We have identified a third member of the junctional adhesion molecule (JAM) family. At the protein level JAM3 displays 36 and 32% identity to JAM2 and JAM1, respectively. The coding region is distributed over 9 exons and maps to chromosome 11q25. The gene shows widespread tissue expression with higher levels apparent in the kidney, brain, and placenta. At the cellular level we show expression of JAM3 transcript within endothelial cells. Our major finding is that JAM3 and JAM2 are binding partners. Thus, JAM3 ectodomain binds firmly to JAM2-Fc. This heterotypic interaction is maintained when JAM3-Fc is used to capture Chinese hamster ovary cells expressing full-length JAM2. In static adhesion assays we show that JAM3 is unable to bind to leukocyte cell lines. This is consistent with the lack of JAM2 expression. However, using JAM2-Fc pull-down experiments in combination with polyclonal anti-JAM3 serum, we demonstrate that JAM3 is the previously uncharacterized 43-kDa counter-receptor that mediates JAM2 adhesion to T cells. Most significantly we demonstrate up-regulation of JAM3 protein on peripheral blood lymphocytes following activation. Finally we show the utility of JAM3 ectodomain as an inhibitor of JAM2 adhesion.

Tight junctions, gap junctions, and adherens junctions contribute to the barrier and communication properties of the vasculature (1). During development, different vascular beds acquire specialized features in accord with organ function (2). Heterogeneity extends to the type and extent of intercellular contacts that form between opposing endothelial cells (3, 4). This results in varying permeabilities throughout the vascular tree. For example, the blood-brain barrier shows high electrical resistance and impedes penetration of proteins, peptides, and drugs from the vasculature into the brain parenchyma (5). This property contrasts with that of the fenestrated endothelia found for example in the kidney peritubular capillaries and ascending vasa recta, which presents a semipermeable barrier (6).

In addition to regulation of solute permeability, intercellular contacts serve to impede leukocyte egress. However, during inflammation leukocytes must pass between endothelial cells to reach the interstitial space. Selective recruitment of leukocytes is achieved by differential expression/up-regulation of adhesion molecules within the microvasculature (7). Similarly, regional expression of homing receptors facilitates lymphocyte targeting to lymphoid organs (8). Adhesion proteins targeted to cell-cell borders are ideally situated to participate in leukocyte emigration. The role of PECAM in this regard is well established (9). More recently the novel junctional adhesion protein (JAM)1 has emerged as a player in this arena. Conceivably, differential expression of intercellular adhesion proteins could act as a filter to regulate leukocyte transmigration.

Partial amino acid sequencing of human junctional adhesion molecule 1 was reported as early as 1995 where it was described as a 32–35-kDa membrane receptor reactive with mAb F11 in platelets (10). Indeed, the ability of this antibody to induce vesicular secretion and aggregation in human platelets was recorded even earlier (11). However, it was not until 1998 that the complete sequence of a mouse homologue was characterized (12, 13). The term junctional adhesion molecule (JAM) stemmed from its localization at tight junctions in endothelial and epithelial cells (12). Participation of JAM1 in the inflammatory process was revealed by the ability of a neutralizing monoclonal antibody to modulate monocyte transmigration through the vessel wall (12). Following this observation, multiple reports detailed the isolation and sequencing of the human JAM1 cDNA (14–18). Presently, JAM1 is proposed to play roles in paracellular permeability, inflammation, and platelet physiology (10–12, 14, 15, 17–21). Most recently it has been described as a receptor for reovirus (22).

In 2000 another member to the junctional adhesion molecule family was described by our group (JAM2, Ref. 23) and Palmeri et al. (VE-JAM; Ref. 24). Northern analysis shows that JAM2 is preferentially expressed in the heart. However, low levels of transcript are detectable within many tissues: a pattern indicative of endothelial expression. Indeed immunostaining confirms this prediction. JAM2 is detected in both large and small vessels of various tissues in addition to high endothelial venules (24). The enhanced expression within the microvasculature of the heart reflects endothelial heterogeneity. Similar to JAM1, immunolocalization defines JAM2 as a protein that is targeted to cell-cell borders (23, 24). It remains to be determined whether JAM2 associates with the tight junctional complex. Evidence that JAM2 functions as an adhesion protein is provided by capture of human T cell lines by JAM2 ectodomain. This property is mediated, at least in part by an uncharacterized 43-kDa cell surface protein (23).

