Homing Receptors on Human and Rodent Lymphocytes—Evidence for a Conserved Carbohydrate-Binding Specificity

By L.M. Stoolman, T.A. Yednock, and S.D. Rosen

Lymphocytes continuously migrate from the bloodstream into lymphoid organs. This process distributes normal lymphocytes throughout the lymphoid system and may facilitate the dissemination of lymphoid malignancies. In peripheral lymph nodes (PNs) and gut-associated lymphoid tissues (GALTs) (ie, Peyer’s patches or appendix), transvenular migration begins with the attachment of circulating lymphocytes to postcapillary venules with a distinctive cuboidal endothelium (the high endothelial venules or HEVs).

Several lines of evidence indicate that this adhesive interaction is organ selective. In the rat, monospecific antisera have been developed that block the attachment of recirculating lymphocytes to the HEVs of either PNs or GALTs. In the mouse and human systems, T cells as a population show preferential binding to the HEVs of PNs whereas B cells bind preferentially to the HEVs of GALTs. In addition, murine lymphomas have been identified that bind to the HEVs of PNs or GALTs with nearly absolute fidelity. These data suggest that the attachment of lymphocytes to postcapillary venules with a distinctive cuboidal endothelium (the high endothelial venules or HEVs) may facilitate the dissemination of lymphoid malignancies.

Candidate adhesion molecules on the lymphocyte surface (referred to as homing receptors) have been isolated from rodents. In mouse, a ubiquinated glycoprotein (gp) termed gp 90 mel-14 appears to be the PN-homing receptor. In the rat, distinct gps with PN-HEVs and GALT-HEVs binding activities have been detected in thoracic duct lymph. Considerable evidence suggests that these factors are shed lymphocytic homing receptors.

We have been interested in the biochemical basis of the adhesive interaction between lymphocytes and the HEVs. Previous work indicates that a carbohydrate-binding receptor on the lymphocyte surface participates in the attachment of rodent lymphoid cells to PN-HEVs in vitro and in vivo. In the mouse, this receptor is either identical or closely related to gp 90 Mel-14. In addition, we have shown directly that carbohydrate moieties on the PN-HEVs are required for lymphocyte attachment. These results support the hypothesis that organ-selective migration across the PN-HEV begins with the binding of a lectin-like receptor on lymphocytes to a carbohydrate-containing ligand on the endothelial surface.

In this article, we present evidence that the attachment of human peripheral blood lymphocytes to PN-HEVs also involves a carbohydrate-binding receptor on the lymphocyte surface. The binding specificity of the receptor is similar to its counterparts on rodent lymphocytes. The preservation of carbohydrate binding specificity across species lines suggests that the recognition/binding domains of the adhesive structures on lymphocytes and HEVs have been conserved through evolution.

MATERIALS AND METHODS

Chemicals

Heparin (H3125), chondroitin sulfate (C3254), hyaluronic acid (H7630), all phosphorylated monosaccharides, bovine serum albumin (BSA) (A7906), trypsin type III (T8253), borax (B9876), boric acid (B0252), cyanogen bromide (C6388), acetyl cysteine (C0250), disodium EDTA (ED2SS), Histopaque (1077), poly-L-lysine (P1524), clostridial neuraminidase (2133), and paraformaldehyde (P6148) were obtained from Sigma Chemical (St Louis). PPME, the polyphosphoammonium core from Hansenula hostii phosphomannan, was generously gifted from Dr M. E. Sliodki (U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL). Lyophilized thrombin was obtained from Parke-Davis (Ann Arbor, MI). Fluorescent microspheres (0.6-μm, FX, 112030-30) were obtained from Duke Scientific, (Palo Alto, CA). Minimal essential medium (MEM) (330-1435) and tricine (845-1398) were purchased from Gibco Laboratories (Grand Island, NY). Powdered FA buffer was obtained from Difco laboratories (Detroit).
Mouse anti-human monoclonal antibodies and FITC-labeled goat-anti-mouse IgG were obtained from Coulter Corporation (Hialeah, FL).

