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Primary Structure of ICAM-1 Demonstrates Interaction between Members of the Immunoglobulin and Integrin Supergene Families

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Summary

Intercellular adhesion molecule 1 (ICAM-1) is a 90 kd inducible surface glycoprotein that promotes adhesion in immunological and inflammatory reactions. ICAM-1 is a ligand of lymphocyte function-associated antigen-1 (LFA-1), an αβ complex that is a member of the integrin family of cell-cell and cell-matrix receptors. ICAM-1 is encoded by an inducible 3.3 kb mRNA. The amino acid sequence specifies an integral membrane protein with an extracellular domain of 453 residues containing five immunoglobulin-like domains. Highest homology is found with neural cell adhesion molecule (NCAM) and myelin-associated glycoprotein (MAG), which also contain five Ig-like domains. NCAM and MAG are nervous system adhesion molecules, but unlike ICAM-1, NCAM is homophilic. The ICAM-1 and LFA-1 interaction is heterophilic and unusual in that it is between members of the immunoglobulin and integrin families. Unlike other integrin ligands, ICAM-1 does not contain an RGD sequence.

Introduction

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface ligand for the lymphocyte function-associated antigen 1 (LFA-1) adhesion receptor (Rothlein et al., 1986; Dustin et al., 1986; Marlin and Springer, 1987; Makgoba et al., 1988a). LFA-1 is a member of a family of three noncovalently associated αβ heterodimers (the leukocyte integrins) that have homologous α subunits, share a common β subunit, and are restricted in expression to leukocytes (Sanchez-Madrid et al., 1982; Sanchez-Madrid et al., 1983). ICAM-1, on the other hand, is a single chain glycoprotein with a polypeptide core of 55 kd that can be expressed on cells of many lineages, although its expression is strongest at specific differentiation stages or after exposure of cells to certain inflammatory mediators in vitro or in inflammatory reactions in vivo (Rothlein et al., 1986; Dustin et al., 1986; Pober et al., 1986; Dustin et al., 1988; Clark et al., 1986). The ICAM-1 polypeptide is heavily glycosylated to between 76 and 114 kd on different cell types (Dustin et al., 1986; Clark et al., 1986). The interaction of LFA-1 and ICAM-1 makes an important contribution to leukocyte adhesion in the execution of immunological and inflammatory functions (Makgoba et al., 1988b; Dougherty et al., 1988), and may contribute to regulating the localization of leukocytes (Pober et al., 1986; Dustin et al., 1986).

The primary structures of the LFA-1 α and β subunits show striking similarities to the corresponding chains of several heterodimeric receptors for extracellular matrix ligands (Kishimoto et al., 1987a; Law et al., 1987; Corbi et al., 1987; R. Larson and T. A. Springer, unpublished data). These homologies place the LFA-1 molecule in a gene family referred to as the integrins (Hynes, 1987). This family includes, among others, the fibronectin receptor and platelet glycoprotein IIb-IIIa, which bind to ligands containing the RGD core sequence (Ruoslahti and Pierschbacher, 1986; Ruoslahti and Pierschbacher, 1987). The leukocyte integrins LFA-1, Mac-1, and p150,95 are the only members of this family known to be involved in intercellular rather than cell-matrix adhesion. While it has been suggested that the interaction of Mac-1 (complement receptor type 3) with the iC3b component of complement involves an RGD sequence (Wright et et al., 1987), the GRGDSP peptide did not inhibit binding of cells to purified ICAM-1 (Marlin and Springer, 1987), suggesting that this exact recognition structure may not be used in LFA-1/ICAM-1 interaction.

The importance of the LFA-1/ICAM-1 adhesion pathway in leukocyte biology and as a receptor–ligand pair mediating intercellular adhesion led us to undertake the cloning of the ICAM-1 cDNA. Here, we describe the cloning and characterization of ICAM-1 cDNAs from the myelo-monocytic cell line HL-60 and LPS-stimulated human umbilical vein endothelial cells. Our approach was to use tryptic peptide sequences obtained with immunoaffinity-purified ICAM-1 to direct the synthesis of oligonucleotides to probe cDNA libraries. Full-length cDNA clones encode identical proteins with a single predicted membrane spanning domain. ICAM-1 shows significant sequence similarity to immunoglobulin family members, especially the neural adhesion molecules myelin associated glycoprotein (MAG) and neural cell adhesion molecule (NCAM). Therefore, leukocyte adhesion is mediated in part by receptor–ligand binding between members of the integrin and immunoglobulin superfamilies.

Results and Discussion

Isolation of ICAM-1 cDNA Clones

ICAM-1 was purified from Sarkosyl/Triton X-100 lysates of spleens from patients with hairy cell leukemia by monoclonal antibody chromatography. From 200 g of spleen, 500 μg of ICAM-1 was obtained which was greater than 99% pure as judged by SDS-PAGE and silver staining (Figure 1). The purified protein was subjected to preparative SDS-polyacrylamide gel electrophoresis and trypsin digestion. ICAM-1 peptide fragments were then purified by reverse-phase HPLC and subjected to gas-phase micro-

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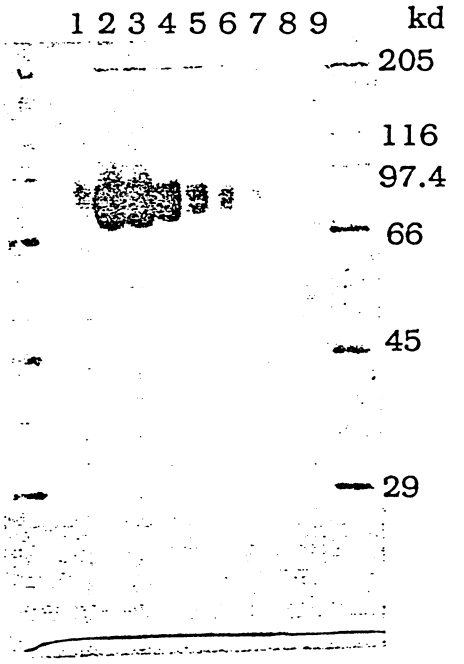

