Endothelial Leukocyte Adhesion Molecule 1: An Inducible Receptor for Neutrophils Related to Complement Regulatory Proteins and Lectins

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Focal adhesion of leukocytes to the blood vessel lining is a key step in inflammation and certain vascular disease processes. Endothelial leukocyte adhesion molecule-1 (ELAM-1), a cell surface glycoprotein expressed by cytokine-activated endothelium, mediates the adhesion of blood neutrophils. A full-length complementary DNA (cDNA) for ELAM-1 has now been isolated by transient expression in COS cells. Cells transfected with the ELAM-1 clone express a surface structure recognized by two ELAM-1 specific monoclonal antibodies (H4/18 and H18/7) and support the adhesion of isolated human neutrophils and the promyelocytic cell line HL-60. Expression of ELAM-1 transcripts in cultured human endothelial cells is induced by cytokines, reaching a maximum at 2 to 4 hours and decaying by 24 hours; cell surface expression of ELAM-1 protein parallels that of the mRNA. The primary sequence of ELAM-1 predicts an amino-terminal lectin-like domain, an EGF domain, and six tandem repetitive motifs (about 60 amino acids each) related to those found in complement regulatory proteins. A similar domain structure is also found in the MEL-14 lymphocyte cell surface homing receptor, and in granule-membrane protein 140, a membrane glycoprotein of platelet and endothelial secretory granules that can be rapidly mobilized (<5 minutes) to the cell surface by thrombin and other stimuli. Thus, ELAM-1 may be a member of a nascent gene family of cell surface molecules involved in the regulation of inflammatory and immunological events at the interface of vessel wall and blood.

THE INFLAMMATORY OR IMMUNE CYTOKINES INTERLEUKIN-1 (IL-1) and tumor necrosis factor (TNF), and bacterial endotoxin, act directly on cultured human vascular endothelium to increase the adhesion of blood leukocytes and related cell lines (*1–10*). Local activation of vascular endothelium also increases leukocyte adhesion and emigration in microvessels in vivo (*11–13*). Two monoclonal antibodies (H4/18 and H18/7) generated against IL-1– or TNF-stimulated human endothelial cells (HEC) (*14, 15*) were shown to react with the same inducible cell surface protein (or proteins) present on cytokine- or endotoxin-stimulated HEC, but not on unstimulated HEC (*15*). One of these, H18/7, significantly blocked the adhesion of both human neutrophils and HL-60 cells to stimulated HEC, thus supporting the designation ELAM-1 (*15*).

Molecular cloning of ELAM-1. A complementary DNA (cDNA) library was constructed (*16, 17*) with RNA obtained from cultured HEC that had been treated for 2.5 hours with recombinant IL-1-beta (rIL-1; 5 U/ml; Biogen). The library was transfected into COS cells, and cells expressing ELAM-1 epitopes were isolated with the use of H18/7 and H4/18. Episomal DNA was recovered, expanded in bacteria (MC1061/p3), and subjected to three more rounds of expression and selection (*16, 17*) to obtain a cDNA clone designated pELAM-1. COS cells transfected with pELAM-1 reacted with both monoclonal antibodies H18/7 and H4/18, but not with control monoclonal antibodies, as demonstrated by indirect immunofluorescence and immunoperoxidase analyses. Two polypeptides of 107 kD and 97 kD were immunoprecipitated from biosynthetically labeled, pELAM-1–transfected COS cells with the use of H18/7 (Fig. 1). The lower molecular mass of the COS cell product versus the endothelial cell product (107 kD compared to 115 kD; higher molecular species, Fig. 1) is consistent with previous observations on transiently expressed cDNA products in COS cells and may reflect altered glycosylation. Treatment of both COS cell-derived and HEC-derived immunoprecipitates with *N*-glycosidase F to remove N-linked carbohydrates, yielded single bands corresponding to 78-kD polypeptides (Fig. 1).

Biosynthesis of ELAM-1. The synthesis of natural and recombinant ELAM-1 protein was examined by biosynthetic labeling experiments with rIL-1–stimulated HEC and pELAM-1 transfected COS cells, respectively (Fig. 2). In both cases, label was first detected in the smaller peptides (97 kD), and subsequently, in the 115-kD or 107-kD forms. In the endothelial cultures, the 115-kD protein was largely lost 3 hours after the labeling was terminated, suggesting a very rapid rate of protein degradation. Taken together, data from *N*-glycosidase and timed labeling studies (Figs. 1 and 2)

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suggest that the two peptides (higher and lower molecular size species) immunoprecipitated with the monoclonal antibodies to ELAM-1(anti–ELAM-1) differ in posttranslational modifications, particularly with respect to carbohydrate processing.

