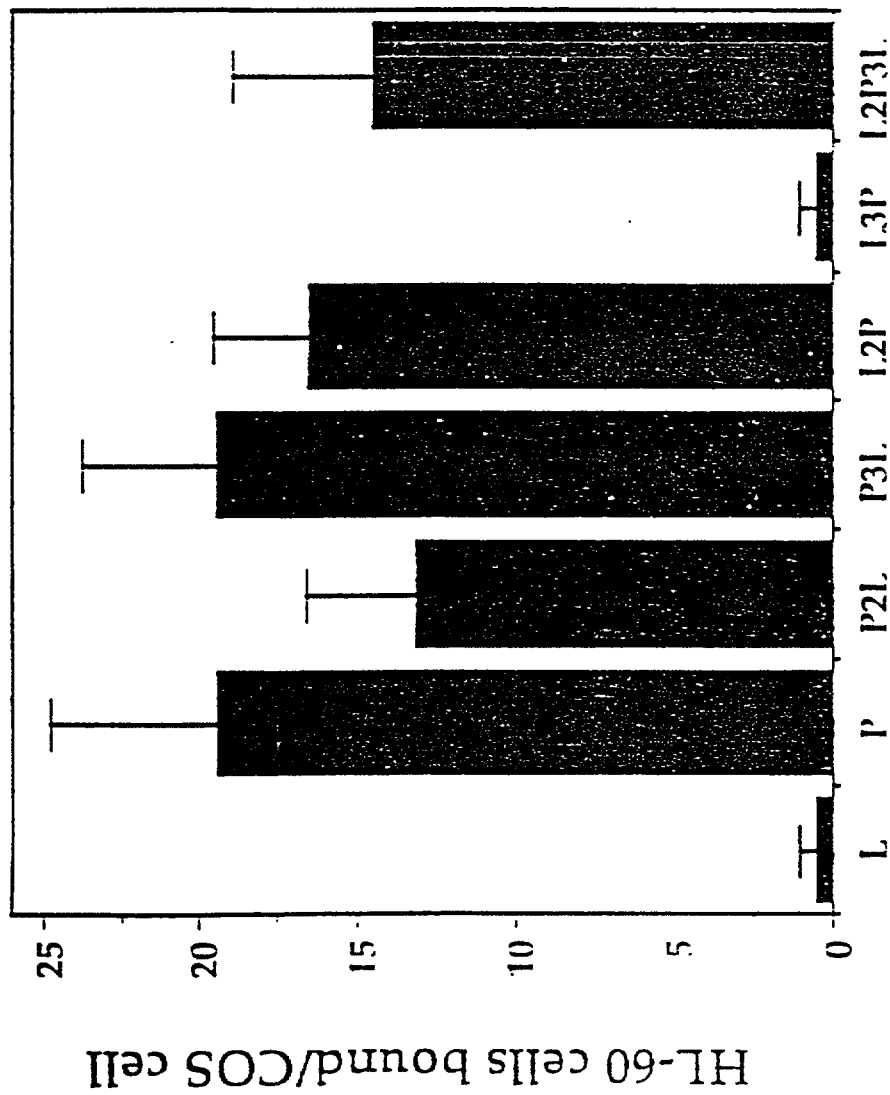


Fig. 2B



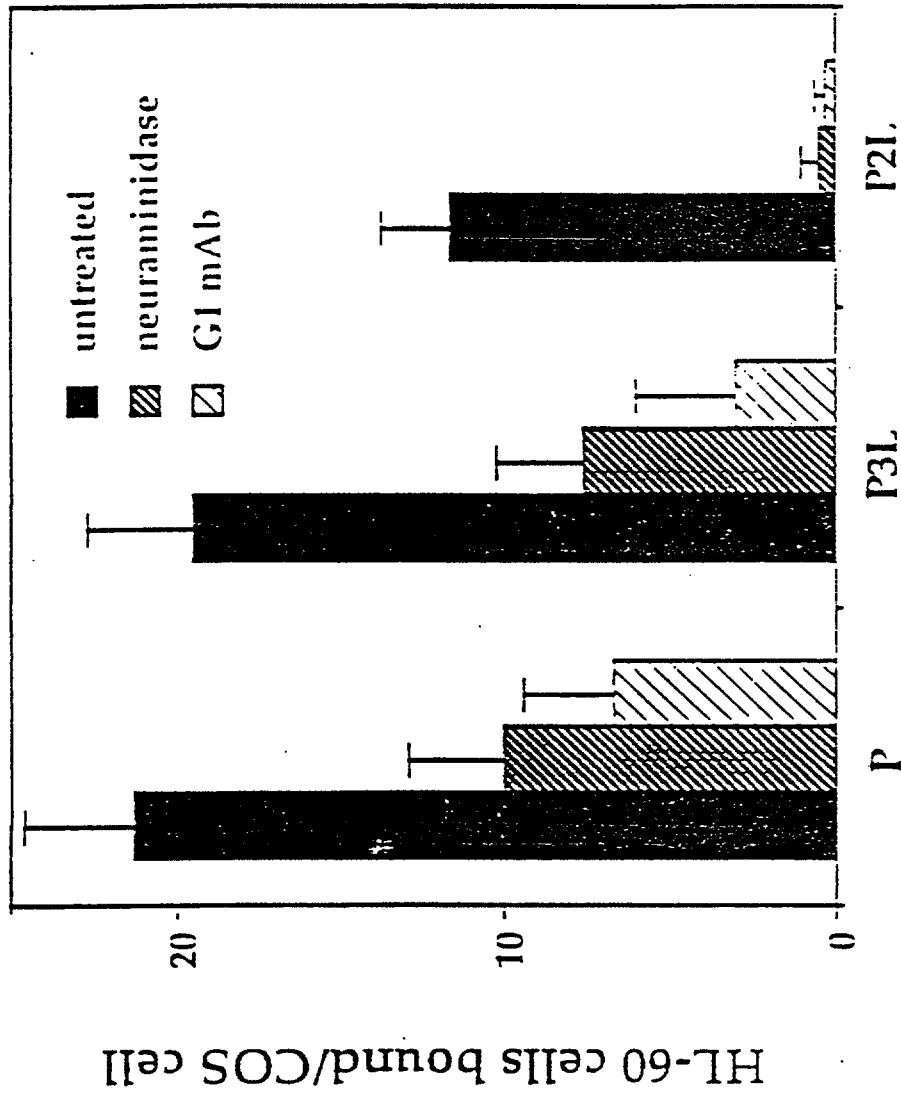


Fig. 3A



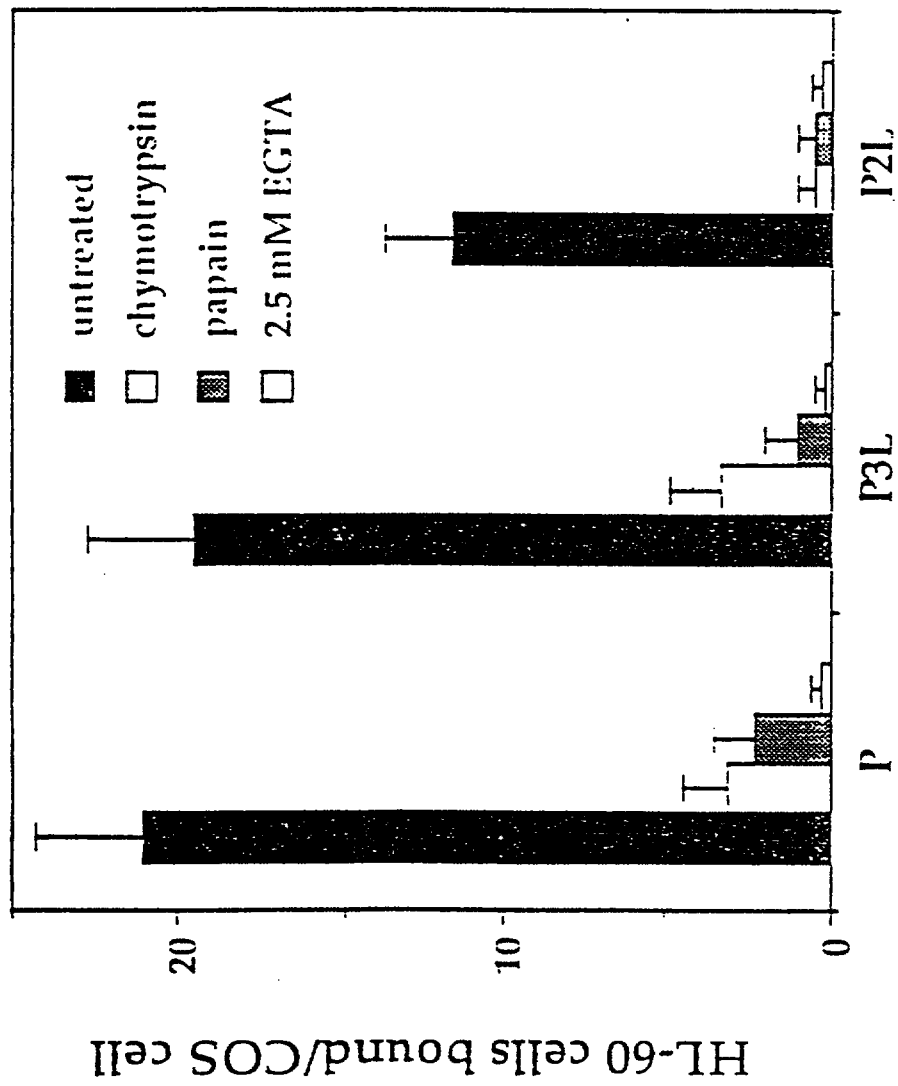


Fig. 3B

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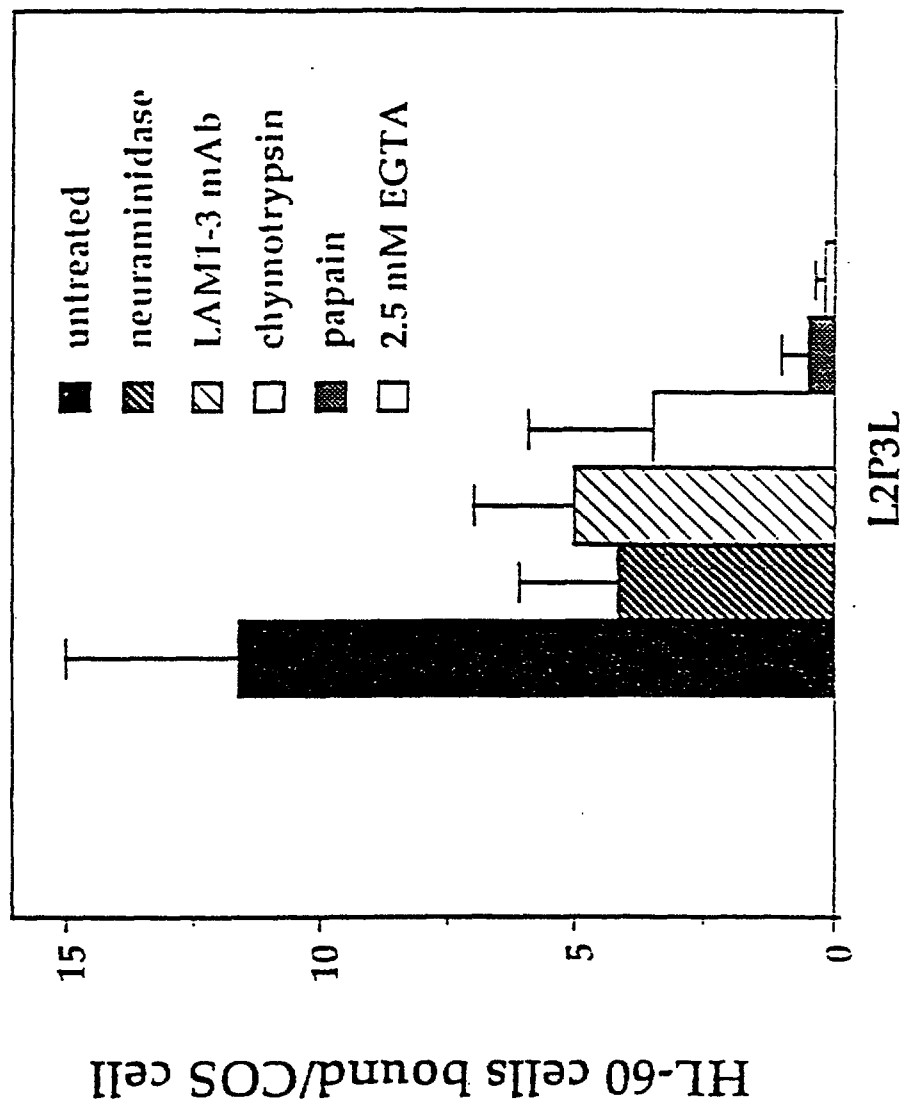


Fig. 3C



1 M G
 gaattcccttt GGGCAAGGAC CTGAGACCCT TGTGCTAAGTCAAGAGGCTCA ATG GGC
 10
 C R R T R E G P S K A M
 TGC AGA AGA ACT AGA GAA GGA CCA AGC AAA GCC ATG 94
 20
 I F P W K C Q S T Q R D L W N I
 ATA TTT CCA TGG AAA TGT CAG AGC ACC CAG AGG GAC TTA TGG AAC ATC 30
 40
 F K L W G W T M L C C D
 TTC AAG TTG TGG GGG TGG ACA ATG CTC TGT TGT GAT 168
 50
 F L A H H G T D C ↓ W T Y H Y S E
 TTC CTG GCA CAT CAT GGA ACC GAC TGC TGG ACT TAC CAT TAT TCT GAA 60
 K P M N W Q R A R R F C
 AAA CCC ATG AAC TGG CAA AGG CCT AGA AGA TTC TGC 262
 80
 R D N Y T D L V A I Q N K A E I
 CGA GAC AAT TAC ACA GAT TTA GTT GCC ATA CAA AAC AAG CCG GAA ATT 90
 E Y L E K T L P F S R S
 GAG TAT CTG GAG AAG ACT CTG CCT TTC AGT CGT TCT 346

FIG. 4A



100 Y Y W I G I R K I G G 110 I W T W
 TAC TAC TGG ATA GGA ATC CGG AAG ATA GGA GGA ATA TGG ACG TGG
 120 V G T N K S L T E E A E N
 GTG GGA ACC AAC AAA TCT CTC ACT GAA GAA GCA GAG AAC 430
 130 W G D G E P N N K K N K E D C
 TGG GGA GAT GGT GAG CCC AAC AAC AAG AAG AAC AAG GAG GAC TGC
 140 V E I Y I K R N K D A G K
 GTG GAG ATC TAT ATC AAG AGA AAC AAA GAT GCA GGC AAA 514
 150 W N D D A C H K L K A A L C Y
 TGG AAC GAT GAC GCC TGC CAC AAA CTA AAG GCA GCC CTC TGT TAC
 160 T A S C Q P W S C S G H G
 ACA GCT TCT TGC CAG CCC TGG TCA TGC AGT GGC CAT GGA 598
 170 E C V E I I N N Y T C N C D V
 GAA TGT GTA GAA ATC ATC AAT AAT TAC ACC TGC AAC TGT GAT GTG
 180 G Y Y G P Q C Q F V I Q C
 GGG TAC TAT GGG CCC CAG TGT CAG TTT GTG ATT CAG TGT 682
 190 E P L E A P E L G T M D C T H
 GAG CCT TTG GAG GCC CCA GAG CTG GGT ACC ATG GAC TGT ACT CAC
 200 P L G N F N F N S Q C A F
 CCT TTG GGA AAC TTC AAC TTC AAC TCA CAG TGT GCC TTC 766
 210 S C S E G T N L T G I E E T T
 AGC TGC TCT GAA GGA ACA AAC TTA ACT GGG ATT GAA GAA ACC ACC
 220 C E P F G N W S S P E P T
 TGT GAA CCA TTT GGA AAC TGG TCA TCT CCA GAA CCA ACC 850
 230 C Q V I Q C E P L S A P D L G
 TGT CAA GTG ATT CAG TGT GAG CCT CTA TCA GCA CCA GAT TTG GGG
 240 I M N C S H P L A S F S F
 ATC ATG AAC TGT AGC CAT CCC CTG GCC AGC TTC AGC TTT 934
 250 T S A C T F I C S E G T E L I
 ACC TCT GCA TGT ACC TTC ATC TGC TCA GAA GGA ACT GAG TTA ATT
 260 G K K K T I C E S S G I W
 GGG AAG AAG AAA ACC ATT TGT GAA TCA TCT GGA ATC TGG 1018
 270 S N P S P I C Q K L D K S F S
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 280 M I K E G D Y N P L F I P
 ATG ATT AAG GAG GGT GAT TAT AAC CCC CTC TTC ATT CCA 1102

FIG. 4B



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V A V M V T A F S G L A F I I
GTG GCA GTC ATG GTT ACT GCA TTC TCT GGG TTG GCA TTT ATC ATT
360
W L A R R L K K G K K S K
TGG CTG GCA AGG AGA TTA AAA AAA GGC AAG AAA TCC AAG 1186
380
R S M N D P Y *
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ATTCTTGGA TACTAAAAAT CATGAGATCC TTAAATCCT TCCATGAAAC 1280
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TCACCCCTCA TTTATCCCTC AACCCCCAGC CCACAGGTCT TTATACAGCT
CAGCTTTTTT TCTTTTCTGA GGAGAAACAA ATAACACCAT AAAGGGAAAG 1480
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TTTTCAGTTT CAATTCAGTG CTGTACTTGA TGACAGACAC TTCTAAATGA 1580
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CAAAAGGAGA GAAGAGAGAA ATAGCCTCCG CTGTTTTTTTA GTTTGGGGGT
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CAACTGACAT CTCTTTGATG TCATATGGAA GAGTTAAAAC AGGTGGAGAA 2080
ATTCCTTGAT TCACAATGAA ATGCTCTCCT TTCCCCTGCC CCCAGACCTT
TTATCCACTT ACCTAGATTC TACATATTCT TTAAATTTCA TCTCAGGCCT 2180
CCCTCAACCC CACCACTTCT TTTATAACTA GTCCTTTACT AATCCAACCC
ATGATGAGCT CCTCTTCCTG GCTTCTTACT GAAAGGTTAC CCTGTAACAT 2280
GCAATTTTGC ATTTGAATAA AGCCTGCTTT TTAAGTGTTA AAAAgaattc 2330

FIG. 4C

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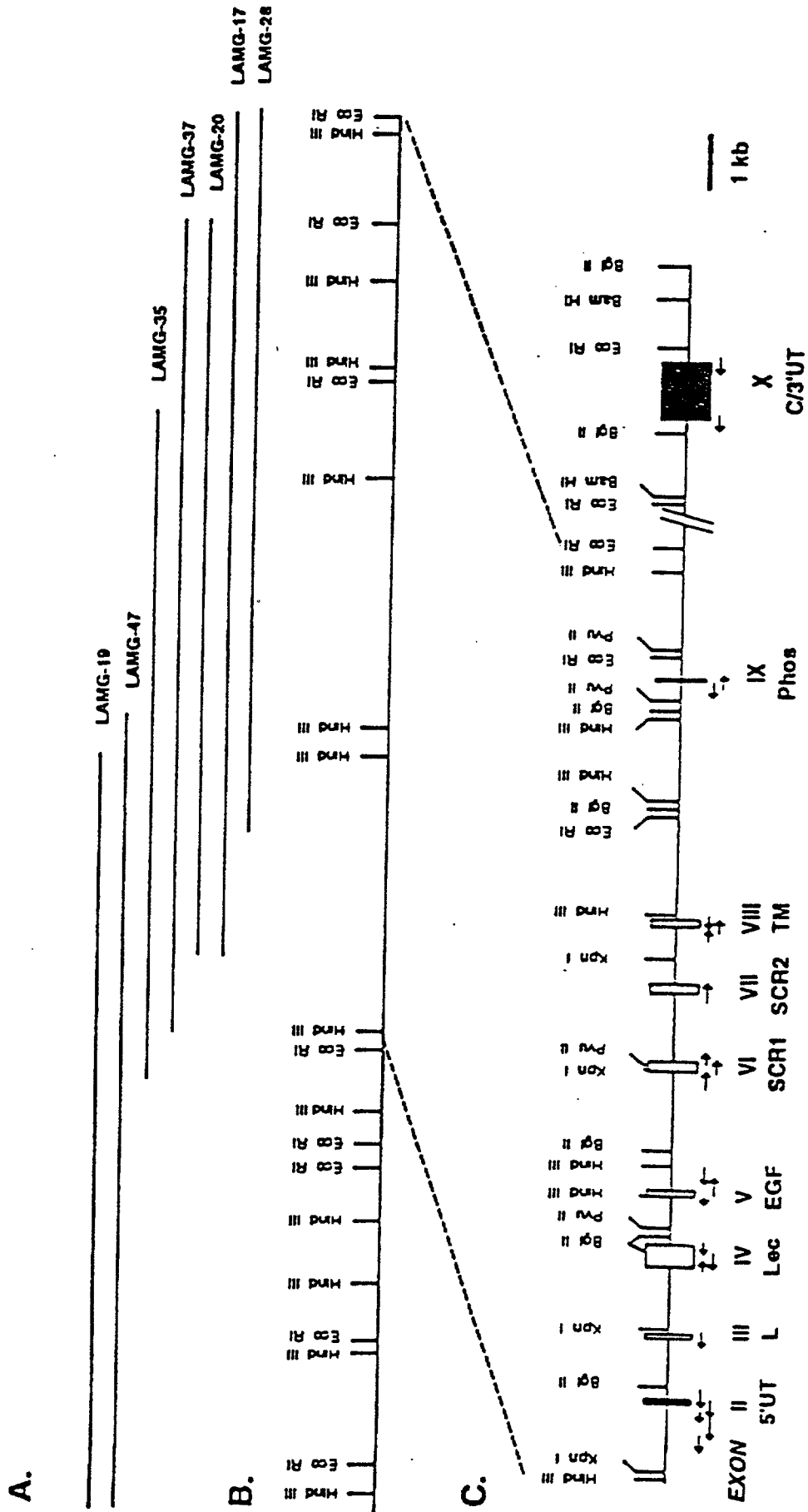


