

LYMPHOCYTE-ASSOCIATED CELL SURFACE PROTEIN

The invention relates to human lymphocyte-associated cell surface proteins.

Background of the Invention

5 Genes exclusively expressed by one cell lineage, but not by others, often define the function of that cell population. The generation of genes by the assembly of functionally independent domains has occurred frequently as new genes have evolved to encode
10 proteins with new functions. An inducible endothelial-leukocyte adhesion molecule (ELAM-1) is expressed on the surface of cytokine-treated endothelial cells. This molecule is thought to be responsible for the accumulation of blood leukocytes at sites of
15 inflammation by mediating the adhesion of cells to the vascular lining (Bevilacqua et al., Proc. Natl. Acad. Sci. USA 84:9238 (1987)). A granule membrane protein found in platelets and endothelial cells, termed GMP-140, has been cloned and is homologous with ELAM-1
20 (Johnston et al., Blood Suppl.1 72:327A (1988)).

Summary of the Invention

The invention generally features a human cDNA sequence encoding lymphocyte-associated cell surface protein LAM-1, which contains domains homologous with
25 binding domains of animal lectins, growth factors, and C3/C4 binding proteins; and the LAM-1 protein encoded by the cDNA sequence or an immunogenic fragment of LAM-1.

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In a preferred embodiment, the cDNA sequence is isolated from a population of B cell-specific cDNAs from a human tonsil cDNA library, and the amino acid sequence of the protein is substantially as indicated in Fig. 2, more preferably 80% homologous with the sequence shown in Fig. 2 and most preferably 90% homologous. (Here "substantially as indicated" defines a sequence close enough to the indicated sequence to have the same function.)

10 In another aspect, the invention features antibody developed against lymphocyte-associated cell surface protein LAM-1, or a fragment thereof, or against a molecule that specifically associates with LAM-1, or a fragment thereof, to generate a functional molecule.

15 In another aspect, the invention features a method of identifying cells that express LAM-1 which involves reacting the antibody just described with a population of cells and isolating those that bind the antibody. Binding of antibody can also be used to block
20 the receptor activity of LAM-1.

 In another aspect, the invention features a method of treating a human patient suffering from a lymphocyte-mobilizing condition which involves administering a therapeutic amount of an antagonist to
25 LAM-1 in a non-toxic pharmaceutical carrier substance. In preferred embodiments of the method the patient is suffering from tissue damage, an autoimmune disorder, or cancer, or the patient is an organ or tissue transplant recipient.

30 In another aspect, the invention features using the cDNA sequence defined above to isolate cross-hybridizing human DNAs.

 In another aspect the invention features using LAM-1 to identify a ligand which will bind to it or to a

molecule that is specifically associated with LAM-1 to generate a functional molecule.

As used herein the term antagonist includes any agent which interacts with LAM-1 and interferes with its function, eg., antibody reactive with LAM-1 or any ligand which binds to LAM-1.

Lymphocyte-associated cell surface protein LAM-1 is a unique receptor protein which has not previously been identified. LAM-1 contains domains that are homologous with those found in several different receptors and is a newly described member of a gene family that includes ELAM-1 and GMP-140, proteins which have been implicated in cell adhesion. LAM-1 most likely serves a similar function but is uniquely expressed by lymphocytes. The isolation of cDNA encoding LAM-1 has allowed the determination of the structure of this molecule; the cDNA has been used to transfer expression of LAM-1 to cells that do not express this gene.

Antibodies reactive with LAM-1 can be used to identify cells that express this receptor and to block its function. In addition, the cDNA protein product can be used to develop antagonistic ligands that can interfere with lymphocyte adhesion and function and thereby be used to treat such conditions as tissue damage and metastasis of cancer cells.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

In the drawings, Figs. 1A and 1B show the structure of the LAM-1 cDNA clone.

Fig. 2 shows the determined cDNA nucleotide sequence and the deduced amino acid sequence of LAM-1.

Figs. 3A, 3B and 3C show the homologies of LAM-1 with other proteins.

B cell-specific cDNAs were isolated from a human tonsil cDNA library (ATCC #37546) using differential hybridization with labeled cDNAs derived from either B cell (RAJI) RNA or T cell (HSB-2) RNA (Tedder et al., Proc. Natl. Acad. Sci. USA 85:208-212 (1988)). Positive plaques were isolated and cloned, and the cDNA inserts were subcloned into the plasmid pSP65 (Promega, Madison, WI). Nucleotide sequences were determined using the method of Maxam and Gilbert (Meth. Enzymol. 65:499 (1980)). Gap penalties of -1 were assessed during homology analysis for each nucleotide or amino acid in the sequence where a gap or deletion occurred. One of the 261 RAJI+ HSB2- cDNA clones isolated, B125, contained a 1.90 kb cDNA insert that hybridized with a 2.4 kb RNA species found in several B cell lines (Tedder et al., supra). However, B125 did not hybridize with any of the other RAJI+ HSB2- clones or with mRNA from several T cell lines. The B125 cDNA clone was characterized by restriction mapping and nucleotide sequence determination. A near-full-length 2.3 kb cDNA that hybridized with B125 was isolated, sequenced, and termed pLAM-1.

