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ISOLATION, CHARACTERIZATION, AND EXPRESSION OF MOUSE ICAM-2 COMPLEMENTARY AND GENOMIC DNA[1]

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Intercellular adhesion molecule-2 (ICAM-2), a cell surface glycoprotein, is a second counter-receptor for lymphocyte function-associated Ag-1 (LFA-1). We report here the isolation and characterization of the cDNA and the gene that encode murine ICAM-2 (Accession numbers X65493 and X65490, respectively). The deduced sequence of the cDNA has 60% amino acid identity with its human counterpart and has the same expression pattern in cells and tissues. Furthermore, COS cells transfected with mouse ICAM-2 complementary and genomic DNA bind to purified human LFA-1, demonstrating the conservation of the function of ICAM-2 as a ligand for LFA-1 and conservation across species of sequences that are critical for binding to human LFA-1. COS cells transfected with the ICAM-2 cDNA do not react with mAb PA3, previously suggested to define ICAM-2 in the mouse. The mouse ICAM-2 gene was isolated and its structural organization determined. The gene is present in a single copy in the mouse genome and contains four exons spanning about 5.0 kb of DNA. The exon/intron architecture correlates to the structural domains of the protein and resembles that of other Ig superfamily members. The gene for ICAM-2, which is constitutively expressed in endothelial cells, has several conserved sequence motifs in its promoter region, including a direct repeat, and lacks transcription factor-binding sites present in the ICAM-1 gene, which is inducible in endothelial cells.

Leukocyte cell surface adhesion molecules play an essential role in inflammatory and immune responses. Important adhesive interactions are mediated by LFA-1[3] and its ligands, ICAM-1, ICAM-2, and ICAM-3 (1–3). LFA-1 is a member of the integrin family that has an α/β heterodimer structure and is expressed on almost all types of leukocytes (1, 4). The α subunit (CD11a) of LFA-1 is unique but the β subunit (CD18) is shared by two other leukocyte integrin molecules, Mac-1 (CD11b/CD18) and p150/95 (CD11c/CD18) (5). The importance of these leukocyte integrins is demonstrated by an inherited human genetic deficiency, leukocyte adhesion deficiency, in which lack of surface expression of LFA-1, Mac-1, and p150,95 results in recurrent life-threatening bacterial infections (6, 7).

ICAM-1 was identified as a counter-receptor of LFA-1 by selecting for mAb to distinct cell-surface structures that could block LFA-1-dependent cell adhesion (8). The presence of a second ligand for LFA-1 was implicated by the observation that antibodies to ICAM-1 only partially inhibited adhesion of leukocytes to some cell types such as resting endothelium, whereas anti-LFA-1 antibodies blocked the adhesion completely (8–10). A second ligand, human ICAM-2, was identified by cloning from endothelial cells a cDNA that when expressed in COS cells conferred binding to purified LFA-1 (2). The two LFA-1 ligands have similar structures; both belong to the Ig superfamily. ICAM-1 has five Ig-like domains (11, 12) whereas ICAM-2 has only two Ig-like domains (2). Remarkably, the two Ig-like domains of ICAM-2 bear 34% identity in amino acid sequence with the two most N-terminal Ig-like domains of ICAM-1, to which the LFA-1-binding site is mapped (2, 13). ICAM-1 and ICAM-2 have different yet overlapping tissue distribution patterns. ICAM-1 is expressed at a low level on a subpopulation of lymphocytes, macrophages, and endothelial cells, but is strongly induced on these cells, and on fibroblasts and epithelial cells, by a number of cytokines and inflammatory mediators such as LPS, IFN-γ, IL-1, and TNF (1, 14). In comparison, ICAM-2 is also expressed on resting lymphocytes and monocytes but its expression in tissues is highly restricted to vascular endothelium. Expression of ICAM-2 on endothelium is much stronger than on leukocytes. The basal level of expression of ICAM-2 on endothelial cells is much higher than that of ICAM-1, and is not further increased by inflammatory mediators (2, 15). A third ligand for LFA-1 has recently been described, and designated ICAM-3 (3). ICAM-3 is restricted to hematopoietic cells and appears to be the major LFA-1 ligand on resting lymphocytes.

