Human Vascular Adhesion Protein-1 (VAP-1) Plays a Critical Role in Lymphocyte–Endothelial Cell Adhesion Cascade Under Shear

Marko Salmi, Sami Tohka, Sirpa Jalkanen

Abstract—Lymphocyte binding to vascular endothelium is a prerequisite for the movement of immune cells from the blood into lymphoid tissues and into sites of inflammation. Human vascular adhesion protein-1 (VAP-1) is an endothelial glycoprotein involved in this interaction. It also displays an enzymatic (monoamine oxidase) activity. Here we examined how recombinant human VAP-1 mediates lymphocyte binding using rotatory and flow chamber binding assays. VAP-1 cDNA transfected into an endothelial cell line, which does not bind lymphocytes, renders the cell line capable of binding lymphocytes in a shear-dependent manner. VAP-1 transfectants bound lymphocytes 5 times better than monocytes with a preference for T killer cells, and no specific granulocyte adherence was detectable. The binding is partially inhibited by anti–VAP-1 monoclonal antibodies or by blocking lymphocyte L-selectin and CD18 integrins, but not by inhibition of several other homing-associated molecules. In contrast, CD44 ligation on lymphocytes markedly upregulates their VAP-1–dependent adhesion, suggesting that the VAP-1 counterreceptor can be activated via CD44. The transfectant model also allowed us to perform detailed structure-function analyses of VAP-1. We show that the exposed integrin-binding motif RGD or the enzymatic activity is not indispensable for VAP-1–dependent adhesion. Together, these data show that VAP-1 can reconstitute the lymphocyte-endothelial adhesion cascade under shear and propose a critical role for VAP-1 in lymphocyte emigration from the blood. (Circ Res. 2000;86:1245-1251.)

Key Words: adhesion ■ leukocyte-endothelial cell interactions ■ cell trafficking ■ recruitment ■ enzymatic activity

Lymphocyte trafficking between the circulation and tissues is essential for the proper function of the immune system. In the extravasation process, the blood-borne cell makes initial contacts with the endothelial lining, which may lead to rolling, activation, firm binding, and finally transmigration.1–3 Multiple adhesion molecules are operative at the different steps of the emigration cascade. Thus, selectins and their sialomucin ligands mediate tethering and rolling interactions under shear, whereas chemokines are thought to be principal mediators of lymphocyte triggering. Thereafter, the activated cells bind stably to their endothelial ligands, which are mainly members of the immunoglobulin superfamily.

Human vascular adhesion protein-1 (VAP-1) is an endothelial adhesion molecule, which in an in vitro frozen section assay mediates lymphocyte binding to high endothelial venules (HEVs),4 the preferred site of lymphocyte extravasation.5 It is a homodimeric transmembrane sialoglycoprotein, which shows significant sequence identity to a subgroup of monoamine oxidases characterized by covalently bound copper and sensitivity to inhibition by semicarbazide.6,7 The physiological function and substrates of these often-soluble enzymes have remained unknown. This enzyme group is clearly distinct from monoamine oxidases A and B with respect to substrates, cofactors, inhibitors, subcellular localization, and protein sequence. Very recently, we have shown that recombinant human VAP-1 is a dual-function molecule; it mediates lymphocyte binding and it also possesses the monoamine oxidase enzyme activity.8

The in vitro Stamper-Woodruff binding assay using frozen sections,9 which has been the only suitable method to study human VAP-1 so far, has certain inherent limitations.1 Therefore, in this study we took advantage of VAP-1 transfectants in dissecting the VAP-1–mediated leukocyte adhesion to endothelial cells. Our results show that transfection of VAP-1 into endothelial cells is able to reconstitute the whole adhesion cascade and that there is cross-talk between lymphocyte homing receptors and endothelial VAP-1. The transfectant model allowed us for the first time to study the role of an RGD sequence of VAP-1, its enzymatic activity, and the effect of laminar shear stress on VAP-1–dependent adherence.