**Preparation of Cell Suspensions**

**Rat cells.** Lymphocytes were obtained from the cervical and mesenteric nodes of Sprague-Dawley rats (female, 180-200 g) after killing by CO₂ anesthesia and cardiac puncture. Thymocytes were obtained from the thymus after dissection away from parathymic lymph nodes. Cells were gently teased free of connective tissue, collected into MEM buffered with 40 mmol/L tricine (pH 7.4) and supplemented with 1 mg/mL BSA or 5% fetal calf serum (FCS) (assay buffer), washed, and resuspended as described for each procedure. All steps were carried out at 4°C.

**Human cells.** Peripheral blood mononuclear cells (PBMCs) were fractionated from the peripheral blood of healthy volunteers using a discontinuous gradient of either Ficoll-Hypaque™ or colloidal silica (Sepradell-MN; Sepratech, Oklahoma City). This procedure removed the bulk of granulocytes, RBCs, and platelets.

Because excessive contamination by platelets resulted in cell clumping during the in vitro binding assay, the cell suspensions were purified further using one of two methods: (a) sequential 200 g, 10 minutes, 4°C centrifugations in calcium, magnesium-free PBS (CMF-PBS), or (b) brief treatment with thrombin as follows. The mononuclear suspension prepared from 60 mL blood was diluted 1:1 with Hank's balanced salt solution (HBS) and centrifuged (~400 g, 15 minutes). The pellet was resuspended in 2 mL of HBS; 10 μL of thrombin solution [1:20 to 1:100 dilution of frozen stock (100 U/mL in HBS)] were then added to 1-mL aliquots of the cell suspension at room temperature. The suspensions were gently agitated (~30 seconds) and centrifuged briefly at 13,000 g to pellet the platelet–fibrin clumps. The mononuclear cells remaining in suspension were pelleted (400 g, 2 minutes, 4°C), the supernatant was discarded, and the cells were resuspended in buffer containing 5% heat-inactivated FCS. Indirect immunofluorescence with T11 (pan-T cell), B1 (pan-B cell), and M01 (monocyte and granulocyte specific) mouse-anti-human monoclonals indicated that PBMC suspensions prepared with either method contained >90% lymphocytes, predominantly mature T cells (data not shown).

T cell lines, isolated after allogeneic stimulation of PBMCs, were provided by Dr Bruce Richardson (Department of Internal Medicine, University of Michigan). Lines were maintained in RPMI 1640 supplemented with 10% calf serum and interleukin 2 (IL 2), pH 7.4, with restimulation by irradiated allogeneic PBMCs at 2-week intervals. Cells were harvested during log-phase growth, washed, and resuspended in assay buffer.

**HEV binding assay.** The binding of lymphocytes to the HEVs of rat lymph nodes was determined essentially as described previously. In brief, suspensions of rat or human lymphocytes were preincubated (15 minutes, 4°C) in the presence of test substances or buffer alone. The suspensions (10 x 10⁶ cells/mL unless otherwise specified) were then applied to paraformaldehyde-fixed, frozen sections of rat cervical lymph nodes and incubated (60 rpm) at 7 to 10°C for 30 minutes. The cell suspensions were then gently decanted, and the sections were fixed in glutaraldehyde (3% in PBS, 20 minutes, 4°C) and stained with toluidine blue. In some experiments, sections were dipped in PBS several times prior to staining to remove unattached dye. The affinity of lymphocytes for HEVs was determined by measuring the percentage of lymphocytes bound to HEVs per 200 x microscopic field. The binding after experimental treatment is expressed as the percentage of binding relative to that in the control (cells exposed to buffer alone). This method of analysis facilitated the pooling of data from independent experiments conducted under the same conditions. SEM for this procedure varied from 10% to 30%.

The binding of human lymphocytes to the HEV of human lymph nodes was determined as above except that PBMCs were routinely preincubated with neuraminidase (0.005 U, 30 minutes, 37°C) before binding assays were conducted. This treatment enhanced the adhesive interaction between human lymphocytes and human HEVs without altering specificity (described in the Results section).

**Human peripheral lymph nodes were snap-frozen in OCT and stored at −70°C until use (2 weeks to 2 months).** Only surgical biopsy specimens showing normal or hyperplastic histology were used in the binding assay.