Little is known about the biologic function of ICAM-2 on lymphocytes and endothelial cells. The interaction of LFA-1 with ICAM-2 seems to be a major component of lymphocyte adhesion to unstimulated cultured endothelium (10, 15). Because ICAM-2 is expressed on both high endothelial venules in lymph nodes and on vascular endothelium in other tissues, it has been hypothesized that ICAM-2 may play an important role in lymphocyte recirculation (15). As a first step to study ICAM-2 function in the mouse, and to understand the mechanisms regulating ICAM-2 expression, we isolated and characterized the cDNA and the gene that encode mouse ICAM-2. We show

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[3] Abbreviations used in this paper: LFA-1, lymphocyte function-associated Ag-1; ICAM, intercellular adhesion molecule; TSM, 25 mM Tris (pH 8.0), 150 mM NaCl, and 2 mM $MgCl_2$.

here that mouse ICAM-2 is highly homologous to human ICAM-2 at the amino acid level (60% identity). We find that mouse ICAM-2, when expressed in COS cells, binds to purified human LFA-1, suggesting that the function of ICAM-2 is conserved in human and mouse tissue. Examination of the promoter and 5′ upstream sequences of the mouse ICAM-2 gene reveals sequence motifs possibly related to its constitutive expression in endothelial cells and a lack of transcription factor-binding sites seen in the ICAM-1 gene.

MATERIALS AND METHODS

Isolation of mouse ICAM-2 cDNA. A cDNA library of the mouse B cell lymphoma line BCL1 constructed in the λZAP XHO-MID phage vector (a gift from Drs. A. Turner and M. Davis at the Department of Microbiology and Immunology, Stanford University, Palo Alto, CA) was screened by cross-hybridization using human ICAM-2 cDNA as a probe. An 800-bp *Xho*I fragment from human ICAM-2 cDNA was labeled with [α-^{32}P]dCTP by nick translation, hybridized to the phage library filters in 5X SSC, 5X Denhardt's solution, 0.1% SDS, 50% formamide, and 50 μg/ml denatured herring sperm DNA at 37°C. Filters were then washed at low stringency with 2X SSC/0.1% SDS twice at 22°C for 15 min and once at 37°C for 15 to 30 min. Hybridizing phages were then purified and the insert-containing plasmids were excised from the phagemid according to the procedure described by Stratagene (La Jolla, CA).

Cloning of mouse ICAM-2 gene. An AKR mouse genomic library in the cosmid vector pWE2 (16), a gift from Dr. Glen Evans at the Salk Institute (San Diego, CA), was screened with the complete mouse ICAM-2 cDNA labeled with [α-^{32}P]dCTP by nick translation. A high stringency screening was performed as previously described (17). Hybridization was carried out in the same solution as described above but at 42°C overnight, filters were washed twice with 1X SSC/0.1% SDS at 37°C for 15 min and then once in 0.1X SSC/0.1% SDS at 68°C for 20 min. Colonies that hybridized to the cDNA were purified and amplified to isolate the cosmid DNA. The isolated pWE2 genomic clones were digested with several restriction enzymes and subjected to Southern blot analysis (17). DNA fragments that hybridized to the cDNA probe were subcloned into plasmid pBluescript KS- (Stratagene) for restriction mapping and sequence analysis.

Sequencing and homology analysis. The nucleotide sequence of the mouse ICAM-2 cDNA and partial sequence of the genomic clones were determined by the dideoxynucleotide chain termination method with modified T7 DNA polymerase (United States Biochemical Corp., Cleveland, OH). Oligonucleotide primers for sequencing reactions were synthesized in an oligonucleotide synthesizer (Applied Biosystems, Foster City, CA) and used without purification. Nucleotide sequence of the cDNA clone was obtained by sequencing both DNA strands. Definitive nucleotide sequence of certain regions was determined using deoxyinosine in place of deoxyguanidine.

Alignment of human and mouse ICAM-2 protein sequences was performed using the Gap program (University of Wisconsin GCG package) (18) followed by inspection. The 5′ upstream region of the gene was searched for the presence of potential transcription factor-binding sites with the transcription factor sites data file of the University of Wisconsin GCG program.

Southern and Northern blot analysis. Genomic DNA was isolated from the AKR mouse strain thymoma cell line BW5147 as previously described (17). Approximately 10 μg of DNA was digested with different restriction endonucleases and separated on a 1% agarose gel and blotted onto nitrocellulose. Poly(A)$^+$ RNA from various cell lines and tissues was isolated using the Invitrogen RNA isolation kit (Invitrogen, San Diego, CA). For Northern blot analysis, 5 μg of poly(A)$^+$ RNA was separated on a 1.2% agarose gel containing formaldehyde, and transferred to a nitrocellulose filter as previously described (17). Both Southern and Northern blots were hybridized to the mouse ICAM-2 cDNA probe as described for the library screening. Filters were washed at high stringency, twice in 1X SSC/0.1% SDS at 37°C, and once in 0.1X SSC/0.1% SDS at 68°C for 15 min.

