A new family of cell adhesion molecules has recently been identified by isolating cDNAs that encode cell surface proteins that uniquely contain domains homologous to those found in animal lectins, epidermal growth factor, and C3/C4 binding proteins (1-4). Members of this family described thus far include the murine lymph node homing receptor (mLHR)\(^1\), expressed by mouse lymphocytes (1), the human endothelial leukocyte adhesion molecule 1 (ELAM-1), expressed by cytokine-stimulated endothelial cells (2), and human GMP-140, expressed by activated platelets (3). In this report, the cloning of a cDNA that encodes a new human lymphocyte-associated cell surface molecule (LAM-1) is described that represents a new member of this family of adhesion proteins. The chromosome localization of the LAM-1 gene suggests that this family of proteins may be encoded by a clustered locus of “adhesion protein” genes.

Materials and Methods

Molecular Cloning. The isolation of human tonsil cDNA clones by differential hybridization has been described (5). Nucleotide sequences were determined using the method of Maxam and Gilbert (6). Gap penalties of -1 were assessed during homology analysis for each nucleotide or amino acid in the sequence where a gap or deletion occurred.

RNA Blot Analysis. For Northern blot analysis, ~2 μg of poly(A)\(^+\) RNA or 15 μg of total cellular RNA was denatured, fractionated by electrophoresis through a 1.1% agarose gel, and transferred to nitrocellulose or nylon membranes as described (5, 7). The pLAM-1 cDNA insert was isolated, nick translated, and hybridized with the filters as described (5, 7).

In Situ Hybridization. The LAM-1 cDNA clone was labeled by nick translation using \(^3\)H nucleotides to a specific activity of 5 × 10\(^5\) cpm/μg. In situ hybridization to metaphase chromosomes from lymphocytes of a normal male individual was carried out using the LAM-1

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1 Abbreviations used in this paper: EGF, epidermal growth factor; ELAM, endothelial leukocyte adhesion molecule; LAM, leukocyte adhesion molecule; LHR, lymph node homing receptor; SCR, short consensus repeat.
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probe at a concentration of 0.02 μg/μl of hybridization mixture as described (8). The slides
were exposed for 7 d.

Results

Isolation of the LAM-1 cDNA Clone. B cell-specific cDNAs were isolated from a
human tonsil cDNA library using differential hybridization with labeled cDNAs de-
derived from either B cell (RAJI) RNA or T cell (HSB-2) RNA (5). One of the 261
RAJI+ HSB2- cDNA clones isolated, B125, contained a 1.9-kb cDNA insert that
hybridized with a 2.4-kb species found in several B cell lines (5). However, B125 did
not hybridize with any of the other RAJI+ HSB2- clones or with mRNA from sev-
eral 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 hy-
bridized with B125 was isolated, sequenced, and termed pLAM-1 (Fig. 1A). This
clone contained a 1,181-bp open reading frame that could encode a protein of 372
amino acids (Fig. 1C).

The amino acid sequence of LAM-1 predicted a structure typical of a membrane
glycoprotein. Two potential translation initiation sites were found at nucleotide po-
tions 53 and 92. The second initiation site confirmed best to the consensus se-
quence for optimal initiation (A/G)CCAUG (9) and was followed by a hydrophobic
region of 27 amino acids that may represent a signal peptide. The algorithm of von
Heijne (10) predicted that the most probable NH2 terminus of the mature protein
would be the Trp at amino acid position 52 (Fig. 1C). The LAM-1 sequence con-
tained a second hydrophobic region between amino acids 346 and 368 that may
be a transmembrane region. The predicted mature LAM-1 protein would have an
extracellular region of ~294 amino acids containing seven potential N-linked car-
bohydrate attachment sites. LAM-1 would have a cytoplasmic tail of 17 amino acids
containing eight basic and one acidic residues. The two cytoplasmic Ser residues
may serve as substrates for phosphorylation since protein kinase C phosphorylates
Ser residues that are on the COOH-terminal side of several basic residues. These
results suggest that the processed LAM-1 protein would have an Mr of at least
50,000.