In this report we describe the isolation and sequencing of yet another member to the junctional adhesion molecule family, namely JAM3. We provide conclusive evidence that JAM3 is
The uncharacterized 43-kDa counter-receptor on HS B cells that mediates JAM2 interactions.

**EXPERIMENTAL PROCEDURES**

**cDNA Cloning**—The human JAM2 sequence (accession number AF162934, H71948, BE873472, BE873711, encoding an incomplete JAM3 molecule, were assembled to form the ecto- and transmembrane domains. The final data were normalized between the three genes.

**JAM3 (sense) 5'-CTGATCATCAACAGGATGAC-3' and coupled with forward primer, 5'-ATGGCGCTGAGGGCCGACG-3', for amplification of the full open reading frame. Human fetal brain mRNA (CLONTECH, Palo Alto, CA) was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Life Technology Inc.) and amplified using Pfu DNA polymerase (Stratagene, La Jolla, CA) by cycling 30 times. The first 17 bp (TTCAAGAAGCCAGGGA) of the assembled sequence at the 3' end was mapped to contig 49048-57168 within GenBank™ accession number AP001775. To complete the intracellular domain, this contig was translated in all three reading frames and the sequence penultimate to all stop codons examined for putative PKD binding motifs. From this analysis, a candidate sequence encoding the C-terminus of the JAM3 protein was identified. A reverse oligonucleotide, was designed, 5'-TCTGATCATCAACAGGATGAC-3' and reverse 5'-TGAGGCTGTTGGTGCTTTGG-3' served as templates for PCR amplification using the Stratagene Primers. Amplicons were sequenced with the following combination of primers: JAM1 forward primer, 5'-TGAGATGGCA-3'; JAM2 reverse, 5'-GATATCAATATGGCGCTGAGGGCCGACG-3' and reverse 5'-6-FAM/3'-TCCCGCCCTCTCTGTCATG-3'.

**Chromosomal Localization, Intron/Exon Structure**—The complete human JAM3 open reading frame was used to retrieve genomic data from the hts database using the Blast tool. The cDNA for JAM1 was processed similarly. The structure of the JAM2 gene had been previously determined (25). The size of the introns and exons were compared between the three genes.

**Northern Blot Analysis**—A 591-bp human JAM3 probe was amplified with Takara Ex Taq™ DNA Polymerase using forward 5'-ATGGCGCTGAGGGCCGACG-3' and reverse 5'-TCTGATCATCAACAGGATGAC-3' primers by cycling 25 times at: 95 °C for 30 s, 58 °C for 30 s, 72 °C for 40 s. The product was radiolabeled with [α-32P]dATP using the Stripping Smarter Kit Inc., Austin, TX) and used to probe normalized multiple tissue Northern blots (CLONTECH) under high stringency conditions. For autoradiography, membranes were exposed to hyperfilm MP (Amersham Pharmacia Biotech, Piscataway, NJ) at ~80 °C.

**QrtPcr**—Total mRNA from human tissues was either purchased from Clontech or prepared from cell lines using RNasey Minikit (Qiagen, Valencia, CA). Quantitative real time PCR assays were performed with TaqMan reagents on an Applied Biosystems 7700 Sequence Detector within the Department of Integrative Biology and Pharmacology at the University of Texas, Houston Medical School. Primers and probes were from IDT, Coralville, IA. Amplicons were derived within JAM1 exon 6 (78 bp), JAM2 exon 5 (71 bp), and JAM3 exon 4-5 (70 bp). TaqMan probes with 5'-FAM/3'-TAMRA were: JAM1: TCTCATCCCTGTGACCTGG-3' (sense), 5'-CCCTGGTCTGATAAAACCCCGATGCTGGC-3' (antisense), JAM2 (sense), 5'-CTGATCATCAACAGGATGAC-3' and (antisense) 5'-GGTTGCGCTCCGCTACTCGTG-3'. Amplification of the JAM3 transcript was performed from fetal brain.