Cell culture. The cell lines used include: mouse thymomas, BW5147 and EL-4 (19, 20); mouse mastocytoma line, P815 (21); mouse monocyte-macrophage-like cell line, P388D1 (21); mouse fibroblasts, NIH 3T3; SV-40-transformed African green monkey kidney fibroblastoid cells, COS (American Culture Type Collection, Rockville, MD); and the human Reed Sternberg line, L428 (22). All cells were maintained in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, and 50 μg/ml gentamicin (complete media).

COS cell transfections and binding assays. The ICAM-2 cDNA was excised from vector with *Xho*I and subcloned into a transient expression vector Ap^{r}M8 (a derivative of CDM8, provided by Dr. Lloyd Klickstein at the Center for Blood Research, Harvard Medical School, Boston, MA). The mouse ICAM-2 genomic DNA, a 6.5-kb *Eco*RI fragment, was also cloned into Ap^{r}M8 in two different orientations. COS cells were transfected with Ap^{r}M8 expression vector constructs containing the mouse ICAM-2 complementary and genomic DNA using DEAE-dextran (23). Mock transfection was performed using vector alone. cDNA of human ICAM-1 (11), ICAM-2 (2), or mouse ICAM-1 (13) in CDM8 were also transfected into COS cells with the same method.

Adhesion assays were performed as previously described (15, 24). LFA-1 was purified from JY cell lysates by immunoaffinity chromatography after detergent solubilization and stored frozen at −70°C in 1% acetyl-β-D-glucopyranoside. LFA-1 diluted 1/20 in TSM was adsorbed to 96-well plastic plates (Linbro-Titertek, Flow Laboratories, McLean, VA) for 2 h at room temperature. Nonspecific binding sites were blocked for 2 h at room temperature with TSM/1% BSA and two washes with PBS/5% FCS/2 mM $MgCl_2$/0.5% BSA (assay medium). The number of LFA-1 sites/microtiter well was determined to be 1100 sites/μm^2 as previously described (25). Inhibition of adhesion to the substrates was performed by pretreatment of the LFA-1-coated 96-well plates with antibodies to the α-subunit of LFA-1 (TS1/22, IgG1) (26) for 45 min at 37°C followed by washing to remove the unbound mAb.

COS cell transfectants were harvested with 10 mM EDTA in HBSS, washed with 10% FCS/RPMI, and labeled with 15 μg/ml of 2′,7′-bis-(2-carboxyethyl)-5(and-6)carboxyfluorescein (BCECF; Molecular Probes, Eugene, OR) for 30 min at 37°C. Cells were then washed twice with 10% FCS/RPMI 1640 and resuspended in assay medium. A total of 4×10^4 labeled cells was added to each well and incubated at 37°C for 1 h. Unbound cells were removed by gravity after inverting and submerging the microtiter plate for 60 min at room temperature in a tank containing 1 liter of assay medium. The fluorescence of the total input cells or the bound cells was determined by a Pandex fluorescence concentration analyzer (Baxter Healthcare Corp., Mundelein, IL).

Flow cytometric analysis. COS cell transfectants were detached from tissue culture dishes with 10 mM EDTA/HBSS and washed with 0.5% FCS/PBS. About 1 to 5×10^6 COS cells or other cell types in 50 μl tissue culture medium were added to either 50 μl mAb supernatant or 50 μl of a 1/200 dilution of mAb ascites fluid and incubated at 4°C for 30 min. Cells were then washed and incubated with 100 μl of a 1/20 dilution of FITC-labeled goat anti-mouse Ig (Zymed Immunochemicals, San Francisco, CA) for 30 min at 4°C. The cells were washed again after 30-min incubation and fixed in 1% paraformaldehyde/PBS. Cell samples were then analyzed by an Epics V (Coulter Diagnostics, Hialeah, FL) flow cytometer. mAb PA3 is a rat IgM that recognizes a 55-kDa cell-surface glycoprotein (27). X63 (myeloma IgG1) supernatant staining was used to control for determining nonspecific fluorescence.

RESULTS

Isolation and analysis of mouse ICAM-2 cDNA. Two independent cDNA clones were isolated from a mouse BCL1 lymphoma library by hybridization with the human ICAM-2 cDNA. The longest with an insert of 1.1 kb, clone mIC2–15, was sequenced on both DNA strands. The sequence of 1168 nucleotides has a single open reading frame of 277 amino acid residues starting with the initiation codon ATG at position 158 and ending with a stop codon TGA at position 989 (Fig. 1). A six-nucleotide polyadenylation signal sequence, AATACA, present in the human ICAM-2 cDNA also appears in the mouse ICAM-2 cDNA. The poly(A) tail is found 14 nucleotides after the polyadenylation signal.