Figure 1. SDS-PAGE of Purified ICAM-1

Lanes 1–9: Fractions of hairy cell ICAM-1 eluted from MAb-Sepharose at pH 12.5 were subjected to SDS 8% PAGE and silver staining. Fractions in lanes 2–4 were pooled for preparative SDS-PAGE and sequencing studies. M_r markers are shown in adjacent lanes.

Table 1. ICAM-1 Tryptic Peptides

Peptide	Residues	
J	14–29	XGSVLVTCSTSCDQPK
U	30–39	LLGIETPL(P) (K)
50	78–85	(T)FLTVYXT
X	89–95	VELAPLP
AA	161–182	XELDLRPQGLELFEXTSAPXQL
K	232–246	LNPTVTYGXDSFSAK
45	282–295	SFPAPNV(T/I) LXKPQ(V/L)
O	313–315	VTL

Parenthesis indicate ambiguity in the sequence. X indicates a blank in sequence. The peptide sequence used to generate oligonucleotide sequence is underlined. The second unidentified residue in AA was predicted to be an asparagine in an N-linked glycosylation site.

sequencing (Table 1). Sequences of low codon redundancy within peptides J and AA were chosen for synthesis of a 32 bp and a 47 bp antisense oligonucleotide, respectively, based on codon usage preference. Both oligonucleotides hybridized to a PMA-inducible 3.3 kb RNA extracted from HL-60 cells, characteristics predicted for an ICAM-1 mRNA. Using each probe separately, six positive phage plaques each were isolated from λgt10 PMA-induced HL-60 and LPS-stimulated endothelial cell cDNA libraries. The cDNA inserts found in eight of the 12 phages were approximately 3 kb in size. These were found to all cross-hybridize under stringent conditions to one of the 3 kb cDNAs isolated from the endothelial cell cDNA library (data not shown). Thus the cDNAs isolated by either probe are identical or closely related.

Nucleotide and Amino Acid Sequence

After subcloning into pGEM 4Z, the cDNA sequence of one HL-60 and one endothelial cell cDNA was established (Figure 2). The 3023 bp sequence contains a short 5′ untranslated region and a 1.3 kb 3′ untranslated region with a consensus polyadenylation signal at position 2966. The longest open reading frame begins with the first ATG at position 58 and ends with a TGA terminating triplet at position 1653. Identity between the translated amino acid sequence and sequences determined from 8 different tryptic peptides totaling 88 amino acids (underlined in Figure 2) confirmed that we had isolated authentic ICAM-1 cDNA clones.

Hydrophobicity analysis (Kyte and Doolittle, 1982) suggests the presence of a 27 residue signal sequence. The assignment of the +1 glutamine is consistent with our inability to obtain N-terminal sequence on three different ICAM-1 protein preparations; glutamine may cyclize to pyroglutamic acid, resulting in a blocked N-terminus. The translated sequence from 1 to 453 is predominantly hydrophilic, followed by a 24 residue hydrophobic putative transmembrane domain. The transmembrane domain is immediately followed by several charged residues contained within a 28 residue putative cytoplasmic domain.

The predicted size of the mature polypeptide chain is 55,219 daltons, in excellent agreement with the observed size of 55,000 for deglycosylated ICAM-1 (Dustin et al., 1986). Eight N-linked glycosylation sites are predicted. Absence of asparagine in the tryptic peptide sequences of two of these sites confirm their glycosylation and their extracellular orientation. Assuming 2,500 daltons per high mannose N-linked carbohydrate, a size of 75,000 daltons is predicted for the ICAM-1 precursor, compared with the observed size of 73,000 daltons (Dustin et al., 1986). After conversion of high mannose to complex carbohydrate, the mature ICAM-1 glycoprotein is 76 to 114 kd, depending on cell type (Dustin et al., 1986; Clark et al., 1986). Thus ICAM-1 is a heavily glycosylated but otherwise typical integral membrane protein.

ICAM-1 mRNA and Genomic DNA

ICAM-1 is an inducible cell surface glycoprotein expressed on cells of both hematopoietic and non-hematopoietic origins. A 3 kb ICAM-1 cDNA probe was hybridized with total RNA, demonstrating higher quantities of a 3.3 kb RNA in HL-60 cells stimulated with 10 μg/ml PMA for 3 days than in unstimulated cells (Figure 3A, lanes 2 and 1, respectively). Hybridization with poly(A)$^+$ mRNA revealed a similar 3.3 kb mRNA in LPS-stimulated endothelial cells and in the epithelial carcinoma cell line EJ (Figure 3A, lanes 3 and 5), but little specific mRNA in the Hep-2 hepatoma line (Figure 3A, lane 4). A second mRNA of 2.4 kb also appeared to be present in smaller quantities in all cell types examined.