**Flow Cytometry**—HL-60, HS B cells, and purified human T cells were analyzed for JAM3 expression. Buffy coats were obtained from the Gulf Coast Regional Blood Center and T cells purified by the method of Woodside et al. (25). T cells were maintained in RPMI (InVitrogen Corp., Carlsbad, CA), and stimulated for 2 days with phorbol 12-myristate 13-acetate (10 ng/ml) and ionomycin (1 μg/ml). Briefly, cells (105) were incubated in phosphate-buffered saline containing either NMS or anti-JAM3 serum (1:500) for 45 min at room temperature, followed by goat anti-mouse-fluorescein isothiocyanate (1:500). Prior to analysis cells were fixed with 1% paraformaldehyde. Samples were analyzed on a COULTER Epics XL machine with 10,000 events recorded in total.

**Adhesion Assay**—The cell lines, HSB, HPB-ALL, Ramos, HL-60, and 293 were loaded with 50 μM calcine-AM (Molecular Probes Inc., Eugene, OR) and adhesion to recombinant human JAM3-Fc, JAM2-Fc, and JAM1-Fc performed in 96-well plates as previously described (23). Binding to mouse IgG2a was used to subtract background. For adhesion to CHO cells we used the stable expressing JAM2 cell line (23). Briefly, incubation with cells was for 90 min at 37 °C in Tris-buffered saline plus 1 μM each of CaCl2, MgCl2, and MnCl2. Following three washes at room temperature, adhered cells were lysed and fluorescence quantified in a Cytofluor with excitation at 485/20 nm and emission at 530/25 nm.

**Pull-down of JAM2 Interacting Proteins**—Intact cells were surface biotinylated with EZ-Link Sulfo-NHS-Biotin (Pierce, Rockford, IL) and lysed in Tris-buffered saline (pH 7.5), 1% Triton X-100 using identical procedures to those previously described (23). For 105 cells, JAM2-Fc (1 μg) was incubated with a protein A-Sepharose slurry at room temperature for 1 h. The recombinant JAM3 ectodomain, was used in combination with protein A-Sepharose to precipitate interacting proteins. For analysis with anti-JAM3 antibody, JAM2 interacting proteins were eluted from the protein A precipitates with 50 μM CHES (pH 9.0). Elutions were then immunoprecipitated with either normal mouse serum (NMS) or anti-JAM3 serum (1:500). Samples were analyzed and detected by Western blotting with streptavidin-horseradish peroxidase (1:14,000) and enhanced chemiluminescence (ECL) (Amersham Pharmacia Biotech).

**Direct Protein-Protein Interactions**—96-Well microtiter plates were coated with purified JAM3 ectodomain at 364 μM. The recombinant ectodomain JAM3-Fc fusion proteins were added at 364 μM in binding buffer (described above) and allowed to interact for 1 h at room temperature. Wells were washed with TBST (1 time) followed by TBS (3 times) and detection achieved with alkaline phosphatase-conjugated goat anti-mouse-IgG (1:2000).

**RESULTS**

Searching of the expressed sequence tag database for sequences similar to JAM3 resulted in the assembly of a partial JAM3 transcript. The expressed sequence tags used for assembly were derived from libraries of fetal brain (AW162934), fetal liver/spleen (H71948), and retinoblastoma (BE873472, BE873711). Amplification of the JAM3 transcript was performed from fetal brain.