The deduced amino acid sequence of the cDNA reveals a transmembrane protein that consists of a hydrophobic N-terminal signal sequence, an extracellular domain, a transmembrane domain, and a cytoplasmic tail. Mouse and human ICAM-2 are 60% identical in amino acid sequence (Fig. 2). The highest homology is observed in the transmembrane and cytoplasmic domains with 75% amino acid identity. The presence of two Ig domains and the position of cysteines are conserved in mouse ICAM-2, along with the presence of four cysteines predicted to

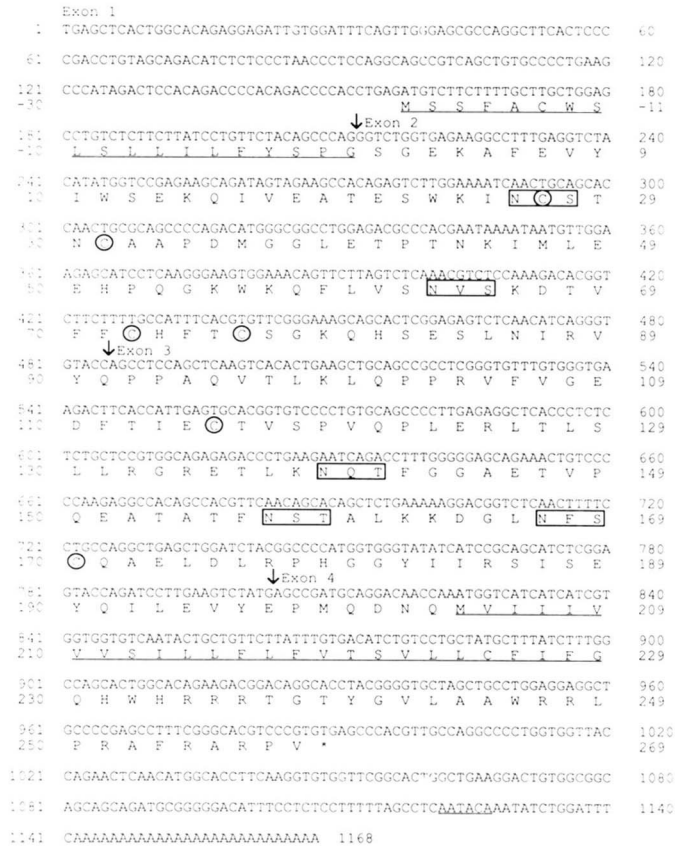


Figure 1. Nucleotide and protein sequence of the mouse ICAM-2 cDNA. The predicted N-terminal signal peptide, the transmembrane region, and the polyadenylation signal sequence are *underlined*. The *boxes* indicate potential *N*-linked glycosylation sites. The cysteine residues in the extracellular domain are *circled*. *Arrows* indicate exon boundaries. The sequence of the cDNA has been submitted to EMBL/GenBank/DDBJ under the accession number X65493.

Figure 2. Comparison of amino acid sequence between mouse and human ICAM-2. Identical amino acid residues are indicated by *match lines*. The predicted two Ig-like domains are shown. *SP*, signal peptide; *TM*, transmembrane domain; *Cyt*, cytoplasmic domain.

form two intradomain disulfide bonds in the first Ig domain, an unusual feature also seen in the N-terminal Ig domains of ICAM-1 (11, 12) and VCAM-1 (28). There are five potential *N*-linked glycosylation sites in the two Ig-like domains of mouse ICAM-2, whereas there are six in human ICAM-2 (Fig. 1).

Expression of mouse ICAM-2 mRNA. Expression of mouse ICAM-2 mRNA was determined by Northern blotting (Fig. 3*A*). A single 1.2-kb RNA species was detected in the T cell lines BW5147 and EL-4, the mastocytoma cell line P815, and the macrophage-like line P388D1. The mouse ICAM-2 message was undetectable in NIH 3T3 fibroblasts and in the human Reed-Sternberg cell line,

