

# The Human Peripheral Lymph Node Vascular Addressin Is a Ligand for LECAM-1, the Peripheral Lymph Node Homing Receptor

Ellen L. Berg, Martyn K. Robinson, R. Aaron Warnock, and Eugene C. Butcher

Department of Pathology, Stanford University, Stanford, California 94305; and The Center for Molecular Biology and Medicine, The Veterans Administration Medical Center, Palo Alto, California 94304

**Abstract.** The trafficking of lymphocytes from the blood and into lymphoid organs is controlled by tissue-selective lymphocyte interactions with specialized endothelial cells lining post capillary venules, in particular the high endothelial venules (HEV) found in lymphoid tissues and sites of chronic inflammation. Lymphocyte interactions with HEV are mediated in part by lymphocyte homing receptors and tissue-specific HEV determinants, the vascular addressins. A peripheral lymph node addressin (PNAd) has been detected immunohistologically in mouse and man by monoclonal antibody MECA-79, which inhibits lymphocyte homing to lymph nodes and lymphocyte binding to lymph node and tonsillar HEV. The human MECA-79 antigen, PNAd, is molecularly distinct from the 65-kD mucosal vascular

addressin. The most abundant iodinated species by SDS-PAGE is 105 kD. When affinity isolated and immobilized on glass slides, MECA-79 immunisolated material binds human and mouse lymphocytes avidly in a calcium dependent manner. Binding is blocked by mAb MECA-79, by antibodies against mouse or human LECAM-1 (the peripheral lymph node homing receptor, the MEL-14 antigen, LAM-1), and by treatment of PNAd with neuraminidase. Expression of LECAM-1 cDNA confers PNAd binding ability on a transfected B cell line. We conclude that LECAM-1 mediates lymphocyte binding to PNAd, an interaction that involves the lectin activity of LECAM-1 and carbohydrate determinants on the addressin.

**A**DHESION to endothelial cells initiates the process of lymphocyte entrance into lymphoid organs and is also one of the initial events in lymphocyte recruitment into tissues at sites of inflammation. Lymphocytes are unique among leukocytes in that they continuously traffic between the blood and lymphatic systems, entering lymphoid organs by adhering to specialized high-walled venules known as high endothelial venules (HEV)<sup>1</sup> (Gowans and Knight, 1964; Marchesi and Gowans, 1964). They exit lymph nodes through efferent lymphatics, passing back to the blood via the thoracic duct. HEV are found preferentially in lymphoid organs and in sites of chronic inflammation. Specific lymphocyte and endothelial cell receptors are thought to mediate the adhesion process, and thus control the type of cells recruited into lymphoid organs and inflammatory sites.

Lymphocyte interactions with HEV are characteristically tissue specific. Lymphocyte subsets have the capacity to distinguish between HEV in peripheral lymph nodes, mucosal lymphoid tissues, the chronically inflamed synovium of ar-

thritic joints, and chronically inflamed skin (reviewed in Butcher et al., 1980; Jalkanen et al., 1986b; Berg et al., 1989; Chin et al., 1988). This capacity is controlled in part by lymphocyte expression of "homing receptors" for particular HEV and by endothelial cell expression of tissue-specific "vascular addressins" (Streeter et al., 1988a,b; Berg et al., 1989).

One of the best characterized lymphocyte homing receptors is involved in lymphocyte recognition of peripheral lymph node HEV. Antibodies to the peripheral lymph node (PLN) homing receptor, LECAM-1, block lymphocyte binding to PLN HEV and not Peyer's patch HEV, and selectively recognize PLN-HEV binding lymphocytes and lymphocyte cell lines (Gallatin et al., 1983). The expression of LECAM-1 is regulated during lymphocyte development, and after activation of lymphocytes *in vivo*, helps determine the capacity of defined virgin versus memory lymphocyte subpopulations to recirculate through peripheral lymph nodes (Butcher, 1986; Picker et al., 1989b). LECAM-1 is a member of the LEC-CAM or selectin gene family (reviewed in Stoolman, 1989) and appears to function as a lectin capable of binding in a neuraminidase sensitive-mechanism to PLN HEV (Imai et al., 1990).

The peripheral lymph node vascular addressin also plays a role in lymphocyte binding to PLN HEV (Streeter et al., 1988b, 1991). The vascular addressins are tissue-specific de-

M. K. Robinson's present address is Celltech Ltd., 216 Bath Road, Slough SCI 4EN Berkshire, England.

1. *Abbreviations used in this paper:* CM, complete medium; HEV, high endothelial venules; MAd, mucosal addressin; PBMC, peripheral blood mononuclear cells; PLN, peripheral lymph node; PNAd, peripheral lymph node addressin.

terminants on high endothelial venules which direct extravasating lymphocytes into the appropriate lymphoid or extralymphoid tissues (Streeter et al., 1988a,b; reviewed in Berg et al., 1989). The peripheral lymph node addressin (PNAd), defined by monoclonal antibody MECA-79, is a tissue-specific endothelial cell antigen found on HEV in both mouse and man (Streeter et al., 1988b, 1991). Immunohistologic studies in the mouse reveal that MECA-79 stains PLN HEV, and a major subset of mesenteric node HEV intensely, but in most mouse strains reacts only weakly with HEV in the mucosal lymphoid organs, the Peyer's patches. Vessels that support lymphocyte extravasation into the small intestinal lamina propria, which express the mucosal addressin (MAd), completely lack the MECA-79 antigen. In humans, HEV in PLN, tonsils, and some sites of extralymphoid chronic inflammation react with mAb MECA-79 (Streeter et al., 1991). Lamina propria venules in the small intestine are negative, as in the mouse, but HEV in the mucosal lymphoid tissues (Peyer's patches and appendix) stain with MECA-79, albeit less intensely than HEV in lymph nodes. In both species, mAb MECA-79 inhibits lymphocyte binding to PLN HEV, but not substantially to HEV in mouse Peyer's patches or human appendix, in which binding via the mucosal addressin predominates (Streeter et al., 1988b, 1991).