Materials and Methods

Cells and Monoclonal Antibodies (mAbs)

An endothelial cell line, Ax, was maintained as described.10 Peripheral blood mononuclear cells (PBMCs), plastic adherent macro-
TABLE 1. mAbs Used

<table>
<thead>
<tr>
<th>Clone</th>
<th>Isotype</th>
<th>Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>Mouse IgM</td>
<td>Human VAP-1</td>
</tr>
<tr>
<td>TK8-14</td>
<td>Mouse IgG2a</td>
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<tr>
<td>TS1/18</td>
<td>Mouse IgG1</td>
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<tr>
<td>TS1/22</td>
<td>Mouse IgG1</td>
<td>Human CD11a</td>
</tr>
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<td>44aacb</td>
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<td>Human CD44</td>
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<td>Dreg-56</td>
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<td>Human L-selectin</td>
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<td>Leu-8</td>
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<td>Human L-selectin</td>
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<tr>
<td>4G4</td>
<td>Mouse IgG1</td>
<td>Human CD73</td>
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<td>Fib 504</td>
<td>Rat IgG2a</td>
<td>Human β2-integrin</td>
</tr>
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<td>HP2/1</td>
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<td>Human β2-integrin</td>
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<td>MRP-1</td>
<td>Mouse Ig</td>
<td>Rat P-selectin</td>
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<tr>
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<td>Mouse IgG1</td>
<td>Rat E-selectin</td>
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<td>1A29</td>
<td>Mouse IgG1</td>
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<td>Rat ICAM-1</td>
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<tr>
<td>Rabbit polyclonal</td>
<td></td>
<td>Rat P-selectin</td>
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<tr>
<td>NS-1</td>
<td>Mouse IgG1</td>
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</tr>
<tr>
<td>7C7</td>
<td>Mouse IgM</td>
<td>Chicken bursal epithelium</td>
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<tr>
<td>MECA-367</td>
<td>Rat IgG2a</td>
<td>Mouse MadCAM-1</td>
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<tr>
<td>HB116</td>
<td>Mouse IgG1</td>
<td>Human HLA class I</td>
</tr>
</tbody>
</table>

MAdCAM-1 indicates mucosal addressin cell adhesion molecule-1.

phages, interleukin-2–activated T-cell lines, and granulocytes were isolated as described.4,11 Namalwa, CA46, Raji, and Jurkat lymphoma cells were from American Type Culture Collection. The antibodies used are listed in Table 1.

Magnetically Activated Cell Sorting (MACS) Selections
T helper and killer cells, L-selectin–positive and –negative lymphocytes, and monocyes were immunomunopurified with MACS as described.12 Neutrophils were separated from other granulocytes using anti-CD16 MACS beads. The purity of the leukocyte subpopulations was always analyzed using fluorescence-activated cell sorting (FACS).

Mutagenesis of VAP-1, Transfections, and Polymerase Chain Reaction (PCR)
VAP-1 mutants were generated by U.S.E. site-directed mutagenesis kit (Pharmacia). A full-length VAP-1 cDNA in pUC19 was used as a template. The mutagenic primers were as follows: ΔRGD, CC4TCTACTTCTCACCTAGGATGCTGG, which deletes the RGD sequence in VAP-1 (amino acids 726 to 728), and MutDA, CTTCCGAGGCGGCAGAGTCTGG, which introduces a single amino acid substitution at position 728. The correctness of the mutations was confirmed by sequencing. Stable VAP-1 transfectants were produced by transfecting the Ax cells with a pcDNA3 expression plasmid containing the full-length VAP-1, VAP-ΔRGD, or VAP-1ΔDVA cDNA as an insert. Ax cell RNAs were isolated and used for reverse transcriptase (RT)–PCR with rat intercellular adhesion molecule-1 (ICAM-1) (GGGTCGTCACATCTCTGAGT and GGATGAAAGCAACTCAGAG) and control β-actin primers.

Adhesion Assays
The nonstatic adhesion assays were performed as described.8,13 In brief, 2×10⁶ leukocytes were incubated for 30 minutes (60 rpm, +7°C) on a slide containing a confluent monolayer of the transfectants within a wax-pen circle. After washings and fixation, the number of bound leukocytes in 9 predefined areas (2.25 mm²) was counted microscopically. The mean of 1 experiment consists of data from 3 independent circles.