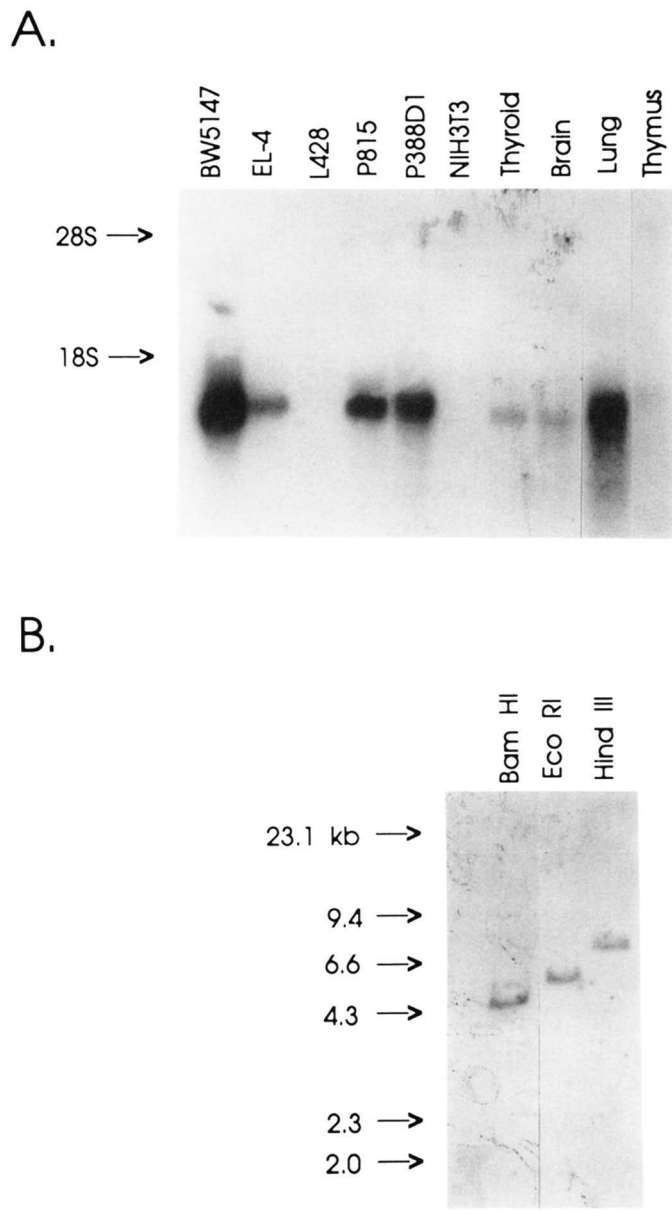


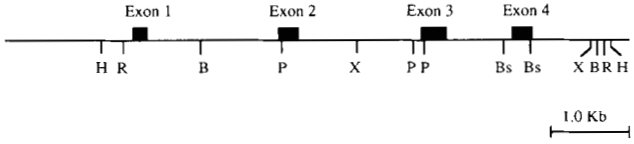
Figure 3. Northern and Southern blot analysis. *A*, poly A$^+$ RNA isolated from the indicated cell lines and tissues was probed with mouse ICAM-2 cDNA. The positions of 28S and 18S rRNA are indicated. *B* genomic DNA isolated from the BW5147 line was digested with the indicated restriction enzymes and probed with mouse ICAM-2 cDNA.

L428. High level expression of mouse ICAM-2 was found in the lung, whereas low level expression was found in the thymus, thyroid, and brain. The expression pattern of mouse ICAM-2 is very similar to that of human ICAM-2, and high expression in the lung is expected because this tissue is rich in endothelium.

Characterization of the mouse ICAM-2 gene. Southern blots of mouse AKR strain DNA digested with restriction endonucleases *Bam*HI, *Eco*RI, and *Hind*III, probed with mouse ICAM-2 cDNA revealed in each case a single band (Fig. 3*B*), suggesting that the mouse ICAM-2 gene is present in a single copy in the mouse genome.

To isolate the mouse ICAM-2 gene, pWE2 cosmids from mouse AKR strain were screened with the cDNA probe under high stringency conditions, and one clone was isolated. A restriction map of the mouse ICAM-2 gene (Fig. 4*A*) was generated by restriction digestion and

A.



B.

