Vascular Endothelial Junction-associated Molecule, a Novel Member of the Immunoglobulin Superfamily, Is Localized to Intercellular Boundaries of Endothelial Cells*

Received for publication, April 13, 2000  
Published, JBC Papers in Press, April 21, 2000, DOI 10.1074/jbc.M003189200

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During the process of lymphocyte homing to secondary lymphoid organs, such as lymph nodes and tonsils, lymphocytes interact with and cross a specialized microvasculature, known as high endothelial venules. There is a great deal of information available about the first steps in the homing cascade, but molecular understanding of lymphocyte transmigration through the intercellular junctions of high endothelial venules is lacking. In analyzing expressed sequence tags from a cDNA library prepared from human tonsillar high endothelial cells, we have identified a cDNA encoding a novel member of the immunoglobulin superfamily. The protein, which we have termed VE-JAM (“vascular endothelial junction-associated molecule”), contains two extracellular immunoglobulin-like domains, a transmembrane domain, and a relatively short cytoplasmic tail. VE-JAM is prominently expressed on high endothelial venules but is also present on the endothelia of other vessels. Strikingly, it is highly localized to the intercellular boundaries of high endothelial cells. VE-JAM is most homologous to a recently identified molecule known as Junctional Adhesion Molecule, which is concentrated at the intercellular boundaries of both epithelial and endothelial cells. Because the Junctional Adhesion Molecule has been strongly implicated in the processes of neutrophil and monocyte transendothelial migration, an analogous function of VE-JAM during lymphocyte homing is plausible.

The endothelial lining of blood vessels serves as a critical interface between blood and tissues, regulating processes such as vascular permeability, blood flow, thrombogenesis, hematogenous metastasis, and leukocyte extravasation (reviewed in Ref. 1). Although all vascular endothelial cells perform certain functions in common, there is a remarkable diversity of specialized functions that depend on the class of the blood vessel and the requirements of the underlying tissue. In many instances, our understanding of these diverse functions is at the phenomenological level without detailed molecular explanations.

One specialized microvasculature that has received a lot of attention are the postcapillary high endothelial venules (HEV) of secondary lymphoid organs (reviewed in Ref. 2). These vessels, which are lined by morphologically distinctive high endothelial cells (HEC), serve as the portal of entry of blood-borne lymphocytes into secondary lymphoid organs, such as lymph nodes and tonsils, allowing lymphocytes of appropriate specificity to respond to sequestered and processed antigens within the organ. The highly selective recruitment process, termed “lymphocyte homing” involves a cascade of steps: rolling of the lymphocyte along the HEV, arrest and flattening on the endothelium, and finally transmigration across the endothelium (reviewed in Refs. 3 and 4). There has been a great deal of progress in our molecular understanding of the first two steps in the homing cascade, i.e. in the identification of primary and secondary adhesion molecules and the elucidation of signaling mechanisms involved in integrin activation (reviewed in Ref. 5). However, considerably less information is available about the molecular basis of lymphocyte transmigration across the HEV.

The capability to isolate primary HEC has opened up new approaches to explore the specialized characteristics of these endothelial cells (6–9). We have taken a gene profile approach by constructing a cDNA library from primary human tonsillar HEC and performing EST sequencing. Several of the sequences that we identified corresponded to a novel member of the immunoglobulin superfamily (IgSF). The encoded protein, which is termed VE-JAM, is prominently expressed on HEC of tonsils but is also present on the endothelium of other vessels. It is most closely related to another member of the IgSF, i.e. JAM, which has been implicated in the recruitment of monocytes and neutrophils across endothelium (10–14).

**EXPERIMENTAL PROCEDURES**

Construction and Sequencing of HEC PCR Select Library—HEC were isolated from surgical specimens of tonsils, and the cDNA library (in pCDNA1.1) was prepared as described previously (15). In parallel, an aliquot of the same HEC cDNA was employed to generate a PCR select library (16). The HEC cDNA was cut into fragments that averaged 400 bp by digestion with RsaI. Subtraction was performed with cDNA fragments (also RsaI-digested) obtained from human umbilical vein

1 The abbreviations used are: HEV, high endothelial venule(s); HEC, high endothelial cell(s); EST, expressed sequence tag; IgSF, immunoglobulin superfamily; VE-JAM, vascular endothelial junction-associated molecule; PCR, polymerase chain reaction; bp, base pairs; PBL, peripheral blood lymphocytes; CHO, Chinese hamster ovary cell; PBS, phosphate-buffered saline; GST, glutathione S-transferase; JAM, junctional adhesion molecule; PECAM-1, platelet endothelial cell adhesion molecule-1.
endothelial cells (HUVEC) and peripheral blood lymphocytes. The sub-
tracted cDNA was amplified by PCR and cloned into pCR-II (Invitrogen,
Carlsbad, CA) to generate the cDNA library. A total of 1049 inserts
were sequenced.