The transfectant monolayer or lymphocytes were subjected to the following 4 sets of different pretreatments: (1) 5 μU neuraminidase (60 minutes, 37°C), 2% paraformaldehyde (30 minutes, 7°C), 50 ng/mL phorbol myristate acetate (PMA) (30 minutes, 37°C), or 10 mmol/L NaNO₃+10 mmol/L 2-deoxyglucose (30 minutes, 7°C); (2) saturating concentrations of function blocking or control mAbs (30 minutes, 37°C); (3) cyclic peptides (P240, CYFRGQDQDAC, or p241, CRDQADFYG) at 1 mg/mL (30 minutes, 7°C); and (4) 5 mmol/L semicarbazide+10 μmol/L hydroxyamine or 5 mmol/L benzylamine (30 minutes, 37°C). Neuraminidase, paraformaldehyde, PMA, and mAbs from lymphocytes were washed away before the binding assays, whereas the other treatments were allowed to be present during the adhesion assay.

Flow Chamber Assay
Confluent transfectants on Petri dishes were pretreated with anti–VAP-1 or control mAbs and mounted into a parallel plate flow chamber device (GlycoTech). Lymphocytes (1×10⁶ cells/mL RPMI 1640 containing 10% FCS and HEPES) were drawn over the plate with a defined laminar shear stress generated by a computer-driven syringe pump. After an initial 5-minute stabilization period, the shear rate was increased at 1-minute intervals and the cell behavior was observed using an inverted microscope coupled to a video camera and VHS recorder and analyzed offline.

Statistical Analyses
Six independent VAP-1, 4 independent mock, and 2 independent RGD mutant lines were used. The results are shown as mean±SEM, and the 2-tailed Student t test was used for comparisons.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results
Leukocyte Subtype–Specific Binding to VAP-1 Transfectants
Recent cloning of VAP-1 cDNA enabled us to study the functional role of VAP-1 in lymphocyte adhesion in a detailed manner. VAP-1 cDNA was stably transfected into Ax cells, which are immortalized endothelial cells from HEVs of rat lymph node. Ax cells maintain many characteristics of HEV-like cells,10,14 but have lost the lymphocyte binding capacity on prolonged culturing.

Ax cells transfected with VAP-1 (Figure 1A) expressed this molecule at the same level as do HEV cells freshly isolated from tonsil.5,15 PBMCs bound very well to VAP-1 or control mAbs and mounted into a parallel plate flow chamber device (GlycoTech). Lymphocytes (1×10⁶ cells/mL RPMI 1640 containing 10% FCS and HEPES) were drawn over the plate with a defined laminar shear stress generated by a computer-driven syringe pump. After an initial 5-minute stabilization period, the shear rate was increased at 1-minute intervals and the cell behavior was observed using an inverted microscope coupled to a video camera and VHS recorder and analyzed offline.

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Ax cells transfected with VAP-1 (Figure 1A) expressed this molecule at the same level as do HEV cells freshly isolated from tonsil.15 PBMCs bound very well to VAP-1 transfectants (relative adherence ratio [RAR]=1.0 by definition) but not to mock-transfected cells (RAR=0.02, n=22, P<0.001) under rotatory conditions (Figures 1B and 1C). Under static conditions, the RAR of PBMC binding to VAP-1 transfectants was 0.13 (n=3, P=0.004) and to mock transfectants 0.08 (n=3, P<0.001). Thus, lymphocyte adhesion to VAP-1 transfectants is optimal under shear conditions, and therefore, all subsequent assays were performed under nonstatic conditions.