The full open reading frame for human JAM3 is encoded by 930 bases (GenBank™ accession number AF356518) and, at the DNA level, displays 53% identity with human JAM2 and JAM1. Multiple sequence alignment of the human JAM proteins reveals 36% identity of JAM3 with JAM2 and 32% identity with JAM1 (Fig. 1A). SignalP V.1.1 predicts that the JAM3 signal sequence will be cleaved after Gly30. The ectodomain possesses two Ig-like folds and sequence profiles lead us to conclude that the N-terminal domain is a V-type while the membrane proximal domain is a C2-type (26, 27). The intrachain disulphide bonds that stabilize each Ig fold are predicted between Cys 53-Cys 115 and Cys 160-Cys 219. The C2-domain also possesses 2 additional cysteine residues, Cys 224 and Cys 230 that may form a second disulphide bridge within the fold (Fig. 1B). These residues are preserved in JAM2. Two potential N-linked glycosylation sites are found at amino acids 104 and 192. The former is conserved only between JAM2 and JAM3 while the latter is preserved in all three adhesion molecules. The short intracellular tail consists of 46 amino acids and possesses a C-terminal binding motif for PDZ domains and a PKC phosphorylation consensus.
JAM3 localizes to 11q25 and the open reading frame is interrupted by 8 introns (Table I). The sequences bordering the splice-site junctions universally follow the GT/AG rule. The open reading frame spans over 88 kilobases of genomic DNA.

Table II compares the gene structure of the JAM family members. For all three genes, the intron separating the signal sequence is considerably larger than the others. The remaining coding exons are all found within a fairly compact region. There is remarkable conservation of gene structure and phase of introns which points to their evolutionary relationship. In the ectodomain, both immunoglobulin folds one and two of all three JAMs are encoded by multiple exons. An atypical phase 0 intron is found between the two half-exons of the C2 Ig-fold in JAMs. Divergence occurs in the intracellular domain where intron 7–8 is absent in JAM3 and the short exon 8 is fused to exon 7.

Northern blot analysis of human JAM3 shows expression within many tissues. More preponderant expression is seen in the placenta, brain, and kidney (Fig. 2A). A more detailed examination of the brain reveals transcription throughout all areas examined (Fig. 2B). To determine whether JAM3 is expressed in endothelial cells, we next probed mRNA derived from primary cultures of human aortic and umbilical vein endothelial cells in addition to the ECV cell line (Fig. 2C). The JAM3 transcript is clearly detectable in primary endothelial cells of both origins but was not detectable in the immortalized ECV cell line.

We have previously shown that human JAM2 is capable of adhering to T cell lines (23). Thus we next tested the ability of immobilized human JAM3 to capture various leukocytes. Fig. 3 shows that JAM3 is unable to adhere to HSB, HPB-ALL, RAMOS, HL-60, or K562 cells under the same conditions that are favorable for JAM2.

JAM1 and JAM2 are capable of forming homotypic interactions (23, 28). Therefore it was reasonable to assume that JAM3 would maintain this characteristic. Using purified recombinant Fc-fusion protein and immobilized JAM3 ectodomain, we were able to detect only weak binding to JAM3-Fc. This amounted to about 2-fold above nonspecific binding to mouse IgG2a or the unrelated adhesion protein, VCAM (Fig. 4). However, most strikingly, when JAM2-Fc was added to the assay a very strong heterotypic interaction was apparent. JAM1 was not functional in this regard.

To extend this observation and show that it can occur at the cell surface, we performed adhesion experiments between immobilized JAM3-Fc ectodomain and stable cell lines of CHO expressing full-length JAM2. Fig. 5 demonstrates that surface expression of JAM2 confers on CHO cells the ability to bind to JAM3.

The predicted mass of JAM3 approximates to 35 kDa and it has the potential to be modified on either of two N-linked glycosylation sites. Our previous studies with JAM2 demonstrated that an uncharacterized 43-kDa surface membrane protein participated in T cell (HSB) binding (23). These observations led us to ask whether JAM3 and the 43-kDa protein were homologous.