The HEC treated for 2 hours with rIL-1 or rTNF contain a single 3.9-kb transcript, which hybridizes with a single-strand pELAM-1 probe and which is absent from unstimulated and immune interferon-stimulated HEC (Fig. 3A). Continuous exposure of HEC monolayers to rIL-1 resulted in a rapid increase in the expression of an ELAM-1 transcript within 1 hour, reaching a maximum at 2 to 4 hours, and declining close to basal levels by 24 hours (Fig. 3B, upper). Endothelial cell surface expression of ELAM-1 protein could be correlated temporally with the expression of ELAM-1 mRNA (Fig. 3B, lower). Rapid induction and high turnover rates of ELAM-1 mRNA and protein contribute to the transient expression of this molecule in activated vascular endothelium. Blot hybridization of genomic DNA from placenta revealed a pattern consistent with a single-copy gene, suggesting that the observed RNA transcript is derived from a single chromosomal locus.

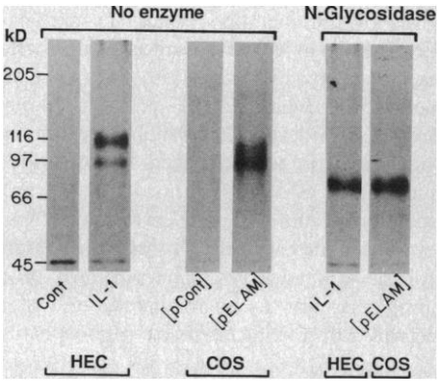

Fig. 1. Immunoprecipitation of ELAM-1 from IL-1–stimulated HEC and cDNA transfected COS cells. HEC and COS cell monolayers were biosynthetically labeled with [^{35}S]cysteine and [^{35}S]methionine (250 μCi per 100-mm dish) for 5 hours and 2 hours, respectively, as described (*15*). Antibody to ELAM-1 (H18/7) and antibody to mouse immunoglobulin coupled to Sepharose-4B (Cooper Biomedicals) were used to immunoprecipitate reactive species from total cell extracts (2 percent Triton X-100) of unstimulated (cont) and IL-1–stimulated (rIL-1 at 5 U/ml, 5 hours) HEC monolayers and COS cells that had been transfected with a plasmid containing an irrelevant cDNA insert [pCont] or the ELAM-1 cDNA [pELAM-1]. After washing (*15*), the beads were boiled for 5 minutes in 0.5 percent SDS with 2-mercaptoethanol (0.1*M*). Samples of the IL-1-HEC and [pELAM]-COS cell immunoprecipitates were diluted to a final concentration of 0.2*M* sodium phosphate (*p*H 8.6) and 1.25 percent NP-40, and treated for 18 hours (37°C) with *N*-glycosidase F (N-Glycanase, Genzyme) at 10 U/ml to remove N-linked carbohydrates. Samples were diluted with 2 times concentrated sample buffer, boiled, and analyzed by 4 to 11 percent SDS–polyacrylamide gel electrophoresis (PAGE), and autoradiographed (*15*).