Reverse Transcriptase PCR—Peripheral blood lymphocytes (PBL)
(10⁵ cells) were isolated as published previously (17). Frozen purified
HEC (10⁶ cells) were solubilized in RNAzol B (Teltest Inc., Friendswood,
TX). Total RNA was isolated as per the manufacturer’s instructions.
First strand cDNA was prepared from 2 μg of RNA primed with random
hexamers using avian myeloblastosis virus reverse transcriptase (Life
Technologies, Inc.). A 539-bp VE-JAM cDNA fragment was amplified
from the cDNA using primers: 5'-GGCTCTTTTCTGCTGCTTCTG
and 3'-TTGAGTTTTATAAGTATTAATTGGT, and the hypoxa-
thine phosphoribosyltransferase primers amplified the appropriate
289-bp fragment from both HEC and PBL (18).

Cloning of VE-JAM—A pool selection technique (15) was employed.
Previously mentioned primers were designed to amplify 539 bp of the
insert of EST clone 1293, which contains sequence toward the 3'-end of
the VE-JAM cDNA (Fig. 1). To favor the identification of a full-length
clone, the individual pools were tested in a PCR reaction using a
5'-primer matching the vector and a 3'-primer matching VE-JAM.
Southern blotting was performed on the PCR reactions to identify
the pools with the longest inserts. The isolated clone was sequenced in both
directions.

Northern Blot Analysis—The probe consisted of the entire insert
of EST clone 1293 (Fig. 1). The probe was labeled with 32P[ATP
(Amersham Pharmacia Biotech) using the Strip-EZ DNA kit (Ambion, Austin,
TX). Multiple tissue and immune system tissue Northern blots (CLON-
TECH, Palo Alto, CA) containing human poly(A)+ mRNA were hybrid-
ized at 60 °C overnight after prehybridization for 2 h in ExpressHyb
hybridization solution (CLONTECH). The blots were then washed twice
in 2× SSC, 0.1% SDS for 15 min at room temperature before autorad-
igraphy. These blots were then stripped and rehybridized with a
β-actin probe that was supplied with the blots.

Flow Cytometry—Chinese hamster ovary (CHO) cells were grown to
80% confluency in 100-cm² flasks and transfected with the empty vector
or the VE-JAM plasmid (5 μg/flask) using LipofectAMINE (Life Tech-
nologies, Inc., 48 h after transfection, the cells were harvested for
4 mM EDTA in PBS without calcium or magnesium and washed once in
0.2% bovine serum albumin. After incubation with fluorescein isothiocyanate
goat-anti-rabbit secondary antibodies (Zymed Laboratories Inc., South
Carlsbad, CA) for 30 min on ice and washing three times with cold PBS
0.2% bovine serum albumin. After incubation with fluorescein isothiocyanate
 goat-anti-rabbit secondary antibodies (Zymed Laboratories Inc., South
San Francisco, CA) for 30 min on ice and washing, flow cytometry was
performed. Peripheral blood was obtained by venipuncture and stained
as above. Leukocyte populations were identified by their forward and
side scatter characteristics. Platelets were identified with an antibody
to aIbß3 (Ib-1, Immunotech, Westbrook, ME). Staining was analyzed
using CellQuest (Becton-Dickinson, Franklin Lakes, NJ).

Protein Purification—A cDNA for the extracellular domain of VE-
JAM (1–717 bp) was cloned into IgG-pEFS vector, which encodes the Fe region of human IgG1 (18). The expression
plasmid containing the VE-JAM/Ig chimera was transfected into COS
cells using serum-free media. After 5 days, the medium was collected.
25 mM Tris-HCl, pH 8.0 (Bio-Rad), was added to the supernatant, which
was agitated overnight at 4 °C with protein A beads (Repligen, Need-
ham, MA). The following day, the beads were poured into a column
equilibrated in PBS. The protein was eluted with 100 mM triethylamine
(pH 11.0) into a tube containing 1/10 the final volume of 3 M Tris-HCl,
8.6. The eluate was concentrated with a Centricon30 (Amicon,
Beverly, MA) and equilibrated with PBS.