**PPME-bead assay.** The conjugation of PPME, the polysaccharide core polysaccharide derived from the yeast Hansenula holstii, to fluorescent microspheres and the procedure for measuring the attachment of conjugated beads to lymphocytes has been presented in detail elsewhere. In brief, 3 to 7 mg PPME was reacted with cyanogen bromide (100 mg/mL in distilled water). The pH of the reaction was monitored and maintained at ~11 by the dropwise addition of NaOH (0.5 mol/L). The cyanogen-bromide-activated PPME was separated from free cyanogen bromide over a G50 Sephadex column in borate buffer (0.2 mol/L, pH 8.0, room temperature). The 2 to 3 1-mL fractions with the highest concentration of carbohydrate (determined by the phenol-sulfuric assay) were pooled, diluted 1:10 with borate buffer, and added to 0.6 μm microspheres (100 μL/1 mL PPME solution). After two- to 24-hour incubations at room temperature, the conjugated beads were washed in distilled water, aliquoted, snap-frozen in liquid nitrogen, and stored at −70°C until use. Conjugated beads prepared in this manner may not withstand long-term storage. Some preparations lost cell binding activity completely within 2 weeks. Therefore, experiments were conducted with freshly thawed aliquots within 7 to 10 days of preparation.

Prior to use, the beads were sonicated to disrupt clumps, washed, and resuspended in assay buffer. The binding assay was initiated by adding 50 μL PPME-beads to 5 x 10⁶ cells in flat-bottomed, 96-well microtiter plates (200 μL final volume; beads diluted 1:32 relative to stock solution). The plates were centrifuged and then allowed to incubate undisturbed for 60 minutes on ice. Cells with attached beads were resuspended, separated from free beads by centrifugation through a 6% BSA solution, and fixed in 1% paraformaldehyde.

Cell-associated fluorescence was quantified on a Coulter Epics V flow cytometer. A narrow low-angle light-scatter gate, set on a control population of cells without attached beads, excluded both cell clumps and free beads from the analysis. A wide 90° light-scatter gate was set to assure that cells with large numbers of attached beads would not be excluded from the analysis. Fluorescence histograms of 5 to 10,000 cells, exposed to either PPME-conjugated or nonconjugated beads, were collected. The fluorescent signal in the latter population was taken as a measure of nonspecific bead attachment and subtracted from the signal measured with PPME-beads (EASY analytic software, Coulter Corp, Hialeah, FL).

The binding of beads to lymphocytes was characterized by measuring both the percentage of fluorescing cells (those with one or more beads attached) and the amount of fluorescence per cell (a measure of the number of beads per cell). Perturbation of the adhesive interaction altered one or both of these parameters. Therefore, a unitless measure of specific bead binding—the product of the percentage of fluorescing cells and the mean fluorescence channel number for the entire population (SBI)—was calculated for each experimental condition. The degree of bead binding in experimental samples was expressed relative to that in control samples (cells exposed to conjugated beads in the absence of additives).
Cell Treatments

Trypsin. Cells were harvested, washed, and resuspended in Dulbecco's PBS. The trypsin digestion was conducted in PBS (10 minutes at 37°C). The reaction was terminated by the addition of excess cold assay buffer. The cells were washed and resuspended in assay buffer and used immediately in binding assays.

EDTA. Prior to the HEV and bead binding assays, cells and beads were washed and resuspended in MEM containing 2 mmol/L disodium EDTA with or without an additional 3 mmol/L calcium or magnesium chloride. The HEV binding assays were initiated by adding cell suspensions directly to tissue sections. The bead-binding assay was initiated by combining cells and beads as described above.

Carbohydrates. Cell suspensions were incubated (15 minutes, 4°C) with carbohydrates prior to the start of the HEV or bead-binding assays. The HEV assays were initiated as described above. Carbohydrates were present throughout the incubation period at the concentrations specified for each experiment.

Neuraminidase. Gradient-purified human PBMCs were incubated (1 x 10⁷ cells/mL, 30 minutes, 37°C) with neuraminidase (0.005 U final concentration in CMF-PBS). The suspensions were subsequently washed in cold assay buffer, resuspended at 5 to 10 x 10⁶ cells/mL, incubated 15 minutes on ice, and then used in lymphocyte-HEV binding experiments. Enzyme treatments were conducted immediately after gradient separation and before exposure to potential inhibitors of the lymphocyte binding interactions (e.g., carbohydrates or EDTA).