The present studies were undertaken to characterize human PNAd; to ask if it binds lymphocytes directly, or instead directs lymphocyte traffic to PLN by other means; to assess the importance of carbohydrate to its function; and ask whether it is the counterreceptor for the lymphocyte PLN homing receptor, LECAM-1.

## Materials and Methods

### Immunoisolation of PNAd

PNAd (the MECA-79 antigen), and control membrane glycoproteins LECAM-1 and H-CAM (the Hermes antigen or CD44; Jalkanen et al., 1986a) were isolated from human tonsils using a two-step procedure. Briefly, NP-40 tissue extracts were prepared as previously described (Streeter et al., 1988a; Nakache et al., 1988), then passed over wheat germ agglutinin-coupled agarose columns (Vector Labs, Inc., Burlingame, CA). Material eluting with 0.5 M *N*-acetylglucosamine was then passed sequentially through 2 ml affinity columns of rat IgM mAb control and Hermes-1 (anti-H-CAM or CD44) (Jalkanen et al., 1986a), or DREG-56 (anti-LECAM-1) (Kishimoto et al., 1990), and then MECA-79 coupled to Sepharose 4B (Pharmacia Inc., Piscataway, NJ). The column wash and elution conditions with  $\beta$ -octylglucoside-containing wash buffer were as previously described for the isolation and functional reconstitution of the mucosal addressin (Nakache et al., 1988). To assess the purity, an aliquot of each of the column eluates was iodinated by standard methods (Iodogen; Pierce Chemical Co., Rockford, IL), desalted to remove free iodine, and analyzed by SDS-PAGE followed by autoradiography. Samples run on SDS-PAGE and stained with silver show the same pattern of species (data not shown). Western blots on immunoisolated PNAd were performed as previously described (Streeter et al., 1988a), except that the second stage used was alkaline phosphatase-conjugated rabbit anti-rat IgG (H+L) from Tago, Inc. (Burlingame, CA). Preliminary studies with Western analysis confirmed that all detectable MECA-79-reactive species in tissue lysates were bound by wheat germ agglutinin.

### Binding of Lymphocytes and Cell Lines to PNAd

Samples of human PNAd or control proteins were absorbed onto glass wells of slides (LABTEK, Wilmington, MA) by dilution in Dulbecco's modified Eagles medium (DMEM; Applied Scientific, San Francisco, CA), followed by a 2-h incubation at room temperature. After blocking with complete medium (CM, 10 mM HEPES, 5% newborn calf serum [Gibco Laboratories,

Grand Island, NY] in DMEM), human peripheral blood mononuclear cells (PBMC), or PLN lymphocytes from normal BALB/c mice ( $1.5 \times 10^6/0.15$  ml CM) were applied to each well. After incubation for 20 min at room temperature on a rotating shaker (50 rpm), the tops of the wells were removed and the slides washed by dipping twice into coplin jars of DMEM and then fixed by incubation in 1.5% glutaraldehyde (Kodak) in DMEM for 1 h. PBMC were prepared by 1 g sedimentation of red blood cells with Dextran T500 (Pharmacia, Inc.) and then centrifugation of the white blood cells on Ficoll-histopaque (Histopaque 1077; Sigma Chemical Co., St. Louis, MO) to separate mononuclear cells from polymorphonuclear leukocytes and then 25% Percoll (Pharmacia, Inc.) to remove residual platelets. Most PNAd adherent cells were lymphocytes, as assessed morphologically and by Wrights stain. Both human and mouse lymphocyte interactions with human PNAd were studied as the tissue specificity of lymphocyte-HEV interactions is retained across species barriers. (Mouse lymphocytes and lymphocyte cell lines can bind to and discriminate between human PLN HEV and appendix HEV with the same specificity as do human lymphocytes, and human lymphocytes adhere to murine PLN HEV and mucosal HEV via the same distinct mechanisms as do mouse lymphocytes [Wu et al., 1988].) Binding of cell lines was carried out as described for lymphocytes. The human Jurkat JS-978 (Stoolman and Ebling, 1989) and mouse 38C13 (Gallatin et al., 1983) cell lines bind well to PLN HEV in frozen sections. The mouse cell line LI-2 does not bind either PLN or mucosal HEV; and mouse lines TK1 and TKJ43 avidly bind mucosal HEV, but do not bind well to PLN HEV (Butcher et al., 1980; Gallatin et al., 1983). LI-2 cells transfected with mouse LECAM-1 cDNA, as described below, were also tested. The amount of PNAd added per well ranged from  $\sim 10$ –100 ng (roughly estimated from comparison on silver-stained gels). In all of the studies presented here, the amount of PNAd added per well was limiting, as increasing the amount of PNAd added per well led to increased lymphocyte attachment. In contrast, increasing the amount of control proteins added to each well (either H-CAM or LECAM-1) never resulted in lymphocyte attachment.