A glutathione S-transferase (GST) fusion protein was prepared by
inserting a cDNA of the extracellular domain of VE-JAM (1–717 bp)
into a modified PEAK 10 vector (Edge Biosystems, Gaithersburg, MD),
which encodes a portion of GST (bases 258–917) (Amersham Pharmacia
Biotech) (18). The VE-JAM/GST fusion protein was produced in
PEAK20™ cells (Edge Biosystems) as recommended by the manufac-
turer. The fusion protein in the conditioned medium was purified with
thiogluthione-agarose (Sigma) by overnight incubation at 4 °C. The beads
were washed into a column with PBS, washed with 10 mM reduced
glutathione (pH 8.0) (Sigma) in PBS, the eluate was concen-
trated with a Centricron30 (Amicon) and equilibrated with PBS.

Antibodies—Polyclonal antibodies were produced by Research Ge-
etics (Huntsville, AL) where 800 μg of VE-JAM/GST fusion protein
was used to immunize two rabbits. Antibody titer was determined by
enzyme-linked immunosorbent assay using VE-JAM/Ig fusion protein
as the antigen (19). Antibodies were affinity purified by chromatogra-
phy on a column of VE-JAM-Ig fusion protein coupled to cyanogen
bromide-activated Sepharose (Sigma) according to standard procedures
(19). Anti-VE-JAM antibody was biotinylated using EZ-link-Sulfo Link
(Pierce) according to the manufacturer’s instructions.

Peptide Microsequencing—10 μg of purified VE-JAM/GST fusion pro-
tein was subjected to SDS-PAGE and stained with Coomassie Brilliant
Blue R-250 (BDH Chemicals Ltd., Poole, United Kingdom), was excised
and subjected to Edman degradation analysis.

Immunohistochemistry—Human tonsil, foreskin, lung, placenta, and
heart were isolated from a newborn infant. Tissue sections were:
fixed in 100% ethanol for 20 min. Sections were blocked with PBS
containing 5% normal goat serum (or 0.2% bovine serum albumin
for placenta and rat heart only). Rabbit polyclonal VE-JAM and anti-CD31
(monoconal antibody 2148, Chemicon, Temecula, CA), rabbit poly-
clonal von Willebrand factor (DAKO, Denmark), and MECA-79 were
used at 0.3, 1.0, and 1.0 μg/ml, respectively. After staining for 1 h at room
temperature, sections were washed two times in PBS. For immunoflu-
orescence, bound antibodies were detected with Cy 3-conjugated goat
anti-rabbit IgG or Cy 2-conjugated goat anti-mouse IgG (1:1000) (Jack-
son Immunoresearch, Westgrove, PA). For immunocytochemistry, bi-
otinylated goat anti-rabbit IgG (Vector, Burlingame, CA) was added to
detect bound rabbit antibodies. This was followed by addition of ELITE
ABC (Vector) mixture, using Nova Red (Vector) as the substrate. Nor-
mal rabbit IgG (Caltag, Burlingame, CA) or mouse IgG1 (Zymed Lab-
oratories Inc.) was used as control.