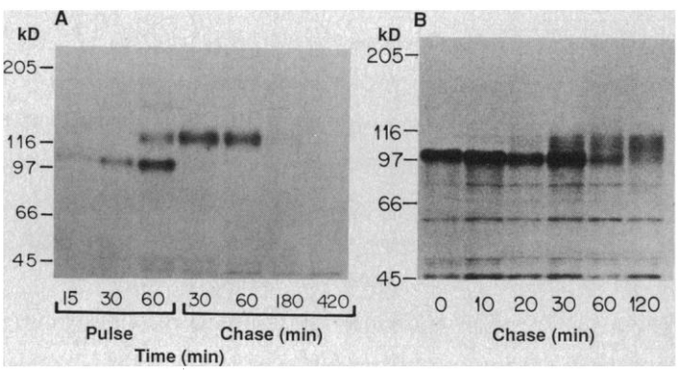

Fig. 2. Kinetics of labeling of endothelial and COS cell ELAM-1. (**A**) HEC monolayers in 35-mm culture dishes were incubated for 30 minutes in RPMI without cysteine or methionine, supplemented with 10 percent dialyzed fetal calf serum (FCS) and with rIL-1 at 5 U/ml. Cultures were then incubated (pulse) for 15, 30, or 60 minutes with [^{35}S]cysteine and [^{35}S]methionine. After the 60-minute period, certain cultures were further incubated (chase) with complete RPMI medium supplemented with fresh IL-1 and undialyzed FCS for the additional time noted. Immunoprecipitation with monoclonal antibody H18/7, SDS-PAGE (4 to 11 percent), and autoradiography were then performed (*15*). (**B**) COS cells that had been transfected 72 hours earlier by means of ELAM-1 cDNA with DEAE-Dextran (*16, 17*), were incubated in RPMI without cysteine or methionine for 30 minutes, in the same medium supplemented with [^{35}S]cysteine and [^{35}S]methionine for 15 minutes, and finally in complete RPMI and undialyzed FCS for the indicated time periods (chase), prior to immunoprecipitation and gel electrophoresis.

Function. To assess the functional capacity of cloned ELAM-1, we examined the adhesion of isolated human peripheral blood neutrophils and HL-60 cells to COS cells transfected with the ELAM-1 cDNA. The pELAM-1–transfected COS cell monolayers supported focal adhesion (rosette formation) of both neutrophils and HL-60 cells, while monolayers similarly transfected with a cDNA encoding a constitutive endothelial or mesenchymal cell surface molecule—about 96 kD, recognized by Mab H4/45 and E1/1.2 (*14, 15*)—did not (Fig. 4). Immunoperoxidase staining of the COS cell monolayers after the adhesion assay revealed that the leukocytes had formed rosettes only on those COS cells expressing ELAM-1 protein. In addition, prior treatment of pELAM-1 transfected COS cells with saturating concentrations of monoclonal antibody H18/7 blocked the adhesion of HL-60 cells (92 to 100 percent inhibition; three experiments), whereas prior treatment with a control monoclonal antibody had no effect.

Primary structure and homologies. The ELAM-1 cDNA was found to be 3.85 kb, consisting of a 116-base 5′ untranslated region, a continuous open reading frame of 1830 bases, and a relatively long 3′ untranslated region (1898 bases) ending in a poly(A) tail (Fig. 5A). Within the 3′ untranslated region, there are multiple AT-rich regions, including eight repeats of the consensus sequence ATTTA, first described in cDNA's encoding various transiently expressed molecules, including cytokines associated with inflammation and

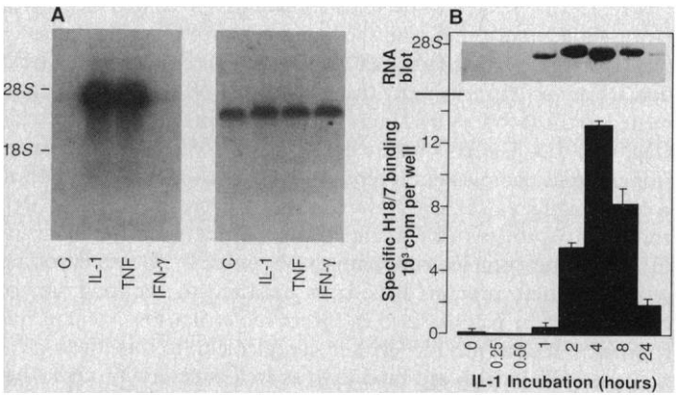

Fig. 3. Inducible expression of ELAM-1 mRNA and protein. (**A**) RNA blot hybridization analysis of ELAM-1 transcripts from HEC that had been cultured for 2 hours in medium (M199–20 percent FCS) alone (C) or medium supplemented with rIL-1 at 5 U/ml, TNF at 200 U/ml, or interferon-γ at 200 U/ml (provided by Biogen). Total RNA (20 μg) was denatured, subjected to electrophoresis through agarose, transferred to nylon membranes (GeneScreen Plus, New England Nuclear), hybridized with the ELAM-1 cDNA, and autoradiographed for 1 hour (left panel) as described (*16, 17*). The same blot was subsequently stripped and again hybridized, but with a cDNA encoding a constitutively expressed endothelial or mesenchymal cell surface antigen—~96 kD, recognized by monoclonal antibody H4/45 and E1/1.2, (*14, 15*). (**B**) The kinetics of expression of ELAM-1 mRNA and cell surface antigen. RNA blot hybridization analysis of ELAM-1 transcripts was performed with total RNA (20 μg) isolated from HEC that had been stimulated with rIL-1 (5 U/ml) for the designated times. In a separate study, cell surface expression of ELAM-1 antigen on intact HEC monolayers was determined for all periods of IL-1 incubation except 0.25 and 0.5 hour with an indirect radioimmunobinding assay (*15*). The data represent the mean ± SEM determined from quadruple experimental wells. Similar results have been obtained in five additional experiments.

immune processes (*18, 19*). The corresponding mRNA sequence AUUUA is thought to confer instability, resulting in rapid degradation, and may explain the decline of the number of ELAM-1 transcripts nearly to preinduction numbers by 24 hours after cytokine treatment.