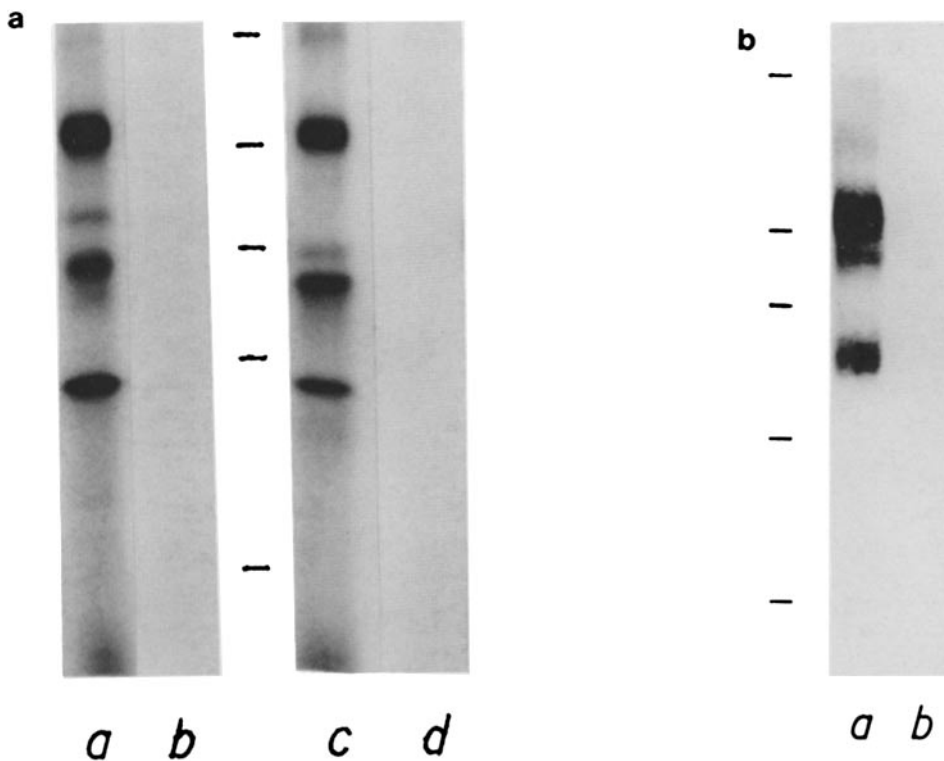
For some binding experiments, the wells containing PNAd were washed and treated either with mAbs MECA-79 or control rat IgM mAb HECA-452 (Duijvestijn et al., 1988) (200  $\mu$ g/ml in CM) for 30 min and then washed in CM before the addition of lymphocytes. Alternatively, wells containing PNAd were treated with *Vibrio cholera* neuraminidase 5 mU/ml (Calbiochem-Behring Corp., Palo Alto, CA) in 50 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 6.0, 2 mM CaCl<sub>2</sub> for 30 min before washing with CM and subsequent addition of lymphocytes. Similar results were obtained using the neuraminidase from *Arthrobacter ureafaciens* (data not shown).

The calcium dependence of the interaction of lymphocytes with PNAd was tested by performing the assay with lymphocytes suspended in HBSS without calcium or magnesium (Applied Scientific) containing 0.5 mM EGTA or HBSS containing 0.5 mM EGTA and 5 mM CaCl<sub>2</sub>.

To test anti-lymphocyte mAbs, human or mouse lymphocytes were preincubated with 100  $\mu$ g/ml/10<sup>7</sup> cells of the mAbs DREG-56 (anti-human LECAM-1) (Kishimoto et al., 1990), Hermes-3 (anti-human H-CAM or CD44) (Jalkanen et al., 1987), MEL-14 (anti-mouse LECAM-1) (Gallatin et al., 1983), RI/2 (anti-mouse VLA-4  $\alpha$  chain, LPAM) (Holzmann et al., 1989), 30-G12 (anti-mouse T2000) (Ledbetter and Herzenberg, 1982), FD441.8 (anti-mouse LFA-1 $\alpha$ , CD11a) (Sarmiento et al., 1982), MJ-64 (anti-mouse CD44), or media (10<sup>7</sup> cells/ml) for 30 min and then washed before the assay.

### Construction of Cell Lines Permanently Transfected with LECAM-1

A cDNA clone encoding LECAM-1 was obtained from a mouse mesenteric lymph node library by polymerase chain reaction amplification. The LECAM-1 gene was inserted downstream of the hCMV promoter in pMRB101 (a derivative of EEb which contains the *Escherichia coli gpt* gene) (Mulligan and Berg, 1981; Stephens and Cockett, 1989). DNA was introduced into LI-2 cells by electroporation (250 V, 1,180  $\mu$  Farads) and the cells selected for resistance to mycophenolic acid. A population of cells staining brightly with MEL-14 were selected by fluorescence activated cell sorting and cloned by limiting dilution. These cells, LI-2/pMRB112 (LI-2<sup>LECAM-1</sup>) and cells transfected with the parent vector alone, LI-2/pMRB101 (LI-2<sup>vector</sup>) were tested for binding to PNAd as described above, except that slides with cells bound were placed directly into fixative without washing. Except for LECAM-1 expression, the phenotypes of LI-2<sup>LECAM-1</sup> and LI-2<sup>vector</sup> cells are similar (T200<sup>+</sup>, LFA-1<sup>+</sup>, and CD44<sup>+</sup>). In addition, the LI-2 cells transfected with LECAM-1, but not the parent cell line or cells transfected with the vector alone, bind PLN HEV in frozen sections (data not shown).



**Figure 1.** SDS-PAGE and Western blot analysis of immunopurified peripheral lymph node addressin (PNAd). (a) Lanes a and c show iodinated samples of PNAd, lanes b and d show similarly treated sample eluates from a control column prepared with an irrelevant class matched control antibody. (b) Lane a shows a nitrocellulose blot of the affinity isolated PNAd or eluate from a control rat IgM column (lane b) probed with MECA-79. Samples were run on 8% (a) or 10% gels (b) under reducing conditions except for those in a, lanes c and d, which were run under nonreducing conditions. (Molecular mass markers are Rainbow Markers from Amersham Corp. (Arlington Heights, IL) which, in our gel system, migrate at 200, 96, 78, 50, and 31 kD).