Human Tonsillar Stromal Preparation—Tonsillar stroma was pre-
pared as described by Baekkevold et al. (7) with a few modifications.
Briefly, surgical specimens were stored for less than 4 h in sterile saline
at 4 °C. Dissection was achieved with Cliniscrub solution (Clinipad
Corp., Rocky Hill, CT) diluted 1/50 in PBS. Tonsils were finely chopped
on a razor blade, and the resulting mince was washed over a cell strainer
by washing with PBS using a Falcon 70-μm cell strainer (Becton Dick-
inson, Franklin Lakes, NJ). The remaining tissue was digested for
15 min at 37 °C in RPMI 1640 medium (Fischer, Pittsburgh, PA) contain-
ing 1 mg/ml collagenase A (Roche Molecular Biochemicals), 1 mg/ml
-dispase (Roche Molecular Biochemicals), and 0.04 mg/ml DNase I
(Roche Molecular Biochemicals). After free lymphocytes were removed
the remaining stroma was washed once with cold PBS and further
lymphocytes were detected with Cy 3-conjugated goat anti-rabbit IgG
by washing with PBS using a Falcon 70-μm cell strainer (Becton Dick-
inson, Franklin Lakes, NJ). The remaining tissue was digested for
15 min at 37 °C in RPMI 1640 medium (Fischer, Pittsburgh, PA) contain-
ing 1 mg/ml collagenase A (Roche Molecular Biochemicals), 1 mg/ml
-dispase (Roche Molecular Biochemicals), and 0.04 mg/ml DNase I
(Roche Molecular Biochemicals). After free lymphocytes were removed
the residual stromal components were digested for an additional 60 min at 37 °C
with fresh enzyme solution. The digested stroma was washed once with RPMI 1640 and plated onto
Vitrogen (Celonix Laboratories, Palo Alto, CA) coated glass slides. Non-
adherent cells were washed away after 90 min, and adherent cells
were cultured in EBM-MV medium (Clonetics, Walkersville, MD).

Immunoprecipitation from Human Tonsil Lysates—A frozen human
tonsil was homogenized in 25 ml of PBS plus 2% Triton X-100 (Roche Molecular Biochemicals),
5 mM EDTA plus 1X Complete™ protease inhibitors (Roche Molecular
Biochemicals). The lysates were spun at 20,000 × g for 20 min at 4 °C.
The supernatants were collected and frozen in 1-ml aliquots. 1 μg
of VE-JAM antibody was added to 1 ml of tonsil lysate and rocked
overnight at 4 °C. 10 μl of protein A beads were added and incubated
for 1 h at 4 °C. The protein A beads (Repligen) were then spun down
and washed five times with PBS. The beads were boiled in 20 μl of 6X
SDS loading buffer and run on 10% SDS-polyacrylamide gel electrophoresis.
The separated proteins were transferred to a Pro-Blott membrane and
probed with 1 μg/ml biotinylated anti-VE-JAM in 3% bovine serum
albumin in PBS for 1 h at room temperature. The blot was incubated
with horseradish peroxidase-conjugated streptavidin (Caltag) for 1/2 h
with detection of specific bands by the ECL method (Amersham Pharmacia
Biotech).

RESULTS
To identify genes that are selectively expressed in HEC, we
prepared a PCR select library (16) from HEC cDNA, which was
subtracted with HUVEC and PBL cDNAs. This approach utilizes
relatively small cDNA fragments (400–600 bp) that are generated with
the RNAi restriction enzyme. We subjected the library to an
EST analysis. A full description of this library and our analysis of
ESTs will be the subject of a future communication.
Of the novel cDNAs present within the library, one appeared five times as partial sequences. A full-length clone was obtained from an unsubtracted HEC cDNA expression library (15) using a pool selection technique in conjunction with PCR (15). The full-length cDNA (Fig. 1) contains a single open reading frame, which is predicted to encode a protein of 298 amino acids followed by a 39 untranslated region of 72 bp and a poly(A) tail. The start codon satisfies the criteria for a strong context for translation initiation (20). A cleavable signal peptide of 28 amino acids was predicted using SignalP (21), which was verified by amino-terminal sequencing of a GST fusion protein expressed in PEAKRapid cells. Overall the cDNA is predicted to encode a type I transmembrane protein with an extracellular portion of 237 amino acids, a transmembrane domain of 23 amino acids, and a cytoplasmic tail of 38 amino acids (GenBank™ accession number AF255910). The protein is hereafter referred to as VE-JAM based on the characterization provided below.

The extracellular region of VE-JAM is comprised of two IgSF domains, a membrane distal V-set domain, and a membrane proximal C2-type domain. It resembles an extensive list of cell surface antigens with two tandem IgSF domains (see “Discussion” below). A subset of these molecules (i.e., ChT1, HCAR, and A33 antigen) contains two additional, similarly spaced cysteines in their C2 type domains, in addition to the conserved pair of cysteines that is characteristic of most IgSF domains (22–24). VE-JAM exhibits these extra cysteines with the identical spacing found in these other proteins (Fig. 2).