FIG. 5



EXON 11 (5' Untranslated Region and Translation Initiation Site)

AGAGAGCTG TTATATAGT TAAACATATG GCGCTGAGC TTCAGAGTIT TIGTGTGTGT TATTATATIT TCCCTAATAT TCCAAATCTG ATTIGCATIT
 GACTCATTA CTACATCTG TTTTATGT CTTTCATCAT ATGGGCTCTA ATTTCACATG CAGCTTATTA AACCACTCTC ATTTATAGT CCAAGATAT
 TAAAGTACT TGTAGGCTCC CAATCTTACA GGTGATAGG CTAGAGGCTA TGGGCTCTCT TCAGGGGTIA ACTTCAGGA GGGCCACTA CAGGGAGCTC CACTAGGCTG
 TGAAGAGGA AAGACGGAGG TGAAGGAGC GAATCGAGTC AATCTCACTG CTTAGCTCTA CTGAGGTIT CTGAAATCTA TAAATATGTC TGAATGCTG TTTIGATTIG
 TAGTATTTG AATTTCCAG GGCATTTAC CACAGGTAGC CAGAGTTAG TTTAGCATIT ATGATATATG TGGTGGTIA GAGGGAGGTG GAGGGAGGAG
 TGAAGAGGA AGAGGAGAGC AAGATCCAT AATATACAGC AATATACAGC AATATACAGC AATATACAGC AATATACAGC AATATACAGC AATATACAGC
 TGAAGAGGA GAGGGTATG GAATGATGCA TAGGAGATG GAGGAGAGG TAGGAGAGG GAGGAGAGG GAGGAGAGG GAGGAGAGG GAGGAGAGG GAGGAGAGG
 TTTGCTAGT GGGAGGCAAT AGAGGAGGAGT CTAGGAGAGG GGTGGGGA GAGGAGAGG AAGTGCAGG GAGGAGAGG GAGGAGAGG GAGGAGAGG GAGGAGAGG
 CTGGGTATG GAATGAGAG AAGGAGAGG TTTGCTAG CATTAGTGC AGTCTAGCTG CAGGAGAGG GAGGAGAGG GAGGAGAGG GAGGAGAGG GAGGAGAGG
 CAGGAGAGG AATGGGCTG AGAGGAGAGG GAGGAGAGG AATGGGCTG AATGGGCTG AATGGGCTG AATGGGCTG AATGGGCTG AATGGGCTG AATGGGCTG
 GGGTGTATG GGCAGGTGGC AAGGATAGG ATCAGGCTT TCAATCTG GTTTATAT AATATAGAGC TAAATATIT CTAGAGGCTT CAAATATAGT

EXON 11 (Leader Domain)

CACTAGAG CTAGGCTTA AATATATAG AATATATAG TGTGATGCTG TGTGATGCTG TGTGATGCTG TGTGATGCTG TGTGATGCTG TGTGATGCTG
 GGTATAGTA CATATGCTT TTTATCTGAT TATTATAGTA GTTATAGTA CCACTCTCA GGTATATAT GTTGGGTIA AGGGATATACA TCTATATATA ATTIGTAT
 TCAATGCTG ATA TTT CCA TGG AAA TGT CAG AGC ACC CAG AGC GAG TTA TGG AAC ATC TTC AAG TGG TGG TGG ACA ATG CAC TGT
 TGT G CAGTATG AATATATAT ATCAGTATG CTATTTAT TATATCTT TTT

FIG. 6A



EXON IV (Leader Domain)
 CT GCGATGATGCG TGGGTCCTTT TTAGCTGTAA CATTTATGTAA GTCGCTGATG GTCACACATGA TGTCTTTCAG AT TTT CTT GCA CAT CAT GCA GCA GCA GCA GCA
 TGG ACT TAC CAT TAT TCT GAA AAA CCC ATG AAC TGG CAA GGG GCT GGA GGA TTT TGG GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA GCA
 CAA AAC AAC GGG GGA ATT GAG TAT GAG
 ATA TGG AAC TGG GAG GAG AAC AAC AAA TCT CTC ACT GAA
 GAG GAG TGG GAG GAG GAG TAT ATC AAC GAA AAC AAA GAT GCA
 TGT TAC GCA G GATGGGAGTG ACAGAGGCG TATGCTGCTT CAGACCTGAG AGGGGCGAG GTTAGAGGAA TACTAGATTT TA

EXON V (EGF Domain)
 AAAATTTTGG CCTATGATTT TTTTGTCTAT GATTTTACCA ATATTAACCTTT TCTGTATTTT TTTTATCTATC TCAAGCAAAA GGTATCTAT GTCATAGAAA ATGACTTTTGA
 GTACTAATAT GTATACAT TAAATATTTT TTTTCTGAC CTCTTAAAG CT TCT TGG GAG
 GAA ATC ATC AAA TAT
 TGTCTTCT TGGTAAAGT CACAGGATC ATTAAGCTT ATCATGAGG TGGTTGGAC AAAATGATAC TGGCCTCTT GAGAAATGG AGGTTTGTAT CAGAAAGCTC
 TGTCTTACCA ATATTTTAC CTATCTGTA AGATTTTATA AGTTTGTAT TGTCTTCTA ACATGCTTT TTAGATACCA CAGGAGGAC AGTGTGGGA
 TAAAGCTGTC AGGTTTACCA TAAAGATTA GATTTGTA TCTGCTCTT CAGGAGGCTT CAGGAGGCTT GAGGTTGTA GATTTTATAG AACCTTAAAG TCTGAGGAA
 ACTATGCTAT TATCATATG GAGGCTGAGT TACCAAGAA GTATCTGCT TTTTCTTCT AGTAGTTCTT TCTTCTTCT CAGTTCTTCA CACTTAAAC ATGTGCTCTG
 TAGCAGCTG ACTTGTGCTG TGGCTTCTC TCTATTTT CACTTGGCA AAAATCAATGT CACTTTTATG ACATTTGTTA AAGACAGTTT CTCTTAAAG GCTT

EXON VI (SCR 1 Domain)
 CTCTGA TGTGATGTT ATTTCCCGAC TAACTGTGTC ATTCCTAGTT ACACCTATTT GGTATTAAGG ATTCCTACCA CAGATTAATAC TGAAGATTAAT ATATATGAGAA
 CTAGCTAATG TTTACTTAGA ATTTCTGATG AGTCAGGCTT TGTCTTAAAG TCTTGTACTT ATGCTAATTT AGTTTCTATA TCAATTTGTAT AAAGATTAACA
 CATTTTCTAT ATTCCTCTTA TATAGATGAA GAAATCTGAG TTTGAGGCTT CAGGATTAACCT TGTATTAAGG CACTTGGTA TCAATGCTGA GGTATCTCA
 GGTACATTC CTCTTAACTC TTTCCCTATAC TCTTCTTCT CCAATTTGAG TGT ATT GAG GCT TTT GAG GCT CCA GAG GCT CCA GAG GCT CCA GAG GCT CCA GAG GCT CCA
 TGT ACT GAG GCT TTT GAG GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG
 GAA AAC GAG GAG GAG TTT GAG GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG TTT GAG
 GGTATTAAGT CAGGATTAATC AGTAGAGGTT TGTCTGAGG TCAATTTTATG GATCTTAAAT TAACTTAACT TTTGTTTAA CTTCTCTGAT GTTCTCTAAA GGTCTTATTC

FIG. 6B

EXOH VII (scr II Domain)

0968

EXON VIII (Transmembrane Domain)

TATCAGAAC TAAGGAGGCT TGGCTGACG GTGACTCTA GGTGATTTT CAGGACCTCT ATGAGCCACA AATCTGGCA TTGATCTCT GTAGCATTTA GACTAGCAAG
GCTGGTCAGT CTTGGCTAT GCTTAGACT CATCAGGGCG CTTCCATGC CAGTTTCTC ATCTGTCAAA TGGCATCATT TGGGCTACTA CTGGAGATG TAGGAGGAA
AAAGATCAAA TATCATGAGA TAGACTAAGG AATATATGCT GGTGGTCTCA TGTATGTGCG CTATCTGATT TCTCTTTCAG AA TTG GAC AAA AGT TTC TCA ATG
ATT AAG GAG GGT GAT TAT AAG CCC CTC TTC ATT CCA GTC GTC ATG GTT ACT GCA TTC TCT GGG TTG GCA TTT ATC ATT TGG CTG
GCA AAG AAG TTA AAG AAA G GTATGTGAGT TTACTTTCG ATGAGAGGAA CAGACCTTTA AGGAGAGAAA GAAAGAGAAA AGGAGAGAGC AGGAGAGTTTAA
TTGAGAGG TTGAGAGGTTT GCGAGATGCC AAGCTT

EXOH IX (Phosphorylation Domain)

TAGTTTACA GTATTGCGCAG CTGTCCCTCA ACCGAGGANTC TGCAGGTGACG ATGGGGTGGG AATGACACTT GAAATGACATA TTGGGTCTTG CAGCCAGGCCA
 AATTGACCAAT TCTGTCTTCT TCGATGCGNC TGGGAGCTCA GAGGAGACTT GCATACACAT GCTCTCTCTG TATTAGTGTG TACCACCTGT TATTATGCCA
 GTTCCCTCAAA TCTCCTTTGA CACAGATAGG GTCCACCTAA CAATATACCA AATATCTCA AAGACACGTT TTGAGAGTGG GAGCTCTCCT TCTCCCTTAC TTGAAACT
 TTTAAATGTC TAAATTTTGC TAAATGCCCTT TACTCTATT TCTATTTCAG GC_AAG_AAA_TCC_AAG_AGA_AG GTAGCTTTTA TTAGTGGCA GAGCTTCCA
 CAGCTGCTGA TTCATTCTCT ACTCTTTAG ACTCTTTGCT TCTNGCTACG CAGATACCA TNGCTGTTAT TNCGGGCTC ATCATGACAT GATAGG

5X HOX5 (Cytoplasmic Tail and 3' Untranslated Region)

[illegible]

FIG. 6C

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A. Lectin domains

	*	#
human-L:	WTYHYSEKPMNWQRARRFCRDNYTDLVAIQNKAEIEYLEKTLPFSSRSYYWIGIRKIGGIW	
mouse-L:	-----EN--K--KQ--N-----R-----N--K-PY-----KM-	
rat-L:	-----RS--EN--KF-KH-----R-----KNPT-----KT-	
bovine-L:	-----KR--P-EK--A--E-----G-----N--Q--T-----VE-V-	
human-E:	-S-NT-TEA-TYDE-SAY-QQR--H-----E-----NSI-SY-P-----VNNV-	
bovine-E:	-S--A-TEM-TFEE--DY-QKT--A-----QE-----NS-FSY-P-----N-T-	
human-P:	-----T-AYS-NIS-KY-QNR-----N--D--N-V--YYS-----NNKT-	
mouse-P:	---N--T-AYS-NNS-V--RHF-----N--AH-NDVI--FN-----NNK-	
bovine-P:	-----N-TYS-NYS-A--QKY--K-----N--A--NE-I-YYN-----NNK-	

	#	#	#	# *	# ###	*	*														
human-L:	TWVG	TNKS	LT	EEA	ENW	GD	GEP	NNK	KNK	EDC	VEI	YIK	RNK	DAG	KWN	DD	ACH	KL	KA	AL	CYT
mouse-L:	-----T--K-----A-----S-----ER-S-----R-----																				
rat-L:	-----T--K-----T-----S-----ER-S-----R-----																				
bovine-L:	-----K--A-----R-S-----S-----A-T-----																				
human-E:	V----	Q-P----	K--AP----	RQ	KD-----	E--V-M----	ER-S-K-L----														
bovine-E:	--I-----K--T--AP-----QSD-----E--S-----EK-T-Q-L----																				
human-P:	-----K-A--N-----A-N-----R-N-----SPSAP-----EH-L-K-H----																				
mouse-P:	-----T-----A-N-----NQ-----S-SAP-----EP-F-R-R----																				
bovine-P:	-----K-T-----A-N-----R-NQ-----SLSAP-----EP-W-R-R----																				

B. EGF domains

	*	*	*	* *	*
human-L:	ASCQPWSCSGHGECVEIINNYTCNC	VDV	GYG	PQC	QF
mouse-L:	-----G--N-R-----T--H--I--A-----Y				
rat-L:	-----E--NR-----T--N--I--P-----Y				
bovine-L:	---K-----Q--V-----L-----E---				
human-E:	-A-TNT-----T-----K--P-FS-LK-EQ				
mouse-E:	---TNA-----I-T--S--K-HP-FL--N-EQ				
bovine-E:	-A-N-TP-GS-----Q-HP-FK-LK-EQ				
human-P:	---DM---KQ---L-T-G---S-YF-F---E-EY				
mouse-P:	---DM---NQ-K-I-T-GS---S-YF-F---E-EY				
bovine-P:	---DM---KQ---I-T-G---S-YF-F---E-EY				

FIG. 7



REPLACED SHEETS		SG
☒ Description	: paged 13, 24 to paged -	
☒ Claim(s)	: paged 37 to paged 41	
☐ Drawing(s)	: paged to paged	
☐ Abstract	: paged to paged - 1 -	
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REPLACEMENT SHEET		SG
☒ Description	: paged 1 to paged 26	
☒ Claim(s)	: paged 37 to paged 41	
☒ Drawing(s)	: paged 114 to paged 119	
☒ Abstract	: paged 42 to paged 42	
DATE OF RECEIPT 23/8/96		

**CHIMERIC SELECTINS AS SIMULTANEOUS BLOCKING AGENTS
FOR COMPONENT SELECTIN FUNCTION**

RELATED APPLICATIONS

10 This application is a continuation-in-part of Tedder,
U.S. Patent Application Serial No. 07/983,606, filed
November 30, 1992, which is a continuation under 37 CFR 1.62
of Serial Nos. 07/730,503, filed July 8, 1991, and 07/313,109,
filed February 21, 1989, both now abandoned. This
15 application also is a continuation-in-part of Tedder, U.S.
Patent Application Serial No. 07/700,773, filed May 15, 1991;
of Tedder, U.S. Patent Application Serial No. 07/737,092,
filed July 29, 1991; of Tedder et al., U.S. Patent
Application Serial No. 07/770,608, filed October 3, 1991; and
20 of Tedder et al., U.S. Patent Application Serial
No. 07/862,483, filed April 2, 1992, the whole of which are
hereby incorporated by reference herein.

FIELD OF THE INVENTION

25 This invention relates to the selectin family of
receptor adhesion molecules and particularly to agents
interfering with selectin function.

GOVERNMENT RIGHTS

Part of the work leading to this invention was made with
United States Government funds. Therefore, the U.S.
Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

30 The ability of leukocytes to leave the circulation and
to migrate into tissues is a critical feature of the immune
response. Normally, the infiltrating leukocytes phagocytize
invading organisms or dead or damaged cells. However, in
35 pathologic inflammation, infiltrating leukocytes can cause

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serious and sometimes deadly damage. Leukocyte-mediated inflammation is implicated in a number of human clinical manifestations, including the adult respiratory distress syndrome, multi-organ failure and reperfusion injury.

5 The selectin family of receptor adhesion molecules mediates the initial interactions of leukocytes with endothelium (Springer, Nature 346:425-434 (1990); Lasky, Science 258:964-969 (1992)). L-selectin (also known as LAM-1) is expressed on the surface of most classes of leukocytes (Griffin et al., J. Immunol. 145:576-584 (1990); Tedder et al., J. Immunol. 144:532-540 (1990)) and mediates the binding of lymphocytes to high endothelial venules (HEV) of lymph nodes and to activated endothelium. P-selectin (also called PADGEM, CD62 or GMP-140) is expressed by activated platelets and endothelial cells (Johnston et al., Cell 56:1033 (1989); Hsu-Lin et al., J. Biol. Chem. 259:9121 (1984)) and mediates adhesion between myeloid cells and activated endothelium or activated platelets (Gallatin et al, Nature 304:30-34 (1983); Spertini et al, J. Immunol. 147:2565-2573 (1991); Larsen et al, Cell 59:305-312 (1989); Geng et al, Nature 343:757-760 (1990)). Another member of the family E-selectin (also known as ELAM-1) is expressed by activated endothelial cells (Bevilacqua et al., Science 243:1160 (1989); Bevilacqua et al., Proc. Nat'l Acad. Sci. USA 84:9238 (1987)) and partially mediates the binding of neutrophils to endothelium at sites of inflammation (Bevilacqua et al., Proc. Nat'l Acad. Sci. USA 84:9238 (1987)). All selectins are derived from evolutionarily related genes (Collins et al., J. Biol. Chem. 266:2466-2478 (1991); Johnston et al., J. Biol. Chem. 34:21381-21385 (1990); Ord et al., J. Biol. Chem. 265:7760-7767 (1990); Watson et al., J. Exp. Med. 172:263-272 (1990)), and are characterized by an NH₂-terminal, Ca²⁺-dependent lectin domain, an epidermal growth factor (EGF)-like domain followed by multiple short consensus repeat (SCR) domains, a transmembrane region, and a cytoplasmic tail. Although the lectin domains are critical for the binding of specific

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carbohydrate ligands (Springer, Nature 346:425-434 (1990); Lasky, Science 258:964-969 (1992)), the role of the conserved EGF-like domains is unknown.

It has been proposed that the treatment of a patient suffering from pathologic inflammation with an antagonist to adhesion receptor function can result in the reduction of leukocyte migration to a level manageable by the target endothelial cells and the subsequent dramatic recovery of the patient. Local administration of therapeutic agents can block competitively the adhesive interactions between leukocytes and the endothelium adjacent to an inflamed region. Therapeutic agents can also be administered on a systemic level for the treatment of a patient suffering from disseminated inflammation (Harlan and Liu, eds., Adhesion: Its Role in Inflammatory Disease, W. H. Freeman (in press)).

SUMMARY OF THE INVENTION

We report here that the EGF-like domains of P-selectin and of E-selectin can participate directly in cell adhesion, having ligand binding sites distinct from those of their respective lectin domains. Cell adhesion mediated at least by the P and E-selectins may be complex, involving interactions between the lectin domain and a carbohydrate ligand, and separately, between the EGF-like domain and one or more proteins or other ligands. Our discovery of a difference in at least one of the ligands recognized by the lectin and EGF domains of a given selectin has permitted the preparation of chimeric polypeptides that combine within one molecule the ability to target two or more different selectin ligands and the use of these agents in therapy or in the preparation of additional classes of antagonists to selectin function.

Thus, the invention generally features chimeric peptides or polypeptides that combine ligand binding portions from within the lectin and EGF domains of two different selectins.