The predicted start codon is embedded in an initiation consensus sequence (*20*), and is preceded by an in-frame stop codon. The translated amino acid sequence predicts a polypeptide of 610 amino acids with features of a type 1 transmembrane protein (Fig. 5, B and C). The initiation methionine is followed by a slightly atypical signal peptide sequence of predominantly hydrophobic amino acids. Comparison with existing signal peptides (*21*) suggests that cleavage precedes the tryptophan (W) residue indicated as position 1 (#) in Fig. 5A; the resulting mature protein would consist of 589 amino acids with a predicted size of 64 kD. The extracellular domain contains 11 potential sites of N-linked glycosylation, sufficient to account for the 37 kD lost after *N*-glycosidase treatment (Fig. 1). Because the product of *N*-glycosidase treatment is 14 kD greater than predicted from the sequence, O-linked sugars or other post-translational modifications, such as ubiquitination, may also be present. The putative transmembrane region (residues 536 to 557) is followed by a cluster of basic amino acids, consistent with a stop transfer signal. The cytoplasmic portion (32 amino acids) contains a tyrosine (Y) residue that has been suggested to be recognized by adaptor proteins mediating internalization into coated pits (*22*). Internalization by this route may account for the short half-life of ELAM-1 at the cell surface.

The extracellular portion of ELAM-1 can be divided into three segments based on homologies to previously described proteins. The NH_2-terminal region (~120 amino acids) is related to several members of the lectin-like protein family, which includes the low-affinity IgE receptor [CD 23 (*23, 24*)] (Fig. 6A), the asialoglycoprotein receptor (*25, 26*), and mannose binding protein (*27*). Residues 121 to 155 are related to proteins containing the epidermal growth factor (EGF) motif (*28*), and show greatest similarity to a sequence present in coagulation factor IX (*29*) (Fig. 6B). Following the EGF domain are six tandem repetitive motifs of ~60 amino acids each, related to those found in a family of complement regulatory proteins (Fig. 6C) (*30–32*). This family includes both cell surface structures [for example, complement receptors 1 and 2 (CR1 and CR2), decay accelerating factor (DAF), and membrane cofactor protein (MCP)], and plasma proteins (for example, C4 binding protein and factor H), that bind complement factors C3b and C4b. Genes encoding several of these proteins have been localized to the long arm of chromosome 1, band q32 (*33*). Other molecules that contain this consensus repeat include certain complement factors themselves (C1r and C1s, which can bind C3), as well as several proteins that have not been shown to interact with complement components including the IL-2 receptor (α chain) (*30–32*).

Two major structural characteristics distinguish ELAM-1 from its previously described relatives: first, the combination of multiple repetitive motifs of the complement regulatory type with lectin and EGF domains, and second, the presence of six cysteines (instead of four) within each of the complement regulatory repeats. Recent evidence suggests that these features of ELAM-1 are shared by two other cell surface proteins, the MEL-14 antigen, a lymphocyte homing receptor (*34*), and granule-membrane protein 140 (GMP-140), a 140-kD glycoprotein that is found in the membranes of secretory granules in platelets and endothelial cells, and that can be rapidly mobilized (<5 minutes) to the cell surface by thrombin and other fast-acting mediators (*35*). An isolated cDNA encoding the murine MEL-14 antigen (*36*) predicts a peptide highly related to ELAM-1, and bearing an NH_2-terminal lectin domain, an EGF domain, and two complement regulatory motifs of ~60 amino acids each; each of these motifs contains six cysteines. The greatest similarity between ELAM-1 and the murine MEL-14 homing receptor is found in the lectin and EGF domains, which share 61 percent identity (Fig. 6D). The recently completed sequence of GMP-140 (*37*) similarly predicts a structure that contains an NH_2-terminal lectin domain, an EGF domain, and nine repetitive motifs like those in ELAM-1. Notably, ELAM-1 does not appear to be related to molecules of the immunoglobulin supergene family (*38, 39*), or the integrin supergene family (*40*); nor does it contain an RGD (Arg-Gly-Asp) sequence, the common recognition site of integrin receptors (*41*).