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-373 AAGCTTGCACACATTCATGCATGCACACACACTTCCTGCATGTGATTTCCCATTGCTTTC -374
-313 CTATCCCCGCACAGGGAGCATGCAACGTGAGAGAACCCTGTTACACCCCTGCTTATCTGC -314
-253 TGCTTCCTGGCTTCCTCTGCATGAATTCTTGAGGCCCTAAAGGCTTGGGAGCTGGTCTGT -194
-193 GCATATTGTTTCCTGATCTCAGATAATTAGAGGAAATGAGCTCACTGGCACAGAGGAGAT -134
-133 TGTGGATTTCAGTTGGGAGCGCCAGGCTTCACTCCCCGACCTGTAGCAGACATCTCTCCC -74
 -73 TAACCCTCCAGGCAGCCGTCAGCTGTGCCCCTGAAGCCCATAGACTCCACAGACCCCACA -14
 -13 GACCCCACCTGAG ATG TCT TCT TTT GCT TGC TGG AGC CTG
                M   S   S   F   A   C   W   S   L
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Figure 4. Structural organization and 5′ upstream sequence of the mouse ICAM-2 gene. *A*, restriction map of the mouse ICAM-2 gene. Locations of the exons indicated by *filled boxes* are shown relative to the restriction sites (*B*, *Bam*HI; *Bs*, *Bst*XI; *H*, *Hin*dIII; *P*, *Pst*I; *R*, *Eco*RI; *X*, *Xba*I). Introns are indicated by thin lines. *B*, DNA sequence of 5′ upstream region of the mouse ICAM-2 gene. The *thick lines* indicate the potential promoter, the inverted CAAT box, and the consensus transcription initiation sequence of non-TATA genes. The genomic sequence has been submitted to EMBL/GenBank/DDBJ under the accession number X65490.

TABLE I

Exons in the mouse ICAM-2 gene

Exon No.	Domain	Exon Length (bp)	Amino Acids	1st Amino Acid of Exons[a]	Intron Phase[b]
1	5′UT[c] + signal peptide	>209	18	Met (−19)	1
2	1st Ig-like domain	273	91	Gly (−1)	1
3	2nd Ig-like domain	318	108	Gln (+91)	1
4	TM[c] + CD + 3′UT	276	60	Glu (+197)	

[a] Amino acids interrupted by intron sequence were assigned to the exon containing two of the three codon nucleotides of the amino acid (also see Fig. 1).
[b] Phase 1 introns split after the first nucleotide of the codon (43).
[c] UT, untranslated; TM, transmembrane; CD, cytoplasmic domain.

Southern blotting and was confirmed by partial sequence analysis. The number and size of exons, exon/intron boundaries, and positions of the exons were also determined (Fig. 1, Fig. 4*A*, and Table I). The coding sequence of the mouse ICAM-2 gene resides in a 5.0-kb region and consists of four exons. Exon 1 encodes the 5′ untranslated sequence and the signal peptide, exons 2 and 3 encode the first and the second Ig-like domains, and exon 4 encodes the transmembrane and the cytoplasmic domain, and the 3′ untranslated region. All splice junctions occur after the first nucleotide of an amino acid codon (type 1).

We have obtained 661 nucleotides of sequence 5′ of the translational start codon. A TATA-like sequence, TATCT, is present at nucleotide −320 (with respect to the translational start codon), (Fig. 4*B*) followed by a consensus sequence, ATTCTT, 31 nucleotides downstream. The latter sequence is similar to the transcription initiation consensus sequence in a number of housekeeping genes having nontypical TATA promoters with transcription initiating at the adenosine of ATTC (29). In an analysis of 60 promoters of a wide variety of genes, the presence of "C" at the forth nucleotide and "T" at the fifth nucleotide of a TATA-like promoter has been reported to be 3% and 22%, respectively (30). An inverted CAAT box (ATTG) is present at nucleotide −412, which is in the expected range of 70 to 80 nucleotides upstream of the transcription initiation site (30–32). No known transcription factor-binding sites were identified within the promoter region. There are, however, interesting features in this region. In particular, the sequence is well conserved between the mouse and human (69% identity from −335 to −43 with respect to the translational start codon), especially around the presumed TATA-like promoter, the predicted transcription initiation site and the inverted CAAT box. In addition, a direct repeat, GCTTCC, which follows TATCT, is identical in the 5′ upstream sequence of the human ICAM-2 gene (J. Garcia-Aguilar, T. A. Springer, unpublished observations).

Binding of mouse ICAM-2 to human LFA-1. To study the function of mouse ICAM-2, we transfected COS cells with mouse ICAM-2 cDNA, and tested the binding of transfected COS cells to human LFA-1. Mouse ICAM-2 cDNA was cloned into a mammalian expression vector Ap^{r}M8. Because of the lack of an antibody to mouse ICAM-2, surface expression of mouse ICAM-2 on COS cells could not be assessed by flow cytometry. However, in parallel, we transfected COS cells with human and mouse ICAM-1, and human ICAM-2 cDNA. Flow cytometric analysis showed that 30 to 50% of transfected COS cells expressed surface molecules (data not shown).