The peptides or polypeptides can be composed solely of the



indicated portions of lectin or EGF domains or they can include portions of any of the remaining domains (SCR, transmembrane or cytoplasmic), or the entire extracellular portion, of a generic selectin molecule. The peptides or polypeptides also can be joined to a carrier protein (e.g., a soluble portion of an immunoglobulin molecule) to increase the serum half-life of the therapeutic agent. In another aspect, the invention features nucleic acid encoding the chimeric polypeptides.

The chimeric polypeptides can be used as therapeutic agents to antagonize selectin function. They are also useful for screening for agents that are simultaneously antagonists of the function of lectin and EGF domains of different selectins.

As used herein, the term "polypeptide" is intended to include shorter molecules, or "peptides." The term "essentially purified" refers to a polypeptide sequence that has been separated or isolated from the environment in which it was prepared or in which it naturally occurs.

BRIEF DESCRIPTION OF THE DRAWINGS

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, taken in conjunction with the accompanying drawings in which:

Fig. 1 shows the structure and expression of L-selectin/P-selectin chimeric proteins (Lectin, lectin domain; EGF, epidermal growth factor-like domain; SCR, short consensus repeat; TM, transmembrane domain; C, cytoplasmic domain);

Fig. 2a shows binding of HL-60 cells to COS cells transfected with natural and chimeric selectins (Original magnification, 200x);

Fig. 2b shows mapping of the domain(s) of L2P responsible for adhesion of HL-60 cells (bars represent the

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means $\pm$ SD, and are representative of at least six experiments);

Fig. 3a shows neuraminidase sensitivity for adhesion mediated by the lectin and EGF domains of P-selectin;

Fig. 3b shows protease sensitivity and Ca^{2+} requirement for adhesion mediated by the lectin and EGF domains of P-selectin;

Fig. 3c shows an analysis of the binding of the chimera L2P3L;

Figs. 4a, 4b, and 4c show the cDNA nucleotide sequence (SEQ ID NO: 1) encoding L-selectin and also show the amino acid sequence of L-selectin (SEQ ID NO: 2);

Fig. 5 shows the restriction map and the exon-intron organization of the *lyam-1* gene;

Figs. 6a, 6b, and 6c show the nucleotide sequence of exons II through X of the *lyam-1* gene; and

Fig. 7 shows the homology among the lectin and EGF domains of L-, P- and E-selectin.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

We have examined the role of the lectin and EGF-like domains of the L-, P-, and E-selectins in cell adhesion and have determined that the EGF-like domains of P- and E-selectin play a direct role in ligand recognition and leukocyte adhesion mediated by the respective selectin. This discovery has permitted the preparation of chimeric polypeptides that combine within one therapeutic agent the ability to target two or more different selectin ligands and the use of these agents in therapy or in the preparation of additional classes of antagonists to selectin function.

Determination of the binding specificity of selectin lectin and EGF domains

The functional activities of the lectin and EGF-like domains of L-, P-, and E-selectin in the adhesive events described above were examined using a panel of

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L-selectin/P-selectin and L-selectin/E-selectin chimeric molecules. Referring to Fig. 1, these hybrid polypeptides were created by exchange of cDNA encoding the lectin, EGF, or both lectin and EGF domains of the desired selectin and expression of the chimeric nucleic acid, as described in the Experimental Procedures section. Many other methods familiar to those of skill in the art can be employed to prepare similar products.

The chimeras conserve the overall polypeptide backbone structure of the selectins, and resulted in minimal changes in the junctions between domains. The functional characterization of these chimeric selectins therefore offered a powerful approach to determining the molecular basis of cell adhesion mediated by these molecules and to preparing diagnostic and therapeutic agents incorporating properties of individual selectins into the same polypeptide.

Expression of the chimeric proteins in COS cells was verified and quantitated using panels of P-, E- and L-selectin domain-specific monoclonal antibodies (mAb) in direct adhesion assays. The chimeric cDNA were subcloned into the pMT-2 vector and used to transiently transfect COS cells as described under Experimental Procedures. Twenty-four hours before analysis, 2.5×10^4 transfected COS cells were replated into 96 well plates. Expression of domain-specific epitopes by chimeric selectins was assessed using mAb reactive with specific domains and a surface immunofluorescence assay as described (Luscinskas et al, J. Immunol. 149:2163-2171 (1992)).

Referring again to Fig. 1, the individual constructs, containing the indicated domains of P-selectin and L-selectin, reacts only with anti-L-selectin and anti-P-selectin monoclonal antibodies recognizing the specific, indicated domains. These results demonstrate that the chimeric cDNA encoded the expected proteins and that each protein was expressed and readily detected on the surface of COS cells. Note that the LAM1-1 mAb fails to recognize

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either the L2P or P2L proteins, and therefore defines an epitope composed of residues in both the lectin and EGF domains. Values given are means of absolute optical density (OD) units from which OD units obtained with a control non-binding mAb were subtracted. Experimental values less than the control values are represented as zero. The values presented are typical of those obtained in three experiments. The standard deviations for these fluorescence measurements were less than 15%.

Characterization of the adhesion reactions mediated by the selectin lectin and EGF domains

The HL-60 myelomonocytic cell line expresses ligands for P-selectin (Larsen et al, Cell 59:305-312 (1989); Geng et al, Nature 343:757-760 (1990)) and E-selectin (Bevilacqua et al., Science 243:1160 (1989); Bevilacqua et al., Proc. Nat'l Acad. Sci. USA 84:9238 (1987)) and was therefore used to assess P-selectin and E-selectin function. Referring to Fig. 2a, HL-60 cells bound at high levels to COS cells expressing P-selectin, but did not bind to COS cells expressing L-selectin. HL-60 cells also bound to COS cells expressing the chimeric protein P2L, in which the lectin domain from P-selectin was substituted for the lectin domain of L-selectin. Therefore, the lectin domain of P-selectin alone, when attached to the EGF-like and other domains from L-selectin, was sufficient to mediate high levels of HL-60 cell adhesion. However, the lectin domain of P-selectin alone was not solely responsible for all HL-60 binding, because HL-60 cells also bound to COS cells expressing L2P, in which the P-selectin lectin domain was replaced with that of L-selectin.

The domain(s) within L2P responsible for HL-60 adhesion were mapped using additional chimeric selectins. Referring to Fig. 2b, HL-60 cells bound to COS cells expressing L2P3L, which contains only the EGF domain from P-selectin, but did not bind to COS cells expressing L3P, which lacks both the

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lectin and EGF-like domains of P-selectin but contains the P-selectin SCR domains. Furthermore, binding of HL-60 cells to P3L, which contains both the lectin and EGF domains from P-selectin, was approximately equivalent to that of native P-selectin and significantly ($P < 0.01$; Student's T test) higher than binding of cells to P2L, even though expression of P2L and P3L were approximately equal. These data indicate that both the lectin and EGF-like domains of P-selectin contain ligand binding sites capable of independently mediating HL-60 cell adhesion.

For study of ligand binding sites in E-selectin, four L-selectin/E-selectin chimeric molecules were constructed. L2E has the lectin of L-selectin and the remainder of E-selectin; E2L is the precise converse. L5E has the amino terminal 88 amino acids of L-selectin (i.e., 74% of the lectin domain) and E5L is the precise converse. Binding of HL-60 cells to COS cells expressing these chimeras was assessed as before. The constructs containing only a portion of a specific lectin domain exhibited zero binding, while the binding to chimeras containing lectin and EGF domains from different selectins was reduced by about a half. Therefore, the overall pattern of binding was similar to the results with L-selectin/P-selectin chimeras.

Determination of the influence of EGF-domains on the adhesive activity or specificity of the lectin domains

Adhesion mediated by P-selectin involves sialic acid-bearing carbohydrates, possibly including the sLe^x and/or related structures, as well as protein determinants (Larsen et al, Cell 63:467-474 (1990); Zhou et al, J. Cell Biol. 115:557-564 (1991); Moore et al, J. Cell. Biol. 118:445-456 (1992); Moore et al, J. Cell Biol. 112:491-499 (1991); Polley et al, Proc. Natl. Acad. Sci. USA 88:6224-6228 (1991); Larsen et al, J. Biol. Chem. 267:11104-11110 (1992)). Therefore, the effects of neuraminidase and protease

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treatment of HL-60 cells on binding to COS cells expressing either P-selectin, P3L, or P2L was examined.

5 Removal of sialic acid residues from HL-60 cells significantly ($P < 0.01$) but incompletely reduced adhesion of HL-60 cells to COS cells expressing native P-selectin or P3L (Fig. 3A). In contrast, removal of sialic acid residues from HL-60 cells nearly completely eliminated adhesion of HL-60 cells to P2L (Fig. 3A). In addition, binding to the E-selectin/L-selectin construct E2L was partially inhibited by pretreatment of the HL-60 cells with CSLEX1 mAb directed against sLex. Similarly, pretreatment of COS cells with mAb directed against the lectin domain of P-selectin significantly ($P < 0.005$), but only partially, inhibited the adhesion of HL-60 cells to P-selectin or P3L, but abolished adhesion to P2L (Fig. 3A).

15 Treatment of HL-60 cells with either chymotrypsin or papain eliminated >90% of binding to COS cells expressing P-selectin, P3L, or P2L (Fig. 3B). In addition, 2.5 mM EGTA abolished adhesion (Fig. 3B). These results confirm the importance of the lectin domain in adhesion, and offer independent evidence that the EGF domain of P-selectin also plays a direct role in adhesion, recognizing a protein ligand.

20 A similar analysis was performed on L2P3L to characterize adhesion mediated by the EGF-like domain of P-selectin. Adhesion of HL-60 cells to COS cells expressing L2P3L was also reduced, but not eliminated, by neuraminidase treatment of the HL-60 cells (Fig. 3C). MAb directed against the L-selectin lectin domain present within L2P3L also significantly ($P < 0.01$) inhibited adhesion. These results suggest that recognition of specific carbohydrate structures on HL-60 cells by the lectin domain of L-selectin, which alone was not sufficient for cell adhesion, contributes to leukocyte adhesion in this system when this interaction is supported by the adhesive activity mediated by the EGF domain of P-selectin. Consistent with this hypothesis, rare

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(~1 plate) rosettes of HL-60 cells were occasionally observed on COS cells transfected with L-selectin (data not shown), and a soluble L-selectin/IgG fusion protein has a measurable affinity for purified, immobilized sLe^x (Foxall et al, J. Cell Biol. 117:895-902 (1992)). Adhesion of HL-60 cells to L2P3L was eliminated by EGTA, indicating that adhesion mediated by the EGF-like domain is also Ca²⁺-dependent, and was significantly ($P < 0.01$) reduced by protease treatment (Fig. 3C).

Determination of the influence of EGF domains on the adhesive activity or specificity of the lectin domains

In order to determine if the EGF-like domains of selectins have any influence on the adhesive activity or specificity of the lectin domain, stable transfectants of the 300.19 murine pre-B cell line expressing L-selectin, P-selectin, L2P or L2P3L were generated. These cells were tested for binding to lymph node HEV using the standard in vitro frozen section assay (Stamper Jr. et al, J. Exp. Med. 144:828-833 (1976)). Transfectants expressing P-selectin did not bind to HEV, whereas transfectants expressing L-selectin, L2P or L2P3L bound to HEV equivalently and at high levels (Table I).

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Table I. Adhesion of cell lines to lymph node HEV is mediated by the lectin domain of L-selectin.

5	<u>cDNA</u>	<u>LAM1-3</u> <u>mAb</u>	<u>expt 1</u>	<u>expt 2</u>	<u>expt 3</u>
	none	-	0.047 ± 0.02	0.12 ± 0.025	<0.01
		+	<0.01	ND	ND
	L-selectin	-	3.43 ± 0.42	6.85 ± 0.45	2.44 ± 0.51
		+	0.05 ± 0.2	ND	<0.01
	L2P	-	3.71 ± 0.26	6.64 ± 0.43	4.40 ± 0.3
		+	0.074 ± 0.03	ND	<0.01
	L2P3L	-	5.71 ± 0.33	6.85 ± 0.18	3.94 ± 0.27
		+	0.08 ± 0.03	ND	<0.01
10	P-selectin	-	<0.01	<0.01	ND

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The lectin domain of L-selectin alone was therefore capable of mediating binding to HEV when attached to the EGF-like and other domains of P-selectin. The L2P and L2P3L chimeric selectins can therefore bind to different types of cells which express ligand(s) for either L- or P-selectin, reflecting the specificities of both of the parent selectins from which they are constructed. This acquisition of novel adhesive properties as a result of the exchange of lectin or EGF-like domains between selectins reinforces our hypothesis that the EGF-like domain of P-selectin can mediate cell adhesion. In addition, these data directly demonstrate the functional independence of the lectin and EGF-like domains, and therefore argue against a role for the EGF-like domain in determining the carbohydrate specificity of the lectin domain.

Structure of the L-selectin gene

As disclosed in U.S. Patent Application Ser. No. 07/983,606, cDNA encoding L-selectin has been cloned and the sequence determined, as shown in Figs. 4a, 4b and 4c (SEQ ID NO: 1). The cDNA encodes a protein of 372 amino acids (SEQ ID NO: 2).

The structure of the *lyam-1* gene, which encodes the LAM-1 protein, was determined by isolating overlapping genomic DNA clones that hybridized with a LAM-1 cDNA probe. The *lyam-1* gene spans greater than 30 kb of DNA and is composed of at least 10 exons. The 5' end of the LAM-1 mRNA was mapped by primer extension analysis, revealing a single initiation region for transcription. Exons II through X contain translated sequences; exon II (SEQ ID NO: 3) encodes the translation initiation codon, residue 14 shown in Fig. 2; exon III (SEQ ID NO: 4) encodes the leader peptide domain, residues 15-41; exon IV (SEQ ID NO: 5) encodes the lectin-like domain, residues 42-170; exon V (SEQ ID NO: 6) encodes the epidermal growth factor-like domain, residues 171-206; exons VI (SEQ ID NO: 7) and VII (SEQ ID NO: 8) encode the

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short consensus repeat unit domains, residues 207-269 and 270-331; exon VIII (SEQ ID NO: 9) encodes the transmembrane region, residues 332-373; exon IX (SEQ ID NO: 10) encodes seven amino acids containing a potential phosphorylation site, residues 374-380; and exon X (SEQ ID NO: 11) encodes the five remaining amino acids of the cytoplasmic tail and the long 3' untranslated region.

The pLAM-1 cDNA was labeled with ^{32}P and used as a probe to isolate hybridizing DNAs from a human leukocyte genomic DNA library. Approximately 1×10^6 plaques were screened, and 13 plaques that hybridized with the cDNA probe were identified and isolated. Seven of these clones were found to contain inserts with unique restriction enzyme maps representing overlapping genomic fragments spanning at least 30 kb. These inserts, LAMG-17, -19, -20, -28, -35, -37, and -47, were further digested and subcloned into plasmids. Detailed restriction maps of these subclones were made and compared to those of intact inserts to determine their correct locations (Figs. 4A and 4B).

The correctness of the restriction map was verified with Southern blot analysis. DNA isolated from two B cell lines, BL and BJAB, and one T cell line, HSB-2, was digested to completion with *Bam* HI, *Bal* II, or *Pvu* II, size-fractionated, and transferred onto nitrocellulose. This filter was probed with the LAM-1 cDNA clone, pLAM-1. All genomic fragments derived from endonuclease digested DNA hybridized with cDNA probe to generate hybridizing bands of the appropriate size.

The pLAM-1 cDNA clone encodes an 85-bp 5' untranslated region. An oligonucleotide homologous with the 5' sequence of the pLAM-1 cDNA was used as a probe for primer extension analysis. This oligonucleotide was hybridized with poly (A⁺) RNA isolated from the human B cell line RAJI, the LAM-1 negative human B cell line Namalwa, the mouse pre-B cell line A20, and yeast tRNA as a control. Complementary DNA was synthesized by extending the primer with reverse transcriptase. The major primer extension product obtained

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using the human LAM-1 positive B cell line RNA was extended 126 nucleotides beyond the translation initiation site. There was a single cluster of transcription initiation sites for the *lyam-1* gene apparent in the reaction with RAJI RNA that was not found with the LAM-1 negative Namalwa RNA. Several primer extension products of size similar to those of the human B cell line RNA were obtained with mouse B cell RNA. Therefore, murine B cells may express an RNA species that cross-hybridizes with the oligonucleotide probe used. No primer extension products were obtained in the yeast tRNA control reactions.

The relationship of the primer extension results to the cloned LAM-1 cDNAs and the most 5' exon of *lyam-1* isolated was used to determine the nucleotide sequence of the exon that encodes the translation initiation AUG codon. This exon ends immediately after the site that encodes the translation initiation codon (Fig. 5) and overlaps precisely with the pLAM-1 cDNA sequence. The length of the cDNA clone obtained by Bowen et al., J. Cell Biol. 109:421-427 (1989) agrees precisely with the primer extension results except for two nucleotides. However, 15 nucleotides before the 5' end of the cDNA the sequence diverges from the genomic sequence at a site homologous with the 3' splice acceptor site consensus sequence. Therefore, it is most likely that this 15-bp region is derived from the exon upstream of this potential splice site. Thus, the primer extension results indicate that exon I would most likely be composed of 15 or fewer base pairs.

A 15-bp oligonucleotide homologous with the 5' nucleotides present in the cDNA clone of Bowen et al., supra but not encoded by exon II, was used to probe the 10 kb of cloned DNAs 5' of exon II; however, specific hybridization was not detected by Southern blot analysis. Under the conditions necessary for hybridization of this oligonucleotide, significant cross-hybridization occurred

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with λ -DNA, making it difficult to use this oligonucleotide to isolate the first exon from a λ -based genomic library.

These results suggest that the exon which encodes the translation initiation site is the second exon of the *lyam-1* gene (Fig. 4C). Consistent with this, the 900 bp upstream of exon II did not contain any apparent "TATA" or "CCAAT" sequences frequently found in promoter regions of eukaryotic genes (Fig. 5). Therefore, it is likely that the transcription initiation region and exon I are further than 10 kb upstream from exon II of the *lyam-1* gene. S1 nuclease protection analysis was carried out using the 5' region of exon II as a labeled probe for hybridization with poly (A⁺) RNA from RAJI, Namalwa, and A20 cells. Two mRNA species were protected in the RAJI mRNA, while no S1 protection was provided by the other RNAs. The length of these fragments was consistent with differential splicing at the two potential CAG/N splice sites located within the potential splice acceptor site in exon II. It is therefore likely that the transcription initiation region has not been identified.

The majority of the exons were localized by comparison of the restriction enzyme maps of the genomic clones and the pLAM-1 cDNA. In cases where this method did not provide definitive results, subcloned DNA fragments were digested with selected restriction enzymes, electrophoresed through agarose gels, and transferred to nitrocellulose. Fragments containing exons were identified by Southern blot analysis using labeled cDNA or oligonucleotide probes. The exon that encodes the 3' untranslated region of the LAM-1 cDNA was not contained within the 30 kb of isolated DNA fragments. Therefore, a labeled 0.9-kb *Dra* I fragment containing most of the 3' untranslated region of the pLAM-1 cDNA was used as a probe to identify a homologous 3.2-kb fragment generated by complete *Eco* RI digestion of genomic DNA. *Eco* RI-digested genomic DNA fragments of this size were used to make a partial λ -gt11 genomic library from which the 3.2-kb *Eco* RI

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fragment was cloned. This 3.2-kb fragment did not overlap with the previously isolated genomic DNAs.

The exact boundaries of the exons were determined by nucleotide sequence analysis. From this analysis, nine exons were identified which make up the entire pLAM-1 cDNA. Exon II encodes the translation initiation codon, and exon III encodes the leader domain of the LAM-1 protein (Fig. 5). Each of the lectin-like, epidermal growth factor-like, transmembrane, and short consensus repeat domains was encoded by a separate exon. The smallest exon, IX, is 19 bp in length and may encode a carboxyl-terminal phosphorylation cassette. The last 5 amino acids of the LAM-1 protein and the 3' untranslated region which includes the poly(A) attachment site, AATAAA, are encoded by exon X as shown in Fig. 5. The nine exons which encode pLAM-1 were split inside codons in all cases, except the junction between exons II and III. In each instance, the consensus sequences of 5' donor splice sites and 3' acceptor splice sites were adhered to. Nucleotide sequence polymorphisms within the coding region were observed between the genomic clones containing exon V that encoded SCR I and the pLAM-1 clone at cDNA nucleotide positions 741 and 747 (A to G), leading to a coding change from Asn to Ser in both cases, and at position 816 (A to G) changing the Glu to a Gly.

