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**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 application also is a continuation-in-part of Tedder, U.S. Patent Application Serial No. 07/700,773, filed May 15, 1991; 15 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 20 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



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, Ca²⁺-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>
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 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
15 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
25 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.
30 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
35 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.

5 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) GTCAA¹⁰²³A 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 ^{99}Tc , ^{123}I , ^{131}I , ^{111}In , ^{87}Ru , ^{67}Cu , ^{67}Ga , ^{68}Ga , ^{72}As , ^{89}Zr , and ^{201}Tl . 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 ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr , and ^{56}Fe . 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:

(A) NAME: Heine, Holliday C.
(B) REGISTRATION NUMBER: 34,346
(C) REFERENCE/DOCKET NUMBER: DFCI-318XX



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

(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:

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	Gly Cys Arg Arg Thr Arg Glu Gly Pro Ser Lys Ala Met Ile Phe Pro	
	5 10 15	
	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	
	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	
	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	
	TCT TAC TAC TGG ATA GGA ATC CGG AAG ATA GGA GGA ATA TGG ACG TGG	391
	Ser Tyr Trp 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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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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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
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	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
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	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	
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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
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	355 360 365	
	AGA TTA AAA AAA GGC AAG AAA TCC AAG AGA AGT ATG AAT GAC CCA TAT	1207
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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 ATTTACACCCC 1387
 TCATTTATCC CTCAACCCCC AGCCCACAGG TGTTTATACA GCTCAGCTTT TTGTCTTTTC 1447
 TGAGGAGAAA CAAATAAGAC CATAAAGGGA AAGGATTCAT GTGGAATATA AAGATGGCTG 1507
 ACTTTGCTCT TTCTTGACTC TTGTTTTTCTG 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 CCTATTTAAC TGGCTTCAGC 1807
 CTCCCCACCT TCTTCAGCCA CCTCTCTTTT TCAGTTGGCT GACTTCCACA CCTAGCATCT 1867
 CATGAGTGCC AAGCAAAAGG AGAGAAGAGA GAAATAGCCT GCGCTGTTTT TTAGTTTGGG 1927
 GGTTTTGCTG TTTCCTTTTA TGAGACCCAT TCCTATTTCT TATAGTCAAT GTTTCTTTTA 1987
 TCACGATATT ATTAGTAAGA AAACATCACT GAAATGCTAG CTGCAACTGA CATCTCTTTG 2047
 15 ATGTCATATG GAAGAGTTAA AACAGGTGGA GAAATTCCTT GATTCACAAT GAAATGCTCT 2107
 CCTTTCCCCT GCCCCCAGAC CTTTTATCCA CTTACCTAGA TTCTACATAT TCTTTAAATT 2167
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 CCCATGATGA GCTCCTCTTC CTGGCTTCTT ACTGAAAGGT TACCCTGTAA CATGCAATTT 2287
 TGCATTGAA 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			
5	Trp	Val	Gly	Thr	Asn	Lys	Ser	Leu	Thr	Glu	Glu	Ala	Glu	Asn	Trp	Gly	
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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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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		
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				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	
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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	TTTGCAATTTG	ACTCATTGAC	120
TCACATCAGT	GGGTCTTCCT	TTTTATTGTC	CTTCATCATA	TGGGTCCTAA	TTTCACATGC	180
AGTCTTATAA	AACCATCTCA	TTTTATAGTC	CAAGAATATT	AAAGGTACTT	GTAGGCTCCC	240
AAACCTACAC	GGTGAAAAGC	TAGAGAGCAT	GGGCTCTCTT	CAGGGGTTAA	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	AAAAACAAGA	ACAAGAACAA	600
GTAGAAGAAG	AGGAGCAGGG	AGGAAAAAGA	AGAGGAAGAA	GAACAGCAAC	AACAATGAGT	660
GAAGGAGGAG	GAGGGTAAGG	AAAGATGCAT	AGGAGAATGG	AAGGAAGGAT	AGAAAGGAGG	720
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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
TAGAAACTCT	TGGGGTTGTA	GAGGCAGGTG	GCAAGGATAG	GAATCACCCC	ATTTCAATTC	1140
TGGTTTTTAA	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 AAACAACTG 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 AGATTTCTCTG 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 TGCAGGCAA TGGAACGATG 420
ACGCCTGCCA CAACTAAAG 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 GGTATCTAT GTCTAAAGAA ATGACTTTGA GTACTAAAAT 120
GTAATCACAT TAAATATTT TTTTCTGAC CTCCTTAAAG CTTCTTGCCA GCCCTGGTCA 180
TGCAGTGGCC ATGGAGAATG TGTAGAAATC ATCAATAATT ACACCTGCAA CTGTGATGTG 240
GGGTACTATG GGCCCCAGTG TCAGTTTGGT AAGTCTCTTT CCTTTCTTG CTTCTTCTTA 300
GGTAAAGTCA CAGGAATCAT TATAGCTTAT CATGAAGCTG GTTGAACAA AATGATACTA 360
GCCACTCTGA GAAATGGGAA GTTTTGATCA GAAAGCTCTG CTTTCACAAT ATTGTTACCT 420
TTCCGTAAAG ATTTTCATAAG TCAGCATGAA GTTTCGATTC ACTTCTCAAC AAGTCTTTTT 480
GAGTACCACA AGAAGCACAG TGTGGGATA AAGCTGTCAG GGTTACAATA AGGAATTAGC 540
ATGGTAGATT CCCGCTCTCA AGAAGCTCAC GATCTAATGA GCTTGTTAGA TTAATTAGAA 600
CTCTAAGGTC TGGAAGAAAC TATGCCATTT ATCATTAGGA GGCTGAGTTA CCCAGAAAGT 660
ATCTTGCTTT TTCCTTCTAG TAGTTCCTTT CCTTCTTGCA 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 TGAATTATGC 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
 5 GACTGTACTC ACCCTTTGGG AACTTCAGC TTCAGCTCAC AGTGTGCCTT CAGCTGCTCT 480
 GAAGGAACAA ACTTAACTGG GATTGAAGAA ACCACCTGTG GACCATTGTTG AACTGGTCA 540
 TCTCCAGAAC CAACCTGTCA AGGTGAGTAA CTTCAGACTA GAGGTTTTGT CATGCAATCC 600
 TGGGCTTACA GTCAGAACAT TCAGTAGAAG TTTGCTGAGA AGTCAAACCTT AGGATCCTAA 660
 TTTAACCTAA CTTTTGTTTA ACCTACTGTG ATGTTTCTCA AAGGACTTAT TC 712
 10 (2) INFORMATION FOR SEQ ID NO:8:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 451 base pairs
 (B) TYPE: nucleic acid
 15 (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 AAATAAAGCC 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 CTTTCATCTGC TCAGAAGGAA CTGAGTTAAT 360
 TGGGAAGAAG AAAACCATTG GTGAATCATC TGGAATCTGG 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
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 TATACTCAA AAGACAGTTT TGAGAGTGGG 300
AGTCTTCCTT CTCCCTTACT TGAAAACTT TAAATTGTCT AATTTTGTCT AATGCCTTTT 360



