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The Role of Two Distinct Endothelial Molecules, Vascular Adhesion Protein-1 and Peripheral Lymph Node Addressin, in the Binding of Lymphocyte Subsets to Human Lymph Nodes

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Lymphocytic binding to high endothelial venules (HEV) in noninflamed peripheral lymph nodes (PLN) relies heavily on two endothelial molecules called vascular adhesion protein-1 (VAP-1) defined by mAb 1B2 and the peripheral lymph node addressins (PNAd) defined by mAb MECA-79. Data from several different groups indicate that these two molecules share several characteristics in expression, biochemical structure, and function, raising the possibility that VAP-1 may be identical to the 170- and 90-kDa species of PNAd glycoproteins. In this study, we show that many PLN HEV coexpress these two molecules. In parallel SDS-PAGE analyses, the m.w. of the 90- and 170-kDa forms of these molecules are indistinguishable. Nevertheless, we show by different metabolic labelings, by reciprocal cross-precipitations, and by immunofluorescence stainings of newly established VAP-1 transfectants that the 90- and 170-kDa species of PNAd and VAP-1 are distinct molecules. In functional terms, VAP-1 is strikingly selective in mediating PLN HEV adhesion of CD8-positive, but not of CD4-positive T cells. In contrast, PNAd contributes to the adhesion of both CD8-positive and CD8-positive cells to these vessels. Together, these data show that initial adhesion of CD8-positive lymphocytes to PLN HEV requires a PNAd- and a VAP-1-dependent step that are both essential and may occur simultaneously or sequentially.
forms of PNAd. In this study, we show that this is not the case but, rather, these two glycoproteins represent two molecularly independent ways for lymphocyte binding to PLN HEV.

Materials and Methods

Abs, tissues, and cells

Function-blocking mAbs 1B2 against human VAP-1 and MECA-79 against mouse and human PNAd have been described (7, 19). mAb TK8-14 is a new IgG2a anti-VAP-1 Ab that was produced by immunizing specific pathogen-free BALB/c mice with affinity-purified VAP-1 obtained from tonsil stromal lysate using 1B2-coupled CNBr-activated Sepharose 4B beads. Immunization, hybridoma production, screening, and testing were done using previously described techniques (19), except that SP-2/0 mouse cells were used as the myeloma partner. The specificity of TK8-14 against VAP-1 was shown by positive staining with VAP-1 (but not with mock) transfecants and by an identical staining pattern with the prototype anti-VAP-1 mAb 1B2 in tissue sections. In reciprocal immunoprecipitations of tonsil lysate, mAbs 1B2 and TK8-14 both depleted all VAP-1 reactivity from the sample when analyzed by immunoblotting. In gel analyses, it was evident that mAb TK8-14 reacts with the dimeric 170-kDa VAP-1 and, under reducing conditions, with the mono-meric 90-kDa form of VAP-1 (data not shown). FITC-conjugated mAbs against CD4 and CD8 were from Becton Dickinson (Palo Alto, CA); mAb 7C7 is a mouse mAb against epithelial cells of bursa of Fabricius in chicken, and mAb 3G6 is a mouse mAb against an Ag expressed on chicken peripheral T cells (produced by Drs. Maara Vuorela and Olli Vainio, respectively (University of Turku)). TIB146 (from American Type Culture Collection, Rockville, MD) is a rat mAb against mouse B cell-specific B220 Ag (24). Mouse mAbs 2C8 against human CD31 (25) and Hermes-3 against human CD44 (26) have been described. In this study, mAbs 7C7, 3G6, and TIB146 served as isotype-matched nonbinding control mAbs, and mAbs 2C8 and Hermes-3 as isotype-matched binding control mAbs.

PBL were isolated from healthy volunteers or from buffy coats. PLN were unaffected nodes removed during surgical operations performed for diagnostic purposes. Tonsils were from tonsillectomies.

At PLN HEV-derived endothelial cell line, Ax, was a kind gift from Dr. M. Miyasaka (University of Osaka, Osaka, Japan), and the cells were maintained as described (27). Stable VAP-1 transfecants were produced by transfecting the Ax cells with a VAP-1 cDNA15 in an eukaryotic expression vector pcDNA3, by lipofection and selecting geneticin-resistant colonies. Mock transfecants were done identically using the VAP-1 cDNA in an inverse orientation in the vector.