RESULTS

The adhesive interaction between human lymphocytes and the HEVs of lymph nodes from humans and rats were examined using an in vitro assay adapted from the work of Stamper and Woodruff.¹⁹,²⁰ We used rat PNs as the routine source of HEVs since previous reports indicated that rodent tissues can substitute for analogous human tissues in the in

Fig 1. Human PBMCs adhering to the HEVs of human (A) and rat (B) peripheral lymph nodes. Exogenous PBMCs (dark cells 7 to 10 μm in diameter) bind to HEVs (vascular structures with defined lumina and prominent lining cells). In contrast to the HEVs, rat sinusoids (not shown) bind significant numbers of both erythrocytes and lymphocytes, indicating that adhesion to this structure is less specific.
vitro adhesion assay. Nonetheless, critical experiments were performed with both human and rat lymph nodes as targets.

Human lymphocytes bound avidly and specifically to the HEVs of paraformaldehyde-fixed, frozen sections of both human and rat lymph nodes (Fig 1A and B). The density of binding over the HEVs was at least 50-fold greater than that to most other regions. This binding interaction could be enhanced by pretreating PBMCs with neuraminidase (Table 1). Such pretreatment did not alter the specificity of inhibitors such as mannose-6-phosphate (M6P) or EDTA (data not shown); it simply increased the baseline density of lymphocyte binding to the HEV.

When tested in parallel at the same cell concentrations, human PBMCs bound 50% to 70% as well as rat nodal lymphocytes to rat HEVs. The magnitude and reproducibility of the binding to rat HEVs far exceeded that to human material, however, and may reflect the lability of the binding sites on HEVs. Rat lymph nodes were snap frozen immediately on removal from the animal and used within hours. Human material could not be frozen as expeditiously, and several weeks generally elapsed before tissues were available for experimental use.

As previously described for rodent lymphocytes, the binding of human lymphocytes to rat HEVs was calcium dependent and trypsin sensitive (Fig 2). Chelation of divalent cations with 2 mmol/L EDTA reduced binding by 70%, the addition of 3 mmol/L Mg increased attachment to a slight degree whereas the same concentration of Ca returned binding to control levels. A 10-minute preincubation of lymphoid cells with 20 mg/mL trypsin reduced binding by 70% whereas 40 mg/mL eliminated binding completely.

The capacity to attach to rat HEVs was not a characteristic of all human lymphocytes examined. Two IL 2-dependent, T4-positive T cell lines failed to bind (Fig 2). In contrast, Navarro et al reported that IL 2-dependent T cell lines bound to human PN-HEVs. This discrepancy may reflect the fact that we examined interspecies rather than intraspecies adhesion. Alternatively, the lines we used may be at a stage of differentiation or proliferation at which the homing receptors were not expressed. Such a relationship has been demonstrated in the murine system. Specifically, immature lymphocytes isolated from the thymus, B lymphoblastoid cells isolated from germinal centers of lymph nodes, and a variety of IL 2-dependent lymphoblastoid cell lines failed to express the Mel 14 antigen, bind to l-IEVs in vitro, or recirculate in vivo. Therefore, the expression of murine homing receptors appears to be regulated. The nature and extent of such regulation in the human requires further study. Nonetheless, the two human lines were useful negative controls in subsequent experiments.

We next examined the effects of various monosaccharides and polysaccharides on the human PBMC-HEV binding

**Table 1. Binding of Human Lymphocytes to Human HEVs**

<table>
<thead>
<tr>
<th>Additive</th>
<th>Neuraminidase Treatment</th>
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<th>+</th>
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<tbody>
<tr>
<td>None</td>
<td>0.7</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Mannose 1P</td>
<td>0.3</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Mannose 6P</td>
<td>0.035</td>
<td>0.8</td>
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</table>

Human PBMC were isolated from peripheral blood as described in the Materials and Methods section. After preincubation (with or without 0.005 U Closstridium perfringens neuraminidase as described in the text), 5 x 10^6 cells/mL were layered on fixed, frozen sections of human peripheral lymph nodes. The mannose-phosphates were present at a concentration of 5 mmol/L. Cell binding to HEVs is reported as the number of cells attached to HEVs per 200 x microscopic field. Neuraminidase treatment also enhances the binding of human PBMCs to HEVs of rat lymph nodes (data not shown).