When compared with PBMCs containing mostly lymphocytes, binding of Percoll-purified granulocytes and plastic-adherent macrophages from the same donors was significantly poorer (Figure 1C; P<0.001 and P=0.002, respectively). CD16-positive neutrophils showed no specific
adhesion to VAP-1 (on average 7 cells/mm² both on VAP-1 and mock-transfected Ax cells). CD14-positive monocytes interacted 5 times ($P<0.001$) less efficiently with the VAP-1 transfectants than did PBMCs (Figures 1B and 1C). This is notable, given that granulocytes and monocytes are generally more adhesive toward endothelial cells than are lymphocytes. Adhesion of all cell types to mock transfectants was negligible (Figure 1B). VAP-1–dependent binding of the T-cell lymphoma line Jurkat and B-cell lymphoma cell lines Raji, Namalwa, and CA46 was minimal when compared with freshly isolated PBMCs (Table 2; $P<0.001$ in all cases). Hence, VAP-1 transfectants display very distinct leukocyte subtype–specific preference in almost solely capturing normal blood lymphocytes under nonstatic conditions.

**Physical Requirements of VAP-1–Mediated Binding**

A marked decrease in PBMC binding to sialidase-treated VAP-1 transfectants showed that the sialoglycoprotein nature of VAP-1 is important for its adhesive function (Table 2). In contrast, removal of sialic acids from the lymphocyte surface resulted in a massive increase in the binding (Table 2 and Figure 1B). Paraformaldehyde and NaN₃ 2-deoxyglucose treatments indicated that both interacting cell types had to be alive and metabolically active to mediate maximal adhesion (Table 2). None of the pretreatments affected lymphocyte

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**Table 2. Adhesion of Leukocytes to VAP-1 Transfectants**

<table>
<thead>
<tr>
<th>Cell</th>
<th>n</th>
<th>VAP-1</th>
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</thead>
<tbody>
<tr>
<td>Jurkat</td>
<td>5</td>
<td>0.17±0.06*</td>
</tr>
<tr>
<td>Raji</td>
<td>5</td>
<td>0.12±0.04</td>
</tr>
<tr>
<td>Namalwa</td>
<td>4</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>CA46</td>
<td>4</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>Tumor-infiltrating lymphocytes</td>
<td>2</td>
<td>0.12±0.03</td>
</tr>
<tr>
<td>PMA-stimulated PBMCs</td>
<td>3</td>
<td>0.69±0.08</td>
</tr>
<tr>
<td>Leu-8⁺ PBMCs</td>
<td>2</td>
<td>2.75±0.32</td>
</tr>
<tr>
<td>Dreg-56⁺ PBMCs</td>
<td>2</td>
<td>0.32±0.04</td>
</tr>
<tr>
<td>Dreg-56⁻ PBMCs</td>
<td>2</td>
<td>0.70±0.16</td>
</tr>
<tr>
<td>Sialidase (endo)†</td>
<td>4</td>
<td>0.49±0.16</td>
</tr>
<tr>
<td>Sialidase (lymph)†</td>
<td>5</td>
<td>7.52±1.84</td>
</tr>
<tr>
<td>Paraformaldehyde (endo)</td>
<td>2</td>
<td>0.39±0.09</td>
</tr>
<tr>
<td>Paraformaldehyde (lymph)</td>
<td>2</td>
<td>0.23±0.07</td>
</tr>
<tr>
<td>Sodium azide+2-deoxyglucose</td>
<td>2</td>
<td>0.53±0.07</td>
</tr>
</tbody>
</table>

*RAR±SEM. RAR of PBMC binding to VAP-1 transfectants was 1.0 by definition; RAR of binding to mock transfectants was <0.10 in all cases (except 0.13±0.08 for CA46).

†endo indicates pretreatment of endothelial cells; lymph, pretreatment of lymphocytes.
binding to mock transfectants (see, eg, sialidase-treated lymphocytes in Figure 1B).