To first determine whether JAM3 was specifically expressed in T cell lines, we performed quantitative real time PCR in the various leukocytes used for adhesion. Expression levels were compared with those of JAM1 and JAM2. Consistent with the binding assay (Fig. 3), Table III shows that JAM3 is only found in the HSB and HPB-ALL T cell lines, and is absent from RAMOS, HL-60, and K562. JAM2 levels are nonexistent throughout, while JAM1 expression is particularly strong.

To provide conclusive proof and to directly probe JAM3 at the protein level, we developed a mouse polyclonal anti-human JAM3 antibody. To test the specificity and utility of the antibody we performed flow cytometric analysis of HSB and HL-60 cells. Fig. 6 shows that anti-JAM3 only labels the T cell line, consistent with transcript analysis. The data also demonstrates surface expression of JAM3 and thus its availability for binding to JAM2.
were viewed by hybridization to human JAM3 (upper panel) and /H9251 lower panel. The gene symbol and name; intestine; lane 10, brain; lane 2, leukocytes. Exposure, 64 h. Lane 3, cerebral cortex; lane 10, thymus; lane 11, spleen; lane 7, kidney; lane 8, liver; lane 9, small intestine; lane 10, placenta; lane 11, lung; lane 12, peripheral blood leukocytes. Exposure, 64 h. B, brain specific: lane 1, cerebellum; lane 2, cerebral cortex; lane 3, medulla; lane 4, spinal cord; lane 5, occipital lobe; lane 6, frontal lobe; lane 7, temporal lobe; lane 8, putamen. Exposure, 64 h. C, endothelial cell: lane 1, HAEC; lane 2, HUVEC; lane 3, ECV. Exposure, 82 h.

To confirm the strong circumstantial evidence for a direct interaction between JAM2-Fc and JAM3 expressed in HSB cells, we again employed the anti-JAM3 serum. As a first step, we captured the 43-kDa HSB biotinylated membrane protein with JAM2-Fc/protein A. Interacting proteins were eluted from JAM2 at pH 9.0 and subjected to immunoprecipitation. Fig. 7 demonstrates that the anti-JAM3 serum, but not the NMS specifically precipitates the 43-kDa band.

We next tested whether soluble JAM3 could act as a competitive inhibitor. In the first approach, JAM2-Fc was used to pull down interacting proteins in the presence or absence of excess JAM3 ectodomain. Fig. 8A shows that the intensity of the 43-kDa band is significantly attenuated in the presence of soluble JAM3. To extend this observation and show inhibition.

Table I: Structural organization of the JAM3 gene

| HGNC EXON description | JAM1 1q21.2-21.3 | JAM2 21q21.2 | JAM3 11q25 |
|-----------------------|-----------------|-------------|----------|
| ATG* signal sequence  | 1 103           | ≥801        | ≥76      |
| Intron 1               | 2-3 167         | 1 5,916     | 1 740    |
| EC, Ig-fold 1          | 3 98            | 1 108       | 1 114    |
| Intron 2               | 3-4 252         | 1 3,782     | 1 3,470  |
| EC, Ig-fold 2          | 4 137           | 1 153       | 1 187    |
| Intron 3               | 4-5 282         | 1 4,768     | 1 398    |
| EC, Ig-fold 3          | 5 203           | 1 203       | 1 203    |
| Intron 4               | 5-6 167         | 0 3,280     | 0 951    |
| EC, Ig-fold 4          | 6 103           | 0 100       | 0 100    |
| Intron 5               | 6-7 128         | 1 3,709     | 1 2,511  |
| Transmembrane          | 7 108           | 1 108       | 1 130    |
| Intron 6               | 8 13            | 16          |         |
| EC, PDZ domain/STOP   | 8-9 304         | 2 2,890     | 2 87     |
| Intron 7               | 9 43            | 49          | 55       |
| Intron 8               | 10-9 136        | 0 2,257     | 0 321    |