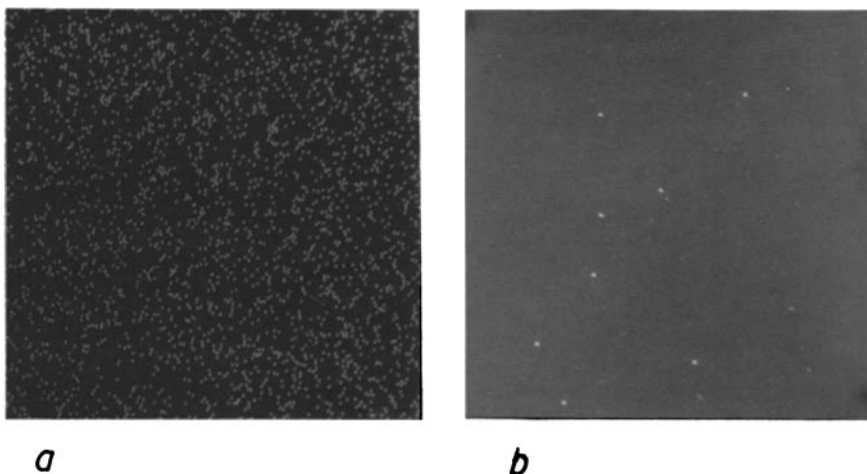
## Results

### Immunoprecipitation of PNAd

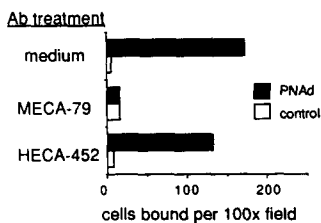
Immunoisolated PNAd, prepared by mAb affinity chromatography from extracts of human tonsil and iodinated, consists of a predominant polypeptide migrating at ~105 kD, and several less intense bands in 10% SDS-PAGE gels (Fig. 1 a, lanes a and c). The 105-kD species (whose appearance on SDS-PAGE ranges from 90 to 105 kD depending on gel conditions) is also the predominant band seen in gels stained with silver (data not shown), although in Western blots (Fig. 1 b, lane a), additional bands at 65, 90, 150, and 200 kD are detected by MECA-79 (note that the smallest immunoprecipitated band of 45 kD is not recognized by MECA-79 by Western blotting).

### Isolated PNAd Is an Adhesion Molecule for Lymphocytes

The MECA-79 antigen, PNAd, could direct lymphocyte extravasation either by binding circulating lymphocytes, or by a less direct mechanism such as activation of adhesion molecules for distinct HEV ligands. PNAd was isolated from human tonsils by sequential wheat germ agglutinin and antibody affinity chromatography and adsorbed onto glass slides. PNAd coated wells bound lymphocytes avidly (Fig. 2 a). In contrast, lymphocytes bound poorly to wells coated with serum or with control membrane proteins, including human CD44 (Fig. 2 b), murine CD44, and mouse or human LECAM-1 homing receptors (not shown). Pretreatment of the PNAd coated wells with mAb MECA-79 blocked lymphocyte attachment (Fig. 3), while control antibodies had no



**Figure 2.** Binding of mouse lymphocytes to (a) immobilized peripheral lymph node addressin, or (b) control protein, H-CAM (CD44).

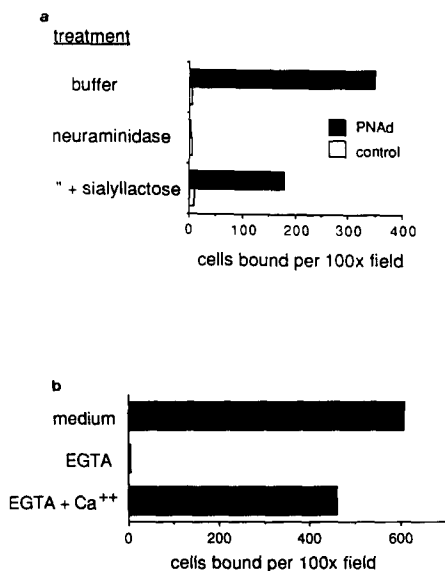


**Figure 3.** Binding of lymphocytes to PNAd is blocked by antibody MECA-79 against PNAd. Experiments were performed as described in Materials and Methods with immobilized PNAd and H-CAM, as control. Six 100 $\times$  fields were counted and the average number of cells bound per field for each sample or condition are reported. The results presented are from a representative experiment of two to four performed with similar results. The standard deviation of the number of cells bound per field was <30% of the number of cells bound ( $n = 6$  fields/sample).

effect on binding, confirming the specificity of the interaction.

### Lymphocyte Binding Ability of PNAd Is Calcium Dependent and Abrogated by Neuraminidase Treatment

Carbohydrate determinants on PLN HEV have previously been implicated in lymphocyte attachment (Stoolman et al., 1984, 1987; Rosen et al., 1985, 1989; Yednock et al., 1987). Certain anionic mono- and polysaccharides block lymphocyte attachment to PLN HEV in frozen sections (Stoolman et al., 1984, 1987; Yednock et al., 1987). In addition, neuraminidase-treated PLN frozen sections do not support lymphocyte binding and neuraminidase treatment in vivo selectively blocks lymphocyte trafficking to PLN (Rosen et al., 1985, 1989). These effects could be due to charge effects on HEV, alteration of accessory mechanisms, or a direct effect on the HEV ligand. As shown in Fig. 4 *a*, treatment of immobilized PNAd with *Vibrio cholera* neuraminidase completely prevents lymphocyte binding. The inclusion of the



**Figure 4.** Binding of lymphocytes to PNAd is blocked by neuraminidase treatment of PNAd (*a*) and is calcium dependent (*b*). Experiments were carried out as described in Materials and Methods with immobilized PNAd or H-CAM as control. Data were treated and reported as in Fig. 3.

sialidase inhibitor sialyllactose blocks the effect of the neuraminidase. This suggests that the ability of neuraminidase to block lymphocyte-HEV interactions is due to a direct effect on PNAd itself and that the interaction of lymphocytes with PNAd involves sialic acid residues. Interestingly, the MECA-79 epitope on PNAd is not affected by neuraminidase treatment (data not shown).