Southern blots using the 3 kb ICAM-1 cDNA probe and

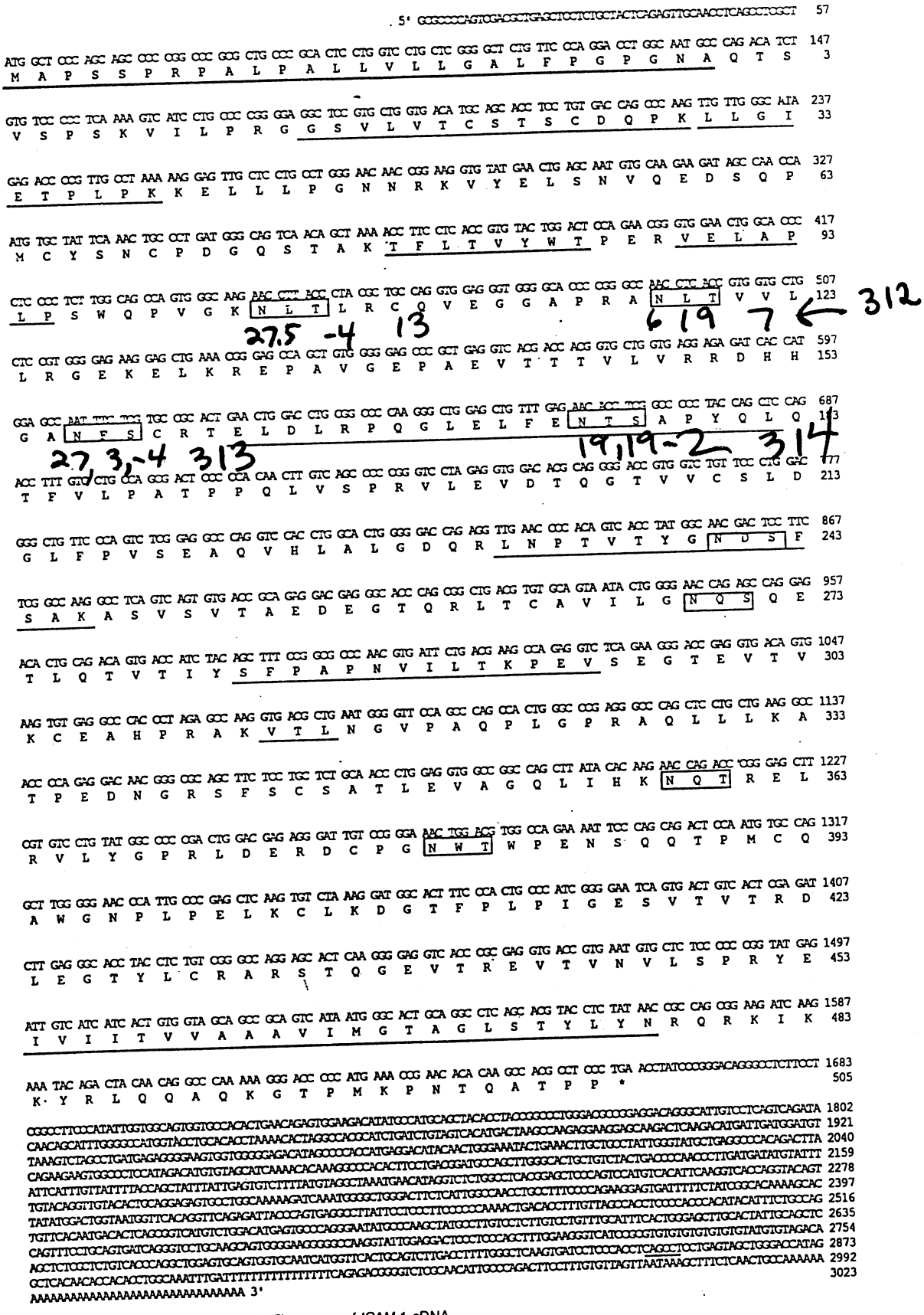

Figure 2. Nucleotide and Amino Acid Sequence of ICAM-1 cDNA

The first ATG is at position 58. Translated sequences corresponding to ICAM-1 tryptic peptides are underlined. The hydrophobic putative signal peptide and transmembrane sequences have a bold underline. N-linked glycosylation sites are boxed. The polyadenylation signal AATAAA at position 2966 is overlined. The sequence shown is for the HL-60 cDNA clone. The sequence of the entire coding region of the endothelial cell cDNA showed only two differences: substitution of A for G at position 778, changing glycine 214 to arginine, and substitution of A for G at position 1281, which is silent (threonine 381).