As shown in Fig. 1A, a restriction map was constructed by the standard single, double or triple digestions of pLAM-1. The putative coding region is shown in black. Arrows indicate the direction and extent of nucleotide sequence determination and the open circles indicate 5'-end labeling. In Fig. 1B, a schematic model of the structure of the LAM-1 mRNA is shown. Thin lines indicate 5' and 3' untranslated sequences (UT), while the thick bar indicates the translated region. The boxes represent the lectin-like

and epidermal growth factor (EGF)-like domains and the two short consensus repeat (SCR) units. The open box indicates the putative transmembrane (TM) region.

5 The expression of LAM-1 mRNA by cell lines of lymphoid and non-lymphoid origin was examined. Northern blot analysis revealed that LAM-1 hybridized strongly to a 2.6 kb RNA species and weakly to a 1.7 kb RNA species in poly(A)+ RNA isolated from the B cell lines Raji, SB, Laz-509, and GK-5. However, RNA isolated from two pre-B
10 cell lines (Nalm-6, PB-697), three B cell lines (Namalwa, Daudi, BJAB), five T cell lines (CEM, Hut-78, HSB-2, Molt-15, Molt-3), a myelomonocytic cell line (U937 and U937 cultured with LPS) and erythroleukemic (K-562) cell line did not hybridize with LAM-1
15 suggesting that expression of this gene was preferentially associated with B lymphocytes.

The B125 cDNA clone contained an 1,181 bp open reading frame that could encode a protein of 372 amino acids as shown in Fig. 2. The numbers shown above the
20 amino acid sequence designate amino acid residue positions. The numbers to the right indicate nucleotide residue positions. Amino acids are designated by the single-letter code, and * indicates the termination codon. The boxed sequences identify possible N-linked
25 glycosylation sites. Hydrophobic regions that may identify signal and transmembrane peptides are underlined. The vertical arrow marks the most probable position of the amino-terminus of the mature protein. (See von Heijne, Nucleic Acids Res. 14:4683 (1986)).

30 The amino acid sequence of LAM-1 predicted a structure typical of a membrane glycoprotein. Two potential translation initiation sites were found at nucleotide positions 53 and 92. The second initiation site conformed best to the consensus sequence for

optimal initiation (A/G)CCAUG (Kozak, Cell 44: 283-292 (1986)) and was followed by a hydrophobic region of 27 amino acids that may represent a signal peptide. The algorithm of von Heijne predicted that the most probable amino-terminus of the mature protein would be the Trp at amino acid position 52. The LAM-1 sequence contained a second hydrophobic region between amino acids 346-368 which may be a transmembrane region. The deduced mature LAM-1 protein would have an extracellular region of about 294 amino acids containing 7 potential N-linked carbohydrate attachment sites. LAM-1 would have a cytoplasmic tail of 17 amino acids containing 8 basic and 1 acidic residues. The two cytoplasmic Ser residues may serve as substrates for phosphorylation since protein kinase C phosphorylates Ser residues that are on the carboxyl-terminal side of several basic residues. These results suggest that the processed LAM-1 protein would have a Mr of at least 50,000. The LAM-1 protein can be isolated by conventional techniques, such as affinity column chromatography with antibody or ligand, from cell lines that normally express this receptor or from transfected cell lines. Or the protein can be synthesized by in vitro translation of the LAM-1 cDNA.

LAM-1 combines previously unrelated domains found in three distinct families of molecules: animal lectins, growth factors, and C3/C4 binding proteins. The proposed extracellular region of LAM-1 contained a high number of Cys residues (7%) with a general structure as diagrammed in Fig. 1B. As indicated in Fig. 3, segments of homologous proteins are shown with the amino acid residue numbers at each end. Homologous amino acids are shown in boxes. Gaps (-) have been inserted in the sequences to maximize homologies. The first 157 amino acids of the protein (Fig. 3A) were

homologous with the low-affinity receptor for IgE (Kikutani et al., Cell 47:657 (1986)), the asialoglycoprotein receptor (Spiess et al., Proc. Natl. Acad. Sci. USA 82:6465 (1985)) and several other carbohydrate-binding proteins (Drickamer et al., J. Biol. Chem. 256:5827 (1981); Ezekowitz et al., J. Exp. Med. 167:1034 (1988); Krusius et al., J. Biol. Chem. 262:13120-13125 (1987); and Takahashi et al., J. Biol. Chem. 260:12228 (1985)). The amino acids conserved among all animal-lectin carbohydrate recognition domains are indicated (*). Although the sequence homologies were less than 30%, all the invariant residues found in animal lectin carbohydrate-recognition domains were conserved (Drickamer, J. Biol. Chem. 263:9557 (1988)).