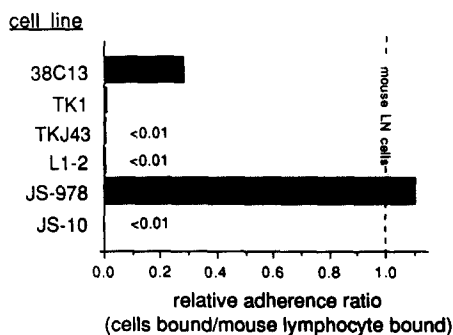
Like lymphocyte attachment to HEV in PLN frozen sections, binding of lymphocytes to PNAd requires calcium, as preincubation of lymphocytes with EGTA inhibits their ability to bind isolated PNAd, while addition of calcium restores it (Fig. 4 *b*).

### PNAd Selectively Binds Lymphoid Cells That Can Bind to PLN HEV

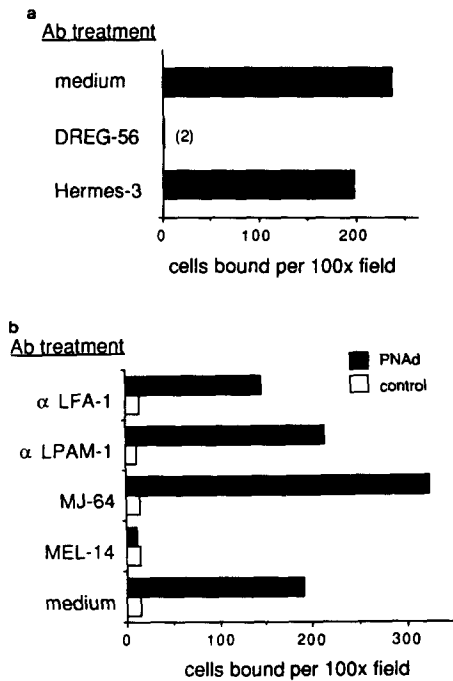
As the original identification of tissue-specific lymphocyte-HEV interactions was based on lymphoid cell lines of restricted binding specificities, we next compared the binding of cell lines that bind well to PLN HEV with that of cells that bind selectively to mucosal HEV, or cell lines which do not bind HEV (Fig. 5). Two variants of the human Jurkat T cell line (generously provided by Lloyd Stoolman, University of Michigan) have been generated which differ dramatically in their expression of LECAM-1 and their capacity to adhere to PLN HEV (Stoolman and Ebling, 1989). Only the high binding cell line, JS-978, which expresses high levels of LECAM-1, was able to adhere to isolated PNAd. The mouse lymphoma cell line, 38C13, an avid PLN HEV-selective binder (Gallatin et al., 1983), which expresses high levels of mouse LECAM-1, also bound well to PNAd. In contrast, cell lines that express little if any LECAM-1, including the mucosal HEV-binding cell lines TKJ43 and TK1 and the HEV nonbinding L1-2 (Butcher et al., 1980; Gallatin et al., 1983) cell line, did not bind to isolated PNAd, although in parallel experiments TKJ43 and TK1 bound well to isolated mucosal addressin (data not shown). These experiments demonstrate the specificity of PNAd for lymphocyte cell lines which express high levels of LECAM-1 and bind PLN HEV.

### PNAd Is a Countereceptor for LECAM-1

Lymphocyte interactions with PLN HEV involve the periph-



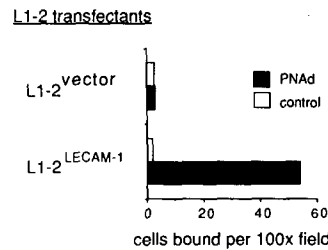
**Figure 5.** Binding of cell lines to PNAd. Cell lines were tested for their binding to PNAd as described in Materials and Methods. Each cell line was tested two to four times with similar results. Data from representative experiments were normalized to that of mouse lymphocytes (binding of mouse lymphocytes ranged from 173 to 570 cells/100 $\times$  field for these experiments). Binding to control protein (human H-CAM/CD44) by these cell lines ranged from one to two cells/100 $\times$  field.



**Figure 6.** Human PBMC (a) or mouse lymphocyte (b) adherence to PNAd is blocked by mAbs against the PLN homing receptor, LECAM-1. Lymphocytes were pretreated with mAbs and tested for PNAd binding as described in Materials and Methods. In b, the control used was H-CAM (CD44). Data were treated and reported as for Fig. 3.

eral lymph node homing receptor LECAM-1, defined by the monoclonal antibodies MEL-14 in the mouse and DREG-56, Leu-8, and others in the human (Gallatin et al., 1983; Tedder et al., 1989; Camerini et al., 1989; Kishimoto et al., 1990). Anti-LECAM-1 antibodies that inhibit lymphocyte-HEV interactions were tested for their ability to block lymphocyte binding to isolated human PNAd. Human lymphocyte binding to PNAd was completely blocked by pretreatment of the cells with DREG-56 (Fig. 6a), and mouse lymphocyte binding was blocked by pretreatment with MEL-14 (Fig. 6b). Binding was not inhibited by antibodies against the leukocyte common antigen, T200 (CD45), or against mouse or human CD44, including Hermes-3 which blocks human lymphocyte attachment to Peyer's patch HEV (Jalkanen et al., 1987). Antibody to LFA-1 (CD11a/CD18) had a slight but reproducible blocking effect on binding to PNAd. Antibody R1/2 to the mouse integrin  $\alpha_4$  chain, which inhibits lymphocyte binding to Peyer's patch but not PLN HEV in vitro (Holzmann et al., 1989), also failed to influence binding to purified PNAd. In contrast to its inhibitory effect on binding to PNAd, MEL-14 had no effect on mouse lymphocyte adhesion to purified MAD (data not shown). The nearly complete inhibition of PNAd binding by antibodies to LECAM-1 suggests that LECAM-1 either binds PNAd or is physically or functionally associated with a PNAd receptor on lymphocytes.