Table II: Comparison of gene structure between JAM family members

Numbers are indicated for exons containing the start and stop codons; HGNC, HUGO Genome Nomenclature Committee, Homo sapiens official gene symbol and name; i-phase of introns; EC, extracellular domain; IC, intracellular domain; Ig-fold, immunoglobulin-like fold; ATG*, JAM1 start codon; 3' Splice: Coding exons (bp) 5' Splice: Introns (bp) 3'-nnnnn/(N)--------(1) ≥76----TCAGGG/gtagt.............≥79,105
..................cttcag/GCTGCC--------(2) 66----TTGAAG/gtaag............740
..................tggtag/GTGTCG--------(3) 114----TTAGG/gtatga...........3,470
..................ctggag/GAGACT--------(4) 153----TGAAG/gtagga.........398
..................ccagag/TGAAGC--------(5) 203----ACTTTG/gtaaga.........951
..................aadacg/GTGTCG--------(6) 100----AGGTCT/gtagt...........2511
..................cttccg/ATGCCT--------(7) 139----AGGAG/gtaga...........87
..................tcagag/TTACAA--------(8) 55----GAGAG/gtaaac...........321
..................tttcag/GGCGAC--------(9) ≥36----NNNNNN/nnnnnn

**Fig. 3.** Screening for JAM3 adhesion to various leukocyte cell lines. Calcein-loaded cells were added to immobilized adhesion proteins captured in 96-well plates. Wells were washed, retained cells lysed, and fluorescence quantitated with a cytofluor at excitation 485/ emission 530 nm. HPB-ALL (A), HSB (B), HL-60 (C), K562 (D), RAMOS (E). Data from a representative experiment. Average ± S.E. (n = 6).
were coated with cleaved recombinant JAM3 ectodomain (364 nM) and probed for direct binding to equimolar concentrations of JAM3, JAM1, JAM2, and VCAM Fc fusion proteins and IgG2a. Interactions were recorded in a spectrofluor following detection with an alkaline phosphatase-conjugated goat anti-mouse IgG2a. A representative experiment. Average with a cytofluor at excitation 485/emission 530 nm. Data are from a representative experiment. Average ± S.E. (n = 4).

**Fig. 4.** Direct recombinant JAM interactions in vitro. Wells (96) were coated with cleaved recombinant JAM3 ectodomain (364 nM) and probed for direct binding to equimolar concentrations of JAM3, JAM1, JAM2, and VCAM Fc fusion proteins and IgG2a. Interactions were recorded in a spectrofluor following detection with an alkaline phosphatase-conjugated goat anti-mouse IgG2a. A representative experiment. Average ± S.E. (n = 4).

**Fig. 5.** JAM3-Fc adheres to CHO cell lines expressing JAM2. Calcein-loaded stable CHO cell lines, either control (CHO wt) or expressing full-length JAM2 (CHO-JAM2) were added to immobilized JAM3-Fc or JAM1-Fc adhesion proteins captured in 96-well plates. Wells were washed, retained cells lysed, and fluorescence quantitated with a cytofluor at excitation 485/emission 530 nm. Data are from a representative experiment. Average ± S.E. (n = 4).

**Fig. 6.** Cell surface expression of JAM3 on HSB T cells as determined by flow cytometry. The HL-60 (upper panel) and HSB (lower panel) leukocyte cell lines were incubated with 1:500 dilution of NMS and mouse anti-human JAM3 (anti-JAM3) polyclonal serum. Specific binding was detected with an fluorescein isothiocyanate-conjugated GAM secondary. A representative profile (n = 3).

**Fig. 7.** Precipitation of JAM3 from HSB cells by JAM2-Fc. Plasma membranes from intact HSB cells were surface biotinylated and specific proteins captured with JAM2-Fc and protein A. Protein was retrieved from the complex by elution at pH 9.0, and the sample was either directly analyzed (lane 1) or subjected to a round of immunoprecipitation using anti-JAM3 serum (lane 2) or NMS (lane 3). Bands were viewed with streptavidin-horseradish peroxidase and ECL following electrophoresis and transfer.