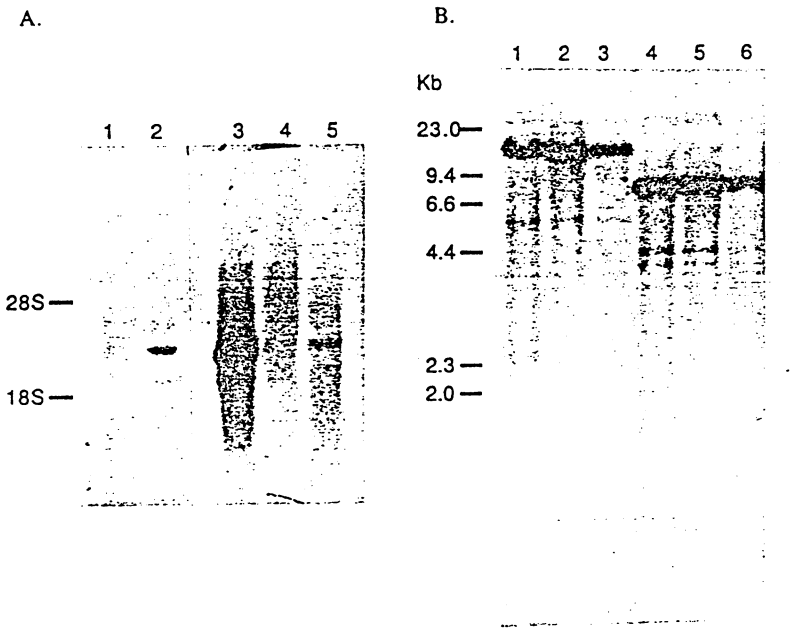

Figure 3. RNA and DNA Blots

Northern (A) and Southern (B) blots were hybridized to a 3 kb ^{32}P-labeled ICAM-1 cDNA. (A) total RNA (20 μg) from HL-60 (lane 1) and HL-60 treated with 10 μg/ml PMA for 3 days (lane 2); poly(A)$^+$ RNA (6 μg) from endothelial cells stimulated with 5 μg/ml LPS for 4 hr (lane 3), a hepatocarcinoma cell line, Hep-2 (lane 4), and an epithelial carcinoma cell line, EJ (lane 5). (B) Genomic DNA (5 μg) from the B cell lines BL2 (lanes 1 and 4), ER-LCL (lanes 2 and 5), and JY (lanes 3 and 6) were digested with BamHI (lanes 1–3) or EcoRI (lanes 4–6) restriction endonucleases.

genomic DNA digested with BamHI and EcoRI showed single predominant hybridizing fragments of 20 and 8 kb, respectively (Figure 3B), suggesting a single gene and suggesting that most of the coding information is present within 8 kb. In blots of three different cell lines there is no evidence of restriction fragment polymorphism.

ICAM-1 Is a Member of the Immunoglobulin Supergene Family

Since ICAM-1 is a ligand of an integrin, it was unexpected that it would be a member of the immunoglobulin supergene family. However, inspection of the ICAM-1 sequence shows that it fulfills all criteria proposed for membership in the immunoglobulin supergene family (Williams and Barclay, 1988). These criteria are discussed below.

The entire extracellular domain of ICAM-1 is constructed from five homologous immunoglobulin-like domains which are shown aligned in Figure 4A. Domains 1–4 are 88, 97, 99, and 101 residues, respectively, and thus are of typical Ig domain size; domain 5 is truncated with 68 residues. Searches of the NBRF protein sequence data bases using the FASTP program revealed significant homologies with members of the immunoglobulin supergene family, including IgM and IgG C domains, T cell receptor α subunit variable domain, and α 1 β glycoprotein (Figure 4B–4D). Based on this information, we compared ICAM-1 to other members of the immunoglobulin supergene family (Williams and Barclay, 1988) and found significant homologies which clearly show that ICAM-1 is a member of this family.

Three types of Ig superfamily domains, V, C1, and C2 have been differentiated (Williams and Barclay, 1988). Both V and C domains are constructed from two β-sheets linked together by the intradomain disulfide bond; V domains contain nine anti-parallel β-strands while C domains have seven. Constant domains were divided into the C1 and C2 sets (Williams and Barclay, 1988) based on characteristic residues shown in Figure 4A. The C1 set includes proteins involved in antigen recognition. The C2 set includes several Fc receptors and proteins involved in cell adhesion including CD2, LFA-3, MAG, and NCAM. ICAM-1 domains have conserved residues that corresponded to conserved residues in both C1 and C2 set domains (Figure 4A), but similarity was highest with the C2 set placing ICAM-1 in this set. Alignments were consistently longest with domains of the C2 set proteins NCAM and MAG; representative alignments with 108 residues of MAG domain 4 and 87 residues of NCAM domain 3 each yielding 32% identity as shown in Figures 4B and 4C. The alignment of ICAM-1 domains with the consensus NCAM domain sequence is shown in Figure 4A. The best alignments with V set and C1 set domains are with T cell receptor Vα (27% identity, 83 residues) and with IgM C domain 3 (34% identity, 81 residues), respectively (Figures 4B and 4D). One of the most important characteristics of immunoglobulin domains is the disulfide-bonded cysteines bridging the B and F β-strands that stabilize the β-sheet sandwich; in ICAM-1 the cysteines are conserved in all cases except in strand f of domain 4, where a leucine is found which may face into the sandwich and stabilize the contact as proposed for some other V and C2 set domains (Williams and Barclay, 1988). The distance between the cysteines (47, 50, 52, and 37 residues) is as described for the C2 set (Williams and Barclay, 1988).

To test for the presence of intrachain disulfide bonds in ICAM-1, endothelial cell ICAM-1 was subjected to SDS-PAGE under reducing and nonreducing conditions. Endothelial cell ICAM-1 was used because it shows less