LAM-1 Contains Multiple Distinct Domains. The proposed extracellular region of
LAM-1 contained a high number of Cys residues (7%) with a general structure,
as depicted in Fig. 1B. The first 157 amino acids of the protein were homologous
with the low affinity receptor for IgE (11), the asialoglycoprotein receptor (12), and
several other carbohydrate-binding proteins (13–16) (Fig. 2A). Although the sequence
homologies were <30%, all the invariant residues found in animal lectin carbohydrate-
recognition domains were conserved (17). The next domain of 36 amino acids was
homologous (36–39%) with epidermal growth factor (EGF) (18) and the EGF-like
repeat units found in factor IX (19) and fibroblast proteoglycan core protein (15)
(Fig. 2B). Immediately after these domains were two tandem domains of 62 amino
acids each that were homologous with the short consensus repeat units (SCR) that
comprise the IL-2-R (20), factor XIII (21), and many C3/C4 binding proteins (22,
23) (Fig. 2C). In contrast to all of the previously described SCR that contain four
conserved Cys residues, these two SCR possessed six Cys residues. A 15-amino acid
spacer preceded the putative transmembrane domain.

Homology of LAM-1 with mLHR, ELAM-1, and GMP140. LAM-1 shares a 77%
FIGURE 1. Structure of the pLAM-1 cDNA clone. (A) The 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. (B) 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 EGF-like domains and the two SCR units. The open box indicates the putative transmembrane (TM) region. (C) The determined nucleotide sequence and predicted amino acid sequence of the LAM-1 cDNA clone. The numbers shown above the 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 an asterisk indicates the termination codon. The boxed sequences identify possible N-linked glycosylation sites. Hydrophobic regions that may identify signal and transmembrane peptides are underlined. The vertical arrow marks the most probable position of the NH2 terminus of the mature protein. These sequence data have been submitted to the EMBL/GenBank Data Library.
LAM-1 shares 67 and 61% sequence homology with GMP-140 and ELAM-1, respectively.

The leader sequence of mLHR shares a 63% amino acid sequence homology with the mouse LHR (Fig. 3). Significant homologies of nucleotide sequences outside of the putative coding regions were not found. The leader sequence of mLHR shares a 63% amino acid sequence homology with LAM-1, while the lectin domains share an 83% homology. The lectin domain of LAM-1 shares a 67 and 61% sequence homology with GMP-140 and ELAM-1, respec-

**Figure 2.** Homologies of LAM-1 domains with other proteins. Segments of homologous proteins are shown with the amino acid residue numbers at each end. Homologous amino acids are shown with the amino acid residues conserved among all animal lectin carbohydrate recognition domains are indicated (*). (A) Lectin-like domain of LAM-1 compared with: FcE-R, the Fc receptor for IgE (11); C-HEL, chicken hepatic lectin (13); H-MBP, human mannose-binding protein (14); F-PGC, fibroblast proteoglycan core protein (15); HHL-1, human hepatic lectin-1 (12); ISL, insect soluble lectin (16). The amino acids conserved among all animal lectin carbohydrate recognition domains are indicated (*). (B) EGF-like domain of LAM-1 compared with: human EGF (18); F-IX, blood clotting factor IX (19); F-PGC, fibroblast proteoglycan core protein (15). (C) Short consensus repeats 1 and 2 of LAM-1 compared with: Ba, proteolytic fragment of factor B (23); CR1, (22), IL-2-R, (20); and F-XIII, blood clotting factor XIII (21). The four conserved Cys residues found in all SCR are indicated by (*), the additional conserved Cys found in LAM-1 are indicated by (○).
Figure 3. Homology of LAM-1 with the mouse lymphocyte homing receptor (1). Identical amino acids are represented (–). Numbers to the right indicate the amino acid residue positions shown in Fig. 1 C. The arrow indicates the predicted NH2 terminus of LAM-1 and the determined NH2 terminus of mLHR. The underlined region indicates the putative transmembrane region.