Experimental Procedures Construction of Chimeric Selectin cDNAs

Four new restriction endonuclease recognition sites were introduced separately into the pLAM-1 cDNA by polymerase chain reaction (PCR)-based site-directed mutagenesis as follows. Oligonucleotides surrounding and including each new restriction sequence were synthesized in both the sense and antisense directions. PCR was carried out using the new restriction site sense oligonucleotide and an antisense oligonucleotide anchor located in the plasmid near the 3' end of the cDNA. In a separate reaction, the new restriction site antisense oligonucleotide plus a sense oligonucleotide

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anchor from the plasmid 5' end were used to amplify the 5' end of the LAM-1 cDNA. The individual PCR products from these two reactions were gel purified, subcloned into the pSP65 vector (Promega Biotec, Madison, WI), digested with the appropriate restriction enzymes, and ligated together. The nucleotide changes were as follows: (a) GA³⁶¹ATCC to GGATCC in the lectin domain, creating a BamHI site, corresponding to amino acid number 53 in the mature protein (Fig. 2); (b) A⁵⁸³TGCAG to CTGCAG in the EGF domain, creating a PstI site, corresponding to amino acid number 127; (c) GAGG⁸⁸¹C⁸⁸²C to GAGCTC in the first SCR domain, creating a SacI site, corresponding to amino acid number 164; and (d) GTCAAA¹⁰²³ to GTCAAC in the second SCR domain, creating a HincII site, corresponding to amino acid number 279. The fidelity of the site-directed mutagenesis was confirmed by restriction mapping and sequence analysis of the cDNAs. Each of these sites is naturally present in the PADGEM cDNA (Larsen et al., Cell 59:305-312 (1984)).

Domain Mapping of Binding Sites

The chimeric selectin cDNAs were subcloned into the PMT-2 expression vector (Kaufman et al, J. Mol. Cell. Biol. 9:946-958 (1989)), and COS cells at ~50% confluency were transiently transfected with 3 µg of the indicated cDNA by the DEAE dextran method. One day after transfection, the COS cells were replated at ~50% confluency onto 35 mm Petri dishes (assay plates) (Becton-Dickinson, Lincoln Park, New Jersey). The following day, HL-60 cells were washed twice in cold RPMI 1640 media (Gibco, Grand Island, NY), resuspended in RPMI 1640 at a final concentration of 3.3 x 10⁶ cells ml, and 0.6 ml (2 x 10⁶ cells) were added to assay plates which had been washed twice with cold RPMI 1640 media. The plates were gently rocked for 20 min at 4°C, washed five times, and the number of HL-60 cells bound to individual COS cells were counted on a minimum of 100 COS cells.

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Assay for leukocyte attachment to HEV

The cDNAs encoding native L-selectin, L2P and L2P3L were subcloned into the Bam HI site of the pZIPneoSV(X) vector (Cepko et al, Cell 37:1053-1062 (1984)), and were used to transfect the mouse pre-B cell line 300.19. Stable transfectants were selected in medium containing 0.5-1.0 mg/ml G418 (geneticin; Sigma), and cells expressing the proteins were selected by panning with the LAM1-3 mAb (Spertini et al, J. Immunol. 147:942-949 (1991)). To generate P-selectin transfectants, the P-selectin cDNA in the pMT-2 vector was cotransfected with the pSV2neo vector containing the neomycin resistance marker. Rat lymph nodes were obtained from freshly euthanized Lewis rats, snap frozen in isopentane/liquid nitrogen, and stored at -70°C in isopentane until use. For the HEV assay, 5×10^6 of each cell type were incubated on three 12 mm sections/ slide at 64 rpm for 25 min at -4°C, the excess cells were gently removed, and the slides were placed vertically in ice-cold fixative (PBS/2.4% glutaraldehyde) overnight. The slides were then counterstained with Gill's hematoxylin, overlaid with glycerol gelatin, and cover slips were applied. Each slide was scored for the number of lymphocytes bound/HEV. Between 100-200 HEV were counted for each experiment. Data are presented as mean $\pm$ standard deviation of lymphocytes bound/HEV. ND, not determined.

Determination of neuraminidase sensitivity, protease sensitivity and Ca^{2+} requirements for adhesion

COS cells were transfected with the indicated cDNA and replated as described above. HL-60 cells were incubated in RPMI 1640 medium at 37°C for two hours with either 0.25 U/ml *Clostridium perfringens* neuraminidase (Type VI, Sigma, St. Louis, MO), 50 mg/ml chymotrypsin or papain (Sigma), washed twice, and the assay was performed as described in the Fig. 2 legend. Neuraminidase treatment of the HL-60 cells was effective in eliminating all reactivity with the CSLEX1

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anti-sLe^x mAb (data not shown). In some groups, COS cells were preincubated with either 5 µg/ml G1 mAb (a gift of Dr. R.P. McEver) (Geng et al, Nature 343:757-760 (1990)), or ascites fluid containing the LAM1-3 mAb (Spertini et al, J. Immunol. 147:942-949 (1991)) diluted 1:200. To determine the effect of EGTA, both the HL-60 cells and the COS cells were washed in RPMI 1640 containing 2.5 mM EGTA, and this medium was also used for the assay. No detachment of COS cells occurred under these conditions. These results are representative of at least four experiments.

Use

The adhesion of leukocytes to the endothelial wall of blood vessels and their infiltration into the surrounding tissues contributes to inflammation of tissue at the cellular level. Normally, the infiltrating leukocytes phagocytize invading organisms or dead or damaged cells. However, in pathologic inflammation, infiltrating leukocytes can cause serious and sometimes deadly damage. Leukocyte-mediated inflammation is involved in a number of human clinical manifestations, including the adult respiratory distress syndrome, multi-organ failure and reperfusion injury.

Local administration of therapeutic agents antagonistic to the function of the selectins can block competitively the adhesive interactions between leukocytes and the endothelium adjacent to an inflamed region. On a systemic level, treatment of a patient in shock (e.g., from a serious injury) with an antagonist to selectin function can result in the reduction of leukocyte migration and adhesion and the subsequent dramatic recovery of the patient.

Chimeric peptides or polypeptides combining ligand binding portions from within the lectin and EGF domains of two different selectins can be used for therapeutic treatment to interfere in the binding of leukocytes at the site of inflammation. Referring to Fig. 7, the close homology among the lectin and EGF domains of L-, P- and E-selectin can be

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seen, and the intron/exon organization of the respective genes encoding P- and E-selectin (Collins et al., J. Biol.Chem. 266:2466 (1991); Johnston et al., J. Biol. Chem.265:21381 (1990)) is the same as that of the *lyam-1* gene encoding L-selectin, disclosed herein. These chimeric agents will offer more efficient targeting than using a single function antagonist. Therapeutic agents of the invention can also be used to block platelet or platelet and mononuclear cell aggregation, thus preventing thrombus formation.

The therapeutic peptides or polypeptides can be composed solely of the indicated portions of lectin or EGF domains or they can include portions of any of the remaining domains (SCR, transmembrane or cytoplasmic), or the entire extracellular portion of a generic selectin molecule. The peptides or polypeptides also can be joined to a carrier protein (e.g., a soluble portion of an immunoglobulin molecule) to increase the serum half-life of the therapeutic agent. Immunoglobulin chimera are easily purified through standard immunochemistry procedures, including IgG-binding protein A-Sepharose chromatography. The chimera have the ability to form an immunoglobulin-like dimer with the concomitant higher avidity and serum half-life.

cdNA sequences encoding the chimeric polypeptides can be incorporated into replicable expression vectors and the vectors transfected into an appropriate host to express the encoded polypeptide. Stop codons can be incorporated into the DNA sequences or any other chain termination method can be used to prepare a soluble polypeptide. The expressed polypeptide can be purified by ordinary techniques well known to those of skill in the art, e.g., by affinity chromatographic methods.

Other agents can also be joined to the therapeutic polypeptides to form a useful product. For example, the ligand binding portions of the lectin and EGF domains of two different selectins can be combined with the toxic portion of a cytotoxin to produce a fusion protein. In addition, the

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selectin chimeric polypeptides may be coupled to a
chemotherapeutic drug or drugs which could simultaneously
bind to cells expressing their respective ligands, to
administer the drug to a site of tissue damage or
inflammation to treat, e.g., acute inflammation or
vasculitis. Such drugs may include, anti-inflammatory agents
or agents that provide regional relief from inflammatory
distress. Syndromes, diseases, and conditions that could be
treated by these agents would include, but not be limited to,
treating inflammation, microbial/parasitic infections, post-
reperfusion injury, leukemia, lymphoma, vasculitis,
inhibition of the metastatic spread of tumor cells, organ
transplantation, or graft rejection. As is well known to
those of skill in the art, the fusion proteins can be
transcribed from a hybrid cDNA molecule, or the agent may be
covalently bonded to the chimeric polypeptide by routine
procedures.

One method of imaging the sites of inflammation in a
patient involves detecting the expression of the L-selectin
ligand on the inflamed endothelium. The method includes
administering to a patient a pharmaceutical composition
consisting of a detectable amount of a labeled ligand binding
fragment of L-selectin, alone or joined to a carrier protein,
including a ligand binding portion of a different selectin
receptor, in a pharmaceutically acceptable carrier.
Sufficient time is allowed for the labeled polypeptide to
localize at the site of L-selectin ligand expression, unbound
polypeptide is permitted to clear from healthy tissue in the
patient, and signal generated by the label is detected and
converted into an image of the site of inflammation. The
amount of labeled ligand binding fragment of L-selectin
preferably would be from 1 pg/kg to 10 µg/kg although higher
or lower doses are possible depending on the imaging agent
label used and the sensitivity of the detection method.

Some of the labels which can be detected externally from
within the body of a human patient include radionuclides,

radiopaque labels, and paramagnetic isotopes. A radionuclide for *in vivo* diagnosis should have a half-life long enough that it is still detectable at the time of maximum uptake, but short enough that after diagnosis unwanted radiation does not remain in the patient. Coupling of radionuclides to antibodies or proteins is well known in the art (see, e.g., Daddona et al., U.S. Pat. No. 5,026,537, the teachings of which are incorporated by reference herein) and is often accomplished either directly or indirectly using an intermediary linking group. Examples of radioisotopes that could be used for *in vivo* diagnosis are ⁹⁹Tc, ¹²³I, ¹³¹I, ¹¹¹In, ⁸⁷Ru, ⁶⁷Cu, ⁶⁷Ga, ⁶⁸Ga, ⁷²As, ⁸⁹Zr, and ²⁰¹Tl. Paramagnetic isotopes for purposes of *in vivo* diagnosis can also be used according to the methods of this invention. Examples of elements that are particularly useful for use in magnetic resonance energy techniques include ¹⁵⁷Gd, ⁵⁵Mn, ¹⁶²Dy, ⁵²Cr, and ⁵⁶Fe. For radiopaque imaging, the LAM-1 protein or ligand binding fragment is coupled to an agent that produces an opaque field at the site of inflammation upon X-ray imaging.

A ligand binding portion of the lectin or EGF-like domains of various selectins can be experimentally determined using this invention. In a typical procedure, fragments of the selectin cDNA can be fused with cDNA encoding a carrier protein, such as immunoglobulin heavy chain or another non-ligand binding portion of a different selectin. These chimeric proteins can be expressed on the cell surface, or as soluble molecules, and their ability to bind ligand assessed. Further segregated fragments of the lectin or EGF-like domain under study can be examined for their ability to bind ligands, as well as their ability to inhibit the binding of the parent selectin molecule to ligand.

Any of the peptides or polypeptides of the invention comprising lectin and EGF ligand binding portions from two different selectin molecules (including portions of any of the remaining domains (SCR, transmembrane or cytoplasmic) of a generic selectin molecule or including a carrier protein)

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can be used to screen for antagonists of lectin or EGF function individually or for agents that can simultaneously antagonize the functions of the lectin and EGF domain of different selectins. This invention will allow the development of pharmacologic reagents composed of peptides, carbohydrate moieties, RNAs or other small molecules which may mimic the ligand of the lectin or EGF domains of the selectins or may mimic the ligand-binding epitopes of the respective selectin domains and thus be useful therapeutic agents.

In another therapeutic method, chimeric selectin polypeptides can be used in combination therapy with any other selectin or any other cell surface molecule, or soluble fragments thereof, involved in adhesion of leukocytes to endothelial surfaces. In addition, antagonists to any of the above receptors or receptor portions (or mAb reactive to the receptors or receptor portions) can be used in the above combinations or as independent antagonist combinations for treatment of a patient. Examples include ICAM-1, VCAM-1, VLA-4, CD18, CD11a, CD11b and CD31, and the mAb reactive with them.

The therapeutic agents may be administered orally, topically, or parenterally, (e.g., intranasally, subcutaneously, intramuscularly, intravenously, or intra-arterially) by routine methods in pharmaceutically acceptable inert carrier substances. Optimal dosage and modes of administration can readily be determined by conventional protocols.

The *lyam-1* gene itself or portions thereof can be used to construct chimeric selectins. The gene can also be used in genetic therapy. Individuals having a genetic defect in the *lyam-1* gene would be unable to produce a fully active L-selectin leukocyte "homing" receptor and thus would be unable to mobilize sufficient leukocytes to a site of inflammation. Individuals suspected of having a congenital defect in the *lyam-1* gene could be screened for this genetic disorder using

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the sequence and structural information described. Treatment of affected individuals would then be possible using the *lyam-1* gene or fragments thereof.

5 The normal regulation of the *lyam-1* gene, as evidenced by the appearance and disappearance of L-selectin on the surface of a specific leukocyte sub-population can be monitored to test the effects of drugs or specific therapies that would alter gene expression.

10 While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection
15 granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Tedder, Thomas F.
Kansas, Geoffrey S.

(ii) TITLE OF INVENTION: CHIMERIC SELECTINS AS SIMULTANEOUS
BLOCKING AGENTS FOR COMPONENT SELECTIN FUNCTION

(iii) NUMBER OF SEQUENCES: 11

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(A) APPLICATION NUMBER: US 07/862,483
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(A) APPLICATION NUMBER: US 07/770,608
(B) FILING DATE: 03-OCT-1991

(vii) PRIOR APPLICATION DATA:

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(B) REGISTRATION NUMBER: 34,346
(C) REFERENCE/DOCKET NUMBER: DFCI-318XX

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(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2330 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 53..1210

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTC	CCCTT	TGGG	CAAGGA	CCTG	AGACCC	TTGT	GCTAAG	TCAAG	AGGCT	CA	ATG	55
											Met	
											1	
GGC	TGC	AGA	AGA	ACT	AGA	GAA	GGA	CCA	AGC	AAA	GCC	103
Gly	Cys	Arg	Arg	Thr	Arg	Glu	Gly	Pro	Ser	Lys	Ala	
											Met	
											Ile	
											Phe	
											Pro	
TGG	AAA	TGT	CAG	AGC	ACC	CAG	AGG	GAC	TTA	TGG	AAC	151
Trp	Lys	Cys	Gln	Ser	Thr	Gln	Arg	Asp	Leu	Trp	Asn	
											Ile	
											Phe	
											Lys	
											Leu	
TGG	GGG	TGG	ACA	ATG	CTC	TGT	TGT	GAT	TTC	CTG	GCA	199
Trp	Gly	Trp	Thr	Met	Leu	Cys	Cys	Asp	Phe	Leu	Ala	
											His	
											His	
											Gly	
											Thr	
GAC	TGC	TGG	ACT	TAC	CAT	TAT	TCT	GAA	AAA	CCC	ATG	247
Asp	Cys	Trp	Thr	Tyr	His	Tyr	Ser	Glu	Lys	Pro	Met	
											Asn	
											Trp	
											Gln	
											Arg	
GCT	AGA	AGA	TTC	TGC	CGA	GAC	AAT	TAC	ACA	GAT	TTA	295
Ala	Arg	Arg	Phe	Cys	Arg	Asp	Asn	Tyr	Thr	Asp	Leu	
											Val	
											Ala	
											Ile	
											Gln	
AAC	AAG	GCG	GAA	ATT	GAG	TAT	CTG	GAG	AAG	ACT	CTG	343
Asn	Lys	Ala	Glu	Ile	Glu	Tyr	Leu	Glu	Lys	Thr	Leu	
											Pro	
											Phe	
											Ser	
											Arg	
TCT	TAC	TAC	TGG	ATA	GGA	ATC	CGG	AAG	ATA	GGA	GGA	391
Ser	Tyr	Tyr	Trp	Ile	Gly	Ile	Arg	Lys	Ile	Gly	Gly	
											Ile	
											Trp	
											Thr	
											Trp	
GTG	GGA	ACC	AAC	AAA	TCT	CTC	ACT	GAA	GAA	GCA	GAG	439
Val	Gly	Thr	Asn	Lys	Ser	Leu	Thr	Glu	Glu	Ala	Glu	
											Asn	
											Trp	
											Gly	
											Asp	

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Date: 23 AUG 1998

	GGT GAG CCC AAC AAC AAG AAG AAC AAG GAG GAC TGC GTG GAG ATC TAT Gly Glu Pro Asn Asn Lys Lys Asn Lys Glu Asp Cys Val Glu Ile Tyr 130 135 140 145	487
5	ATC AAG AGA AAC AAA GAT GCA GGC AAA TGG AAC GAT GAC GCC TGC CAC Ile Lys Arg Asn Lys Asp Ala Gly Lys Trp Asn Asp Asp Ala Cys His 150 155 160	535
	AAA CTA AAG GCA GCC CTC TGT TAC ACA GCT TCT TGC CAG CCC TGG TCA Lys Leu Lys Ala Ala Leu Cys Tyr Thr Ala Ser Cys Gln Pro Trp Ser 165 170 175	583
10	TGC AGT GGC CAT GGA GAA TGT GTA GAA ATC ATC AAT AAT TAC ACC TGC Cys Ser Gly His Gly Glu Cys Val Glu Ile Ile Asn Asn Tyr Thr Cys 180 185 190	631
15	AAC TGT GAT GTG GGG TAC TAT GGG CCC CAG TGT CAG TTT GTG ATT CAG Asn Cys Asp Val Gly Tyr Tyr Gly Pro Gln Cys Gln Phe Val Ile Gln 195 200 205	679
	TGT GAG CCT TTG GAG GCC CCA GAG CTG GGT ACC ATG GAC TGT ACT CAC Cys Glu Pro Leu Glu Ala Pro Glu Leu Gly Thr Met Asp Cys Thr His 210 215 220 225	727
20	CCT TTG GGA AAC TTC AAC TTC AAC TCA CAG TGT GCC TTC AGC TGC TCT Pro Leu Gly Asn Phe Asn Phe Asn Ser Gln Cys Ala Phe Ser Cys Ser 230 235 240	775
	GAA GGA ACA AAC TTA ACT GGG ATT GAA GAA ACC ACC TGT GAA CCA TTT Glu Gly Thr Asn Leu Thr Gly Ile Glu Glu Thr Thr Cys Glu Pro Phe 245 250 255	823
25	GGA AAC TGG TCA TCT CCA GAA CCA ACC TGT CAA GTG ATT CAG TGT GAG Gly Asn Trp Ser Ser Pro Glu Pro Thr Cys Gln Val Ile Gln Cys Glu 260 265 270	871
30	CCT CTA TCA GCA CCA GAT TTG GGG ATC ATG AAC TGT AGC CAT CCC CTG Pro Leu Ser Ala Pro Asp Leu Gly Ile Met Asn Cys Ser His Pro Leu 275 280 285	919
	GCC AGC TTC AGC TTT ACC TCT GCA TGT ACC TTC ATC TGC TCA GAA GGA Ala Ser Phe Ser Phe Thr Ser Ala Cys Thr Phe Ile Cys Ser Glu Gly 290 295 300 305	967
35	ACT GAG TTA ATT GGG AAG AAG AAA ACC ATT TGT GAA TCA TCT GGA ATC Thr Glu Leu Ile Gly Lys Lys Lys Thr Ile Cys Glu Ser Ser Gly Ile 310 315 320	1015
	TGG TCA AAT CCT AGT CCA ATA TGT CAA AAA TTG GAC AAA AGT TTC TCA Trp Ser Asn Pro Ser Pro Ile Cys Gln Lys Leu Asp Lys Ser Phe Ser 325 330 335	1063
40	ATG ATT AAG GAG GGT GAT TAT AAC CCC CTC TTC ATT CCA GTG GCA GTC Met Ile Lys Glu Gly Asp Tyr Asn Pro Leu Phe Ile Pro Val Ala Val 340 345 350	1111
45	ATG GTT ACT GCA TTC TCT GGG TTG GCA TTT ATC ATT TGG CTG GCA AGG Met Val Thr Ala Phe Ser Gly Leu Ala Phe Ile Ile Trp Leu Ala Arg 355 360 365	1159
	AGA TTA AAA AAA GGC AAG AAA TCC AAG AGA AGT ATG AAT GAC CCA TAT Arg Leu Lys Lys Gly Lys Lys Ser Lys Arg Ser Met Asn Asp Pro Tyr 370 375 380 385	1207