Immunostainings

Normal indirect immunoperoxidase staining of acetone-fixed frozen sections was done exactly as described (19). For two-color immunofluorescence stainings, the sections were sequentially overlaid with mAb 1B2, FITC-conjugated sheep anti-mouse Ig supplemented with 5% AB serum, mAb MECA-79, and finally, phycocyanin-conjugated mouse anti-rat Ig light-chain specific mAb supplemented with 5% normal mouse serum. In a negative control staining, isotype-matched nonbinding mAbs were used in the first and third stage. Between each incubation, the slides were washed twice in PBS. Coverslips were attached with Fluoromount. Sections were analyzed under epillumination and GR filter block by Leica, allowing simultaneous visualization of both fluorochromes.

Ax transfecants were harvested from tissue culture flasks by a trypsin-EDTA incubation. After washings, the cells were sequentially incubated with primary Abs (10 µg/ml) and then with an appropriate FITC-conjugated anti-mouse or anti-rat second stage reagent. Cells were analyzed using FACSscan and CellQuest software (Becton Dickinson).

Metabolic labelings

Metabolic labelings of tonsil in an in vitro culture system were done essentially as described (21). Briefly, small tonsil cubes were starved in an appropriate medium, labeled with NaH18SO4 or [35S]methionine/cysteine, and lysed in the lysis buffer (150 mM NaCl, 1.5 mM MgCl2, 10 mM Tris-base (pH 7.2), 1% Nonidet P-40, 1% aprotinin, and 1 mM PMSF). The Ags were immunoprecipitated from the precleared lysates with protein A beads armed with mAbs 1B2, 3G6, 2C8, MECA-79, and TIB146. After washings, the Ags were eluted in reducing Laemmli’s sample buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, and 0.01% bromphenol blue containing 5% 2-ME) and loaded in 5 to 12.5% gradient SDS-PAGE gels. All samples bearing the same label were analyzed in parallel lanes of one gel. After electrophoresis, the gels were fixed, soaked in Enlightening (DuPont, Wilmington, DE), dried, and subjected to autoradiography.

Immunoblottings and cross-precipitations

Immunoblottings were done essentially as described (21). Briefly, Ig-depleted Nonidet P-40 lysate from tonsil stroma was mixed with nonreducing Laemmli’s sample buffer. After mild heating (30 min at 37°C), the samples were resolved in SDS-PAGE and the Ags were transferred onto Hybond enhanced chemiluminescence (ECL) nitrocellulose filters for immunoblotting with the enhanced chemiluminescence system (Amersham, Arlington Heights, IL). For cross-precipitations, mAbs 1B2, TK8-14, MECA-79, 2C8, and TIB146 were coupled to rabbit anti-mouse- or rabbit anti-rat-conjugated protein A-Sepharose. Ig-depleted tonsil lysate was subjected to two rounds of preclearing with the specific protein A beads. Thereafter, the beads were discarded and the supernatants were depleted of residual Ig molecules by a 2-h incubation with protein G beads. Finally, the supernatants were filtered to remove any residual Ig-containing beads, mixed with Laemmli’s sample buffer, and subjected to SDS-PAGE and immunoblotting, as described above.

All samples for immunoblottings were analyzed in parallel lanes of the same gel whenever possible. In all cases, m.w. markers (Rainbow; Amersham) were included in all strips to allow precise juxtaposition of the lanes.

Peptide maps of VAP-1

VAP-1 was purified from precleared tonsil lysate by immunoadfinity chromatography using cyanogen bromide-activated Sepharose 4CL beads (Pharmacia, Piscataway, NJ), as suggested by the manufacturer. Bound VAP-1 Ag was eluted from the matrix with triethylamine (pH 11.5) and lyophilized. The purified Ag was resuspended in nonreducing Laemmli’s sample buffer, and a portion of it was loaded in SDS-PAGE gels that were subsequently silver stained for analyzing the purity of the preparation.