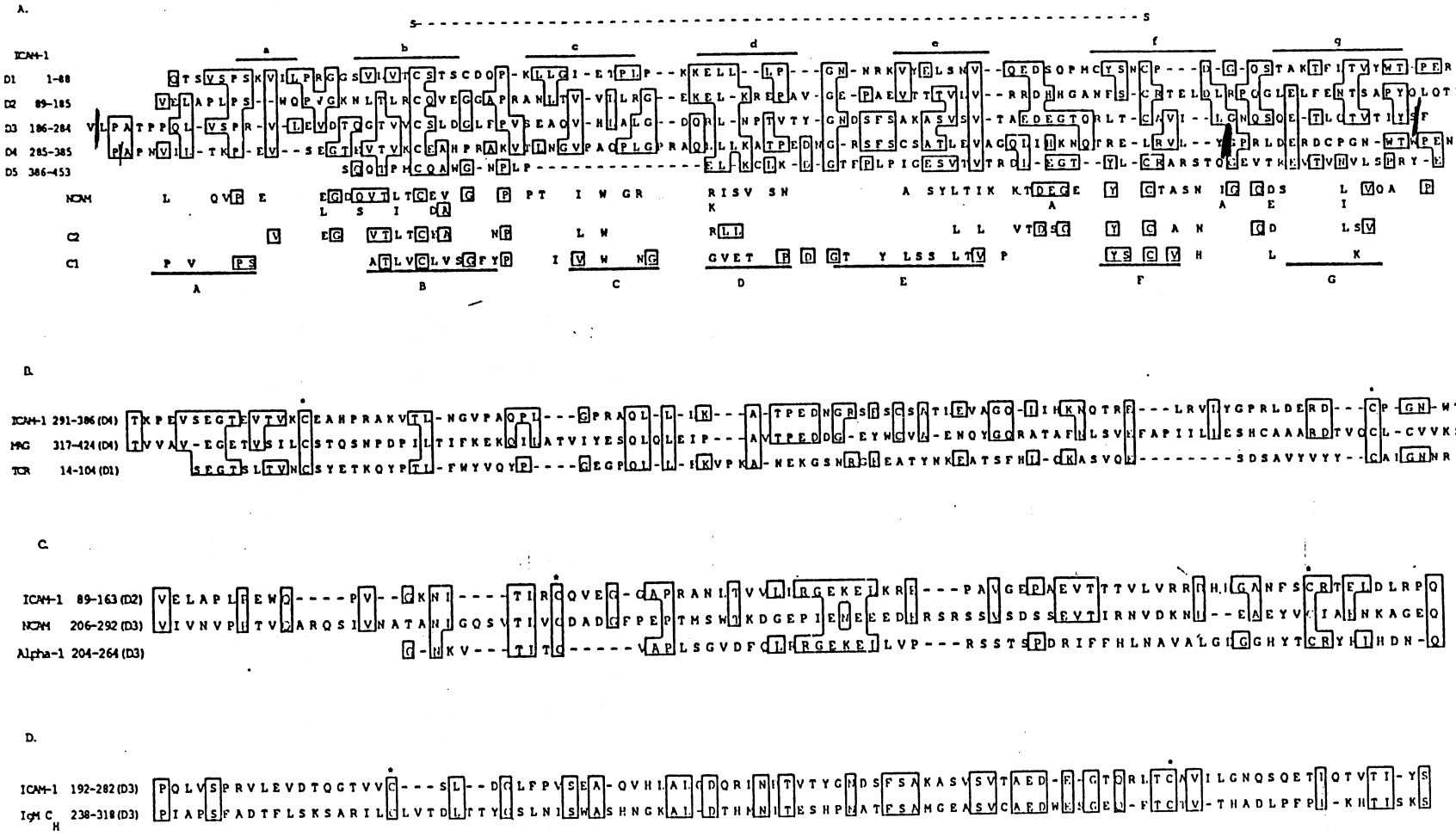

Figure 4. The ICAM-1 Homologous Domains and Relationship to the Immunoglobulin Supergene Family

(A) Alignment of five homologous domains (D1–5). Sequences were aligned using protein analysis programs (see Experimental Procedures) and by inspection. Two or more identical residues that aligned are boxed. Residues conserved two or more times in NCAM (Cunningham et al., 1987) domains, as well as residues conserved in more than four of ten domains of the sets C2 and C1 (Williams and Barclay, 1988), were aligned with the ICAM-1 internal repeats and are boxed when aligned with residues conserved in two or more ICAM-1 domains. The location of the predicted β-strands in the ICAM-1 domain is marked with bars and lowercase letters above the alignments and the known locations of β-strands in immunoglobulin C domains are marked with bars and capital letters below the alignment. The position of the putative disulfide bridge within ICAM-1 domains is indicated by S-S.

(B–D) Alignment of protein domains homologous to ICAM-1 domains; proteins were initially aligned by searching NBRF databases using the FASTP program. The protein sequences are MAG (Salzer et al., 1987), NCAM (Cunningham et al., 1987), T cell receptor α subunit V domain (Becker et al., 1985), IgM μ chain (Bernstein et al., 1984), and α 1 β glycoprotein (Ishioka et al., 1986).

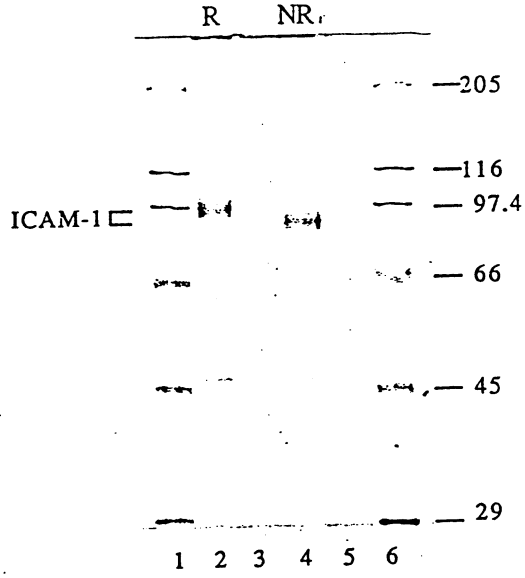

Figure 5. SDS-8% PAGE of Endothelial Cell ICAM-1 under Reducing and Nonreducing Conditions

ICAM-1 was heated in sample buffer with either 2-mercaptoethanol (lane 2, R) or iodoacetamide (lane 4, NR). M_r markers in lanes 1 and 6 were in sample buffer containing 2-mercaptoethanol. The intervening lanes between reduced and nonreduced samples (3 and 5) contained sample buffer without mercaptoethanol or iodoacetamide.

glycosylation heterogeneity than JY or hairy cell splenic ICAM-1 and allows greater sensitivity to shifts in M_r. Endothelial cell ICAM-1 had an M_r of 100 kd under reducing conditions and 96 kd under nonreducing conditions (Figure 5), strongly suggesting the presence of intrachain disulfides in native ICAM-1.