Expression of LAM-1 mRNA. Northern 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 (Fig. 4 A), Laz-509, and GK-5 (data not shown). The 1.7-kb RNA species may result from alternate use of the potential poly(A) signal sequence, ATATAAA, at position 1493 (Fig. 1 C), which serves as
Figure 5. Regional localization of the \textit{LAM-1} gene to human chromosome 1 band q23-q25. (a) Distribution of 181 in situ sites of hybridization on human chromosomes. (b) Distribution of autoradiographic grains on a diagram of chromosome 1.
a poly(A) attachment signal in the mouse LHR cDNA (1). RNA isolated from two pre-B 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 an erythroleukemic cell line (K-562) did not hybridize with LAM-1, suggesting that expression of this gene was preferentially associated with B lymphoblastoid cell lines (data not shown). However, analysis of mRNA isolated from blood lymphocytes and purified blood T cells revealed that LAM-1 mRNA was readily detected in T cells and increased after mitogen stimulation (Fig. 4B). Both the 2.6- and 1.7-kb RNA species that hybridized with pLAM-1 were expressed by T cells. Low levels of LAM-1 mRNA were detected in some monocyte preparations, but not in others, suggesting that LAM-1 may also be expressed by monocytes at amounts far below those observed in T lymphocytes. Alternatively, low numbers of lymphocytes (<2%) contaminating the monocyte preparations may account for this variability. Treatment of monocytes with Bryostatin 1 or LPS did not induce LAM-1 mRNA, and LAM-1 mRNA was not detected in malignant cells from two patients with chronic myelogenous leukemia (data not shown). In addition, a human fibroblast cell line (Wi-38 and Wi-38 stimulated with LPS) and primary cultures of human fibroblasts transfected with the EJ-ras oncogene did not express detectable LAM-1 mRNA. Human epithelial keratinocytes, untreated or cultured with LPS or TNF, also failed to express detectable LAM-1 mRNA.

Chromosome Location of the LAM-1 Gene. The LAM-1 gene was localized on human metaphase chromosomes by in situ hybridization using the LAM-1 cDNA clone as a labeled probe. A total of 181 sites of hybridization in 48 metaphase cells were scored (Fig. 5a). Of the 181 sites of hybridization, 48 (27%) were located between bands q22 and q25 of the long arm of chromosome 1. There was no significant hybridization to other chromosomes (Fig. 5a). The largest number of grains on chromosome 1 were located at bands q23 and q24, with significant hybridization to band q25 (Fig. 5b).

Discussion

The generation of genes by the assembly of functionally independent domains has occurred frequently as new genes evolved to encode proteins with new functions. However, LAM-1 combines previously unrelated domains found in three distinct families of molecules: animal lectins, growth factors, and C3/C4 binding proteins. The LAM-1 lectin-like domain has homology with mammalian lectins specific for glycans with terminal galactosyl, N-acetylgalactosaminyl, and mannosyl residues (17), and homology with the lectin-like domain of the low affinity Fc receptor for IgE (11). Most animal carbohydrate-binding receptors, however, differ from LAM-1 since their COOH terminus contains the lectin domain and is on the outside of the membrane, while their NH2 terminus is inside the cell (17). An exception, like LAM-1, is the galactose-binding protein from fly hemolymph that has the lectin domain at the NH2-terminal end of the protein (16). Although the carbohydrate-binding domain in fly hemolymph lectin diverged a considerable time ago from human LAM-1, it is homologous (23%) with the LAM-1 lectin-like domain (Fig. 2A). Interestingly, it has been proposed that the lectin isolated from fly hemolymph may be involved in a primitive immune response (24). The homology of another LAM-1 domain with proteins that contain EGF-like sequences, such as blood clotting factors,
suggests that this domain could also interact with other proteins (19). EGF-like repeat units are also found in juxtaposition with the lectin domain of fibroblast proteoglycan core protein, suggesting that this protein and LAM-1 may be evolutionarily related (15). The SCR domains may also have receptor function, since the extracellular domains of receptors for many complement components and IL-2 are composed of SCR domains (20–23).