The rest of the sample was separated in another SDS-PAGE gel, electroblotted onto Problott polyvinylidene difluoride membrane (Applied Biosystems, Foster City, CA), and stained with Coomassie brilliant blue. The specific 90- and 170-kDa bands were excised separately, treated with polyvinylpyrrolidone (PVP-40; Sigma, St. Louis, MO), and subjected to digestion with 1 μg of sequencing-grade trypsin (Boehringer Mannheim, Indianapolis, IN). The membrane digestion was performed overnight using the protocol of Fernandez (28). The generated trypsin peptides were separated by HPLC equipped with a Vydac C18 column (2.1 mm × 150 mm) reverse-phase column (The Separations Group, Hesperia, CA). The solvent gradient used was 2 to 30% B (0–63 min), (A = 0.1% trifluoroacetic acid/H2O, B = 0.08% trifluoroacetic acid/acetonitrile), 30 to 60% B (63–95 min), 60 to 80% B (95–105 min), 80% B (105–110 min), and 80 to 2% B (110–115 min), with a flow rate of 150 μl/min. The detection wavelength was 220 nm.

Immunomagnetic cell separations and adhesion assays

CD4-positive and CD8-positive PBL were isolated using MACS (Miltenyi Biotech). A total of 100 to 300 × 106 cells was incubated with anti-CD4 or anti-CD8 MACS superparamagnetic microbeads and isolated in a B-type column in a MACS apparatus, according to the manufacturer’s instructions. Specificity of the isolation was always confirmed by staining the cells with FITC-conjugated anti-CD4 and anti-CD8 mAbs against different epitopes.

The HEV adhesion assays were done as described (29). Briefly, the target tissue was pretreated with the anti-endothelial or control mAbs for 30 min, and then the PBL subpopulations (3 × 105 cells/section) were added. The whole assay was done under nonstatic (rotation) conditions on an orbital shaker (60 rpm). After 30 min, the nonadherent cells were tilted off and the adherent cells were fixed to the sections by an overnight incubation in ice-cold PBS containing 1% glutaraldehyde. The sections were analyzed under dark-field microscopy, and the number of cells adherent to HEV was scored. Each adhesion assay was done two to five times, and at least 120 HEV per target tissue from at least two different donors were counted. The results are expressed as percentage of control binding, which is defined by the number of HEV-bound lymphocytes in control mAb-treated sections.

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**Results**

**VAP-1 and PNAd are expressed in an overlapping set of HEV.**

In PLN, both VAP-1 and PNAd are expressed in most HEV (data not shown). Therefore tonsil, which represents a mixed-type lymphatic organ containing both PLN- and mucosa-type HEV, was analyzed to study the colocalization of these two Ags. In many areas of tonsil, the vessels were positive for both Ags, but, in the same specimens, VAP-1 PNAd and VAP-1 PNAd HEV were also detected (Figs. 1 and 2). In certain HEV, both VAP-1 and PNAd were preferentially expressed luminally, and in others, expression was found throughout the whole thickness of the HEV cells. VAP-1 was also found in the dendritic cells of germinal centers and in the smooth muscle cells of vessels, whereas MECA-79 epitopes were absent from these cell types. Instead, fine irregular septae within lymphatic tissue stained positively with mAb MECA-79, but not with an anti-VAP-1 mAb 1B2. Thus, VAP-1 and PNAd are coexpressed prominently in many paracortical HEV that support most lymphocyte trafficking in vivo. Nevertheless, unique expression of one or the other of these molecules in certain HEV indicates that even adjacent HEV can have different adhesive functions.

**PNAd and VAP-1 have identical m.w.**

Data from different laboratories show that mAb MECA-79 recognizes several different protein cores, including a ~150 to 170- and a 90-kDa form (5, 9), which have a similar m.w. as we have reported for VAP-1 (21). We first wanted to see whether the electrophoretic mobilities of PNAd and VAP-1 are identical when analyzed in parallel under the same conditions. Avid incorporation of sulfate is a characteristic of many forms of PNAd (12), and therefore, small pieces of human tonsil were labeled in vitro with inorganic $^{35}$S sulfate. From these lysates, immunoprecipitation with mAb MECA-79 yielded prominent smeared bands of about 50, 105, and 170 kDa, and weaker, diffuse, 40- and 200-kDa bands (Fig. 3A). In contrast, no labeled molecules were precipitated with mAb 1B2.

When $[^{35}\text{S}]$methionine/cysteine was used as a label, weak signals from the 170-, 105-, and 50-kDa PNAd species were observed (Fig. 3B). From $[^{35}\text{S}]$methionine/cysteine lysates, the 90- and 170-kDa...
forms of VAP-1 were clearly seen. The unavoidable immunoprecipitation of nonspecific components at the 105- and 50-kDa range (similar in size to the CD34/Sgp90 and GlyCAM-1 components of PNAd) with an isotype-matched control mAb TIB146 rendered \[^{35}\text{S}\]methionine/cysteine labeling unfeasible for further experimentation.