Use of the primary sequence to predict secondary structure (Chou and Fasman, 1974) showed the seven expected β-strands in each ICAM-1 domain, labeled a–g in Figure 4A (upper), exactly fulfilling the prediction for an immunoglobulin domain and corresponding to the positions of strands A–G in immunoglobulins (Figure 4A, lower). Domain 5 lacks the A and C strands, but since these form edges of the sheets, the sheets could still form, perhaps with strand D taking the place of strand C as proposed for some other C2 domains (Williams and Barclay, 1988), and the characteristic disulfide bond between the B and F strands would be unaffected. Thus, the criteria for domain size, sequence homology, conserved cysteines forming the putative intradomain disulfide bond, presence of disulfide bonds, and predicted β-sheet structure are all met for inclusion of ICAM-1 in the immunoglobulin supergene family.

ICAM-1 was found to be most strongly homologous with the NCAM and MAG glycoproteins of the C2 set. This is of particular interest since both NCAM and MAG mediate cell–cell adhesion. NCAM is important in neuron–neuron and neuro–muscular interactions (Cunningham et al., 1987), while MAG is important in neuron–oligodendrocyte and oligodendocyte–oligodendrocyte interactions during myelination (Poltorak et al., 1987). The cell surface expression of NCAM and MAG is developmentally regulated during nervous system formation and myelination, respectively, in analogy to the regulated induction of ICAM-1 in inflammation (Springer et al., 1987). ICAM-1, NCAM (Cunningham et al., 1987), and MAG (Salzer et al., 1987) are similar in overall structure as well as homologous, since each is an integral membrane glycoprotein constructed from five C2 domains forming the N-terminal extracellular region, although in NCAM some additional non-Ig-like sequence is present between the last C2 domain and the transmembrane domain. ICAM-1 aligns over its entire length, including the transmembrane and cytoplasmic domains with MAG with 21% identity; the same percent identity is found comparing the five domains of ICAM-1 and NCAM-1. Domain by domain comparisons show that the level of homology between domains within the ICAM-1 and NCAM molecules ($\bar{x}$ ± s.d. 21 ± 2.8% and 18.6 ± 3.8%, respectively) is the same as the level of homology comparing ICAM-1 domains to NCAM and MAG domains (20.4 ± 3.7 and 21.9 ± 2.7, respectively). Although there is evidence for alternative splicing in the C-terminal regions of NCAM (Cunningham et al., 1987; Barthels et al., 1987) and MAG (Lai et al., 1987), we have not found any evidence for this in the sequencing of endothelial or HL-60 ICAM-1 clones or in studies on the ICAM-1 protein backbone and precursor in a variety of cell types (Dustin et al., 1986).

Conclusions

ICAM-1 functions as a ligand for LFA-1 in lymphocyte interactions with a number of different cell types. Lymphocytes bind to ICAM-1 incorporated in artificial membrane bilayers, and this requires LFA-1 on the lymphocyte, directly demonstrating LFA-1 interaction with ICAM-1 (Marlin and Springer, 1987; Makgoba et al., 1988a). LFA-1 is a leukocyte integrin and has no immunoglobulin-like features. Leukocyte integrins comprise one integrin subfamily. The other two subfamilies mediate cell-matrix interactions and recognize the sequence RGD within their ligands, which include fibronectin, vitronectin, collagen, and fibrinogen (Hynes, 1987; Ruoslahti and Pierschbacher, 1987). The leukocyte integrins are only expressed on leukocytes, are involved in cell–cell interactions and the only known ligands are ICAM-1 and iC3b, a fragment of the complement component C3 that shows no immunoglobulin-like features and is recognized by Mac-1 (Kishimoto et al., 1987b; Springer et al., 1987; Anderson and Springer, 1987).

ICAM-1 is the first example of a member of the immunoglobulin supergene family that binds to an integrin. Although both of these families play an important role in cell adhesion, interaction between them previously had not been expected. In contrast, interactions within the immunoglobulin gene superfamily are quite common (Williams and Barclay, 1988). It is quite possible that further examples of interactions between the integrin and immunoglobulin families will be found. LFA-1 recognizes a ligand distinct from ICAM-1 (Springer et al., 1987), and the leukocyte

integrin Mac-1 recognizes a ligand distinct from iC3b in neutrophil–neutrophil adhesion (Anderson and Springer, 1987). Furthermore, purified MAG-containing vesicles bind to neurites that are MAG$^-$ and thus MAG must be capable of heterophilic interaction with a distinct receptor (Poltorak et al., 1987).

NCAM's role in neural–neural and neural–muscular cell interactions has been suggested to be the result of homophilic NCAM–NCAM interactions (Cunningham et al., 1987). The important role of MAG in interactions between adjacent turning loops of Schwann cells enveloping axons during myelin sheath formation might be due to interaction with a distinct receptor or due to homophilic MAG-MAG interactions. The homology with NCAM and the frequent occurrence of domain–domain interactions within the immunoglobulin supergene family raises the possibility that ICAM-1 could engage in homophilic interactions as well as ICAM-1–LFA-1 heterophilic interactions. However, binding of B lymphoblast cells that co-express similar densities of LFA-1 and ICAM-1 to ICAM-1 in artificial or cellular monolayers can be completely inhibited by pretreatment of the B lymphoblast with LFA-1 MAb, while adherence is unaffected by B lymphoblast pretreatment with ICAM-1 MAb. Pretreatment of the monolayer with ICAM-1 MAb completely abolishes binding (Dustin et al., 1986; Marlin and Springer, 1987; Dustin et al., 1988). These findings show that if ICAM-1 homophilic interactions occur at all, they must be much weaker than heterophilic interaction with LFA-1.