We measured binding of transfected COS cells to purified LFA-1. An earlier study has demonstrated that human LFA-1 can interact with mouse ICAM-1 and another mouse counter-receptor, possibly mouse ICAM-2 (33); therefore, we tested binding to purified human LFA-1. COS cell transfectants expressing either human or mouse ICAM-1 bound to purified human LFA-1 on plastic (Fig. 5). Similarly, COS cells transfected separately with human ICAM-2 cDNA, mouse ICAM-2 cDNA, or mouse

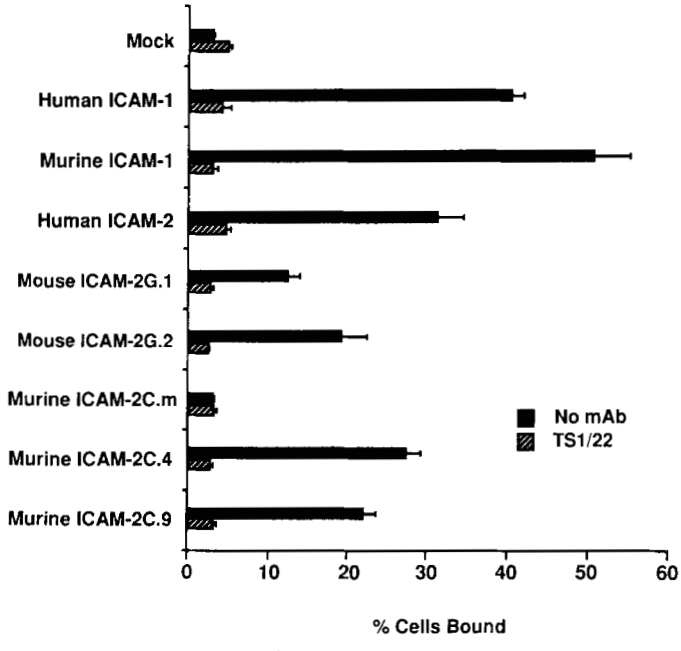

Figure 5. Binding to purified human LFA-1 of COS cells expressing ICAM. ICAM-2C.4 and ICAM-2C.9 indicate two ICAM-2 cDNA clones used in transfection, whereas ICAM-2G.1 and ICAM-2G.2 indicate two genomic clones. COS cells transfected with vector only (*Mock*) or a mutant form of mouse ICAM-2 cDNA (*ICAM-2C.m*) are used as control. mAb TS1/22 to human LFA-1 is used to block binding. Data shown are mean and SD and are representative of three independent experiments.

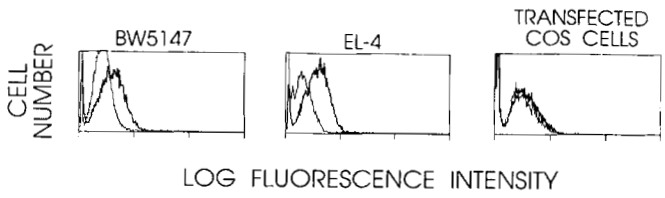

Figure 6. Flow cytometric analysis of thymoma lines, BW5147 and EL4, and COS cells transfected with mouse ICAM-2 cDNA labeled with mAb PA3 (*thick line*) and nonbinding control antibody X63 (*thin line*).

ICAM-2 genomic DNA also bound to human LFA-1. The binding was specific because antibodies to LFA-1 inhibited the binding to control levels. COS cells transfected with vector only, or with a mutated form of mouse ICAM-2 cDNA showed very little binding to purified LFA-1. The mutant ICAM-2 cDNA was cloned from another library and carries a deletion of two nucleotides, A804 and G805, at the splice site between intron 3 and exon 4. This mutation results in a frame shift of the amino acid sequence from the glutamic acid at residue 198, seven amino acids before the transmembrane domain.

Examination of relationship between mAb PA3 and mouse ICAM-2. Golde et al. defined with the PA3 mAb a functionally important molecule on mouse cells with attributes that suggested it could be a ligand for LFA-1 (27). The PA3 mAb recognizes a 55-kDa cell-surface glycoprotein and blocks the LFA-1-dependent T cell response to Ag/MHC presented by B cells and macrophages. The m.w. of mouse PA3 Ag and human ICAM-2 are very similar. We examined the relationship between PA3 and mouse ICAM-2. PA3 mAb did not stain COS cells transfected with mouse ICAM-2 cDNA that in parallel experiments were shown to bind to LFA-1 (Fig. 6). The PA3 mAb was also unable to inhibit binding of COS cells transfected with mouse ICAM-2 cDNA to human LFA-1 (data not shown).

DISCUSSION

We have identified the mouse ICAM-2 cDNA and genomic clone, and characterized the structural organization of the gene. We have also shown that mouse ICAM-2 has an expression pattern similar to human ICAM-2, and when expressed in COS cells can bind to human LFA-1.