**Table 2. Binding of Human Lymphocytes to HEVs: Inhibition by Carbohydrates**

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Binding Relative to Control</th>
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<tbody>
<tr>
<td>Monosaccharides</td>
<td></td>
</tr>
<tr>
<td>Mannose 6P</td>
<td>36 (9.5)</td>
</tr>
<tr>
<td>Mannose 1P</td>
<td>84 (8.5)</td>
</tr>
<tr>
<td>Fructose 6P</td>
<td>—</td>
</tr>
<tr>
<td>Fructose 1P</td>
<td>—</td>
</tr>
<tr>
<td>Glucose 6P</td>
<td>—</td>
</tr>
<tr>
<td>Glucose 1P</td>
<td>—</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td></td>
</tr>
<tr>
<td>PPSME</td>
<td>11 (3.4)</td>
</tr>
<tr>
<td>Fucoidin</td>
<td>15 (4)</td>
</tr>
<tr>
<td>Heparin</td>
<td>89 (20)</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>91 (18)</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>—</td>
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</tbody>
</table>

Human PBMCs were prepared as described in the Materials and Methods section; 1 x 10^6 cells/mL were incubated over sections of either human or rat peripheral lymph nodes, with or without added carbohydrates. All monosaccharides were present at 5 mmol/L concentration. Polysaccharides were at 0.1 mg/mL in experiments with human tissue sections and 1.0 mg/mL in experiments with rat tissue sections. The means and SEM (in parentheses) for binding relative to the buffer control were based on a minimum of four replicate samples.
interaction using both rat and human HEV as targets. In keeping with previous observations in both the rat and mouse systems, the attachment of human lymphocytes was inhibited by M6P, the M6P-rich mannan PPME and the sulfated, fucose-rich polysaccharide fucoidin (Table 2). Mannose-1-phosphate (M1P) and a variety of anionic polysaccharides that contain neither fucose nor mannose residues showed significantly less activity. In addition, fructose-1-phosphate, a structural homologue of M6P, inhibited attachment to rat HEVs whereas fructose-6-phosphate and the glucose-phosphates had minimal activity. Thus, human and rodent lymphocytes appeared to use similar carbohydrate binding receptor(s) to attach to HEVs.

The presence of this receptor was confirmed with fluorescent microspheres covalently linked to PPME (PPME-beads). As shown in Fig 3, PPME-beads bound avidly and specifically to human PBMCs. In general, 45% to 75% of the PBMCs bound to PPME-conjugated beads whereas <5% bound to the nonconjugated beads. The percentages of cells that bound PPME-beads (% POS) and the average number of PPME-beads/cell (MCN) varied considerably from one experiment to the next. This variability may reflect batch-to-batch inconsistencies in the conjugation process, leaching of PPME from the bead surface during storage, or modulation of the receptor on the PBMC surface. Physiologic parameters that may alter the expression of the carbohydrate binding receptor(s) are currently under investigation.

The binding of human PBMCs to PPME-beads paralleled the PBMC-HEV adhesive interaction in several respects. First, PPME-bead attachment was calcium dependent and trypsin sensitive (Fig 4). Second, the two T cell lines that failed to attach to rat HEV also failed to express receptors for PPME-beads (Fig 4). Third, the monosaccharide and polysaccharides that inhibited binding to HEV were also effective against PPME-beads (Table 3). Finally, the dose-response curves for the mannose-phosphates, PPME, and fucoidin revealed identical relative potencies in the two adhesion assays. Specifically, M6P was four to fivefold more potent than M1P as an inhibitor of attachment to both HEV and PPME-beads (Fig 5), whereas fucoidin and PPME were equally potent in both assays (Fig 6).

These results parallel those reported by us in mouse and, in Table 4, we report similar findings in the rat. Specifically, the attachment of PPME-beads to rat cervical lymph node lymphocytes was calcium dependent, trypsin sensitive, and inhibited to a greater extent by M6P than M1P. Thymocytes, a nonrecirculating population of cells that do not bind to the HEVs, showed minimal binding to the PPME-beads.

**DISCUSSION**

In summary, the attachment of normal human PBMCs to PN-HEVs involves a carbohydrate-binding receptor with affinity for M6P and related compounds. Direct analysis, using fluorescent microspheres derivatized with a high-

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**Fig 3.** Binding of PPME-conjugated and nonconjugated fluorescent beads to PBMC. (A through C) Fluorescence intensity (256 channels; three-decade log scale) is plotted on the x-axis, and the number of cells at each intensity level is registered on the y-axis. (A) Distribution of cell-associated fluorescence with PPME-conjugated beads. (B) Distribution of fluorescence with nonconjugated beads. (C) Subtraction histogram (A and B) generated by the IMMUNO analytic software (described in Materials and Methods section). The percentage of the cell population with attached beads (% POS), the mean channel number of the fluorescence distribution (MCN) and the specific binding index (SBI) are calculated from the subtraction histogram; % binding relative to control is the measure used to compare the effects of the various experimental conditions.