The identification of three cell surface molecules of similar, complex domain structure appears to describe a new gene family. Two of the members, ELAM-1 and MEL-14 antigen, have a defined role in leukocyte-endothelial adhesion. ELAM-1, a molecule expressed on activated endothelium, mediates the adhesion of blood neutrophils, while MEL-14 antigen, a molecule expressed by granulocytes, monocytes, and lymphocyte subsets (*42*), mediates lymphocyte adhesion to specialized lymph node endothelium (*34*). The function of GMP-140 is not known at present, but its presence in secretory granules of platelets and endothelium and its rapid redistribution to the cell surface by activating stimuli are consistent with a role in cell adhesion. The adhesive properties of the MEL-14 antigen appear to be mediated, at least in part, by a lectin-like activity recognizing mannose 6-phosphate and 6-phosphomannose

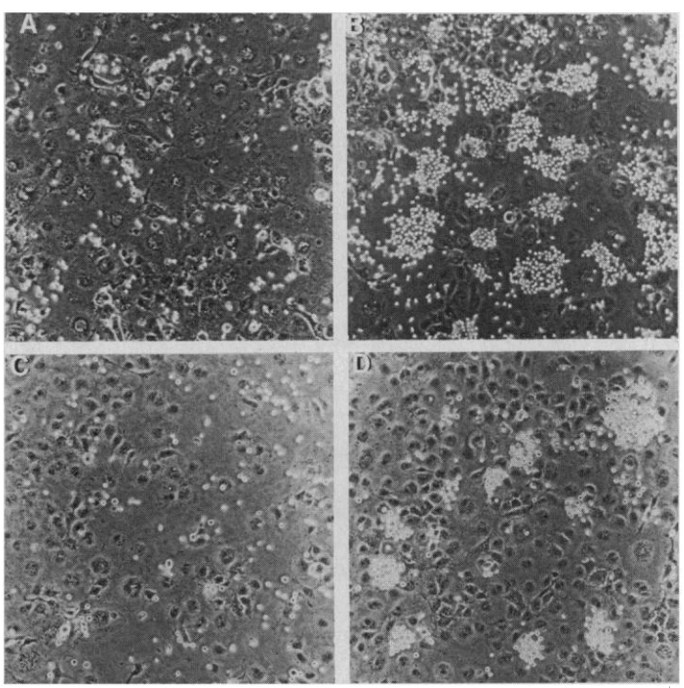

Fig. 4. Adhesion of isolated human neutrophils (**A**, **B**) and HL-60 cells (**C**, **D**) to cDNA-transfected COS cells. COS cells were transfected with DEAE-Dextran and a control cDNA (A, C) or the ELAM-1 cDNA (B, D), trypsinized and transferred to the experimental wells 48 hours before the assay. Human blood neutrophils were isolated (*15*), resuspended to 5.0×10^6 per milliliter in Hanks' balanced salt solution with calcium and magnesium (M.A. Bioproducts) and 1 percent bovine serum albumen (Sigma). HL-60 cells were maintained in RPMI-1640 with 10 percent FCS (*15*), and resuspended to 1.0×10^6 in RPMI–1 percent FCS for the assay. Leukocytes were coincubated with the COS cell monolayers for 15 to 30 minutes at 25°C to allow adhesion. The control cDNA encodes a 96-kD glycoprotein that is constitutively expressed on vascular endothelium as well as several other cell types. This protein has been referred to as mesenchymal cell antigen (*14*) and p96 (*15*). COS cell expression of ELAM-1 or p96 antigens was confirmed by immunofluorescence analysis, with monoclonal antibodies H18/7 or E1/1.2, respectively.