**TABLE III**
Quantitation of JAM transcripts in leukocytes by qrtPCR

| JAM3 | JAM2 | JAM1 |
|------|------|------|
| HSB  | 0.61 ± 0.11 | 0.00 ± 0.00 | 0.39 ± 0.06 |
| HPB-ALL | 0.49 ± 0.17 | 0.00 ± 0.00 | 0.41 ± 0.17 |
| RAMOS | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.37 ± 0.07 |
| HL60 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.28 ± 0.06 |
| K562 | 0.00 ± 0.00 | 0.00 ± 0.00 | 1.46 ± 0.11 |

at the level of cell adhesion, binding of JAM2-Fc to HSB cells was performed with the inclusion of soluble JAM3 in the binding buffer. Fig. 8B shows that JAM3 is very effective at preventing JAM2 capture of HSB cells. That this inhibition was dependent upon integrin (29, 30).

**Fig. 8.** The level of JAM3 expression affects the ability of JAM2 to bind to HSB cells. The level of JAM2 binding to HSB cells was determined by flow cytometry using the 7700 Sequence Detector (Applied Biosystems) and TaqMan reagents. Numbers represent average ± S.E. from three independent RNA isolations.

**DISCUSSION**

In this article we describe the third member of the human junctional adhesion molecule family. JAM3 maps to chromosome 11q25 while JAM2 is found at 21q21.2 and JAM1 at 1q21.2-q21.3. While the structural organization of the JAM3 extracellular domain (V/C2) is shared by several CD molecules, the most closely related proteins are those of the CTX family, A33 antigen, the coxsackie and adenovirus receptor (CAR) and the recently described endothelial cell-selective adhesion molecule, ESAM (reviewed in Ref. 31) (32–35). All of these molecules contain additional cysteine residues within the C2 domain in strands A and G, which align with those in JAM3 and JAM2. An atypical feature that is shared with the CTX family, is the formation of the C2 Ig domain by two separate exons. However, even more unusual is the apparent formation of the first Ig-fold from three separate exons. The third coding exon initiates within strand B of Ig fold 1 and contains the...
terms of tissue expression. All three genes are expressed well in the placenta. Other preponderant areas for JAM3 transcripts are the brain and kidney while JAM2 is mainly expressed in the heart and JAM1 in the kidney, liver, and lung (12). While all three JAMS are found within endothelial cells, JAM1 is clearly not restricted to this cell type. In this article we report on JAM3 expression in T cell lines. Furthermore, we provide evidence that JAM3 is up-regulated following T cell activation. Although human JAM1 is also expressed in peripheral blood leukocytes, levels are similar between resting and stimulated cells (16).

Most recently, Aurrand-Lions et al. (36) reported on the isolation of a novel mouse JAM which is the likely homologue of the human JAM3 reported here. This group describes expression of this protein as purely endothelial and reports a lack of expression in the mouse brain. This contrasts sharply with the human JAM3 expression where transcription in the brain is clearly apparent throughout. An anomaly of this type also occurs between mouse and human JAM1 where expression in leukocytes appears specific for the human protein. These discrepancies will be important to consider when attempting to extrapolate the function of these molecules across species and when using animal models for validation studies.

Dimerization of JAM1 has been demonstrated in CHO cells and analytical ultracentrifugation detects oligomerization of recombinant soluble ectodomain (28). Several groups have demonstrated that JAM1 homotypic binding between cells results in decreased paracellular permeability (12, 15). That this characteristic is also a property of JAM3 and JAM2 is highly likely. Indeed, as reported most recently for the mouse homologue of JAM3, expression in CHO cells attenuates movement of fluorescein isothiocyanate-dextran across confluent monolayers (36). It was not the aim of this study to measure affinity constants for the homotypic and heterotypic JAM-JAM binding possibilities: assays were not designed accordingly. Nevertheless, we can conclude that under identical conditions, JAM3 homotypic interactions are weaker than heterotypic binding to JAM2. This is evident when testing binding directly between the recombinant purified proteins and also when examining cellular adhesion. Thus, JAM3-Fc is unable to promote efficient HSB cell adhesion despite its expression in this T cell line (Fig. 3). Using this standard solid phase adhesion assay, others too have reported on the inability to identify JAM1 homotypic binding (16). Additionally, expression of JAM1 in CHO cells does not promote aggregation of cells in suspension (12). Solid phase adhesion assays mimic clustering for only the receptor performing capture. While this seems sufficient for the JAM2-JAM3 interaction, clustering may be required on both sides of the assay to detect weaker homotypic binding. In addition to avidity, it has been suggested that the correct binding of intracellular components that would occur at tight junctions, but not following their disruption, are required (12, 16).