To examine the LECAM-1-PNAd interaction directly, the mouse pre-B cell line, L1-2, (which does not express LECAM-1, Gallatin et al., 1983) was transfected with cDNA encoding mouse LECAM-1 and the transfectants tested for



**Figure 7.** Mouse L1-2 cells transfected with LECAM-1 cDNA bind to PNAd. Experiment was carried out as described in Materials and Methods with immobilized PNAd or control protein H-CAM (CD44). Data were treated and reported as in Fig. 3.

their PNAd-binding capacity. As seen in Fig. 7, L1-2 cells transfected with cDNA containing the LECAM-1 gene, L1-2<sup>LECAM-1</sup>, but not L1-2 cells transfected with the vector alone, L1-2<sup>vector</sup>, bound specifically to isolated PNAd adsorbed onto glass slides. These results indicate that the peripheral lymph node addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor.

## Discussion

The results presented here demonstrate that the peripheral lymph node addressin mediates lymphocyte attachment through LECAM-1, and suggest that carbohydrate determinants on one or more of the PNAd species, including neuraminidase-sensitive sialic acid residues, comprise an important part of the physiological ligand(s) for LECAM-1. A number of glycoprotein species of distinct molecular weights bear the anti-PNAd mAb MECA-79 epitope; the predominant iodinated species is ~105 kD under reducing and nonreducing conditions. In the mouse, MECA-79 recognizes a similar pattern of species by Western blot, with two predominant glycoproteins of 90 and 115 kD, a minor 65-kD species, and additional species reproducibly detected at 75, 170, and 200 kD (Berg, E., P. Streeter, and E. C. Butcher, manuscript in preparation). Interestingly, in the mouse, MECA-79 also reacts with an ~50-kD species that incorporates <sup>35</sup>S<sub>4</sub> (metabolically labeled by incubation of lymph node fragments), but labels poorly if at all with iodine. In independent studies, Rosen and co-workers have confirmed that this component of mouse PNAd is also a ligand for LECAM-1. It is precipitated by a chimeric antibody containing LECAM-1 and the hinge and constant regions of the human immunoglobulin heavy chains (Watson et al., 1990), as is the dominant 105-kD major PNAd polypeptide described here (Berg, E., unpublished results). It remains to be determined which molecular species identified by MECA-79 represent the dominant LECAM-1 binding element or elements; whether one, a subset, or all can interact with the lectin; and whether any functionally significant species recognized by LECAM-1 lack the MECA-79 epitope. However, our results raise the possibility that both the MECA-79 epitope and LECAM-1 binding ability are determined not primarily by a single glycoprotein species or receptor, but rather by unique PLN HEV-specific glycosyltransferases or other mechanisms of posttranslational modification that can decorate more than one HEV surface acceptor molecule with mAb MECA-79 and/or LECAM-1 binding sites.

This model is consistent with the known importance of the lectin domain of LECAM-1 in recognition of HEV, and with our emerging understanding of the structure/function relationships of carbohydrate ligands of two related lectins in-

volved in leukocyte-endothelial cell interactions, ELAM-1 and GMP-140. These molecules are members of a subfamily of C-type (calcium-dependent) lectins, the LEC-CAMs or selectins, characterized structurally by an NH<sub>2</sub>-terminal domain homologous to C-type lectins, an epidermal growth factor-like domain, a variable number of repeating units related to complement regulatory sequences, a transmembrane domain and a short cytoplasmic tail (Siegelman et al., 1989; Lasky et al., 1989; Tedder et al., 1989; Camerini et al., 1989; Bevilacqua et al., 1989; Johnston et al., 1989). The lectin domains of these molecules are important to their function. Recognition of neutrophils by GMP-140 and ELAM-1, like recognition of PNA<sub>d</sub> by LECAM-1, is calcium dependent and sensitive to ligand treatment with neuraminidase (Phillips et al., 1990; Walz et al., 1990; Corral et al., 1990; Moore et al., 1991; Berg, E., personal observations). A major neutrophil carbohydrate ligand for ELAM-1 has been identified as the sialated Lewis X antigen, sLe<sup>x</sup> (Lowe et al., 1990; Phillips et al., 1990; Walz et al., 1990), and neuraminidase-sensitive determinants, perhaps associated with the Lewis X antigen itself, Le<sup>x</sup>, have been implicated as ligands for GMP-140 (Larsen et al., 1990; Corral et al., 1990; Moore et al., 1991). sLe<sup>x</sup> and Le<sup>x</sup> are known to modify multiple glycoprotein species as well as glycolipids on neutrophils and the expression of these carbohydrate determinants by neutrophils may be determined, in part, by expression of a specific alpha (1,3) fucosyltransferase (Goelz et al., 1990). It is attractive to hypothesize that the mAb MECA-79 may recognize a sialic acid-independent carbohydrate structure whose expression is conferred by a PLN HEV-specific glycosyltransferase or other carbohydrate-modifying enzyme, and that comprises the core of the sialic acid-modified LECAM-1 recognition site. It is also possible, however, that the LECAM-1 ligand is an independent carbohydrate structure expressed in close proximity to the MECA-79 epitope.

It is worth noting that sLe<sup>x</sup> and Le<sup>x</sup> are unlikely to be natural HEV ligands for LECAM-1 as HEV do not express appreciable levels of either antigen, at least when examined with specific monoclonal antibodies. Furthermore, monoclonal anti-sLe<sup>x</sup>, which inhibits neutrophil adhesion to ELAM-1 cDNA transfectants, has no effect on lymphocyte binding to isolated PNA<sub>d</sub> (Berg, E., and T. Yoshino, unpublished results).