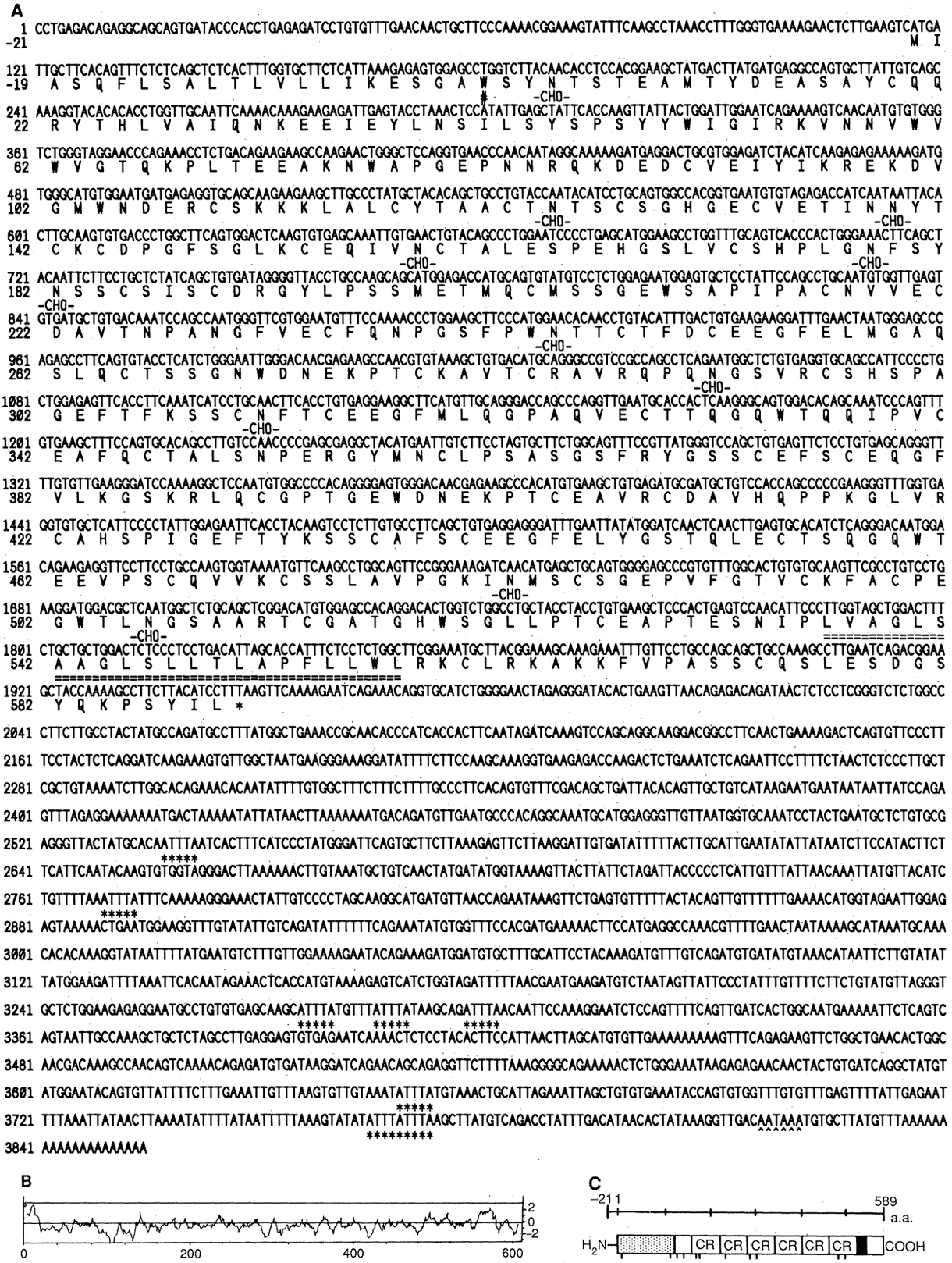

Fig. 5. (**A**) Nucleotide sequence of the ELAM-1 cDNA and predicted amino acid sequence of the ELAM-1 protein. Nucleotide and amino acid numbering are given at the left. Within the 3′ untranslated region of the cDNA, the mRNA decay consensus sequence motifs ATTTA (*****) (*18, 19*) and the polyadenylation signal (^^^^^^) are underscored. The predicted NH_2-terminal amino acid (W) is indicated by a number sign (#), sites of potential N-linked glycosylation are denoted by the symbol -CHO-, and the predicted transmembrane (TM) sequence is underscored (====). (**B**) Hydropathicity profile of the predicted amino acid sequence of ELAM-1. (**C**) Schematic diagram of the ELAM-1 protein depicting the signal peptide (horizontal line), NH_2-terminal lectin-like domain (stippled), the EGF-like domain (open), six ~60 amino acid consensus repeats (CR), transmembrane domain (solid), and the COOH-terminal cytoplasmic tail. Single letter abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