**VAP-1 Blockade Diminishes Lymphocyte Binding**

A small, but statistically significant ($P<0.02$), inhibition of lymphocyte adhesion to VAP-1 transfectants treated with anti-VAP-1 mAbs 1B2 and TK8-14 (both block function in HEV assays and are against separate epitopes$^{4,16}$) was seen (Figure 2A). It prompted us to study which molecules could mediate the residual binding. Native Ax cells and mock-transfected Ax cells lacked E- and P-selectin but were brightly positive for ICAM-1 (Figure 2B). VAP-1-transfected cells also lacked both selectins but, surprisingly, lost ICAM-1 expression. This phenotypic change was observed in all independent transfectant cell lines and was confirmed with a second anti-rat ICAM-1 mAb (data not shown). We further analyzed by RT-PCR whether VAP-1–transfected Ax cells lose the ICAM-1 transcription. Native Ax cells as well as 2 independent mock-transfected lines gave the expected signal for rat ICAM-1 in PCR, whereas 2 independent VAP-1–transfected lines showed a complete absence of ICAM-1 mRNA (Figure 2C). Thus, VAP-1 transfection into Ax cells leads to downregulation of endogeneous ICAM-1 mRNA and surface protein synthesis.

**Lymphocytes Use L-Selectin and Leukocyte Function–Associated Antigen (LFA)–1 to Adhere to VAP-1 Transfectants**

Because, as a result of the lack of available reagents, we were unable to study other rat endothelial adhesion molecules, the role of human PBMC adhesion molecules in this interaction was analyzed. mAbs against $\alpha_4$, $\beta_7$, and $\beta_1$ integrins or CD73 had no effect in this assay (Figure 2D). In contrast, mAbs against L-selectin reduced the number of bound cells by 45% ($P<0.008$). In addition, when CD11a and CD18 integrins were blocked by a combination of mAbs, a 30% decrease in the number of adherent cells was seen ($P<0.01$). To study which leukocyte integrin mediates the adhesion, the role of different CD11 chains and CD18 was analyzed separately. The results showed that blockade of CD18 contributed most to the binding and that both CD11a and, to a lesser extent, CD11b were used in the adhesion, whereas CD11c played no role. When L-selectin+$\beta_1$ integrins on lymphocytes, VAP-1 on endothelial cells+$\beta_1$ integrins on lymphocytes, or VAP-1 on endothelial cells+$\beta_1$ integrins and L-selectin on lymphocytes were blocked simultaneously, only a marginal additive effect was seen at best (Figure 2D and data not shown).

The enormous increase in binding of desialylated lymphocytes to VAP-1 transfectants was clearly VAP-1 dependent, because no increase in binding to mock-transfected cell lines was ever observed. Blocking of VAP-1 and L-selectin showed that their relative contribution in the adhesion is the same with normal and desialylated lymphocytes (Figure 2E).

**Lymphocyte CD44 Triggers VAP-1–Dependent Binding**

Quite surprisingly, preincubation of lymphocytes with 2 different mAbs against CD44, as a pool or separately, increased the number of bound cells 2-fold ($P<0.006$; Figure 3). No change in adhesion to mock cells was observed. Preincubation of the VAP-1 transfectants with anti-VAP-1 mAbs completely reversed the increase in binding seen with anti-CD44–treated lymphocytes (Figure 3). These data suggest that ligation of CD44 on lymphocytes with antibodies

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**Figure 2.** Lymphocyte binding to VAP-1 transfectants is mediated mainly by L-selectin and LFA-1. A, Anti–VAP-1 mAbs inhibit lymphocyte adhesion to VAP-1 transfectants. The endothelial monolayer (Transf.) was pretreated with the indicated mAbs before the adhesion assay. B, Expression of endothelial adhesion molecules on native Ax (nat), mock, and VAP-1–transfected cells was determined by immunofluorescence stainings and FACS analyses. C, Synthesis of rat ICAM-1 mRNA in independent Ax transfectant lines and parental cells was measured by RT-PCR and agarose gel electrophoresis. The expected ICAM-1 product from cDNA is a 662-bp fragment. Rat $\beta$-actin (predicted size from cDNA is 621 bp) signals are shown as positive controls. Lanes 1, water control; lanes 2, native Ax; lanes 3, Ax mock 1; lanes 4, Ax mock 2; lanes 5, Ax VAP-1/1; and lanes 6, Ax VAP-1/2. Note the absence of ICAM-1 signal in the VAP-1 transfectants. D and E, Adhesion of PBMCs (Leuk.) to VAP-1 transfectants. Cells were pretreated with the indicated function-blocking mAbs, and their binding to Ax VAP-1 transfectants was determined. E, Lymphocytes were pretreated with sialidase (Sial) as indicated. Neg. co indicates negative control.
triggers an increase in the adhesiveness of the cells toward VAP-1 transfectants in a VAP-1–dependent manner.