Are immunoglobulin domains simply a convenient scaffold for RGD-like sequences on cell surfaces that are recognized by those integrins that mediate cell–cell interactions, or does recognition of ICAM-1 occur in a way fundamentally different from binding to RGD in extracellular matrix components? The possibility that the leukocyte integrins recognize ligands in a fundamentally different way is consistent with the presence of a 180 residue sequence in their α subunits which may be important in ligand binding and which is not present in the RGD-recognizing integrins (Corbi et al., 1987; Larson et al., unpublished data). Although Mac-1 has been proposed to recognize an RGD sequence present in iC3b (Wright et al., 1987), there is no RGD sequence in ICAM-1 (Figure 2). This is in agreement with the failure of the fibronectin peptide GRGDSP and the control peptide GRGESP to inhibit ICAM-1–LFA-1 adhesion (Marlin and Springer, 1987). However, related sequences such as PRGGS and RGEKE are present in ICAM-1 in regions predicted to loop between β-strands a and b of domain 1 and c and d of domain 2, respectively (Figure 4), and thus may be accessible for recognition. The sequence PERVE is present between domains 1 and 2. It is tantalizing that the homologous MAG molecule contains an RGD sequence between domains 1 and 2 (Poltorak et al., 1987; Salzer et al., 1987). The complete amino acid sequence of ICAM-1 now allows testing of whether short amino acid segments are critical in recognition, as for matrix component recognition by integrins (Ruoslahti and Pierschbacher, 1986), or whether recognition is much more complex and conformation-dependent, as for interaction between immunoglobulin domains.

Experimental Procedures

Purification of ICAM-1 and Peptide Sequencing

ICAM-1 was purified from detergent lysates of spleens from patients with hairy cell leukemia by affinity chromatography using the anti-ICAM-1 monoclonal antibody RR1/1. The previously described purification procedure (Marlin and Springer, 1987) was modified by inclusion of Tween 40 and N-lauroyl sarcosine detergent extraction steps to improve purity. Frozen human spleen (200 g fragments) were thawed on ice in 200 ml Tris-saline (0.14 M NaCl, 50 mM Tris-HCl [pH 7.4] at 4°C) containing 1 mM PMSF, 0.2 U/ml aprotinin and 5 mM iodoacetamide. The tissue was homogenized with a Tekmar power homogenizer. To 300 ml of homogenate, 100 ml of 10% Tween 40 in Tris-saline was added to obtain a final concentration of 2.5%. To prepare membranes, the homogenate was extracted using three strokes of a Teflon Potter Elvejhem homogenizer and then centrifuged at 1000 × g for 15 min. The insoluble material was re-extracted with 200 ml of 2.5% Tween 40 in Tris-saline. Both extractions were combined and centrifuged at 150,000 × g for 1 hr to pellet the membranes. The membranes were washed in Tris-saline, homogenized, and N-lauroyl sarcosine was added to a final concentration of 1%. Insoluble material was removed by centrifugation at 150,000 × g for 1 hr. Triton X-100 was added to the supernatant to a final concentration of 2%. Affinity chromatography was performed as described previously (Miller and Springer, 1987; Marlin and Springer, 1987). Briefly, the detergent lysate was passed through a precolumn of rat IgG-Sepharose-CL4B and a RR1/1 Sepharose column attached in series. The RR1/1 column was washed sequentially with lysis buffer; 0.14 M NaCl, 20 mM Tris (pH 8), 0.1% Triton X-100; 20 mM glycine-NaOH (pH 10.0), 0.1% Triton X-100; and 50 mM triethylamine(pH 11.0), 0.1% Triton X-100; and 50 mM triethylamine-HCl (pH 11.0), 1% octylglucoside. The bound ICAM-1 was eluted with 50 mM triethylamine (pH 12.5), 1% octylglucoside. Purified ICAM-1 was then subjected to preparative SDS-PAGE and electroelution (Hunkapiller et al., 1983). ICAM-1 was then reduced with 2 mM dithiothreitol/2% SDS, alkylated with 5 mM iodoacetic acid, and precipitated. The protein was dissolved in 0.1 M ammonium bicarbonate (pH 8.0), containing 0.1 mM calcium chloride and 0.1% zwittergent 3–14 (Calbiochem) and digested with 1% w/w trypsin at 37°C for 4 hr, followed by a second 12 hr digestion. The tryptic peptides were purified by reverse-phase HPLC using a C4 column (Vydac) and eluted with a gradient of 0%–60% acetonitrile in 0.1% TFA (Kishimoto et al., 1987a). Selected peptides were subjected to microsequencing on a gas-phase sequenator (Applied Biosystems).

SDS-PAGE of Endothelial Cell ICAM-1

ICAM-1 was purified from 16 hr LPS (5 μg/ml) stimulated umbilical vein endothelial cell cultures (Dustin and Springer, submitted) by immunoaffinity chromatography as described above. Acetone precipitated ICAM-1 was resuspended in sample buffer (Laemmli, 1970) with 0.25% 2-mercaptoethanol or 25 mM iodoacetamide and brought to 100°C for 5 min. The samples were subjected to SDS-PAGE (Laemmli, 1970) and silver staining (Morrissey, 1981).