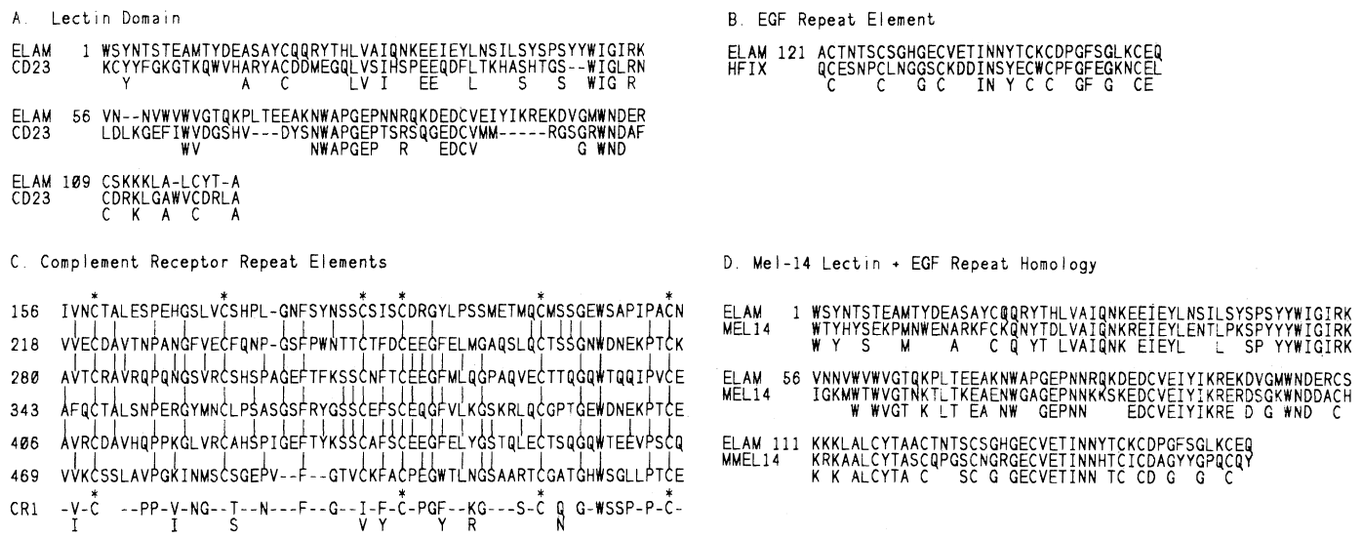

Fig. 6. (**A**) Optimal alignment of the NH_2-terminal 120 amino acids of ELAM-1 with the lectin-related portion (amino acids 173 to 286) of the low affinity IgE receptor (CD23) by the ALIGN program of the Protein Identification Resource. Conserved residues are displayed beneath the aligned sequences. The lectin-like domain of ELAM-1 demonstrated a higher relatedness to this CD23 domain than to any other member of this class of lectin-like molecules (*23–27*). Unlike ELAM-1, CD23 and several related lectins are type 2 transmembrane proteins, with a COOH-terminal extracellular domain and an NH_2-terminal cytoplasmic tail (*23–27*). (**B**) Optimal alignment of ELAM-1 amino acids 121 to 155 with the EGF-repeat element of coagulation factor IX (amino acids 96 to 130). (**C**) Optimal alignment of individual ~60 amino acid repetitive motifs within ELAM-1 (top six lines) and comparison to the most conserved residues of the complement binding motifs from complement receptor 1 (CR 1, bottom two lines) as determined by Klickstein *et al.* (*32*). Asterisks denote the six cysteines within the consensus repeat of ELAM-1, and the four cysteines within that of complement receptor 1. (**D**) Optimal alignment of the lectin-like and EGF domains of ELAM-1 with those of the murine MEL-14 lymphocyte homing receptor. The extracellular portion of the homing receptor also contains two complement regulatory motifs with six cysteines each.

polymers (*43*). The greatest similarity between the three molecules lies in the combined lectin and EGF-repeat domains.