LAM-1 is a new member of a family of recently described cellular adhesion proteins (1–3). This family includes the mouse LHR that has a 77% amino acid sequence homology with LAM-1 (Fig. 3). Northern analysis of LAM-1 expression demonstrated a predominantly lymphoid distribution in humans (Fig. 4) that is also similar to the cell surface expression of the mLHR (1). Since pretreatment of mouse lymphocytes with specific polysaccharides inhibits their adherence to high endothelial venules in lymph nodes, the lectin-like domain of LAM-1 may also function in the adherence of lymphocytes to endothelial cells (25). The lectin-like and EGF-like domains of LAM-1 and mLHR shared considerable homology (83–80%), but the area of highest homology (95%) was found in the putative transmembrane region, suggesting that these domains may be most critical to the function of LAM-1 and mLHR. Although LAM-1 and mLHR are structurally homologous, it is not known whether LAM-1 functions as the LHR in man. However, preliminary studies demonstrate that the cell lines that express high levels of LAM-1 mRNA (Fig. 4) bind to human high endothelial venules, while most of those that do not express LAM-1 mRNA do not bind (A. Freedman and T. F. Tedder, unpublished observations). Therefore, it is possible that LAM-1 serves a function in man similar to that of the LHR in mouse.

Other members of this new adhesion molecule family that are homologous with LAM-1 include the inducible ELAM-1, which is expressed on the surface of cytokine-treated endothelial cells (2). This molecule is thought to be responsible for the accumulation of blood leukocytes at sites of inflammation by mediating the adhesion of cells to the vascular lining. LAM-1 and ELAM-1 exhibit homologous structural features, including the presence of lectin- and EGF-like domains followed by SCR domains that contain six conserved Cys residues. In addition, a granule membrane protein found in platelets and endothelial cells, termed GMP-140, is also homologous with LAM-1 in that it contains lectin, EGF, and SCR domains (3). These proteins and LAM-1 thus appear to define a new family of homologous structures that are each expressed by different human cell lineages.

The LAM-1 gene is located on human chromosome 1 at band q23-25 (Fig. 5). The gene for GMP-140 has also been mapped to chromosome 1 q21-24 (3, McEver, R., G. Johnston, and M. Le Beau, personal communication). These findings suggest that a cluster of “adhesion molecule” loci may exist on the long arm of chromosome 1 in the region q21-25, which contains at least two of the three members of this human gene family. This locus is distinct from the “complement receptor” locus at band 1q32, which encodes the SCR containing proteins, CR1, CR2, C4-binding protein, and factor H (26). The finding that two of these new genes map to the same band of human chromosome 1, in addition to their common structural motif, further demonstrates that these proteins are members of a closely related family of proteins.
Summary

A cDNA encoding a new human lymphocyte cell surface molecule has been isolated and shown to identify a fourth member of a recently discovered family of adhesion proteins. This lymphocyte-associated molecule (LAM-1) is uniquely composed of multiple distinct domains, one domain homologous with animal lectins, one homologous with epidermal growth factor, and two short consensus repeat units similar to those found in C3/C4 binding proteins. This cDNA clone hybridized with RNAs found in B cell lines and T lymphocytes, but not with RNA from other cell types. The amino acid sequence of LAM-1 is 77% homologous with the sequence of the mouse lymphocyte homing receptor, suggesting that LAM-1 may function in human lymphocyte adhesion. The LAM-1 gene is located on chromosome 1q23-25, as is another member of this adhesion family, suggesting that this new family of proteins may be encoded by a cluster of "adhesion protein" loci.

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Note added in proof: The cloning of GMP-140 by Johnston et al. has been published (27), and the cloning of the mouse LHR by Lasky et al. has been published (28).

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