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 CTGCTCCTC TTCTCATCTC TAATGAATAT 240
 TTACCTTTAC TACTAACACT CCAAGTTTTG CAATTTTAA ACTCTTATTA TCTTTTGTTT 300
 TTCTTTTCAGT ATGAATGACC CATATTAAAT CGCCCTTGGT GAAAGAAAAT TCTTGGAATA 360
 20 CTAAAAATCA TGAGATCCTT TAAATCCTC CATGAAACGT TTTGTGTGGT GGCACCTCCT 420
 ACGTCAAACA TGAAGTGTGT TTCCTTCAGT GCATCTGGGA AGATTTCTAC CTGACCAACA 480
 GTTCCTTCAG CTTCCATTTT ACCCCTCATT TATCCCTCAA CCCCAGCCC ACAGGTGTTT 540
 ATACAGCTCA GCTTTTTGTC TTTCTGAGG AGAAACAAAT AAGACCATAA AGGGAAAGGA 600
 TTCATGTGGA ATATAAGAT GGCTGACTTT GCTCTTCTT GACTCTTGTT TTCAGTTTCA 660
 25 ATTCAGTGCT GTACTTGATG ACAGACACTT CTAAATGAAG TGCAAATTTG ATACATATGT 720
 GAATATGGAC TCAGTTTTCT TGCAGATCAA ATTCGCGTC GTCTTCTGTA TACGTGGAGG 780
 TACACTCTAT GAAGTCAAAA GTCTACGCTC TCCTTTCTTT CTAACCTCAG TGAAGTAATG 840
 GGGTCCTGCT CAAGTTGAAA GAGTCCTATT TGCACTGTAG CCTCGCCGTC TGTGAATTGG 900
 ACCATCCTAT TTAAGTGGCT TCAGCCTCCC CACCTTCTTC AGCCACCTCT CTTTTTCAGT 960
 30 TGGCTGACTT CCACACCTAG CATCTCATGA GTGCCAAGCA AAAGGAGAGA AGAGAGAAAT 1020
 AGCCTGCGCT GTTTTTTAGT TTGGGGGTTT TGCTGTTTCC TTTTATGAGA CCCATTCTTA 1080
 TTTCTTATAG TCAATGTTTC TTTTATCAG ATATTATTAG TAAGAAAACA TCACTGAAAT 1140
 GCTAGCTGCA ACTGACATCT CTTGATGTC ATATGGAAGA GTTAAAACAG GTGGAGAAAT 1200
 TCCTTGATTC 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 TTGAATAAAG CCTGCTTTTT AAGTGTTAAC	1440
TAGTTTGCCT 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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