Since metabolic labelings can selectively label different species and since neither label gave equally strong signals for VAP-1 and PNAd glycoproteins, we sought to determine the m.w. by another method. To that end, tonsil lysate was separated in SDS-PAGE and probed with mAbs MECA-79 and 1B2 in immunoblotting experiments. mAb 1B2 specifically reacted with a 170-kDa molecule that was indistinguishable in size from one of those seen by mAb MECA-79 (Fig. 3C). The 90-kDa form of VAP-1 was not detectable in immunoblots with mAb 1B2. Large-scale immunoisolation of tonsil VAP-1 yielded enough material from both the 90- and 170-kDa forms of VAP-1 for peptide mapping (Fig. 4B). The HPLC elution profiles include peptides that are released from the membrane during trypsin digestion. Especially large peptides are efficiently retained by the membrane and are not detected in the chromatogram, which reduces the complexity of the tryptic peptide mixtures. The positions and intensities of separated selected peptides, as shown in Figure 4A, strongly suggest that they originate from molecules with identical primary structures. Thus, the 170-kDa mature VAP-1 is a homodimer and the monomeric 90-kDa form of VAP-1 loses the 1B2 epitope.

Hence, using three different methods, a prominent 105-kDa (probably including CD34) and 170-kDa species and a less abundant 50-kDa (probably soluble GlyCAM-1) form of PNAd were observed. The sizes of VAP-1-specific bands were similar to those of the two PNAd glycoforms, but they lacked detectable sulfate incorporation.

mAb MECA-79 does not recognize VAP-1 transfectants

The staining and m.w. analyses would be compatible with the possibility that mAb 1B2 recognizes 90- and 170-kDa forms of PNAd. To further elucidate the molecular identity of VAP-1 and PNAd, we used novel VAP-1 cDNA expression transfectants made in a rat HEV-like endothelial cell line Ax. As shown in Figure 5, the original anti-VAP-1 mAb 1B2 and a novel anti-VAP-1 mAb TK8-14 brightly stain VAP-1, but not mock, transfectants, whereas control mAbs were negative. In contrast, the anti-PNAd mAb MECA-79 did not recognize VAP-1 transfectants. These results suggest that VAP-1 and PNAd are not identical molecules. However, the transfectant data do not prove the distinct molecular nature of VAP-1 and PNAd, since mAb MECA-79 recognizes an oligosaccharide-dependent epitope (14) and Ax cells may not be able to decorate rVAP-1 with the same modifications as occur in high endothelial cells in vivo.

The 170- and 90-kDa forms of VAP-1 and PNAd are distinct molecular species

To show directly that natural VAP-1 is not identical with the 170- and 90-kDa species of PNAd, cross-precipitations were performed. As shown in Figure 6, the 170-kDa species of VAP-1 was effectively and specifically depleted from the lysate with the protein A-Sepharose-1B2 beads \(lane\ 13\), whereas no change in MECA-79 reactivity \(lane\ 5\) was observed. In a complementary set of experiments, protein A-MECA-79 beads were used to deplete tonsil lysates of PNAd, and the precleared lysate was used for immunoblotting. The efficacy of preclearing was shown by the fact that all MECA-79-reactive bands were absent from this lysate (Fig. 6, lane 7). Importantly, the 170-kDa species of VAP-1 remained intact after removal of all forms of PNAd (Fig. 6, lane 15).
To study the identity of the 90-kDa VAP-1 and PNAd species, we had to use a novel anti-VAP-1 mAb, TK8-14, which detects monomeric VAP-1 in immunoblotting under reducing conditions. This is because mAb 1B2 detects a conformation-specific epitope present only in the dimeric form of VAP-1. When tonsil lysate was precleared with mAb TK8-14, the 90- to 100-kDa VAP-1 species disappeared (Fig. 7, lane 8), but the 90- to 100-kDa MECA-79 molecules remained intact (lane 4). In a reciprocal setup, depletion of all MECA-79-reactive species (lane 2) did not affect the detection of the 90-kDa VAP-1 molecule (lane 6). Control precipitations with irrelevant isotype-matched mAbs did not affect the reactivity of either VAP-1 or PNAd. Furthermore, mAb MECA-79 does not recognize purified VAP-1 Ag (data not shown). Thus, these studies unambiguously show that the 170- and 90-kDa species of PNAd and VAP-1 are indeed distinct molecular species.