It is conceivable that the pattern of JAM expression within different vascular beds affects the barrier properties of the vessels within respective tissues. This would depend upon the various affinities of binding for the homotypic and heterotypic interactions and the availability of JAM molecules for partnering. In endothelial cells, where JAM2 and JAM3 may be co-expressed, the opportunity clearly arises for heterotypic interaction. Within brain endothelium, JAM3/JAM2 engagement may contribute to the blood-brain barrier in collaboration with the classical tight junction components. Possibly the most exciting aspect of this work is the potential

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2 M. P. Arrate, J. M. Rodriguez, T. M. Tran, T. A. Brock, and S. A. Cunningham, unpublished observations.
for endothelial JAM2 interaction with JAM3 expressed in T cells during inflammation. Indeed we show that JAM3 is up-regulated following T cell activation, further supporting this hypothesis. JAM1 facilitates transmigration of monocytes/neutrophils across the vessel wall (12, 21). During inflammation, endothelial cell selectins and VCAM/ICAM perform the initial capture and firm adhesion of leukocytes to the vessel wall, respectively (7). It is likely that the JAM molecules only then become available for interactions as the leukocyte moves between endothelial cells. We would hypothesize that in the appropriate vascular beds, intercellular JAM2 participates in T cell migration by binding with JAM3. Furthermore, it would be necessary for the JAM2-JAM3 interaction to be fairly weak and transient to maintain leukocyte motility. JAM1 is reported to redistribute to the cell surface following inflammatory stimuli (17). If a similar phenomenon were to occur for JAM2, then JAM2/JAM3 interactions could potentially participate in the adhesion process within the vessel lumen. We would predict that JAM3 and JAM2 might contribute to pathologies with T cell involvement such as multiple sclerosis, organ transplant, and arthritis.

At present we have no knowledge about the physiological consequences of JAM2/JAM3 engagement. All members of the JAM family contain consensus sequences for phosphorylation. Precedence for JAM phosphorylation by PKC following physiological stimuli is provided by JAM1 following thrombin stimulation of platelets (18, 19). Modifications of this type on JAM2 and/or JAM3 could potentially affect the adhesion between these proteins. JAM1 associates with the tight junction components ZO-1 and AF-6 via their PDZ domains (37, 38). More recently the calcium/calmodulin-dependent serine protein kinase (CASK/LIN-2) has also been shown to bind the C terminus of JAM1 (39). The PDZ binding motif is conserved in JAM2 and JAM3. A future focus will be identification of molecules that bind JAM2 and JAM3 intracellular domains prior and during heterotypic engagement. Indeed, consequences of binding different intracellular components may be alteration of JAM1/2/3 cellular localization and/or affinities of the ectodomains for homotypic and/or heterotypic binding.

Whether additional interactions occur between JAMs and ESAM, or JAMs and the CTX family members will be an interesting avenue to explore. Furthermore, it is possible that JAM2 and/or JAM3 may interact with viral proteins to mediate viral cell entry as has been described for the JAM1 and CAR receptors.

In summary, this is the first report describing the human JAM3 sequence and identification of JAM2 as its binding partner. This discovery will greatly facilitate research focused on delineating the function of these novel adhesion proteins in endothelial cells and their contribution to the inflammatory process.

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Cloning of Human Junctional Adhesion Molecule 3 (JAM3) and Its Identification as the JAM2 Counter-receptor
M. Pia Arrate, Jose M. Rodriguez, Tuan M. Tran, Tommy A. Brock and Sonia A. Cunningham

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