In addition to PNA<sub>d</sub>, two other vascular addressins have been identified. The mucosal addressin (MA<sub>d</sub>) is a 60-kD glycoprotein involved in trafficking of mucosa-homing lymphocytes and is preferentially expressed on HEV in mucosal lymphoid tissues and venules within the gut lamina propria (Streeter et al., 1988a). Like PNA<sub>d</sub>, MA<sub>d</sub> is an adhesion molecule for lymphocytes, however, it selectively binds lymphocytes and lymphoid cell lines that interact with mucosal HEV (Nakache et al., 1988). ELAM-1, first characterized as an inducible endothelial cell adhesion molecule for neutrophils (Bevilacqua et al., 1987), plays a second role as a skin vascular addressin. In the setting of chronic inflammation, it is preferentially expressed in cutaneous sites, and it selectively binds a skin-associated memory T cell subset defined by expression of the cutaneous lymphocyte antigen (Picker et al., 1990a, 1991). Thus, the importance of the LEC-CAM/selectin gene family to lymphocyte traffic is underscored by the involvement of two of its members, LECAM-1

and ELAM-1, in tissue specific lymphocyte-endothelial cell recognition.

In conclusion, our results define a tissue-specific endothelial cell adhesion mechanism capable of directing the traffic of lymphocytes in man, and suggest that this mechanism involves a lectin-carbohydrate interaction between LECAM-1 and one or more species immunisolated with the PNA<sub>d</sub>-specific mAb, MECA-79.

We thank R. Bargatze, P. Falanga, R. Hallman, K. Kishimoto, S. Michie, and L. Picker for critical review of this manuscript and for helpful discussions; L. Stoolman for human Jurkat cell lines; T. K. Kishimoto for the mouse mesenteric lymph node cDNA library; and L. Picker for generous provision of human tissues.

E. Berg was supported by a postdoctoral fellowship from the American Cancer Society and is a Special Fellow of the Leukemia Society of America. E. Butcher is an Established Investigator of the American Heart Association. This work was supported in part by grants GM37734, GM41965, and AI19957 from the National Institutes of Health and by an award from the Veterans Administration.

Received for publication 25 January 1991 and in revised form 18 April 1991.

### References

- Berg, E. L., L. A. Goldstein, M. A. Jutila, M. Nakache, L. J. Picker, P. R. Streeter, N. W. Wu, D. Zhou, and E. C. Butcher. 1989. Homing receptors and vascular addressins: cell adhesion molecules that direct lymphocyte traffic. *Immunol. Rev.* 108:5-18.
- Bevilacqua, M. A., J. S. Pober, D. L. Mendrick, R. S. Cotran, and M. A. Gimbrone, Jr. 1987. Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 84:9238-9242.
- Bevilacqua, M., S. Stenglein, M. Gimbrone, and B. Seed. 1989. Endothelial-leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science (Wash. DC).* 243:1160-1165.
- Butcher, E. C. 1986. The regulation of lymphocyte traffic. *Curr. Top. Microbiol. Immunol.* 128:85-122.
- Butcher, E. C., R. G. Scollay, and I. L. Weissman. 1980. Organ specificity of lymphocyte migration: mediation by highly selective lymphocyte interaction with organ-specific determinants on high endothelial venules. *Eur. J. Immunol.* 10:556-561.
- Camerini, D., S. P. James, I. Stamenkovic, and B. Seed. 1989. Leu-8/TQ1 is the human equivalent of the MEL-14 lymph node homing receptor. *Nature (Lond.).* 280:496-498.
- Chin, Y.-H., V. Falanga, J. W. Streilein, and R. Sackstein. 1988. Specific lymphocyte-endothelial cell interactions regulate migration into lymph nodes, Peyer's patches and skin. *Reg. Immunol.* 1:78-83.
- Corral, L., M. S. Singer, B. A. Macher, and S. D. Rosen. 1990. Requirement for sialic acid on neutrophils in a GMP-140 (PADGEM) mediated adhesive interaction with activated platelets. *Biochem. Biophys. Res. Commun.* 172:1349-1356.
- Duijvestijn, A., E. Horst, S. T. Pals, B. N. Rouse, A. C. Steere, L. J. Picker, C. J. Meijer, and E. C. Butcher. 1988. High endothelial differentiation in human lymphoid and inflammatory tissues defined by monoclonal antibody HECA-452. *Am. J. Path.* 130:147-155.
- Gallatin, W. M., I. L. Weissman, and E. C. Butcher. 1983. Cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature (Lond.).* 304:30-34.
- Goelz, S. E., C. Hession, D. Goff, B. Griffiths, R. Tizard, B. Newman, G. Chi-Rosso, and R. Lobb. 1990. ELFT: a gene that directs the expression of an ELAM-1 ligand. *Cell.* 63:1349-1356.
- Gowans, J. L., and E. J. Knight. 1964. The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. B. Biol. Sci.* 159:257-282.
- Holzmann, B., B. McIntyre, and I. Weissman. 1989. Identification of a murine Peyer's patch-specific lymphocyte homing receptor as an integrin molecule with an alpha chain homologous to human VLA-4 alpha. *Cell.* 56:37-46.
- Imai, Y., D. D. True, M. S. Singer, and S. D. Rosen. 1990. Direct demonstration of the lectin activity of gp90<sup>MEL</sup>, a lymphocyte homing receptor. *J. Cell Biol.* 111:1225-1232.
- Jalkanen, S., R. F. Bargatze, L. Herron, and E. C. Butcher. 1986a. A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. *Eur. J. Immunol.* 16:1195-1202.
- Jalkanen, S., A. C. Steere, R. I. Fox, and E. C. Butcher. 1986b. A distinct endothelial cell recognition system that controls lymphocyte traffic into inflamed synovium. *Science (Wash. DC).* 233:556-558.