Different T cell subtypes utilize VAP-1 and PNAd differently in PLN HEV adhesion

We have shown recently that VAP-1 mediates the binding of CD8-positive and CD16-positive lymphocytes, but not that of CD4-positive or CD19-positive lymphocytes or CD14-positive monocytes, to PLN HEV (20). We therefore wanted to compare whether the prototype peripheral lymph node addressin, PNAd, would also display lymphocyte subtype-selective adhesion pattern. To this end, binding of immunomagnetically purified CD4-positive and CD8-positive PBL to PLN HEV was analyzed. The purity of the CD4-positive and CD8-positive cells was 97 to 100% when analyzed by FACS. Anti-VAP-1 mAb 1B2 very efficiently blocked the adhesion of CD8-positive cells to PLN HEV, but it did not affect binding of CD4-positive cells (Fig. 8).

**FIGURE 4.** VAP-1 is a homodimeric ~170-kDa protein composed of two 90-kDa subunits. A, Immunoisolated VAP-1 from tonsil was resolved in SDS-PAGE gel that was subjected to silver staining. The two specific bands at 90 and 170 kDa are marked by an arrow and arrowhead, respectively. B, The 90- and 170-kDa bands were isolated, digested with trypsin, and separated by HPLC, as described in Materials and Methods. Note that the peptide elution profiles from both forms of VAP-1 are identical. PVP-40 elutes as a large peak in the end of the gradient.

**FIGURE 5.** rVAP-1 does not display MECA-79 epitopes. VAP-1 cDNA transfectants and mock-transfected controls in Ax cells were stained with the indicated mAbs (7C7 and TIB146 are negative controls, 1B2 and TK8-14 are against VAP-1, and MECA-79 is against PNAd) and analyzed using immunofluorescence and FACS. The x-axis is the relative fluorescence in a log scale, and the y-axis is the relative number of cells.
The anti-PNAd mAb, MECA-79, inhibited by 60% the adherence of CD8-positive cells to PLN HEV, but it also efficiently reduced interactions between PLN HEV and CD4-positive cells. In those experiments in which 1B2, MECA-79, and 1B2 + MECA-79 inhibitions were done in parallel using the same donor cells and the same target tissues, combined blocking of VAP-1 and PNAd did not augment the inhibition of PLN HEV adhesion of CD4-positive cells seen in the presence of mAb MECA-79. In these assays, 1B2 and MECA-79 showed marginal additive effects in PLN HEV binding of CD8-positive cells. This tendency, however, failed to reach statistical significance and, when the results from other experiments are combined, it is no longer detectable due to interindividual variation in the adhesion (Fig. 8). Thus, these data strongly indicate that mAbs 1B2 and MECA-79 recognize separate endothelial adhesion molecules and that CD4 cells only use PNAd. In contrast, both molecules are needed for the interactions between CD8-positive cells and PLN HEV, suggesting that these molecules mediate successive or overlapping steps in the adhesion cascade.

Discussion

We show in this study that VAP-1 and PNAd are often coexpressed in the same HEV and they include both ~90- and 170-kDa protein species. It has been shown earlier that these two adhesion molecules display similar characteristics both in tissue distribution and inducibility at similar sites during chronic inflammation (22, 23). Moreover, both molecules are sialoglycoproteins, the adhesive function of these two endothelial adhesion molecules depends on proper sialic acid modifications, and they both mediate early interactions between lymphocytes and the vessel wall (11, 14, 20, 21). Based on these similarities, we tested a hypothesis that VAP-1 would consist of the same 90- and 170-kDa molecules that are included among the six or more glycoproteins comprising the PNAd Ags. Our results show, however, that the Ags recognized by anti-VAP-1 and anti-PNAd Abs are molecularly distinct. We acknowledge that our present experiments cannot formally rule out the possibility that anti-VAP-1 mAbs would recognize an activation-dependent epitope of PNAd (if such exists). Thus, most likely, the present results reveal two molecularly independent adhesion mechanisms that mediate the binding of distinct lymphocyte subpopulations to PLN HEV in humans.