Using the human sequence VE-JAM cDNA sequence as a probe, we identified a homologous mouse sequence in the NCBI dbEST data base. When the clone containing the EST was retrieved and fully sequenced, we obtained a full-length cDNA (GenBank™ mEST AI154320) (Fig. 1). The sequence predicts a protein of 298 amino acids, which is 80% identical to human VE-JAM and exhibits the same general features; therefore, this protein is very likely to be the murine ortholog of VE-JAM.
Sequence comparisons with the GenBank™ NR data base revealed that VE-JAM is most homologous to JAM (Fig. 2), a recently identified protein that is localized to intercellular boundaries of epithelial and endothelial cells and that is strongly implicated in transendothelial migration of myeloid cells (10–14). Homology between VE-JAM and JAM is seen throughout the predicted proteins with an overall identity of 34%. Two stretches of 9 and 11 amino acids in the second IgSF domains are identical. In addition, the potential glycosylation sites in the second IgSF domain of VE-JAM are conserved in mouse and human JAM. However, the extra pair of cysteines is not present in either mouse or human JAM (Fig. 2B).

A Northern analysis on a multitissue blot (human) was carried out with a 509-bp probe corresponding to the 3’-end of the VE-JAM cDNA (Fig. 3A). Three major species of ~1.2, 1.4, and 4.4 kilobases were detected in several tissues, the smallest of which is consistent with the minimum expected message size. Variable expression in different tissues was observed with highest expression in the heart followed by the placenta and lymph node.

Because cDNAs corresponding to VE-JAM were initially detected in a library derived from HEC mRNA, we wished to confirm the presence of transcripts in HEC. As shown in Fig. 3B, VE-JAM-specific primers amplified a band in a PCR reaction using cDNA made from an independent preparation of HEC. There was no specific band when reverse transcriptase was not included in the preparation of template, proving that RNA, and not genomic DNA, was responsible for the amplification. There was no detectable signal from PBL cDNA.

To study VE-JAM at the protein level, we prepared a rabbit polyclonal antibody to the ectodomain of the protein. A GST fusion protein containing the entire extracellular domain of the human VE-JAM was expressed in mammalian cells, purified on a glutathione-agarose column, and used to immunize rabbits. Specific antibodies were affinity purified on a column of an immunoglobulin chimera of VE-JAM, which contained the extracellular domain of VE-JAM fused to the Fc portion of IgG1. The purified antibodies stained CHO cells that were transfected with the VE-JAM cDNA but not the parental cells (Fig. 3A). Normal IgG was negative on both populations of cells. CHO cells transfected with a cDNA encoding human JAM were not stained by the VE-JAM antibody, establishing that the antibody was not cross-reactive to the related protein (data not shown).

To visualize the protein encoded by the VE-JAM cDNA, we carried out a Western blot analysis on VE-JAM-transfected CHO cells. A single band of ~40 kDa was detected in lysates of transfected cells but not in the parental cells (Fig. 3B). Normal rabbit IgG did not react with any bands. The same molecular weight was observed when VE-JAM was labeled at the cell surface by surface biotinylation (not shown). The unmodified mature protein is predicted to have a molecular mass of 33 kDa. The extra mass is likely attributable to glycosylation at the potential sites for N-linked modifications.

To determine the cellular expression pattern of VE-JAM, we carried out immunocytochemistry on cryostat sections of sev-
veral human tissues with the polyclonal VE-JAM antibody. Staining of human tonsil sections demonstrated the VE-JAM was strongly expressed in HEV (Fig. 5, A and B), consistent with the occurrence of ESTs in the HEC cDNA library and the reverse transcriptase-PCR experiments. Specificity was established by demonstrating that all reactivity was blocked when the antibody was mixed with purified VE-JAM-IgG (not shown). In human and rat heart, staining with the VE-JAM antibody was observed on the endothelium of endocardium, arteries, venules, and capillaries (Fig. 5, C and D). Co-staining for PECAM-1 or von Willebrand factor, widely used markers for vascular endothelium (reviewed in Refs. 25 and 26), verified that VE-JAM was present on the endothelial lining of endocardium and the vessels (not shown). A survey of other human tissues (lung, placenta, and foreskin) established staining on the endothelia of both large and small vessels. Interestingly, VE-JAM was not detected in oral epithelium, epidermis of skin, respiratory epithelium of bronchus (not shown), or in mesothelium of the pericardium (Fig. 5C). This result is in contrast to the expression pattern of JAM, which is broadly distributed on both simple and stratified epithelia, as well as vascular endothelium (10, 11). Additionally in flow cytometry experiments, VE-JAM was not detected on the surface of platelets or leukocytes isolated from blood (Table I). This latter finding was consistent with the failure to detect VE-JAM mRNA in RNA of PBL (Fig. 5A) and the absence of staining in T- and B-cell zones of tonsils (Fig. 5, A and B).