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Date: 23 AUG 1956

5 TAAATCGCCC TTGGTGAAAG AAAATTCTTG GAATACTAAA AATCATGAGA TCCTTTAAAT 1267
 CCTTCCATGA AACGTTTTGT GTGGTGGCAC CTCCTACGTC AAACATGAAG TGTGTTTCCT 1327
 TCAGTGCATC TGGGAAGATT TCTACCTGAC CAACAGTTCC TTCAGCTTCC ATTTACCCCC 1387
 TCATTTATCC CTCAACCCCC AGCCACAGG TGTATTATACA GCTCAGCTTT TTGTCTTTTC 1447
 10 TGAGGAGAAA CAAATAAGAC CATAAAGGGA AAGGATTCAT GTGGAATATA AAGATGGCTG 1507
 ACTTTGCTCT TTCTTGACTC TTGTTTTAG TTTCAATTCA GTGCTGTACT TGATGACAGA 1567
 CACTTCTAAA TGAAGTGCAA ATTTGATACA TATGTGAATA TGGACTCAGT TTTCTTGACG 1627
 ATCAAATTTT GCGTCGTCTT CTGTATACGT GGAGGTACAC TCTATGAAGT CAAAAGTCTA 1687
 CGCTCTCCTT TCTTTCTAAC TCCAGTGAAG TAATGGGGTC CTGCTCAAGT TGAAAGAGTC 1747
 CTATTTGCAC TGTAGCCTCG CCGTCTGTGA ATTGGACCAT CCTATTTAAC TGGCTTCAGC 1807
 CTCCCCACCT TCTTCAGCCA CCTCTCTTTT TCAGTTGGCT GACTTCCACA CCTAGCATCT 1867
 CATGAGTGCC AAGCAAAGG AGAGAAGAGA GAAATAGCCT GCGCTGTTTT TTAGTTTGGG 1927
 GGTTTTGCTG TTTCCTTTTA TGAGACCCAT TCCTATTTCT TATAGTCAAT GTTTCTTTTA 1987
 TCACGATATT ATTAGTAAGA AACATCACT GAAATGCTAG CTGCAACTGA CATCTCTTTG 2047
 15 ATGTCATATG GAAGAGTTAA AACAGGTGGA GAAATTCCTT GATTCACAAT GAAATGCTCT 2107
 CCTTTCCCCT GCCCCAGAC CTTTTATCCA CTTACCTAGA TTCTACATAT TCTTTAAATT 2167
 TCATCTCAGG CCTCCCTCAA CCCACCACT TCTTTTATAA CTAGTCCTTT ACTAATCCAA 2227
 CCCATGATGA GCTCCTCTTC CTGGCTTCTT ACTGAAAGGT TACCCTGTAA CATGCAATTT 2287
 TGCATTTGAA TAAAGCCTGC TTTTAAAGTG TAAAAAGAA TTC 2330

20 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 385 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Cys Arg Arg Thr Arg Glu Gly Pro Ser Lys Ala Met Ile Phe
 1 5 10 15
 30 Pro Trp Lys Cys Gln Ser Thr Gln Arg Asp Leu Trp Asn Ile Phe Lys
 20 25 30
 Leu Trp Gly Trp Thr Met Leu Cys Cys Asp Phe Leu Ala His His Gly
 35 40 45
 Thr Asp Cys Trp Thr Tyr His Tyr Ser Glu Lys Pro Met Asn Trp Gln
 50 55 60
 35 Arg Ala Arg Arg Phe Cys Arg Asp Asn Tyr Thr Asp Leu Val Ala Ile
 65 70 75 80

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	Gln	Asn	Lys	Ala	Glu	Ile	Glu	Tyr	Leu	Glu	Lys	Thr	Leu	Pro	Phe	Ser	
					85					90					95		
	Arg	Ser	Tyr	Tyr	Trp	Ile	Gly	Ile	Arg	Lys	Ile	Gly	Gly	Ile	Trp	Thr	
				100					105					110			
5	Trp	Val	Gly	Thr	Asn	Lys	Ser	Leu	Thr	Glu	Glu	Ala	Glu	Asn	Trp	Gly	
			115					120					125				
	Asp	Gly	Glu	Pro	Asn	Asn	Lys	Lys	Asn	Lys	Glu	Asp	Cys	Val	Glu	Ile	
		130					135					140					
10	Tyr	Ile	Lys	Arg	Asn	Lys	Asp	Ala	Gly	Lys	Trp	Asn	Asp	Asp	Ala	Cys	
		145				150					155					160	
	His	Lys	Leu	Lys	Ala	Ala	Leu	Cys	Tyr	Thr	Ala	Ser	Cys	Gln	Pro	Trp	
					165					170					175		
	Ser	Cys	Ser	Gly	His	Gly	Glu	Cys	Val	Glu	Ile	Ile	Asn	Asn	Tyr	Thr	
				180					185					190			
15	Cys	Asn	Cys	Asp	Val	Gly	Tyr	Tyr	Gly	Pro	Gln	Cys	Gln	Phe	Val	Ile	
			195					200					205				
	Gln	Cys	Glu	Pro	Leu	Glu	Ala	Pro	Glu	Leu	Gly	Thr	Met	Asp	Cys	Thr	
		210					215					220					
20	His	Pro	Leu	Gly	Asn	Phe	Asn	Phe	Asn	Ser	Gln	Cys	Ala	Phe	Ser	Cys	
		225				230					235					240	
	Ser	Glu	Gly	Thr	Asn	Leu	Thr	Gly	Ile	Glu	Glu	Thr	Thr	Cys	Glu	Pro	
					245					250					255		
	Phe	Gly	Asn	Trp	Ser	Ser	Pro	Glu	Pro	Thr	Cys	Gln	Val	Ile	Gln	Cys	
				260					265					270			
25	Glu	Pro	Leu	Ser	Ala	Pro	Asp	Leu	Gly	Ile	Met	Asn	Cys	Ser	His	Pro	
			275					280					285				
	Leu	Ala	Ser	Phe	Ser	Phe	Thr	Ser	Ala	Cys	Thr	Phe	Ile	Cys	Ser	Glu	
		290					295					300					
30	Gly	Thr	Glu	Leu	Ile	Gly	Lys	Lys	Lys	Thr	Ile	Cys	Glu	Ser	Ser	Gly	
		305				310					315					320	
	Ile	Trp	Ser	Asn	Pro	Ser	Pro	Ile	Cys	Gln	Lys	Leu	Asp	Lys	Ser	Phe	
					325					330					335		
	Ser	Met	Ile	Lys	Glu	Gly	Asp	Tyr	Asn	Pro	Leu	Phe	Ile	Pro	Val	Ala	
				340					345					350			
35	Val	Met	Val	Thr	Ala	Phe	Ser	Gly	Leu	Ala	Phe	Ile	Ile	Trp	Leu	Ala	
			355					360					365				
	Arg	Arg	Leu	Lys	Lys	Gly	Lys	Lys	Ser	Lys	Arg	Ser	Met	Asn	Asp	Pro	
		370					375						380				
40	Tyr																
	385																

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Date: 23 AUG 1966

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1192 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGAGAGCTGT TATAAAGATT AAACAATATA ATAAATATGG CGCGTGAGCT TCAGAGTTTT	60
TGTTGTTGTT ATTATTATTT TCCTAAAAAT GCAAATCTGA TTTGCATTTG ACTCATTGAC	120
TCACATCAGT GGGTCTTCCT TTTTATTGTC CTTCATCATA TGGGTCCTAA TTTCACATGC	180
AGTCTTATAA AACCATCTCA TTTTATAGTC CAAGAATATT AAAGGTACTT GTAGGCTCCC	240
AAACCTACAC GGTGAAAAGC TAGAGAGCAT GGGCTCTCTT CAGGGGTAA CTTCAGGAAG	300
TGCCACTAAC AAGGACGTCC ACTAGGTGGT GAGCAAGGAA AGACGGAGGT GAAGGAACCG	360
AAACGAGTCA AGTCCACTGC TTAGCTCTAC TGAAGTTTGT CAAACATCAT AAATATGTCT	420
GAAATGCAGT TTTGATTTGT AGTATTGCA ATTTCCAAGG GCCATTTACC ACAGGTAGCC	480
AAGAGTTAGT TTAGCATTTA TGAAAAGAT AGGGGAGGGT GGTGGTTAAG AAGGAGGTGG	540
AGGAGAGAGT GAAGGAGGAA GAGGAGAACA AGAACCAAAC AAAACAAGA ACAAGAACAA	600
GTAGAAGAAG AGGAGCAGGG AGGAAAAGA AGAGGAAGAA GAACAGCAAC AACAATGAGT	660
GAAGGAGGAG GAGGGTAAGG AAAGATGCAT AGGAGAATGG AAGGAAGGAT AGAAAGGAGG	720
GAAGGAAGAG AGAATCTAGT CACATTACTT TCTGATCAGC AGTTCATTTT TGTCTCAGTG	780
GGAGGCAATA GAGGCCAGTC TAGGAAAGGG GTGGGGAAAG AGGAAAGAGA AGTGCAGGAG	840
GAAGGGGAGG CCCAAGGGGA GGAGGAGGAG GATGTGAGAC TGGGTTAGAG AAATGAAAGA	900
AAGCAAGGCT TTCTGTTGAC ATTCAAGTCA GTCTACCTGC AGCACAGCAC ACTCCCTTTG	960
GGCAAGGACC TGAGACCCTT GTGCTAAGTC AAGAGGCTCA ATGGGCTGCA GAAGAACTAG	1020
AGAAGGACCA AGCAAAGCCA TGGTGAGCCT TTCAGCCTAA AAGACGTTTA GATGCTCAGA	1080
TAGAAACTCT TGGGGTTGTA GAGGCAGGTG GCAAGGATAG GAATCACCCC ATTTCAATTC	1140
TGGTTTTAAA TAATATAGAA ACTAAACATT TTCTCAGACC CTCAAAAAAA GT	1192

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Date:

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 363 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CACTGAGACT AAGCGTAAAA TAAATAGAAC AAACAAACTG TGCATCAGTT CTGATGTAAA 60
TTTGAAGTAA TTTTCATCTA TGTCTGAGAA ACCTGTTACC TCAGACAGGG TTAGTAGACA 120
TATGTGTTTT ATTCTGATTA TTAAGAAAGT TGTAAGCACC ACCTCAAAGG CTATAAATGT 180
GTGGTTTAAAG GGTATACATC TAAATATAAT TTTGTATTTT ATTTGCAGAT ATTTCCATGG 240
AAATGTCAGA GCACCCAGAG GGACTTATGG AACATCTTCA AGTTGTGGGG GTGGACAATG 300
CTCTGTTGTG GTATGTTATG ATATTTATAT ATCACTAAGT CTATTTTACT TATATTCATT 360
TTT 363

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 531 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGGAGTAGT GCTAGGTTCT TTTTAGCTGT AACATTATGT AAGTCTGCAT AGGTCACACT 60
GATGTCTTGC AGATTTTCTG GCACATCATG GAACCGACTG CTGGACTTAC CATTATTCTG 120
AAAAACCCAT GAACTGGCAA AGGGCTAGAA GATTCTGCCG AGACAATTAC ACAGATTTAG 180
TTGCCATACA AAACAAGGCG GAAATTGAGT ATCTGGAGAA GACTCTGCCT TTCAGTCGTT 240
CTTACTACTG GATAGGAATC CGGAAGATAG GAGGAATATG GACGTGGGTG GGAACCAACA 300
AATCTCTCAC TGAAGAAGCA GAGAACTGGG GAGATGGTGA GCCCAACAAC AAGAAGAACA 360
AGGAGGACTG CGTGGAGATC TATATCAAGA GAAACAAAGA TGCAGGCAAA TGAACGATG 420
ACGCCTGCCA CAACTAAAG GCAGCCCTCT GTTACACAGG TAGGGAGTGA CAAGACGGCT 480
ATGCTGCCTC AGACTCAGGA AGGGCCACGG TTAAGAGAAT ACTCAGATTT A 531

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Date: 23 AUG 1996

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AAAATTTTAG CCATATGATT TTTATGCTAT GAATTTACCA AATAAACCTT TCCTGATTAT 60
TTAATCATC TCAGACAAAA GGTATCTAT GTCTAAAGAA ATGACTTTGA GTACTAAAAT 120
GTAATCACAT TAAAATATTT TTTTCTGAC CTCCTTAAAG CTTCTTGCCA GCCCTGGTCA 180
TGCAGTGGCC ATGGAGAATG TGTAGAAATC ATCAATAATT ACACCTGCAA CTGTGATGTG 240
GGGTACTATG GGCCCCAGTG TCAGTTTGGT AAGTCTCTTT CTTTCTTTG CTTCTTCTTA 300
GGTAAAGTCA CAGGAATCAT TATAGCTTAT CATGAAGCTG GTTGGACAA AATGATACTA 360
GCCACTCTGA GAAATGGGAA GTTTTGATCA GAAAGCTCTG CTTTCACAAT ATTGTTACCT 420
TTCCGTAAAG ATTCATAAG TCAGCATGAA GTTCGATTG ACTTCTCAAC AAGTCTTTTT 480
GAGTACCACA AGAAGCACAG TGTGGGATA AAGCTGTCAG GGTTACAATA AGGAATTAGC 540
ATGGTAGATT CCCGCTCTCA AGAAGCTCAC GATCTAATGA GCTTGTTAGA TTAATTAGAA 600
CTCTAAGGTC TGAAGAAAC TATGCCATTT ATCATTAGGA GGCTGAGTTA CCCAGAAAGT 660
ATCTTGCTTT TTCCTTCTAG TAGTTCCTTT CTTCTTGCA GTTCTCCACA CTTAACACAT 720
GTGCTCTGTA GCACACTGAC TTTGCTGGTG GCCTTCTCTC TCATTTTGCA CATGGCCAAA 780
AAACATGTCA TCTTTAAGAC ATTGTTCAAA GACAGTTTCT TCTAGGAAGC TT 832

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 712 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCTGATGTG ATAGTTATTT CCCGACTAAG CTGGTCATTC CCAGTTACAC CTATTGGCT 60
TTAAGGATTC TCACTACAGA TAATACTGAA GATAATAATA TGAAGACTAG CTAATGTTTA 120
CTTAGAATTT CTGATGAGTC AGGCTTTGTT CTAACGTCCT TGACTTATGC TAATTGAATT 180

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5 ACATTTAGTT TCCATATCAA TTTGATAAAG ATAACACAAT TTCATTATTC CTCTTATATA 240
 GATGAAGAAA CTGAAGTTGG AGGGTTCAAG TAACCTTGTT TAAAGGCACA TG GTTATCAA 300
 GTGGCAGGGC TAGGATTCAA ATCCAGGCGT CAGTTCCTCT TAACTCTTCC CCATACTGTT 360
 TCTTCCCTA TTGAAGTGAT TCAGTGTGAG CCTTTGGAGG CCCCAGAGCT GGGTACCATG 420
 GACTGTACTC ACCCTTTGGG AACTTCAGC TTCAGCTCAC AGTGTGCCTT CAGCTGCTCT 480
 GAAGGAACAA ACTTAACTGG GATTGAAGAA ACCACCTGTG GACCATTGG AACTGGTCA 540
 TCTCCAGAAC CAACCTGTCA AGGTGAGTAA CTTCACTA GAGGTTTTGT CATGCAATCC 600
 TGGGCTTACA GTCAGAACAT TCAGTAGAAG TTTGCTGAGA AGTCAAACCTT AGGATCCTAA 660
 TTTAACCTAA CTTTGTGTTA ACCTACTGTG ATGTTTCTCA AAGGACTTAT TC 712

10 (2) INFORMATION FOR SEQ ID NO:8:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 451 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

20 GAGGGTCACC TTAGCTAGGG CAGCAGCCTG GAGTAGCTAC TCCTCTCCCC ACAGCTTTCA 60
 ATGCTTCCTT GCCTTCATCT CTCATTCACC ACCCACCATC ATTCTCAAGA AAATAAGCC 120
 TGGAAGCAAT ATCACAAGTA ATGTAGTCAG GCAGCTTTGG CTAAAAATCC AAAGCTCAAG 180
 GGAGGGTCTC TACTCAGAAA TACTGTTTTG TCTTTTTTTT TTTTCTTTT TCATTGAAGT 240
 GATTCAGTGT GAGCCTCTAT CAGCACCAGA TTTGGGGATC ATGAACTGTA GCCATCCCCT 300
 25 GGCCAGCTTC AGCTTTACCT CTGCATGTAC CTTATCTGC TCAGAAGGAA CTGAGTTAAT 360
 TGGGAAGAAG AAAACCATTT GTGAATCATC TGAATCTGG TCAAATCCTA GTCCAATATG 420
 TCAAAGTGAG TAAGTTTGTC CTGGAAGTGA A 451

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Date: 23 AUG 1996

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 544 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TATCAGAACT AAGAAAGCTT GGGCTGCAGG TCGACTCTAG GTGCATTTTC AGGAACTCTA	60
TGAACCACAA ATCTGGGCAT TGAGATTCTG TAGGCATTAG ACTAGCAAGG CTGGTCAGTC	120
TTTGCCTATG CTGTAGACTC ATCAGGGGCC TTCCCATGCC AGTTTCCTCA TCTGTCAAAT	180
GGCATCATTT GGGCTACTAC TGGGAGATGT AAGGAGGAAA AAAGTCAAAT ATCATGAGAT	240
AGACTAAGGA AATAATGCTG GTGGTCTCAT GCTATGTGCC TTACTGATTT CTCTTTCAGA	300
ATTGGACAAA AGTTTCTCAA TGATTAAGGA GGGTGATTAT AACCCCCTCT TCATTCCAGT	360
GGCAGTCATG GTTACTGCAT TCTCTGGGTT GGCATTTATC ATTTGGCTGG CAAGGAGATT	420
AAAAAAAGGT ATGTGAGTTT AACTTCACAT GAAAAGAACA CAACTTTAAA GTGAAAAAGA	480
AAAAAAAAG AAACCCACAG GAAATTAAAT GTGATAGATT CAACACAAGC AGGATGCCAA	540
GCTT	544

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 524 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TAGTTTACAG TATTAGCAGC TGTCCCTCAA GGAAGAATCT GCAGGTAGAT GAGATGCAGA	60
TTGGGTGGGA TAAACACTTG AATGACATAT TGGGTCTTGC CACCAGGCAA TTTAGCAATT	120
CTGTCTTCTT GAGTAGCAGG GAGATGGAAT GGAACCTCAG GAGGCATCTG CATCAACATG	180
TCTGTTCTGT ATTAGTGTCT ACCACTGTTT ATTAAGCCAG TTCCTCAAAT CTCCTTTGAC	240
ACAGATAGGG TCCACCTAAC AAATACCTAA TATACTTCAA AAGACAGTTT TGAGAGTGGG	300
AGTCTTCCTT CTCCTTACT TGAAAACTT TAAATTGTCT AATTTTGTCT AATGCCTTTT	360

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TCTCTATTTT CTATTTTCAGG CAAGAAATCC AAGAGAAGGT AAGTTTTATT AGTGGCGAGG 420
AGTTTCCACA TCTGCTGATT CATTCTCTAC TTCTTAAGTT ACTTCTGCTC TAGCTAGACA 480
CATACCCATA GTAGTTATTA CTGGGTCTAT CAATGACAGA TAGG 524

(2) INFORMATION FOR SEQ ID NO:11:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1696 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