- Jalkanen, S., R. F. Bargatze, J. Toyos, and E. C. Butcher. 1987. Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85-95-kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. *J. Cell Biol.* 105:983-990.
- Johnston, G. I., R. G. Cook, and R. P. McEver. 1989. Cloning of gmp 140, a granule membrane protein of platelets and the endothelium: sequence similarity to proteins involved in cell adhesion and inflammation. *Cell.* 56:1033-1044.
- Kishimoto, T. K., M. A. Jutila, and E. C. Butcher. 1990. Identification of a human peripheral lymph node homing receptor: a rapidly down-regulated molecule. *Proc. Natl. Acad. Sci. USA.* 87:2244-2248.
- Lasky, L. A., M. Singer, T. Yednock, D. Dowbenko, C. Fennie, H. Rodriguez, T. Nguyen, S. Stachel, and S. D. Rosen. 1989. Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell.* 56:1045-1055.
- Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47:63-90.
- Lowe, J. B., L. M. Stoolman, R. P. Nair, R. D. Larsen, T. L. Berhend, and R. M. Marks. 1990. ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell.* 63:475-484.
- Marchesi, V. T., and J. L. Gowans. 1984. The migration of lymphocytes through the endothelium of venules in lymph nodes. *Proc. R. Soc. Lond. B Biol. Sci.* 159:283-290.
- Moore, K. L., A. Varki, and R. P. McEver. 1991. GMP-140 binds to a glycoprotein receptor on human neutrophils: evidence for a lectin-like interaction. *J. Cell Biol.* 112:491-499.
- Mulligan, R. C., and P. Berg. 1981. Selection for animal cells that express the Escherichia coli gene coding for xanthine-guanine phosphoribosyltransferase. *Proc. Natl. Acad. Sci. USA.* 78:2072-2076.
- Nakache, M., E. L. Berg, P. R. Streeter, and E. C. Butcher. 1988. The mucosal vascular addressin is a tissue-specific endothelial cell adhesion molecule for circulating lymphocytes. *Nature (Lond.).* 337:179-181.
- Phillips, M. L., E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S.-I. Hakomori, and J. C. Paulson. 1990. ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, sialyl-Le<sup>x</sup>. *Science (Wash. DC).* 250:1130-1132.
- Picker, L. J., S. A. Michie, L. S. Rott, and E. C. Butcher. 1990a. A unique phenotype of skin-associated lymphocytes in humans. *Am. J. Pathol.* 136:1053-1068.
- Picker, L. J., L. W. M. Terstappen, L. S. Rott, P. R. Streeter, H. Stein, and E. C. Butcher. 1990b. Differential expression of homing associated adhesion molecules by T cell subsets in man. *J. Immunol.* 145:3247-3255.
- Picker, L. J., T. K. Kishimoto, C. W. Smith, R. A. Warnock, and E. C. Butcher. 1991. ELAM-1 is an adhesion molecule for skin-homing T cells. *Nature (Lond.).* 349:796-799.
- Rosen, S., M. Singer, T. A. Yednock, and L. M. Stoolman. 1985. Involvement of sialic acid on endothelial cells in organ-specific lymphocyte recirculation. *Science (Wash. DC).* 228:1005-1007.
- Rosen, S. D., S. I. Chi, D. D. True, M. S. Singer, and T. A. Yednock. 1989. Intravenously injected sialidase inactivates attachment sites for lymphocytes on high endothelial venules. *J. Immunol.* 142:1895-1902.
- Sarmiento, M., D. P. Dialynas, D. W. Lancki, K. A. Wall, M. I. Lorber, M. R. Loken, and F. W. Fitch. 1982. Cloned T lymphocytes and monoclonal antibodies as probes for cell surface molecules active in T cell-mediated cytotoxicity. *Immunol. Rev.* 68:135-169.
- Siegelman, M., M. van de Rijn, and I. Weissman. 1989. Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science (Wash. DC).* 243:1165-1172.
- Stephens, P. E., and M. I. Cockett. 1989. Construction of a highly efficient and versatile set of mammalian expression vectors. *Nucleic Acids Res.* 17:7110.
- Stoolman, L. M. 1989. Adhesion molecules controlling lymphocyte migration. *Cell.* 56:907-910.
- Stoolman, L. M., and H. Ebling. 1989. Adhesion molecules of cultured hematopoietic malignancies. A calcium-dependent lectin is the principle mediator of binding to the high endothelial venule of lymph nodes. *J. Clin. Invest.* 84:1196-1205.
- Stoolman, L. M., T. S. Tenforde, and S. D. Rosen. 1984. Phosphomannosyl receptors may participate in the adhesive interaction between lymphocytes and high endothelial venules. *J. Cell Biol.* 99:1535-1540.
- Stoolman, L. M., T. A. Yednock, and S. D. Rosen. 1987. Homing receptors on human and rodent lymphocytes: evidence for a conserved carbohydrate-binding specificity. *Blood.* 70:1842-1850.
- Streeter, P. R., E. L. Berg, B. T. Rouse, R. F. Bargatze, and E. C. Butcher. 1988a. A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature (Lond.).* 331:41-46.
- Streeter, P. R., B. T. Rouse, and E. C. Butcher. 1988b. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol.* 107:1853-1862.
- Streeter, P. R., L. J. Picker, S. Michie, and E. C. Butcher. 1991. A human peripheral lymph node vascular addressin. *Cell Reg.* In press.
- Tedder, T. F., C. M. Isaacs, T. J. Ernst, G. Demetri, D. Adler, and C. M. Distech. 1989. Isolation and chromosomal localization of cDNAs encoding a novel human lymphocyte cell surface molecule, LAM-1. *J. Exp. Med.* 170:123-133.
- Walz, G., A. Aruffo, W. Kolanus, M. Bevilacqua, and B. Seed. 1990. Recognition by ELAM-1 of the sialyl-Le<sup>x</sup> determinant on myeloid and tumor cells. *Science (Wash. DC).* 250:1132-1135.
- Wu, N. W., S. Jalkanen, P. R. Streeter, and E. C. Butcher. 1988. Evolutionary conservation of tissue-specific lymphocyte-endothelial cell recognition mechanisms involved in lymphocyte homing. *J. Cell Biol.* 107:1845-1851.
- Yednock, T. A., E. C. Butcher, L. M. Stoolman, and S. D. Rosen. 1987. Receptors involved in lymphocyte homing: relationship between a carbohydrate-binding receptor and the MEL-14 antigen. *J. Cell Biol.* 104:725-731.