In many of the HEV, VE-JAM appeared to be concentrated at lateral boundaries of HEC (Fig. 5B). To study the distribution of VE-JAM on these cells in more detail, we isolated tonsillar stroma and placed it in tissue culture. During the first several days of culture, distinct clusters of endothelial cells were evident, as indicated by staining with PECAM-1 (Fig. 5, A and B).

To characterize the naturally occurring form of VE-JAM, we took advantage of its strong expression in the HEV of tonsils. We immunoprecipitated a detergent lysate with the VE-JAM antibody and then performed Western blotting on the precipitate with a biotinylated version of the same antibody (Fig. 6).
Identification of VE-JAM

single band was visualized, which ran at ~40 kDa under both reducing and nonreducing conditions. This result argues that VE-JAM is unlikely to use the extra pair of cysteines in its C2-type IgSF domain for interchain disulfide bonds.

The chromosomal location of the VE-JAM gene was determined by searching for human genomic clones in the NCBI GenBank™ NR data base. The VE-JAM cDNA sequence was distributed across several genomic clones (AP000223, AP000225, and AP000226), which are tandemly arrayed along the long arm of chromosome 21 (21q21.2). Only two differences were noted between the genomic sequences and our cDNA, neither of which affects the amino acid sequence of the mature protein. It should be noted that mutations, deletions, and duplications in the long arm of chromosome 21 are associated with a number of disorders including Usher syndrome, Down syndrome, and Alzheimer’s disease. (28–30).

DISCUSSION

Taking advantage of procedures for the isolation of primary HEC from secondary lymphoid organs, a number of recent efforts have been directed at the identification of genes that are differentially expressed in HEC (6, 8, 9). Our approach has been to apply EST sequencing to a subtracted tonsillar cDNA library. We identified sequences corresponding to a novel member of IgSF, for which we obtained a full-length cDNA in both mouse and human. Because of its highly restricted expression in vascular endothelium and its association with intercellular boundaries, we have termed the molecule encoded by this cDNA as VE-JAM for vascular endothelial junction-associated molecule.

VE-JAM is predicted to be a type I integral membrane protein with two tandem extracellular IgSF domains of the V-set (membrane distal) and C2-set (membrane proximal). This organization is characteristic of the CD2 subgroup (CD2, CD58, CD48, 2B4, and CDw150) and as well as other members of the IgSF (CD80, CD86, OX-2, A-33, HCAR, and ChT1) (22–24, 31). Interestingly, many of these have been implicated as mediators of cell-cell interactions, generally through heterophilic interactions with other IgSF members (31, 32). A further feature of VE-JAM, shared by only three of these proteins (A-33, HCAR, and ChT1), is an extra pair of cysteines separated by the same spacing in the C2 domain (22–24).

Consistent with the occurrence of VE-JAM ESTs in the HEC cDNA library, reverse transcriptase-PCR analysis detected VE-JAM transcripts in RNA prepared from independently isolated HEC. Moreover, our immunohistochemical and immunoprecipitation experiments confirmed the expression of VE-JAM at the protein level in HEC. Staining of primary cultures of HEC and CHO cell transfectants established that VE-JAM was expressed at the cell surface.

Although VE-JAM was highly expressed in HEC, it was not restricted to this site, as first indicated by the presence of transcripts in a variety of nonlymphoid organs, but most conspicuously in heart and placenta. Immunocytochemistry indicated the presence of VE-JAM in endothelium of blood vessels in human and rat heart, human placenta, lung, and foreskin. Staining was observed in examples of capillaries, venules, and large vessels. Further studies are needed to determine whether there is variation in the expression of the protein in different vascular beds under basal conditions and whether there might be modulation of expression during inflammatory reactions.