The pattern of leukocyte emigration in inflammatory processes may be determined, in part, by the leukocyte selectivity and kinetics of expression of endothelial-leukocyte adhesion molecules. ELAM-1 was identified by monoclonal antibody blocking of leukocyte adhesion to activated endothelial monolayers (*8, 15*), which appear to express various pro-adhesive (*1–10*) and anti-adhesive (*44*) activities. Our data here show that expression of recombinant ELAM-1 in COS cells is sufficient to promote the adhesion of isolated human neutrophils and HL-60 cells. Preliminary studies have suggested that ELAM-1 plays a lesser role in the adhesion of monocytes or lymphocytes (*45*). Thus, focal expression of ELAM-1 at sites of endothelial activation may promote neutrophil adhesion and emigration. The rapid induction and high turnover rates of ELAM-1 mRNA and protein are consistent with the transient nature of the neutrophil influx during acute inflammation. A second inducible endothelial molecule, intercellular adhesion molecule–1 (ICAM-1), appears to contribute to both neutrophil (*46*) and lymphocyte (*9*) adhesion in vitro. Unlike ELAM-1, ICAM-1 is expressed on multiple leukocytic and nonleukocytic cell types, and is involved in a variety of other cell-cell interactions, including lymphocyte aggregation and lymphocyte-fibroblast adhesion (*47, 48*). Treatment of cultured endothelial cells with IL-1, TNF, and endotoxin results in an increase in ICAM-1 expression that is more prolonged (maintained > 48 hours) (*49*) than that observed with ELAM-1 (see Fig. 3), suggesting a role in chronic inflammatory processes. Tissue-specific endothelial-leukocyte adhesion molecules, designated vascular addressins, appear to play a role in lymphocyte recirculation, and may also contribute to leukocyte influx in certain inflammatory conditions (*50, 51*). The availability of monoclonal antibody probes, recombinant proteins, and peptide fragments should enable assessment of the contributions of ELAM-1, ICAM-1, vascular addressins, and other, as yet undescribed, endothelial cell adhesion molecules to various physiologic and pathologic processes. In the case of ELAM-1, such approaches may provide insights and potentially new therapeutic interventions in acute inflammatory disease states, such as adult respiratory distress syndrome and post-reperfusion myocardial injury, in which neutrophil-mediated tissue damage appears to play a role (*52, 53*).

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Mouse Lymph Node Homing Receptor cDNA Clone Encodes a Glycoprotein Revealing Tandem Interaction Domains

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Isolation of a clone encoding the mouse lymph node homing receptor reveals a deduced protein with an unusual protein mosaic architecture, containing a separate carbohydrate-binding (lectin) domain, an epidermal growth factor-like (EGF) domain, and an extracellular precisely duplicated repeat unit, which preserves the motif seen in the homologous repeat structure of complement regulatory proteins and other proteins. The receptor molecule is potentially highly glycosylated, and contains an apparent transmembrane region. Analysis of messenger RNA transcripts reveals a predominantly lymphoid distribution in direct relation to the cell surface expression of the MEL-14 determinant, and the cDNA clone is shown to confer the MEL-14 epitope in heterologous cells. The many novel features, including ubiquitination, embodied in this single receptor molecule form the basis for numerous approaches to the study of cell-cell interactions.

A FUNDAMENTAL EVENT REQUIRED FOR THE DEVELOPMENT of lymphoid organs and for appropriate progression of the immune response resides at the interface between a lymphocyte's mobile circulating phase and its relatively sessile phase within a particular lymphoid organ. The specific portal of entry of lymphocytes from bloodstream into peripheral lymphoid organs was identified as specialized postcapillary venules bearing unusually high-walled endothelia (*1–3*), and named high endothelial venules (HEV's) (*4*). The fundamental role of HEV-lymphocyte interaction in lymphocyte trafficking has been demonstrated (*5, 6*). Recirculating lymphocytes, but not other blood-borne cells, specifically recognize and adhere to luminal walls, and migrate through this highly specialized endothelium into the lymphoid organ parenchyma. Both B and T lymphocytes enter lymphoid organs via common HEV's (*5–8*), and thereafter migrate to T cell or B cell domains (*9–11*). Recirculation of lymphocytes from bloodstream to particular sites has been called "homing," and the cell surface structures mediating recognition and adherence to lymphoid organ HEV's have been called "homing receptors" (*12*).

Migration of lymphocytes from blood to lymphoid organs occurs nonrandomly. Peripheral node lymphocytes exhibit binding preference for peripheral node HEV's, while binding to Peyer's patch HEV is favored by lymphocytes derived from Peyer's patches (*13*). Specificity of homing results from the particular array of homing receptors actively expressed by any one lymphocyte (*9, 14, 15*). Subsequent studies of lymphocyte populations and cell lines showed mutually exclusive homing to either lymph node HEV or Peyer's patch HEV, an indication that the two specificities are mediated by independent cell surface structures. A significant proportion of murine lymphomas are unispecific with regard to Peyer's patch or peripheral lymph node HEV binding (*12*), while others recognize neither venule type. Therefore, lymphocyte homing appears to be

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