**RGD Motif of VAP-1 Is Not Required for Adhesion**

In the VAP-1 amino acid sequence, there is a classical RGD motif for integrin binding at positions 726 to 728. In molecular models, that segment protrudes from the extracellular domain of the molecule and hence could be of functional significance. Pretreatment of the lymphocytes with a cyclic nonapeptide containing the RGD sequence and the same amino acids that are in the juxtaposition in VAP-1 sequence caused no significant decrease in the number of lymphocytes bound to VAP-1 transfectants (86 ± 6% binding, n = 3) when compared with the lymphocytes treated with the scrambled control peptide.

We next deleted the RGD sequence or changed it into an RGA sequence and produced stable transfectants expressing comparable levels of standard VAP-1 and the mutant versions (Figure 4A). Lymphocyte adhesion was diminished to VAP-1 DRGD (P = 0.01) but not to VAP-1728 D/A transfectants, when compared with normal VAP-1 transfectants (Figure 4B). However, lymphocyte binding to all 3 transfectants was inhibited by anti–VAP-1 mAbs to the same extent (Figure 4C). Hence, a gross alteration (deletion) but not a minor change (D/A mutation) in the VAP-1 RGD sequence appears to diminish lymphocyte binding to VAP-1 transfectants, which nevertheless still takes place in the complete absence of the RGD motif.

**Enzymatic and Adhesive Functions of VAP-1 Are Distinct**

VAP-1 also possesses a copper-containing semicarbazide-sensitive monoamine oxidase activity, which can be inhibited by semicarbazide and hydroxylamine. At the concentrations used, these carbonyl reactive compounds completely abrogated all enzyme activity without having any adverse effect on the viability of lymphocytes or the transfectant monolayer (data not shown). The number of bound lymphocytes remained essentially the same in nontreated (100%, n = 7) and semicarbazide-treated (84.5 ± 8.4%, n = 6) or hydroxylamine-treated (92.2 ± 10.5%, n = 5) samples.

To induce the catalysis of the biologically active reaction products, the transfectants were preincubated with a VAP-1 substrate for 30 minutes before performing the adhesion assay. Lymphocyte binding was the same to both nontreated and benzylamine-treated monolayers (100% and 96.0 ± 23.7% adhesion, respectively, n = 4). Together, the inhibition and induction studies clearly demonstrate that the enzymatic activity of VAP-1 is not necessary for its adhesive function in this adhesion assay.

**VAP-1 Transfectants Interact With Lymphocytes Under Laminar Shear**

The importance of defined laminar shear stress in peripheral blood lymphocyte tethering to VAP-1 transfectants was tested using an in vitro flow chamber assay. Lymphocytes interacted with VAP-1 transfectants at shear stresses of 0.3 to 0.7 dyn/cm². Notably, the contacts were blocked by neutralizing VAP-1 with an mAb (Figure 5). Thus, VAP-1 can support lymphocyte–endothelial cell interactions under physiologically relevant laminar flow.

**Discussion**

In this study, we show that VAP-1 is able to reconstitute the adhesion cascade in an endothelial cell line. The adhesive function of VAP-1 was characterized by a leukocyte subtype–selective binding under shear, with T killer cells being the preferred target population. Lymphocytes bound to VAP-1 very poorly under static conditions, whereas good specific binding was observed with the same cells under rotatory conditions. Most importantly, the in vitro flow chamber
model showed that VAP-1 transfectants can support lymphocyte–endothelial cell interactions up to laminar shear stresses of 0.7 dyn/cm². This shear is at the lower range of those seen in vivo and comparable with those used for demonstration of shear-dependent binding to other adhesion molecules.