The next domain of 36 amino acids (Fig. 3B) was homologous (36-39%) with epidermal growth factor (EGF) (Gregory, Nature 257:325 (1975)) and the EGF-like repeat units found in Factor IX (Yoshitake et al., Biochem. 25:3736 (1985)) and fibroblast proteoglycan core protein (Krusius et al., supra).

Immediately following these domains were two tandem domains of 62 amino acids each (Fig. 3C) that were homologous with the short consensus repeat units (SCR) that comprise the IL-2 receptor (Leonard et al., Nature 311:626 (1984)), Factor XIII (Ichinose et al., Biochem. 25:4633 (1986)) and many C3/C4 binding proteins (Klickstein et al., J. Exp. Med. 165:1095 (1987); and Morley et al., EMBO J. 3:153 (1984)). In contrast with all of the previously described SCR that contain four conserved Cys residues, these two SCR possessed six Cys residues. The four conserved Cys residues found in all SCR are indicated in Fig. 3C by (*); the additional conserved Cys found in LAM-1 are indicated by (+). Of the multiple SCR present in each of these proteins, the

SCR with the highest homology to LAM-1 is diagrammed. A 15 amino acid spacer preceded the putative transmembrane domain.

The deduced amino acid sequence of LAM-1 is homologous with that of ELAM-1 and GMP-140. Thus these two proteins and LAM-1 define a new family of homologous structures that are expressed by different cell lineages and that can function as receptors in cellular interactions.

Use

As lymphocyte migration and infiltration into areas of tissue damage or injury to tissue transplant can cause or increase pathology, agents produced in accordance with the present invention that impede these processes are useful for therapeutic treatment. LAM-1 is used as an antigen to produce antibodies against this protein and to develop antagonistic ligands that can interfere with lymphocyte adhesions and function. The use of these reagents in research will permit the determination of the 3-dimensional structure of LAM-1 and clarify its role in lymphocyte function. The administration of these reagents to patients can be used to block or reduce pathology. As an example, subpopulations of malignant cells that express this antigen would allow the receptor to function in metastasis of tumour cells. Agents developed to block receptor function can inhibit the metastasis and homing of malignant cells.



Claims

1. A human cDNA sequence encoding lymphocyte-associated cell surface protein LAM-1, said protein containing domains homologous with binding domains of animal lectins, growth factors, and C3/C4 binding proteins.
2. The cDNA sequence of claim 1 isolated from a human tonsil cDNA library.
3. The cDNA sequence of claim 1 isolated from a population of B cell-specific cDNAs.
4. The cDNA sequence of claim 1, having a nucleotide sequence substantially as indicated in Fig. 2.
5. Lymphocyte cell surface protein LAM-1 substantially as encoded by the cDNA sequence of any one of claims 1-4, or an immunogenic fragment of said protein.
6. An essentially purified human lymphocyte-associated cell surface protein LAM-1, containing domains homologous with binding domains of animal lectins, growth factors, and C3/C4 binding proteins; or an immunogenic fragment thereof.
7. The protein of claim 6, having an amino acid sequence substantially as indicated in Fig. 2.
8. The protein of claim 6, having 80% or greater homology with the amino acid sequence indicated in Fig. 2.

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9. The protein of claim 6 having 90% or greater homology with the amino acid sequence indicated in Fig. 2.

10. Antibody developed against the protein of any one of claims 5-9 or against a fragment thereof.

11. Antibody developed against a molecule that specifically associates with the protein of any one of claims 5-9, or with a fragment thereof, to generate a functional molecule.

12. A method of identifying cells expressing LAM-1 comprising reacting the antibody of claim 10 with a population of cells and isolating cells to which said antibody binds.

13. A method of blocking the receptor activity of LAM-1 comprising reacting antibody of claim 10 with a population of cells expressing said LAM-1.

14. The use of the protein of any one of Claims 5-9 to identify a ligand that binds to said protein.

15. The use of the protein of any one of Claims 5-9 to identify a ligand that binds to a molecule specifically associated with said protein, or a fragment thereof, to generate a functional molecule.

16. A pharmaceutical preparation for use in the treatment of a patient suffering from a lymphocyte-mobilizing condition, comprising a therapeutic amount of an antagonist to a protein according to any of Claims 5 to 9 in a non-toxic pharmaceutical carrier.

17. A preparation according to Claim 16, wherein said antagonist comprises a ligand, or a portion thereof, identified using the procedure of Claims 14 or 15.

18. An antagonist to protein according to any of Claims 5 to 9, for use in the treatment of a patient suffering from a lymphocyte-mobilizing condition such as tissue damage, an auto immune disorder, cancer, or organ or tissue transplantation.

19. The use of the cDNA sequence of any one of Claims 1-4 to isolate human DNAs that cross-hybridize with said cDNA sequence.

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