15 ATAAGCATCA CTAAAGAGCT TGTTAGGGGT GCAGAATCTC AGGCTCCACT CAGACCTACT 60
GAATCAGAGT CTGCATTTTA ACACCATCTC TGAGTGGTAA GGACATGAAA ATCTGAGAAG 120
TGCTGCTACT AGGGTTTGCT TACATTTGTT CATCTTCAGA GGTTCCTAAA GCCTGGCCTC 180
TTGTCTGAGA TTTCCAGCTG AAAGCATTTT CTTGCTCCTC TTCTCATCTC TAATGAATAT 240
TTACCTTTAC TACTAACACT CCAAGTTTTG CAATTTTTAA ACTCTTATTA TCTTTTGTTT 300
TTCTTTTCAGT ATGAATGACC CATATTAAAT CGCCCTTGGT GAAAGAAAAT TCTTGGAATA 360
20 CTAAAAATCA TGAGATCCTT TAAATCCTTC CATGAAACGT TTTGTGTGGT GGCACCTCCT 420
ACGTCAAACA TGAAGTGTGT TTCCTTCAGT GCATCTGGGA AGATTCTCTAC CTGACCAACA 480
GTTCCCTCAG CTTCCATTTT ACCCCTCATT TATCCCTCAA CCCCAGCCC ACAGGTGTTT 540
ATACAGCTCA GCTTTTTGTC TTTTCTGAGG AGAAACAAAT AAGACCATAA AGGGAAAGGA 600
TTCATGTGGA ATATAAAGAT GGCTGACTTT GCTCTTTCTT GACTCTTGTT TTCAGTTTCA 660
25 ATTCAGTGCT GTACTTGATG ACAGACACTT CTAAATGAAG TGCAAATTTG ATACATATGT 720
GAATATGGAC TCAGTTTTCT TGCAGATCAA ATTCGCGTC GTCTTCTGTA TACGTGGAGG 780
TACACTCTAT GAAGTCAAAA GTCTACGCTC TCCTTTCTTT CTAAGTCCAG TGAAGTAATG 840
GGGTCTGCT CAAGTTGAAA GAGTCCTATT TGCAGTGTAG CCTCGCCGTC TGTGAATTGG 900
ACCATCCTAT TTAAGTGGCT TCAGCCTCCC CACCTTCTTC AGCCACCTCT CTTTTTCAGT 960
30 TGGCTGACTT CCACACCTAG CATCTCATGA GTGCCAAGCA AAAGGAGAGA AGAGAGAAAT 1020
AGCCTGCGCT GTTTTTTAGT TTGGGGGTTT TGCTGTTTCC TTTTATGAGA CCCATTCCCTA 1080
TTTCTTATAG TCAATGTTTC TTTTATCAG ATATTATTAG TAAGAAAACA TCACTGAAAT 1140
GCTAGCTGCA ACTGACATCT CTTTGATGTC ATATGGAAGA GTTAAACAG GTGGAGAAAT 1200
TCCTTGATTG ACAATGAAAT GCTCTCCTTT CCCCTGCCCC CAGACCTTTT ATCCACTTAC 1260
35 CTAGATTCTA CATATTCTTT AAATTCATC TCAGGCCTCC CTCAACCCCA CCACTTCTTT 1320

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TATAACTAGT CCTTTACTAA TCCAACCCAT GATGAGCTCC TCTTCCTGGC TTCTTACTGA 1380
AAGGTTACCC TGTAACATGC AATTTTGCAT TTGAATAAG CCTGCTTTTT AAGTGTTAAC 1440
TAGTTTGCCT AGTTTGTTAT TTTGAAAATT GATCATATGT TTTGTTTTCT CCCCAGTGAG 1500
TTACATGCTC CTTCAGGGCA GAGTTTGTGT CAGATCCCTG GAGTATCTAG TGCATTACTT 1560
GACACTCAAT AAATGAATGT TCAAATAAT CAGAAAGAGC ATACAGTGCA CTGCTGATAT 1620
AAGTTTCAGC ATCCCTCTTT CTCTATGGCA TCTGATGACC TGGGTCAGAT ATCACCTAAT 1680
GTCAACAGCT GAATTC 1696

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Date: 23 AUG 1996



CLAIMS

What is claimed is:

1. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
P-selectin and
a ligand binding portion found within the lectin domain
of L-selectin.
2. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
E-selectin and
a ligand binding portion found within the lectin domain
of L-selectin.
3. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
P-selectin and
a ligand binding portion found within the lectin domain
of E-selectin.
4. A bifunctional ligand binding polypeptide comprising
a ligand binding portion found within the EGF domain of
E-selectin and
a ligand binding portion found within the lectin domain
of P-selectin.
5. The polypeptide of claim 1 wherein
the ligand binding portion found within the EGF domain
of P-selectin is the entire EGF domain of P-selectin and
wherein
the ligand binding portion found within the lectin
domain of L-selectin is the entire lectin domain of L-
selectin.

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6. The polypeptide of claim 2 wherein
the ligand binding portion found within the EGF domain
of E-selectin is the entire EGF domain of E-selectin and
wherein
the ligand binding portion found within the lectin
domain of L-selectin is the entire lectin domain of L-
selectin.
7. The polypeptide of claim 3 wherein
the ligand binding portion found within the EGF domain
of P-selectin is the entire EGF domain of P-selectin and
wherein
the ligand binding portion found within the lectin
domain of E-selectin is the entire lectin domain of E-
selectin.
8. The polypeptide of claim 4 wherein
the ligand binding portion found within the EGF domain
of E-selectin is the entire EGF domain of E-selectin and
wherein
the ligand binding portion found within the lectin
domain of P-selectin is the entire lectin domain of P-
selectin.
9. The polypeptide of any one of claims 1-4 further
comprising a selectin SCR domain and a selectin transmembrane
domain.
10. The polypeptide of any one of claims 1-4 further
comprising a
carrier region/joined to a carrier protein.
11. The polypeptide of claim 10 wherein said carrier protein
comprises an immunoglobulin heavy chain constant region.

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12. The polypeptide of claim 10 wherein said carrier protein comprises non-ligand binding portions of a selectin molecule.
13. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of P-selectin and
a ligand binding portion found within the lectin domain of L-selectin.
14. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of E-selectin and
a ligand binding portion found within the lectin domain of L-selectin.
15. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of P-selectin and
a ligand binding portion found within the lectin domain of E-selectin.
16. A DNA isolate encoding a polypeptide comprising
a ligand binding portion found within the EGF domain of E-selectin and
a ligand binding portion found within the lectin domain of P-selectin.
17. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising
the exon encoding the EGF domain of P-selectin and
the exon encoding the lectin domain of L-selectin.
18. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising
the exon encoding the EGF domain of E-selectin and
the exon encoding the lectin domain of L-selectin.





19. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising the exon encoding the EGF domain of P-selectin and the exon encoding the lectin domain of E-selectin.
20. A DNA isolate able to hybridize under stringent conditions to the complement of a sequence comprising the exon encoding the EGF domain of E-selectin and the exon encoding the lectin domain of P-selectin.
21. A pharmaceutical composition for use in treating the polypeptide of any one of claims 1-4 in a pharmaceutically acceptable carrier substance.
22. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said condition is tissue damage or an autoimmune disorder.
23. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said condition is a post-reperfusion injury.
24. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said condition is an organ or tissue transplant recipient.
25. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said polypeptide is joined to a therapeutic agent.
26. The pharmaceutical composition of leukocyte-mobilizing claim 21 wherein said polypeptide is joined to a carrier protein.

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27. The pharmaceutical composition of leukocyte-mobilizing claim 25 wherein said therapeutic agent comprises a chemotherapeutic drug or an anti-inflammatory agent.

28. The use of the polypeptide of any one of claims 1-4 to identify a ligand or ligands that bind to said polypeptide.

29. The use of the polypeptide of any one of claims 1-4 to screen for antagonists of selectin EGF domain function.

30. The use of the polypeptide of any one of claims 1-4 to screen for simultaneous antagonists of the function of lectin and EGF domains of different selectins.

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**CHIMERIC SELECTINS AS SIMULTANEOUS BLOCKING AGENTS
FOR COMPONENT SELECTIN FUNCTION**

ABSTRACT

Chimeric peptides or polypeptides that combine ligand binding portions from within the lectin and EGF domains of two different selectins are disclosed. The peptides or polypeptides can be constructed solely of the indicated portions of lectin or EGF domains or they can include portions of any of the remaining domains (SCR, transmembrane or cytoplasmic), or the entire extracellular portion, of a generic selectin molecule. The peptides or polypeptides also can be joined to a carrier protein (e.g., a soluble portion of an immunoglobulin molecule) to increase the serum half-life of the therapeutic agent. The chimeric polypeptides can be used as therapeutic agents to antagonize selectin function. They are also useful for screening for agents that are simultaneously antagonists of the function of lectin and EGF domains of different selectins.

FIG. 1

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MONOCLONAL ANTIBODY REACTIVITY

DESIGNATION	G1 (P LECTIN)	LAM1-3 (L LECTIN)	LAM1-1 (L LECTIN/EGF)	LAM1-14 (L SCR)	SI2 (P SCR)
L-SELECTIN (L)	0	525	374	576	0
P-SELECTIN (P)	1712	0	0	0	1059
P2L	839	0	68	419	0
P3L	714	0	0	350	0
L2P	19	1484	0	0	1093
L3P	0	1779	1503	0	1018
L2P3L	0	363	0	266	0

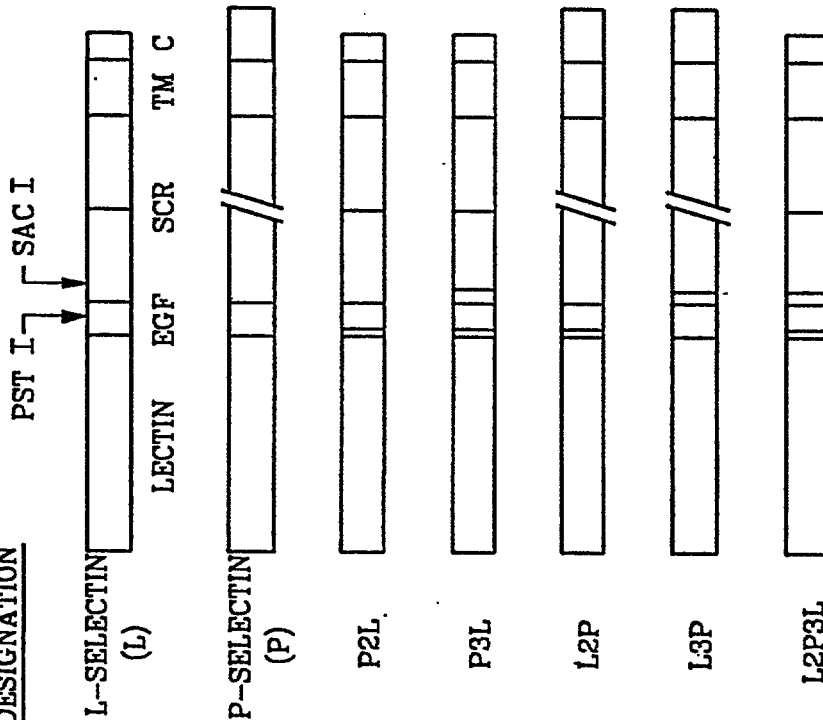


FIG. 1

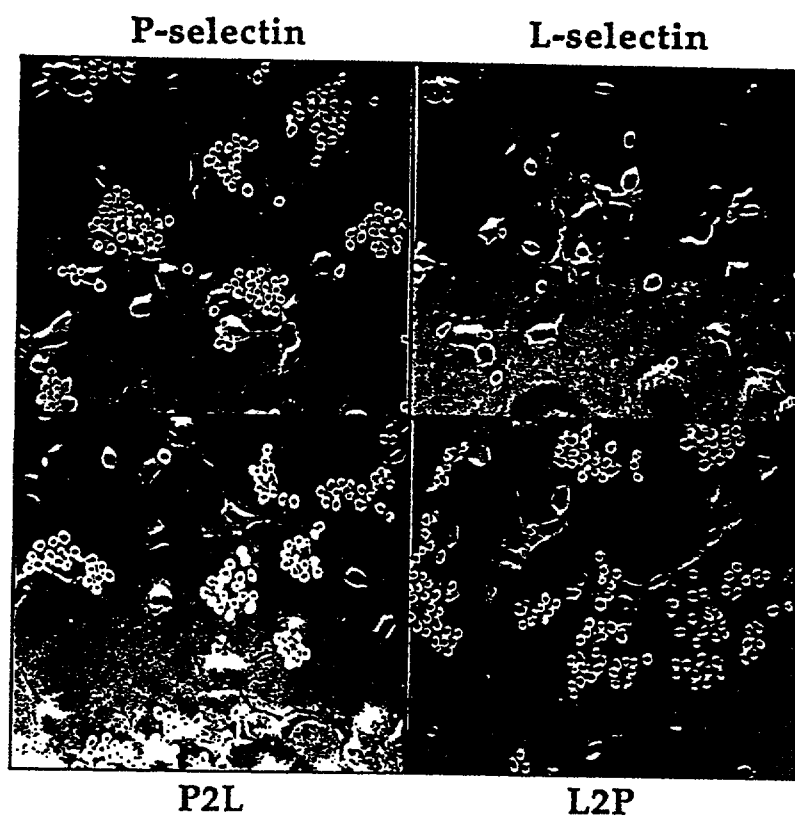


FIG. 2A

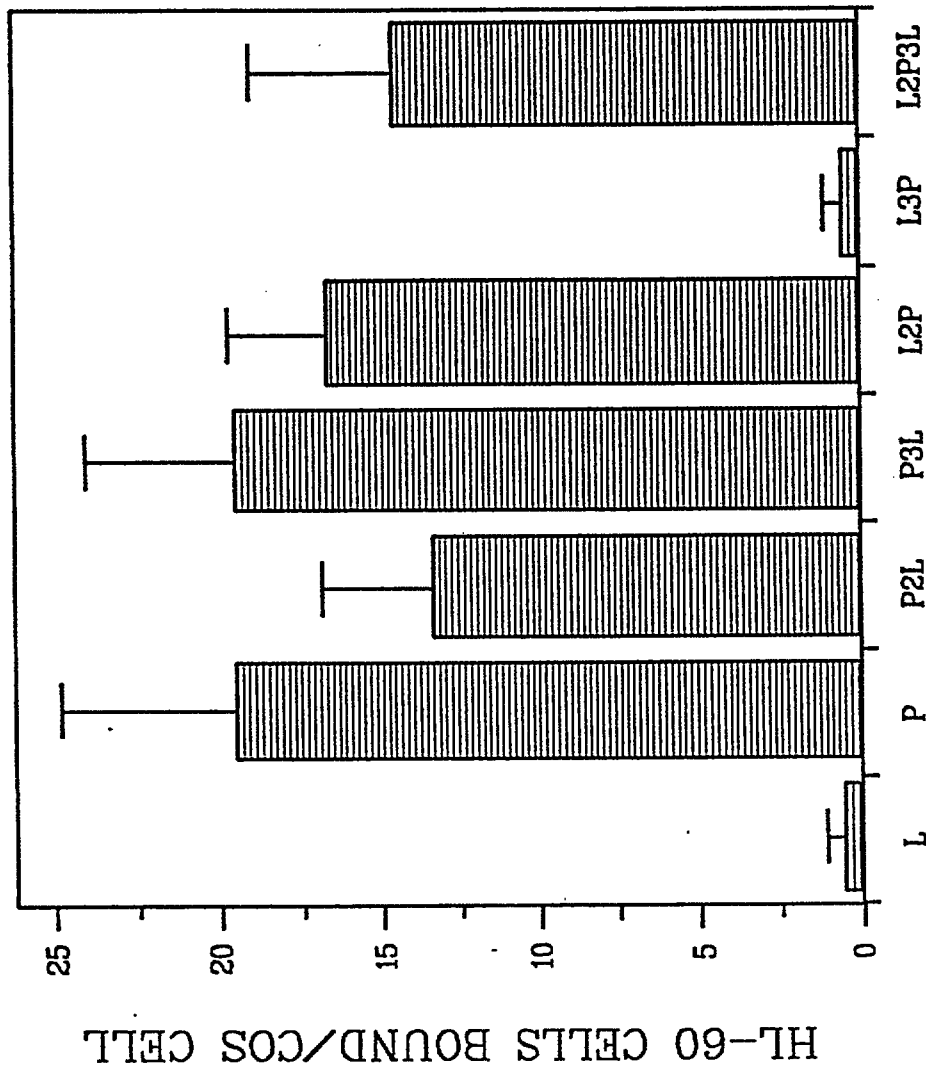


FIG. 2B

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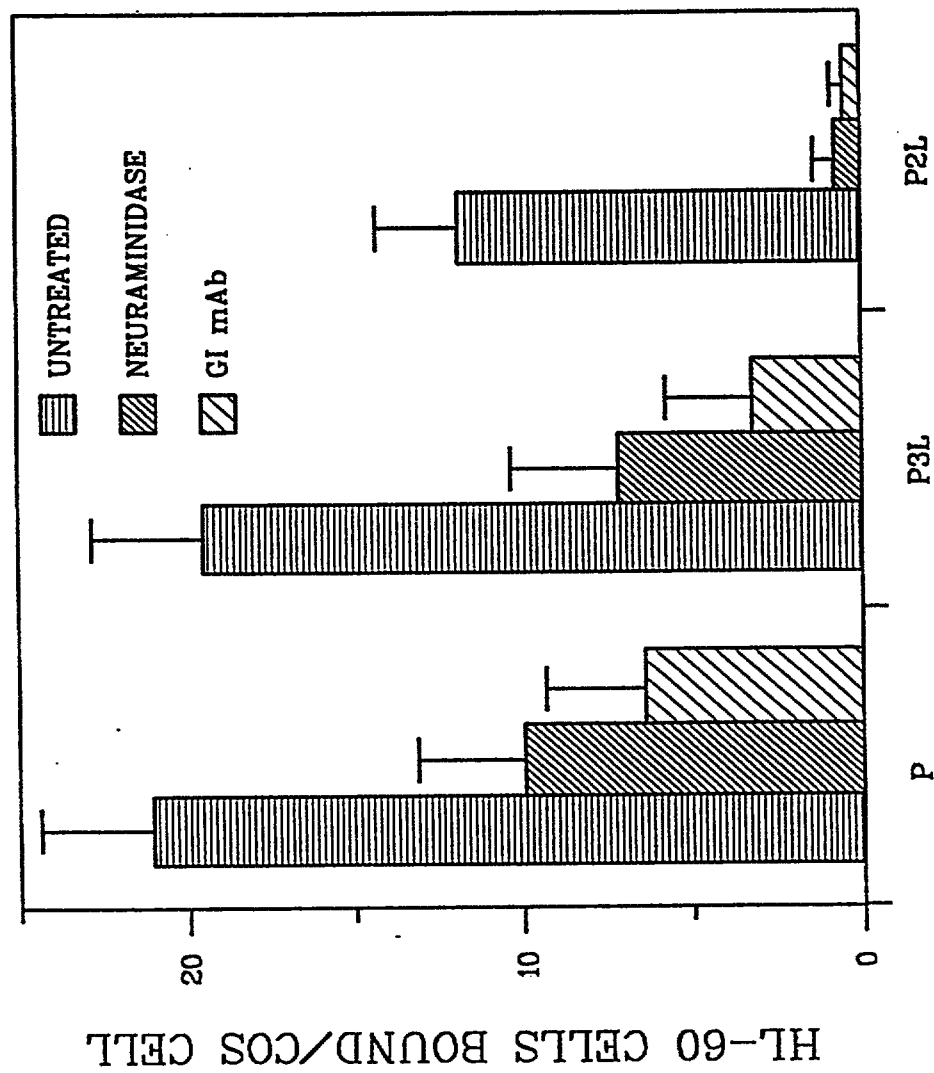