Construction and Screening of cDNA Libraries

The generation of a size-selected cDNA library using poly(A)$^+$ RNA from PMA-induced HL-60 cells and the method of Gubler and Hoffman (1983) was described previously (Corbi et al., 1987). Similarly, a cDNA library was generated using poly(A)$^+$ RNA extracted from LPS-stimulated umbilical vein endothelial cells. Briefly, total RNA was isolated from endothelial cells that had been stimulated for 4 hr with LPS (5 μg/ml) by homogenizing in 4 M guanidinium isothiocyanate and ultracentrifugation through a CsCl gradient (Chirgwin et al., 1979). Poly(A)$^+$ RNA was isolated by oligo (dT)-cellulose (type 3, Collaborative Research) chromatography (Aviv and Leder, 1972). First strand cDNA was synthesized using 8 μg of poly(A)$^+$ RNA, avian myeloblastosis virus reverse transcriptase (Life Sciences), and an oligo(dT) primer. The hybrid was digested with RNase H (BRL) and the second strand synthesized with DNA polymerase I (New England Biolabs).

The product was methylated with EcoRI methylase (New England Biolabs), blunt-end ligated to EcoRI linkers (New England Biolabs), digested with EcoRI, and size selected on a low melting point agarose gel. cDNA greater than 500 bp were ligated to λgt10 which had previously been EcoRI digested and dephosphorylated (Stratagene). The product of the ligation was then packaged (Stratagene gold). The endothelial and HL-60 cDNA libraries were then plated at 20,000 pfu/150 mm plate. Recombinant DNA was transferred in duplicate to nitrocellulose filters, denatured in 0.5 M NaOH/1.5M NaCl, neutralized in 1 M Tris (pH 7.5)/1.5 M NaCl and baked at 80°C for 2 hr (Benton and Davis, 1977). Filters were prehybridized and hybridized in 5× SSC containing 5× Denhardt's, 50 mM $NaPO_4$ and 1 mg/ml salmon sperm DNA. Prehybridization was carried out at 45°C for 1 hr. Hybridization was carried out using 32 bp (5'-TTGGGCTGGTCACAGGAGGTGGAGCAGGTGAC) or 47 bp (5'-GAGGTGTTCTCAAACAGCTCCAGGCCCTGGGGCCGC-AGGTCCAGCTC) anti-sense oligonucleotides based on the ICAM-1 tryptic peptides J and AA, respectively (Lathe, 1985). Oligonucleotides were end labeled with $\gamma[^{32}P]$-ATP using T4 polynucleotide kinase and conditions recommended by the manufacturer (New England Biolabs). Following overnight hybridization, the filters were washed twice with 2× SSC/0.1% SDS for 30 min at 45°C. Positive phage were isolated by succesive replating and rescreening.

Sequencing of cDNAs

Phage DNA from positive clones were digested with EcoRI and examined by Southern analysis using a cDNA from one clone as a probe. Maximal size cDNA inserts that cross-hybridized were subcloned into the EcoRI site of plasmid vector pGEM 4Z (Promega). HL-60 subclones containing the cDNA in both orientations were deleted by exonuclease III digestion (Henikoff, 1984) according to the manufacturer's recommendations (Erase-a-Base, Promega). Progressively deleted cDNAs were then cloned and subjected to dideoxynucleotide chain termination sequencing (Sanger et al., 1977) according to the manufacturer's recommendations (Sequenase, U. S. Biochemical). The HL-60 cDNA 5' and coding regions were sequenced completely on both strands and the 3' region was sequenced approximately 70% on both strands. A representative endothelial cDNA was sequenced on both strands over its coding sequence by shotgun cloning of 4 bp-recognition restriction enzyme fragments.

Southern and Northern Blots

Southern blots were performed using 5 μg of genomic DNA extracted from three cell lines: BL2, a Burkitt lymphoma cell line (a gift from Dr. Gilbert Lenoir), JY, and ER-LCL, EBV transformed B-lymphoblastoid cell lines.

The DNAs were digested with five times the manufacturer's recommended quantity of BamHI and EcoRI endonucleases (New England Biolabs). Following electrophoresis through a 0.8% agarose gel, the DNAs were transferred to a nylon membrane (Zeta Probe, BioRad). The filter was prehybridized and hybridized following standard procedures (Maniatis et al., 1982) using ICAM cDNA from HL-60 labeled with $\alpha[^{32}P]$d XTP's by random priming (Boehringer Mannheim). Northern blots were performed using 20 μg of total RNA or 6 μg of poly(A)$^+$ RNA. RNA was denatured and electrophoresed through a 1% agarose-formaldehyde gel (Maniatis et al., 1982) and electrotransferred to Zeta Probe. Filters were prehybridized and hybridized as described previously (Staunton and Thorley-Lawson, 1987) using the HL-60 cDNA probe or ^{32}P-labeled oligonucleotide probes (described above).

Sequence Homologies

Alignment of ICAM-1 internal repeats was performed using the Microgenie protein alignment program (Queen and Korn, 1984) followed by inspection. Alignment of ICAM-1 to IgM, N-CAM, and MAG was carried out using Microgenie and the ALIGN program (Dayhoff et al., 1983). Four protein sequence databases, maintained by the National Biomedical Research Foundation, were searched for protein sequence similarities using the FASTP program of Lipman and Pearson (Lipman and Pearson, 1985).

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Note Added in Proof

Since this manuscript was submitted, Simmons et al. (1988. Nature *331*, 624–627) reported on ICAM-1 cDNA isolated by expression in COS cells. The sequence of 1846 nucleotides is identical to nucleotides 45–1890 of our 3023 bp sequence.