The following 2 lines of evidence suggest that lymphocyte adherence to Ax cells is directly dependent on VAP-1. (1) There was a mean 65-fold increase (n = 22) in PBMC binding to 6 independent VAP-transfectant cell lines (not clones) when compared with 4 independent mock-transfected Ax cell lines. (2) In naturally occurring revertants, which have lost VAP-1 expression, the lymphocyte binding capacity was always concomitantly lost. It is equally clear, however, that VAP-1 alone is not sufficient for lymphocyte binding. Chinese hamster ovary transfectants expressing high levels of VAP-1 but lacking all other relevant adhesion molecules did not bind lymphocytes under these assay conditions. We interpret the data so that VAP-1 is able to reconstitute the adhesion cascade on Ax cells (lacking P- and E-selectin) by allowing early contacts of lymphocytes with endothelial cells via VAP-1, which then leads to binding mostly via other endothelial adhesion molecules (Figure 6). L-Selectin, LFA-1, and Mac-1 ligands were shown to be important in this interaction, but other pathways must also contribute to the binding. These may include, eg, CD11d/CD18 adhesion to VCAM-1. Remarkably, in the absence of VAP-1, neither lymphocyte LFA-1, Mac-1, nor L-selectin was sufficient for them to adhere to the same endothelial cells. The inhibition studies suggest that the relative functional importance of the VAP-1 epitopes recognized by the anti–VAP-1 mAbs used is more significant under low shear (<0.7 dyn/cm²) than at high laminar or rotatory shear.

Triggering of lymphocyte CD44 through antibody ligation dramatically increased the number of cells bound to VAP-1 transfectants, but it had no effect on binding to mock cells. The antibodies used inhibit CD44 interaction with hyaluronate and mucosal addressin. Nevertheless, antibodies can also trigger this multifunctional adhesion molecule to a higher avidity state. The anti-CD44 mAb–triggered increase in adhesion was absolutely and directly VAP-1 dependent, given that pretreatment of the VAP-1 transfectants with anti-VAP-1 mAbs completely nullified the effect. CD44 does not apparently directly bind to VAP-1, given that several cell lines (eg, granulocytes, monocytes, TIL cells, and Jurkat cells) expressing high levels of CD44 failed to adhere properly to VAP-1 transfectants. Together, the results suggest that CD44 ligation on lymphocytes induces a functional upregulation of a receptor(s), which then interacts with the VAP-1 molecule to trigger the adhesion between the 2 cell types (Figure 6C).

VAP-1 displays adhesive and enzymatic properties, but it has remained completely unknown whether these 2 functions are interconnected. Chemical inhibition or promotion of the monoamine oxidase activity of VAP-1 had no effect on lymphocyte adhesion in our assays with Ax cells. On the basis of the molecular modeling, we hypothesize that the oligosaccharides extending from the outer planar surface of VAP-1 are mediating the interactions with the unknown ligands on lymphocytes, whereas the enzymatic reaction takes place independently within the deeply buried catalytic center of the molecule. These data, however, do not rule out the possibility that the biologically active reaction products could modulate the expression or function of other adhesion molecules in vivo or in vitro in primary endothelial cells. This hypothesis is supported by the fact that transfection of VAP-1 into Ax cells led to suppression of endogenous ICAM-1 mRNA and protein synthesis. Hence, putative effects of VAP-1 on the synthesis of some other adhesion molecules, fucosyltransferases or sulfotransferases, might partially account for the dramatically increased ability of VAP-1 transfectants to support lymphocyte adherence.

In conclusion, we have shown with the transfectant model that VAP-1 has dual functions. It is a new membrane-bound...
semicarbazide-sensitive monoamine oxidase that catalyzes a reaction of which the physiological substrate and biological function are not understood at the moment. On the other hand, VAP-1 alone is sufficient to reconstitute the leukocyte–endothelial cell adhesion cascade when expressed in a recombinant form in transfectants. Endothelial VAP-1–dependent adhesion mediates leukocyte subtype–selective binding under shear. Structure-function analyses revealed that the RGD motif or enzymatic activity of VAP-1 is not absolutely required for its adhesive function. These data suggest that VAP-1 will be an important determinant in mediating and regulating physiological recirculation of lymphocytes and, in particular, in controlling the lymphocyte efflux from the blood into sites of inflammation.

Acknowledgments

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References

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