Both mAbs 1B2 and MECA-79 detect abundantly sialylated 90- and 170-kDa glycoprotein species from endothelial cells. The 170- and 90-kDa forms of PNAd are sulfated, and at least the 90-kDa form is also fucosylated, whereas VAP-1 apparently lacks these modifications (14, 21). The 170-kDa form of PNAd has not been molecularly cloned, but the 90-kDa species of PNAd was shown to be identical to CD34. Surprisingly, however, generation of CD34 null mice revealed that a 90-kDa MECA-79-reactive glycoprotein was still found in these animals (17). Thus, the 90-kDa form of PNAd consists of at least two distinct molecular species, CD34 and an uncharacterized sialoglycoprotein. In this study, we have used reciprocal immunoprecipitations and staining of VAP-1 transfectants to prove that the 170- and 90-kDa Ags of VAP-1 have different protein scaffolds from the PNAd glycosforms of similar size that react with mAb MECA-79.

Both VAP-1 and PNAd are preferentially expressed in the same set of HEV in vivo under normal conditions. Most PLN-type HEV synthesize both VAP-1 and PNAd, but exclusive expression of VAP-1 or PNAd in certain HEV offers a possibility to fine tune tissue-selective lymphocyte-endothelial cell interactions, since the lymphocyte counterreceptors of these two molecules are separate. L-selectin is the only known lymphocyte receptor for PNAd (5), whereas the lymphocyte receptor for VAP-1 is currently unknown. Nevertheless, we have shown that L-selectin is neither sufficient nor necessary to mediate the adhesion of PBL to PLN HEV via VAP-1 (20). Thus, in two morphologically indistinguishable venules, L-selectin-positive lymphocytes can bind either to VAP-1- or PNAd-positive HEV, whereas L-selectin-negative cells cannot interact via PNAd.

VAP-1 mediates T cell subtype-selective recognition of endothelial cells, since anti-VAP-1 mAb largely abolished PLN HEV adhesion of CD8-positive T cells, but had no effect on the binding of CD4-positive cells. In contrast, PNAd is important in PLN HEV recognition of both T cell subtypes, although adhesion of CD8-positive T cells was more efficiently blocked with
mAb MECA-79 than that of CD4-positive cells. In the case of PNAd-mediated adhesion, this may relate to the fact that L-selectin is expressed at significantly higher levels on CD8-positive cells than on CD4-positive lymphocytes (30). Recently, data have been emerging that different T cell subpopulations may rely on quite distinctive endothelial adhesion molecules during the first steps of the adhesion cascade. Th1-type CD4 cells, but not Th2-type T cells, have been shown to express a functionally intact P-selectin glycoprotein ligand-1 that allows them to bind to P-selectin and E-selectin and invade inflammatory sites in which a Th1-type response predominates (31, 32). On the other hand, memory-type RO-positive CD4-positive T cells, but not naive CD4-positive cells, have functionally active adhesion receptors that allow them to bind to E- and P-selectin and VCAM-1 under flow conditions (33). Moreover, our results directly imply that CD4-positive cells have to utilize an additional distinct adhesion pathway for PLN HEV binding since the binding of CD4-positive cells was only partially inhibited by anti-PNAd treatment and it was unaffected by mAbs against VAP-1. Together these data show that the specificity of the adhesion cascade may be critically controlled at the lymphocyte subpopulation level, possibly by selective expression of functional receptors for endothelial adhesion ligands.

Based on our results, we propose that during the multistep adhesion cascade most L-selectin-positive CD8-positive cells make simultaneous or successive contacts in PLN HEV between L-selectin and PNAd, and between a non-L-selectin receptor and VAP-1. Normal L-selectin-positive CD8-positive cells probably need both of these contacts for successful adhesion, since inhibition of either interaction leads to pronounced abrogation of PLN adhesion. On the other hand, L-selectin-negative effector cells (recently activated cells that have shed L-selectin and a subpopulation of memory cells (34)) may rely directly on VAP-1-dependent mechanisms in PLN HEV recognition. There is direct evidence that both VAP-1 and PNAd are involved in the early steps of the adhesion cascade that take place under conditions of shear (11, 20). Currently, we believe that PNAd may initiate the contacts between the blood-borne CD8-positive L-selectin-positive cells and endothelium more efficiently than VAP-1, but that VAP-1 is also brought into the play very quickly before the signaling step.

In conclusion, we have shown that VAP-1 and PNAd are distinct adhesion molecules. However, these molecules act in concert to mediate PLN HEV recognition of CD8-positive PBL in humans. These findings cast new light on the regulation of tissue-selective endothelial binding of distinct lymphocyte subpopulations during the adhesion cascade.


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