FIG. 3A

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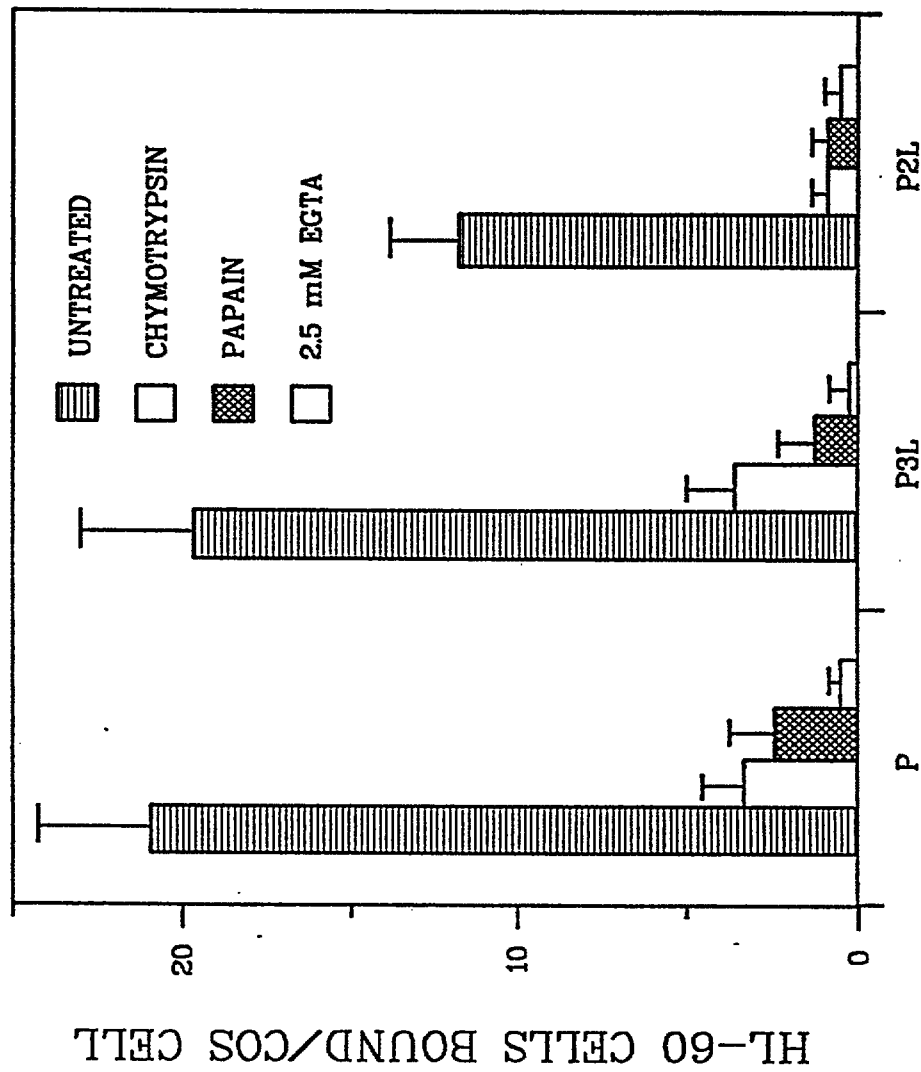


FIG. 3B

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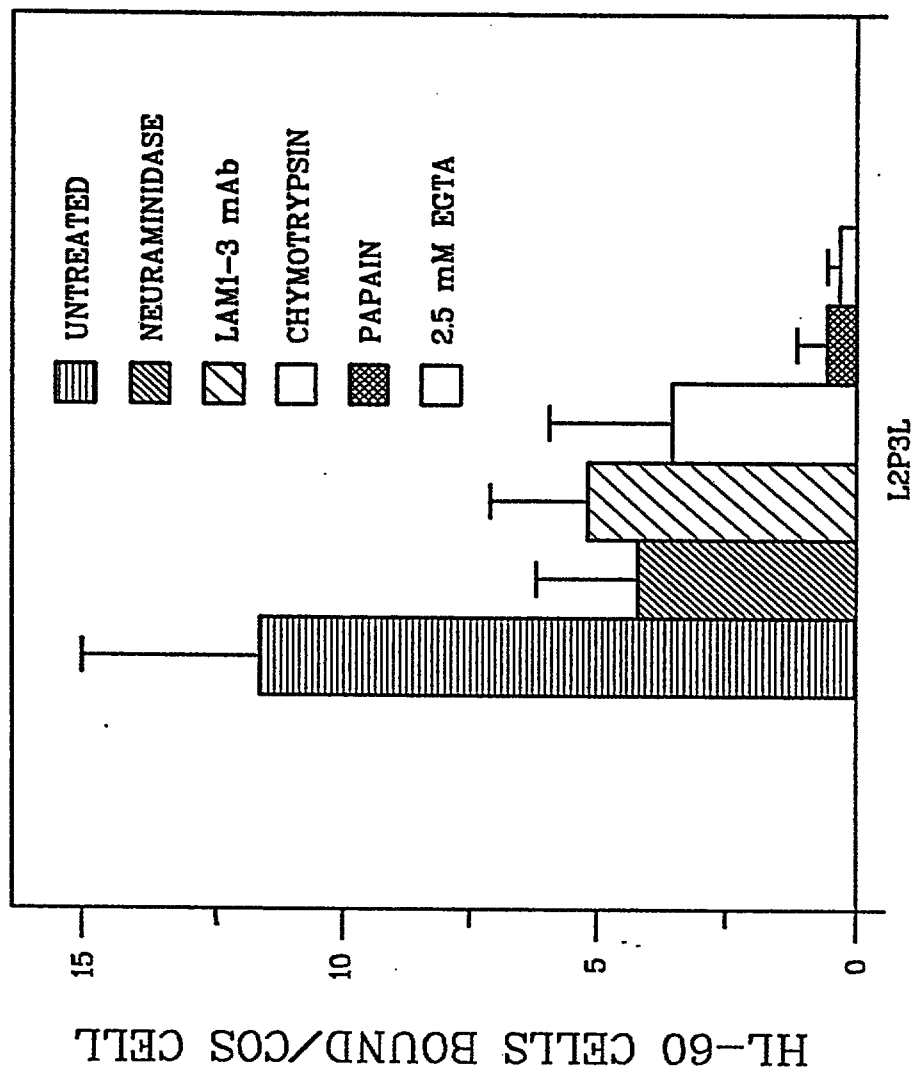


FIG. 3C

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I	M	G
gaattcCCTTT	GGCAAGGAC	CTGAGACCCT TGTCCTAAGTCAAGAGGCTCA ATG GGC
C R R T R E G P S K A M		
TGC AGA AGA ACT AGA GAA GGA CCA AGC AAA GCC ATG	94	
I F P W K C Q S T Q R D L W N I	20	30
ATA TTT CCA TGG AAA TGT CAG AGC ACC CAG AGG GAC TTA TGG AAC ATC		
F K L W G W T M L C C D	40	
TTC AAG TTG TGG GGG TGG ACA ATG CTC TGT TGT GAT	168	
F L A H H G T D C ↓ W T Y H Y S E	50	
TTC CTG GCA CAT CAT GGA ACC GAC TGC TGG ACT TAC CAT TAT TCT TCT GAA		
K P M N W Q R A R R F C		
AAA CCC ATG AAC TGG CAA AGG GCT AGA AGA TTC TGC	262	
R D N Y T D L V A I Q N K A E I	80	
CGA GAC AAT TAC ACA GAT TTA GTT GCC ATA CAA AAC AAG CGG GAA ATT		
E Y L E K T L P F S R S	90	
GAG TAT CTG GAG AAG ACT CTG CCT TTC AGT CGT TCT	346	

FIG. 4A

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100										110				
Y	Y	W	I	G	I	R	K	I	G	G	I	W	T	W
TAC	TAC	TGG	ATA	GGA	ATC	CGG	AAG	ATA	GGA	GGA	ATA	TGG	ACG	TGG
120														
V	G	T	N	K	S	L	T	E	E	A	E	N		
GTG	GGA	ACC	AAC	AAA	TCT	CTC	ACT	GAA	GAA	GCA	GAG	AAC	430	
130														
W	G	D	G	E	P	N	N	K	K	N	K	E	D	C
TGG	GGA	GAT	GGT	GAG	CCC	AAC	AAC	AAG	AAG	AAC	AAG	GAG	GAC	TGC
140														
V	E	I	Y	I	K	R	N	K	D	A	G	K		
GTG	GAG	ATC	TAT	ATC	AAG	AGA	AAC	AAA	GAT	GCA	GGC	AAA	514	
150														
W	N	D	D	A	C	H	K	L	K	A	A	L	C	Y
TGG	AAC	GAT	GAC	GCC	TGC	CAC	AAA	CTA	AAG	GCA	GCC	CTC	TGT	TAC
160														
T	A	S	C	Q	P	W	S	C	S	G	H	G		
ACA	GCT	TCT	TGC	CAG	CCC	TGG	TCA	TGC	AGT	GGC	CAT	GGA	598	
170														
E	C	V	E	I	I	N	N	Y	T	C	N	C	D	V
GAA	TGT	GTA	GAA	ATC	ATC	AAT	AAT	TAC	ACC	TGC	AAC	TGT	GAT	GTG
180														
G	Y	Y	G	P	Q	C	Q	F	V	I	Q	C		
GGG	TAC	TAT	GGG	CCC	CAG	TGT	CAG	TTT	GTG	ATT	CAG	TGT	682	
190														
E	P	L	E	A	P	E	L	G	T	M	D	C	T	H
GAG	CCT	TTG	GAG	GCC	CCA	GAG	CTG	GGT	ACC	ATG	GAC	TGT	ACT	CAC
200														
P	L	G	N	F	N	F	N	S	Q	C	A	F		
CCT	TTG	GGA	AAC	TTC	AAC	TTC	AAC	TCA	CAG	TGT	GCC	TTC	766	
210														
S	C	S	E	G	T	N	L	T	G	I	E	E	T	T
AGC	TGC	TCT	GAA	GGA	ACA	AAC	TTA	ACT	GGG	ATT	GAA	GAA	ACC	ACC
220														
C	E	P	F	G	N	W	S	S	P	E	P	T		
TGT	GAA	CCA	TTT	GGA	AAC	TGG	TCA	TCT	CCA	GAA	CCA	ACC	850	
230														
C	Q	V	I	Q	C	E	P	L	S	A	P	D	L	G
TGT	CAA	GTG	ATT	CAG	TGT	GAG	CCT	CTA	TCA	GCA	CCA	GAT	TTG	GGG
240														
I	M	N	C	S	H	P	L	A	S	F	S	F		
ATC	ATG	AAC	TGT	AGC	CAT	CCC	CTG	GCC	AGC	TTC	AGC	TTT	934	
250														
T	S	A	C	T	F	I	C	S	E	G	T	E	L	I
ACC	TCT	GCA	TGT	ACC	TTC	ATC	TGC	TCA	GAA	GGA	ACT	GAG	TTA	ATT
260														
G	K	K	K	T	I	C	E	S	S	G	I	W		
GGG	AAG	AAG	AAA	ACC	ATT	TGT	GAA	TCA	TCT	GGA	ATC	TGG	1018	
270														
S	N	P	S	P	I	C	Q	K	L	D	K	S	F	S
TCA	AAT	CCT	AGT	CCA	ATA	TGT	CAA	AAA	TTG	GAC	AAA	AGT	TTC	TCA
280														
M	I	K	E	G	D	Y	N	P	L	F	I	P		
ATG	ATT	AAG	GAG	GGT	GAT	TAT	AAC	CCC	CTC	TTC	ATT	CCA	1102	
290														
300														
310														
320														
330														
340														
350														

FIG. 4B

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V A V M V T A F S G L A F I I
 GTG GCA GTC ATG GTT ACT GCA TTC TCT GGG TTG GCA TTT ATC ATT
 360
 W L A R R L K K G K K S K
 TGG CTG GCA AGG AGA TTA AAA AAA GGC AAG AAA TCC AAG 1186
 370
 R S M N D P Y *
 AGA AGT ATG AAT GAC CCA TAT TAA ATCGCCCTTG GTGAAAGAAA
 ATTCTTGGA TACTAAAAAT CATGAGATCC TTTAAATCCT TCCATGAAAC 1280
 GTTTTGTGTG GTGGCACCTC CTACGTCAAA CATGAAGTGT GTTTCCTTCA
 GTGCATCTGG GAAGATTTCT ACCTGACCAA GAGTTCCTTC AGCTTCCATT 1380
 TCACCCCTCA TTTATCCCTC AACCCCCAGC CCACAGGTCT TTATACAGCT
 CAGCTTTTTC TCTTTTCTGA GGAGAAACAA ATAACACCAT AAAGGGAAAG 1480
 GATTCATGTG GAATATAAAG ATGGCTGACT TTGCTCTTTC TTGACTCTTG
 TTTTCAGTTT CAATTCAGTG CTGTACTTGA TGACAGACAC TTCTAAATGA 1580
 AGTGCAAATT TGATACATAT GTGAATATGG ACTCAGTTTT CTTGCAGATC
 AAATTTTCGCG TCGTCTTCTG TATACGTCCA GGTACACTCT ATGAAGTCAA 1680
 AAGTCTACGC TCTCCTTTCT TTCTAACTCC AGTGAAGTAA TGGGGTCCTG
 CTCAAGTTGA AAGAGTCCTA TTTGCACTGT AGCCTCGCCG TCTGTGAATT 1780
 GGACCATCCT ATTTAACTGG CTTCAGCCTC CCCACCTTCT TCAGCCACCT
 CTCTTTTTC A GTTGGCTGAC TTCCACACCT AGCATCTCAT GAGTGCCAAG 1880
 CAAAAGGAGA GAAGAGAGAA ATAGCCTCCG CTGTTTTTTA GTTTGGGGGT
 TTTGCTGTTT CCTTTTATGA GACCCATTCC TATTTCTTAT AGTCAATGTT 1980
 TCTTTTATCA CGATATTATT AGTAAGAAAA CATCACTGAA ATGCTAGCTG
 CAACTGACAT CTCTTTGATG TCATATGGAA GAGTTAAAC AGGTGGAGAA 2080
 ATTCCTTGAT TCACAATGAA ATGCTCTCCT TTCCCCTGCC CCCAGACCTT
 TTATCCACTT ACCTAGATTC TACATATTCT TTAAATTTCA TCTCAGGCCT 2180
 CCCTCAACCC CACCACTTCT TTTATAACTA GTCCTTTACT AATCCAACCC
 ATGATGAGCT CCTCTTCCTG GCTTCTTACT GAAAGGTTAC CCTGTAACAT 2280
 GCAATTTTGC ATTTGAATAA AGCCTGCTTT TTAAGTGTTA AAAAgaattc 2330

FIG. 4C

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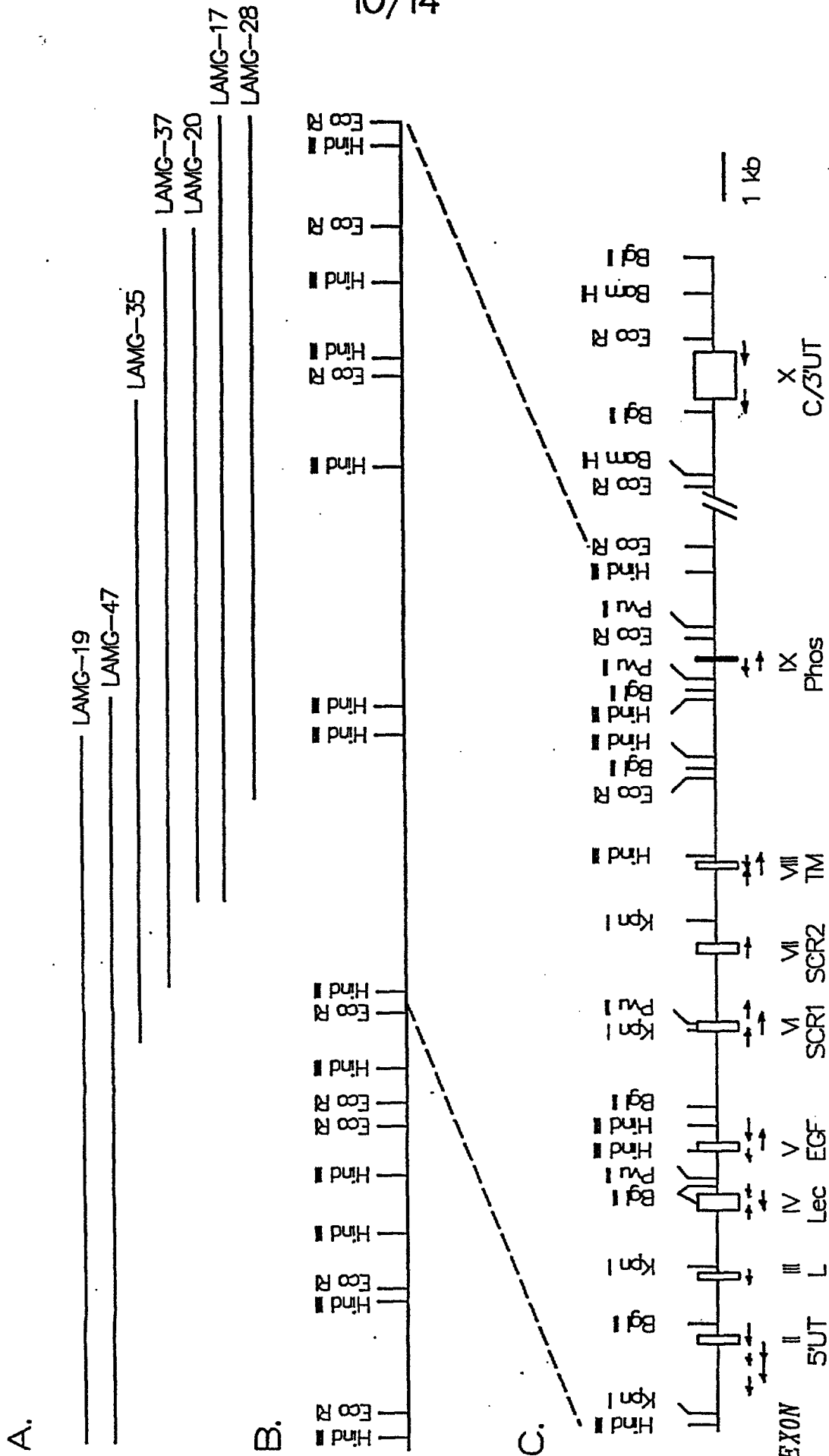


FIG. 5

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EXON II (5' Untranslated Region and Translation Initiation Site)

AGAGAGCTG TTATAAAGAT TAAACAATAT AATAAATATG GCGCTGAGC TTCAGAGTTT TTGTTGTTGT TATTATTATT TTCTAATAA TGCAATCTG ATTTGCATTT
 GACTCATTA CTCACATCAG TGGGTCTTCC TTTTATTGT CTTTCATCAT ATGGTCTCTA ATTTCACATG CAGTCTTATA AAACCATCTC ATTTATAGT CCAAGAATAT
 TAAAGGTACT TGTAGGCTCC CAACCTTACA CGGTGAAGA CTTAGAGAGCA TGGGCTCTCT TCAGGGGTTA ACTTCAGGAA GTGCCACTAA CAAGGACGTC CACTAGGTGG
 TGAGCAAGGA AAGACGGAGG TGAAGGAACC GAAACGAGTC AAGTCCACTG CTTAGCTCTA CTGAAGTTT GCAAACTCA TAAATATGTC TGAATGTCAG TTTTGTATTG
 TAGTATTGC AATTCCAG GGCATTAC CACAGGTAG CACAGTTAG TTTAGCATTT ATGAAAAGA TAGGGAGGG TGGTGGTTAA GAAGAGGTG GAGGAGAGG
 TGAAGGAGGA AGAGGAGAAC AAGAACCRAA AAGAACAAG AACAGAACA ACTAGAAGAA GAGGAGCAG GAGAAAAG GAGAACTAG TCACATTACT TTCTGATCAG CAGTTCAATT
 TGAAGGAGGA GGAGGGTAAG GAAAGATGCA TAGGAGATG GAAAGAGGAG GGAAGGAGA GGAATCTAG TCACATTACT TTCTGATCAG CAGTTCAATT
 TTGTCTCAGT GGAGGCAAT AGAGGCCACT CTAGGAAAGG. GGTGGGAAA GAGGAGGAG GGAAGGAGG GCGCAAGGG AGGAGGAGGA GGATGTGAGA
 CTGGGTTAGA GAAATGAAG AAAGCAAGGC TTTCTGTTGA CATTGAGTGC AGTCTACCTG CAGCACAGCA CACTCCCTTT GGGCAAGGAC CTGAGACCCCT TGTGCTAAGT
 CAAGAGGCTC AATGGGCTGC AGAAGAACTA GAGAGGACC AAGCAAGCC ATG GTGAGCCTTT CAGCCTAAA GACGTTTAGA TGCTCAGATA GAAACTCTTG
 GGGTTGTAGA GCGAGGTGC AAGGATAGGA ATCACCCCAT TTCAATTCTG GTTTTAAATA ATATAGAAAC TAAACATTTT CTCAGACCCCT CAAAAAAGT

EXON III (Leader Domain)

CACTGAGA CTAAGCGTAA AATAAATAGA ACAACAAC TGTGCATCAG TTCTGATGTA AATTGAAAT AATTTTCATC TATGCTGAG AAACCTGTTA CCTCAGACAG
 GGTAGTAGA CATATGTGTT TTATCTGAT TATTANGAA GTTGAAGCA CCACCTCANA GGCTATTAAT GTGTGCTTTA AGGTATACA TCTAANTATA ATTTTGTATT
 TCATTTCAG ATA TTT CCA TGG AAA TGT CAG AGC ACC CAG AGG GAC TTA TGG AAC ATC TTC AAG TTG TGG GGG TGG ACA ATG CTC TGT
 TGT G GTATGTTATG ATATTATAT ATCACTAAGT CTATTTTACT TATATTCAAT TTT