The cloning and analysis of the mouse ICAM-2 cDNA demonstrates that there is a striking structural and functional similarity between human and mouse ICAM-2. The overall homology between human and mouse ICAM-2 (60% amino acid identity) is higher than that for ICAM-1 (53% amino acid identity) (34, 35). Interestingly, there is higher homology between human and mouse ICAM-2 in the transmembrane and the cytoplasmic domains (75% amino acid identity) than in the extracellular domain (57% amino acid identity). In contrast, the transmembrane and cytoplasmic domains of human and mouse ICAM-1 are less conserved (49% amino acid identity) in comparison with the extracellular domain (54% amino acid identity). This may suggest a functional importance of the transmembrane and cytoplasmic regions of ICAM-2.

The function of ICAM-2 of binding to LFA-1 is also conserved in the mouse. Our finding that COS cells transfected with either mouse ICAM-1 or ICAM-2 cDNA bind to human LFA-1 confirmed previous observations that mouse ICAM-1 and a second mouse ICAM, either ICAM-2 or ICAM-3, interact with human LFA-1 (33). The fact that both mouse ICAM-1 and ICAM-2 bind to human LFA-1 as do human ICAM-1 and ICAM-2 suggests that the binding site for LFA-1 may be presented in a similar fashion on all four ICAM molecules. It has been demonstrated that a glutamic acid at residue 34 and a glutamine at residue 73 in the first Ig-like domain of human ICAM-1 are required for binding to LFA-1 (13). Interestingly, both these residues are conserved in all four ICAM molecules.

The tissue distribution of murine ICAM-2 as determined by Northern blotting is similar to that of human ICAM-2, which is restricted to leukocytes and endothelium (2, 15). Mouse ICAM-2 is expressed on T lymphoma, macrophage, and mastocytoma cell lines. Among several tissues examined, murine ICAM-2 is most strongly expressed in lung, which is rich in endothelium.

Determination of the genomic structure of mouse ICAM-2 has revealed that mouse ICAM-2 shares many common features with other members of the Ig superfamily. There is a good correlation between exon/intron organization of mouse ICAM-2 and the structural domains of the protein. Like other members of the Ig superfamily, all exons of the mouse ICAM-2 gene are separated by type 1 introns (36). This uniform intron phase implies that exons could be spliced in and out of mRNA without altering the reading frame, and therefore, may be important for gene duplication.

Analysis of the promoter region of the mouse ICAM-2 gene revealed a TATA-like sequence, TATCT, an inverted CAAT box, and a consensus transcription initiation sequence, but no known transcription factor-binding sites. In contrast, transcription factor NF-κB and AP1-binding sites have been identified in the 5′ upstream region of the ICAM-1 gene (37). Because ICAM-2 expression on the cell surface is not inducible, it is not surprising that the mouse ICAM-2 gene lacks transcription factor NF-κB or AP1 sites that have been shown to be important in inducible genes (37–41).

Mouse ICAM-2 appears to be distinct from the Ag defined by the mAb PA3. This is an important distinction, because the PA3 Ag has properties expected of a ligand for LFA-1 (27), and in a recent publication (42) had been assumed to be mouse ICAM-2. PA3 antibody failed to interact with mouse ICAM-2 transfectants, even though it interacted with surface molecules on two mouse thymoma lines (Fig. 6) and on the mastocytoma line P815 (not shown). The distribution of murine ICAM-2 mRNA and human ICAM-2 Ag also supports a distinction from PA3 Ag. The 55-kDa membrane protein that PA3 recognizes was found not only on T cells and B cells, but also on three different fibroblast lines (27). In contrast, we did not detect any mouse ICAM-2 mRNA expression in mouse fibroblasts in Northern blots. Furthermore, human ICAM-2 is expressed on lymphocytes and endothelial cells but is absent from transformed fibroblasts and from fibroblasts in a variety of tissues (15). A further distinction is that mitogens stimulate increased expression of PA3 Ag on mouse T cells, but not ICAM-2 on human T cells (15, 27).

In conclusion, we have isolated mouse ICAM-2 cDNA and genomic clones. The authenticity of mouse ICAM-2 cDNA is exhibited by 1) nucleotide and amino acid sequence homology to human ICAM-2, 2) a similar expression pattern to that of human ICAM-2, and 3) binding of

COS cells transfected with the cDNA to human LFA-1. Therefore, mouse ICAM-2 is both structurally and functionally conserved across species. Further studies on murine ICAM-2 may illuminate its function in vivo.

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