VE-JAM is most homologous to JAM, a member of the IgSF

TABLE I

| Blood cell | VE-JAM | Normal IgG |
|------------|--------|------------|
| Granulocytes | 15.2   | 10.3       |
| Monocytes | 4.0    | 3.3        |
| Platelets | 22.0   | 10.2       |
| Lymphocytes | 2.2    | 2.1        |

FIG. 5. Expression of VE-JAM on endothelium. Frozen sections (10 µm) were made from human tonsils (A and B) and rat heart (C and D). Total stroma was isolated from fresh surgical specimens of human tonsils (E and F). A and B, staining for VE-JAM by immunoperoxidase histochemistry; C, D, and F, staining for VE-JAM and (E) PECAM-1 by immunofluorescence. The bar in each panel represents 20 µm. In C, the large vessel at the upper left is an artery, and the vessel at the lower right is a vein. The arrows in C indicate the mesothelium on the outer surface of the pericardium (not stained). In D, the arrow indicates the endothelial lining of endocardium, and the arrowhead indicates one example of a capillary. The arrow in F points to a free boundary of the cluster of endothelial cells.

Fig. 6. Visualization of VE-JAM from human tonsils. A detergent lysate of tonsils was reacted with VE-JAM antibody or normal rabbit IgG. The precipitates were electrophoresed on a 10% SDS gel, with or without reduction, and Western blotted with biotinylated VE-JAM antibody as the probe. Without reduction, the apparent molecular weight of VE-JAM was less than with reduction, consistent with the presence of disulfide bonds.

A sample of blood was stained with VE-JAM antibody or normal rabbit IgG and analyzed by flow cytometry as described under “Experimental Procedures.” Values for the mean fluorescent intensity are indicated. The slight signal seen with platelets was not observed in an independent experiment with purified cells.
containing two V-set domains. The proteins, which are virtually the same length (298 or 299 amino acids), show the same basic organization with homology from the extracellular domains through the cytoplasmic tails. Like VE-JAM, JAM is expressed on endothelial cells of many blood vessels (10). However, JAM is also distributed on a wide variety of epithelia. Although our limited survey of human tissues cannot exclude the possibility that VE-JAM may be present on some epithelia, we failed to detect VE-JAM on several examples of epithelial cells (simple and stratified) where strong JAM expression has been documented (10). Furthermore, whereas JAM is on platelets (10, 11), we did not detect VE-JAM on these cells or any other blood cells.

A striking feature of JAM is that it is concentrated at intercellular boundaries of endothelial monolayers (9, 10). A number of other molecules, including PECAM-1, and various proteins associated with tight and adherens junctions also exhibit this distribution (reviewed in Ref. 33). Dejana and co-workers (10, 13) have shown that an antibody to mouse JAM can block transendothelial migration of monocytes both in vitro and in vivo. Both spontaneous and chemokine-induced transendothelial migration were found to be inhibited. Importantly, the antibody did not block adhesion of monocytes to cultured endothelium but prevented their transit across the endothelium, indicating the involvement of JAM in the transmigration process (10). A more recent study showed that administration of the antibody in a mouse experimental meningitis model attenuated monocyte and neutrophil recruitment into both cerebrospinal fluid and the brain parenchyma (13). The inhibitory effects of the JAM antibody on transmigration were incomplete, suggesting the involvement of other endothelial molecules in the process. PECAM-1, also concentrated at intercellular boundaries and implicated in the transmigration of myeloid cells across endothelia (34, 35), is an obvious candidate for collaboration with the JAM system.

As reviewed in the Introduction, HEV are specialized for recruitment of lymphocytes into secondary lymphoid organs. Although many of the early events associated with lymphocyte rolling and arrest on HEV have been elucidated at the molecular level, the process of transmigration is poorly understood. JAM is expressed on these vessels but at a relatively low level (10). The prominent expression of VE-JAM in HEV and its distribution at interendothelial boundaries are, therefore, very intriguing findings. Given the many parallels between VE-JAM and JAM, future experiments should be directed at the investigation of the potential role of VE-JAM in lymphocyte transmigration across HEV.

Acknowledgments—We thank Mark Singer for his assistance with histology. We thank Jin Kyu Lee for assistance with the Northern analysis. Useful advice was provided by Chris Sassetti, Mark Singer, Yan Zhou, and the Rosen laboratory members in many technical aspects of the experiment. We are also grateful to Rebecca Newton, Jeffrey Golden, Susan Fisher, Vedang Londhe, Phil Ursel, and Jerome Hester for assistance in obtaining the human and rat tissues. We would also like to thank Dr. David Simmons for the plg1 plasmid.

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