FIG. 6A

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EXON IV (Lectin Domain)

CT GGAGTAGTGC TAGGTCTTT TTAGCTGTAA CATTATGTAA GTCTGCATAG GTCACACTGA TGCTTCGAG AT TTC CTG GCA CAT CAT GGA ACC GAC TGC
 TGG ACT TAC CAT TAT TCT GAA AAA CCC ATG AAC TGG CAA AGG GCT AGA AGA TTC TGC CGA GAC AAT TAC ACA GAT TTA GTT GCC ATA
 CAA AAC AAG GCG GAA ATT GAG TAT CTG GAG AAG ACT CTG CCT TTC AGT CGT TCT TAC TAC TGG ATA GGA ATC CGG AAG ATA GGA GGA
 ATA TGG ACG TGG GTG GGA ACC AAC AAA TCT CTC ACT GAA GCA GAG AAC TGG GGA GAT GGT GAG CCC AAC AAC AAG AAG AAC AAG
 GAG GAC TGC GTG GAG ATC TAT ATC AAG AGA AAC AAA GAT GCA GGC AAA TGG AAC GAT GAC GCC TGC CAC AAA CTA AAG GCA GCC CTC
 TGT TAC ACA G GTAGGGAGTG ACAAGACGGC TATGCTGCCT CAGACTCAGG AAGGCCACG GTTAAGAGAA TACTCAGATT TA

EXON V (EGF Domain)

AAAATTTTAG CCAATATGATT TTTATGCTAT GAATTPACCA AATAAACCTT TCCTGATAT TTAATCATC TCAGACAAA GGTATCTAT GTCTAAAGAA ATGACTTTGA
 GACTAAAT GTATACACAT TAAATATTT TTTTCTGAC CTCCTTAAAG CT TCT TGC CAG CCC TGG TCA TGC AGT GGC CAT GGA GAA TGT GTA
 GAA ATC ATC AAT AAT TAC ACC TGC AAC TGT GAT GTG GGG TAC TAT GGT CCC CAG TGT CAG TTT G GTAAGTCTCT TTCCTTTCTT CAGAAAAGCTC
 TGCTTCTTCT TAGGTAAAGT CACAGGAATC ATTATAGCTT ATCATGAAGC TGGTTGGAAC AATATGATAC TAGCCACTCT GAGAAATGGG AAGTTTGTGAT
 TGCTTTCACA ATATTGTAC CTTTCCGTAA AGATTTCATA AGTCAGCATG AAGTTTCGAT TCAGTTCTCA ACAGTCTTT TTGAGTACCA CAAGAAGCAC AGTGTGGGA
 TAAAGCTGC AGGTTACAA TAAGGAATTA GCATGGTGA TTCCCGCTCT CAAGAAGCTC ACGATCTAAT GAGCTTGTGA GATTAATTAG AACTCTAAG TCTGGAAGAA
 ACTATGCCAT TTATCATTAG GAGGCTGAGT TACCCAGAAA GTATCTTGCT TTTTCTTCT AGTAGTTCTT TTCCTTCTTG CAGTTCTCCA CACTTAACAC ATGTGCTCTG
 TAGCACACTG ACTTTGCTGG TGGCCCTTCTC TCTCATTTTG CACATGGSCCA AAAACATGT CATCTTTAAG ACATTGTTC AAGACAGTTT CTTCTAGGAA GCTT

EXON VI (SCR I Domain)

CTCTGA TGTGATAGTT ATTTCCCGAC TAAGCTGGTC ATTCCAGTT ACACCTATTT GGTTTAAGG ATTTCTACTA CAGATAATAC TGAAGATAAT AATATGAAGA
 CTAGCTAATG TTACTCTAGA ATTTCTGATG AGTCAGGCTT TGTTCTAAGC TCCTTGACTT ATGTAAATG AATTACATTT AGTTTCCATA TCAATTTGAT AAAGATAACA
 CAATTTCAAT ATTCTCTCTTA TATAGATGAA GAACTGAAG TTGGAGGGTT CAAGTAACCT TGTTTAAGG CACATGGTTA TCAAGTGGA GGGTAGGAT TCAATCCAG
 GCGTCAGTTC CTCTTAATC TTCCCCATAC TGTTCCTTTC CCTATTGAAG TG ATT CAG TGT GAG CCT TTG GAG GCC CCA GAG CTG GGT ACC ATG GAC
 TGT ACT CAC CCT TTG GGA AAC TTC AGC TTC AGC TCA CAG TGT GCC TTC AGC TGC TCT GAA GGA ACA AAC TTA ACT GGG ATT GAA
 GAA ACC ACC TGT GGA CCA TTT GGA AAC TGG TCA TCT CCA GAA CCA ACC TGT CAA G GTGAGTAAT TCAGACTAGA GGTTCGTCA TGCATTCCTG
 GGCTTACAT CAGACATTC AGTAGAAGT TCGTGAGAG TCAACTTAG GATCCTAAT TAACCTAAT TTTGTTTAACT TTTGTTTAACT CTACTGTGAT GTTCTCTCAA GGACTTATTC

FIG. 6B

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EXON VII (SCR II Domain)
 GAGGTCAC CTTAGCTAGG GCACAGCCT GGAGTAGCTA CTCTCTCTCC CACAGCTTTC AATGCTTCTT TGCCTTCATC TCTCATTCAC CACCCACCAT CATCTCTCAAG
 AAAATAAAGC CTGGAAGCAA TATCACAAGT AATGTAGTCA GGCAGCTTTG GCTAAATATC CAAAGCTCAA GGGAGGGTCT CTACTCAGAA ATACTGTTTT GTCTTTTTTT
 TTTTCTCTTTT TTCTATGAAG TG ATT CAG TGT GAG CCT CTA TCA GCA CCA GAT TTG GGG ATC ATG AAC TGT AGC CAT CCC CTG GCC AGC
 TTC AGC TTT ACC TCT GCA TGT ACC TTC ATC TGC TCA GAA GGA ACT GAG TTA ATT GGG AAG AAG AAA ACC ATT TGT GAA TCA TCT
 GGA ATC TGG TCA AAT CCT AGT CCA ATA TGT CAA A GTGAGTAAAT TTGCTCTGGA ACTGAA

EXON VIII (Transmembrane Domain)
 TATCAGAAC TAAGAAAGCT TGGGCTGCAG GTCTGACTCTA GTTGCATTTT CAGGAATCTT ATGAACCACA AATCTGGGCA TTGAGATTCT GTAGGCATTA GACTAGCAAG
 GCTGGTCAGT. CTTTGCTTCT GCTGTAGACT CATCAGGGGC CTTCCCATGC CAGTTCTCTC ATCTGTCAA TGGCATCATT TGGGTACTA CTGGGAGATG TAAGGAGGAA
 AAAAGTCAAA TATCATGAGA TAGACTAAGG AAATAATGCT GGTGGTCTCA TGCTATGTGC CTTACTGATT TCTCTTTCAG AA TTG GAC AAA AGT TTC TCA ATG
 ATT AAG GAG GGT GAT TAT AAC CCC CTC ATT CCA GTG GCA GTC ATG GTT ACT GCA TTC TCT GGG TTG GCA TTT ATC ATT TGG CTG
 GCA AGG AGA TTA AAA AAA G GTATGTGAGT TTAACCTTCA CACAATTTA AGTGAAAAA GAAAAAAA AGAAACCCAC AGGAATTTAA
 ATGTGATAGA TTCAACACAA GCAGATGCC AGCTT

EXON IX (Phosphorylation Domain)
 TAGTTTACA GTATTAGCAG CTGTCCCTCA AGGAAGAATC TGCAGGTAGA TGAGATGCAG ATTGGGTGGG ATAAACACTT GAATGACATA TTGGGTCTTG CCACCAGGCA
 ATTTAGCAAT TCTGTCTTCT TGAGTAGCAC GGAGATGGAA TGGAACTTCA GGAGGATCT GCATCAACAT GTCTGTCTG TATTAGTGC TACCACATGT TATTAAGCCA
 GTTCTCAAA TCTCCTTTGA CACAGATAGG GTCCACCTAA CAAATACCTA ATATACCTCA AAAGACAGTT TTGAGAGTGG GAGTCTTCT TCTCCTTAC TTGAAAAACT
 TTAATATGTC TAATTTTTTC TAATGCTTT TAATGCTTT TCTCTATTT TCTATTCTAG GC AAG AAA TCC AAG AGA AG GTAAATTTA TTAGTGGCA GGAGTTTCCA
 CATCTGCTGA TTCTATTTCT ACTTCTTAAG TTACTTCTGC TCTAGCTAGA CACATACCCA TAGTAGTTAT TACTGGGTCT ATCAATGACA GATAGG

EXON X (Cytoplasmic Tail and 3' Untranslated Region)
 ATAGCATC ACTAAGAGC TTGTTAGGGG TGCAGAATCT CAGGCTCCAC TCAGACCTAC TGAATCAGAG TCTGCATTTT AACACCATCT CTGAGTGGTA AGGACATGAA
 AATCTGAGAA GTGCTGCTAC TAGGGTTGC TTACATTTGT TCATCTTCAG AGGTTCTTAA AGCCTGGCCT CTTGCTGAG ATTTCCAGCT GAAAGCATTT CCTTGCTCCT
 CTTCTCATCT CTAATGAATA TTACCTTTA CTAATAACAC TCCAAAGTTT GCAATTTTAA AACTCTTATT ATCTTTTGT TTTCTTTGAG T ATG AAT GAC CCA
 TAT TAA ATCGCCCTTG GTGAAGAAA ATTCTTGGAA TACTPAAAAAT CATGAGATCC TTAAATCTCT TCCATGAAC GTTTTGTGTG GTGGCAC(983 BASES)G
 CAAATTTGCA TTGTGAATAA GCTGCTTTT TAAGTGTAA CTAGTTTGC TAGTTTGTAA TTTTGAAAAAT TGATCATATG TTTTGTGTTT TCCCCAGTGA GTTACATGCT
 TAAGTTTCAG CATCCCTCTT TCTCTATGGC ATCTGATGAC CTGGGTGAGA TATCAGCTAA TGTCAACAGC TGAATTC

FIG. 6C

This is the replacement sheet

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A. Lectin domains

human-L: WTYHYSEKPMNWQARRRCRDNYTDLVAIQKAEIYLEKTLPPFSRSYYWIGIRKIGGIW
 mouse-L: -----EN-K-KQ-----N-----R-----N-K-PY-----KM-
 rat-L: -----RS-----EN-KF-KH-----R-----R-----KNPT-----KT-
 bovine-L: -----KR-P-EK-A-E-----G-----N-Q-----T-----VE-V-
 human-E: -S-NT-TEA-TYDE-SAY-QQR--H-----E-----NSI-SY-P-----VNVV-
 bovine-E: -S-A-TEM-TFEE-DY-QKT--A-----QE-----NS-FSY-P-----N-T-
 human-P: -----T-AYS-NIS-KY-QNR-----N-D-N-V-YYS-----NNKT-
 mouse-P: -----N-T-AYS-NNS-V---RHF-----N-AH-NDVI--FN-----NNK-
 bovine-P: -----N-TYS-NYS-A--QKY--K-----N-A-NE-I-YYN-----NNK-

human-L: TWVGTNKSLEEEAENWGDGEPNNKKNKEDCVEIYIKRNKDAGKWNDDACHKLKALCYT
 mouse-L: -----T-K-----A-----S-----ER-S-----R-----
 rat-L: -----T-K-----T-----S-----ER-S-----R-----
 bovine-L: -----K-A-----R-S-----S-----A-T-----
 human-E: V-----Q-P-----K-AP-----RQKD-----E-V-M-----ER-S-K-L-----
 bovine-E: -I-----K-T-AP-----QSD-----E-S-----EK-T-Q-L-----K
 human-P: -----K-A-N-----A-N-----R-N-----SPSAP-----EH-L-K-H-----
 mouse-P: -----T-----A-N-----N-Q-----S-SAP-----EP-F-R-R-----
 bovine-P: -----K-T-----A-N-----R-NQ-----SLSAP-----EP-W-R-R-----R

B. EGF domains

human-L: ASCQPWSCSGHGECEIINNYTCNCVDVGYGPQCQF
 mouse-L: -----G-N-R-----T-----H-I-A-----Y
 rat-L: -----E-NR-----T-----N-I-P-----Y
 bovine-L: -----K-----Q-V-----L-----E-----
 human-E: -A-TNT-----T-----K-P-FS-LK-EQ
 mouse-E: -----TNA-----I-T-S-----K-HP-FL--N-EQ
 bovine-E: -A-N-TP-G-----Q-HP-FK-LK-EQ
 human-P: -----DM---KQ---L-T-G-----S-YP-F---E-EY
 mouse-P: -----DM---NQ-K-I-T-GS-----S-YP-F---E-EY
 bovine-P: -----DM---KQ---I-T-G-----S-YP-F---E-EY

FIG. 7

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carbohydrate ligands (Springer, Nature 346:425-434 (1990); Lasky, Science 258:964-969 (1992)), the role of the conserved EGF-like domains is unknown.

It has been proposed that the treatment of a patient suffering from pathologic inflammation with an antagonist to adhesion receptor function can result in the reduction of leukocyte migration to a level manageable by the target endothelial cells and the subsequent dramatic recovery of the patient. Local administration of therapeutic agents can block competitively the adhesive interactions between leukocytes and the endothelium adjacent to an inflamed region. Therapeutic agents can also be administered on a systemic level for the treatment of a patient suffering from disseminated inflammation (Harlan and Liu, eds., Adhesion: Its Role in Inflammatory Disease, W. H. Freeman (in press)).

A chimeric selectin combining the EGF domain of P-selectin and the lectin binding domain of L-selectin has been disclosed (Kansas, G. S. et al., The J of Cell Biology, vol. 114/(2), 1991, pp. 357 - 358) but the ligand binding properties of the EGF domain were not appreciated. Kansas, G.S., APMIS, vol 100, 1992, pp. 287 - 293 disclosed a rationale for producing selectin chimeras in which various domains of selectin were to be replaced by the homologous domains of another family member of the selectin family. Again a ligand binding property of the EGF domain was not appreciated.

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SUMMARY OF THE INVENTION

We report here that the EGF-like domains of P-selectin and of E-selectin can participate directly in cell adhesion, having ligand binding sites distinct from those of their respective lectin domains. Cell adhesion mediated at least by the P and E-selectins may be complex, involving interactions between the lectin domain and a carbohydrate ligand, and separately, between the EGF-like domain and one or more proteins or other ligands. Our discovery of a difference in at least one of the ligands recognized by the lectin and EGF domains of a given selectin has permitted the preparation of chimeric polypeptides that combine within one molecule the ability to target two or more different selectin ligands and the use of these agents in therapy or in the preparation of additional classes of antagonists to selectin function.

Thus, the invention generally features chimeric peptides or polypeptides that combine ligand binding portions from within the lectin and EGF domains of two different selectins. The peptides or polypeptides can be composed solely of the

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the sequence and structural information described. Treatment of affected individuals would then be possible using the *lyam-1* gene or fragments thereof.

5 The normal regulation of the *lyam-1* gene, as evidenced by the appearance and disappearance of L-selectin on the surface of a specific leukocyte sub-population can be monitored to test the effects of drugs or specific therapies that would alter gene expression.

10 While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection
15 granted hereon be limited only by appended claims.

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Date: 05 AUG 1998

CLAIMS:

1. A bifunctional ligand binding polypeptide comprising a ligand binding portion found within the lectin domain of a first selectin and a ligand binding portion found within the EGF domain of a second selectin, wherein the polypeptide binds ligands of the first selectin and ligands of the second selectin, but wherein the polypeptide is not a polypeptide comprising a lectin domain of L-selectin and an EGF domain of P- selectin.
2. The polypeptide according to claim 1, wherein the ligand binding portion found within the EGF domain is the entire EGF domain and wherein the ligand binding portion found within the lectin domain is the entire lectin domain.
3. The polypeptide according to claims 1 or 2 further comprising a selectin SCR domain and a selectin transmembrane domain.
4. The polypeptide according to any one of claims 1 to 3 joined to a carrier protein.
5. The polypeptide according to claim 4, wherein said carrier protein comprises an immunoglobulin heavy chain constant region.
6. The polypeptide according to claim 4, wherein said carrier protein comprises non-ligand binding portions of a selectin molecule.

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7. A DNA sequence encoding a polypeptide according to any one of claims 1 to 3.
8. A DNA sequence selected from the group consisting of:
 - (a) a DNA sequence able to hybridise under stringent conditions to the complement of a sequence encoding the EGF domain of E-selectin and to the complement of a sequence encoding the lectin domain of L-selectin.
 - (b) a DNA sequence able to hybridise under stringent conditions to the complement of a sequence encoding the EGF domain of a P-selectin and to the complement of a sequence encoding the lectin domain of E-selectin; and
 - (c) a DNA sequence able to hybridise under stringent conditions to the complement of a sequence encoding the EGF domain of E-selectin and to the complement of a sequence encoding the lectin domain of P-selectin.
9. Use of a bifunctional ligand binding polypeptide comprising a ligand binding portion found within the lectin domain of a first selectin and a ligand binding portion found within the EGF domain of a second selectin, wherein the polypeptide binds ligands of the first selectin and ligands of the second selectin, in the preparation of a medicament for simultaneously inhibiting selectin mediated adhesion, migration and infiltration into tissues of cells expressing the first selectin and cells expressing the second selectin.
10. A pharmaceutical composition comprising a therapeutic amount of the bifunctional ligand binding polypeptide of any one of claims 1-6 in a pharmaceutically acceptable carrier substance.

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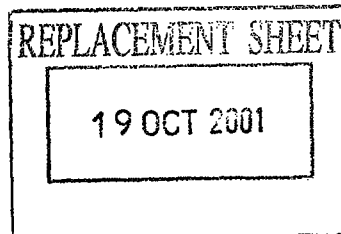
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11. The pharmaceutical composition according to claim 10, for use in treating a leukocyte-mobilising condition.
12. The pharmaceutical composition according to claim 11, wherein said leukocyte-mobilising condition is selecting from the group consisting of
 - (a) tissue damage;
 - (b) an autoimmune disorder; and
 - (c) a post-reperfusion injury.
13. The pharmaceutical composition according to claim 11, wherein said leukocyte-mobilising condition exists in an organ or tissue transplant recipient.
14. The pharmaceutical composition according to any one of claims 10 to 13, wherein said polypeptide is joined to an additional therapeutic agent.
15. The pharmaceutical composition according to claim 14, wherein said therapeutic agent comprises a chemotherapeutic drug or an anti-inflammatory agent.
16. A diagnostic composition comprising the polypeptide according to any one of claims 1 to 3.
17. The use of a bifunctional ligand binding polypeptide according to any one of claims 1 to 6, to screen for simultaneous antagonists to the ligand binding activity of said first and second selectins.

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CHIMERIC L- AND P-SELECTIN BY EXCHANGE OF DOMAINS-USES
THEREOF

RELATED APPLICATIONS

This application is a continuation-in-part of Tedder, U.S. Patent Application Serial No. 07/983,606, filed November 30, 1992, which is a continuation under 37 CFR 1.62 of Serial Nos. 07/730,503, filed July 8, 1991, and 07/313,109, filed February 21, 1989, both now abandoned. This application also is a continuation-in-part of Tedder, U.S. Patent Application Serial No. 07/700,773, filed May 15, 1991; of Tedder, U.S. Patent Application Serial No. 07/737,092, filed July 29, 1991; of Tedder et al., U.S. Patent Application Serial No. 07/770,608, filed October 3, 1991; and of Tedder et al., U.S. Patent Application Serial No. 07/862,483, filed April 2, 1992, the whole of which are hereby incorporated by reference herein.

FIELD OF THE INVENTION

This invention relates to the selectin family of receptor adhesion molecules and particularly to agents interfering with selectin function.

GOVERNMENT RIGHTS

Part of the work leading to this invention was made with United States Government funds. Therefore, the U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

The ability of leukocytes to leave the circulation and to migrate into tissues is a critical feature of the immune response. Normally, the infiltrating leukocytes phagocytize invading organisms or dead or damaged cells. However, in pathologic inflammation, infiltrating leukocytes can cause