**Fig 4.** Binding of PBMCs to PPME-conjugated beads—divalent cation requirements, trypsin sensitivity, and the interaction with two T cell lines. The assay was conducted as described in the Materials and Methods section and the legend to Fig 2. Means and SEM were based on a minimum of four replicate samples.
Table 3. Binding of Human Lymphocytes to PPME-Beads: Inhibition by Carbohydrates

<table>
<thead>
<tr>
<th>Carbohydrate</th>
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<tbody>
<tr>
<td>Monosaccharides</td>
<td></td>
</tr>
<tr>
<td>Mannose 6P</td>
<td>19 (5)</td>
</tr>
<tr>
<td>Mannose 1P</td>
<td>97 (22)</td>
</tr>
<tr>
<td>Fructose 6P</td>
<td>96 (3)</td>
</tr>
<tr>
<td>Fructose 1P</td>
<td>35 (7)</td>
</tr>
<tr>
<td>Glucose 6P</td>
<td>91 (5)</td>
</tr>
<tr>
<td>Glucose 1P</td>
<td>86 (6)</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td></td>
</tr>
<tr>
<td>PPME</td>
<td>15 (8)</td>
</tr>
<tr>
<td>Fucoidin</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Heparin</td>
<td>74 (10)</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>81 (7)</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>92 (7)</td>
</tr>
</tbody>
</table>

Effects of carbohydrates on the binding of human PBMCs to PPME-conjugated beads. All monosaccharides were present at 5 mM/L; PPME and fucoidin at 1 μg/mL; heparin at 10 μg/mL; chondroitin sulfate and hyaluronic acid at 100 μg/mL. Means and SEM (in parentheses) were based on a minimum of four replicate samples.

The attachment of human PBMCs to HEVs from either human or rat peripheral lymph nodes suggests that the ligand for the PN-homing receptor on the HEVs is similarly conserved during evolution. Previous studies have reached similar conclusions. Jalkanen et al isolated EBV-transformed human lymphoblastoid lines that show identical tissue binding specificities toward human and mouse HEVs. Woodruff et al reported a polyclonal rabbit anti-human lymphocyte antibody that blocks the attachment of human PBMCs to the HEVs of human tonsil and rat cervical lymph node. This antibody failed to inhibit the attachment of human PBMCs to rodent mesenteric lymph nodes. Thus, several independent investigations indicate that the organ selective determinants on HEVs are preserved across species lines.

Neuraminidase-treated PBMCs were used to enhance the sensitivity and reproducibility of the human lymphocyte-human HEV binding assay. Although not used extensively in the current study, this treatment similarly accentuated specific binding to both rat HEVs and PPME-beads (data not shown). Therefore, it has become our standard method for conducting all binding assays with human PBMCs.

Mild neuraminidase treatment appeared to enhance adhesion without altering the interaction of the PN-homing receptor with carbohydrate ligands. This is in keeping with the work of Samlowski et al showing that the murine PN-homing receptor functions normally in vivo after neuraminidase treatment ex vivo if one inhibits the sequestration of infused lymphocytes in the liver. Desialation of cell-surface structures may enhance their interactions with specific ligands, as documented in the homotypic interaction of purified neural cell adhesion molecules (N-CAMs) with endogenous N-CAMs on intact cells. In addition, generalized removal of sialic acid residues from the cell surface reduces the net negative charge, resulting in nonspecific accentuation of adhesive phenomena. Therefore, neuraminidase treatment in vitro may directly alter the activity of the lymphocyte homing receptor or indirectly enhance adhesion by reducing surface charge.

The accentuation of lymphocyte binding to HEVs after treatment of the lymphocytes contrasts sharply with the complete inhibition of the binding interaction after neuraminidase treatment of the HEVs. The latter result indicates that sialic acid may be part of the lymphocyte-binding site on the HEVs. Thus, sialic acid moieties on both the lymphocyte and the HEVs can influence their adhesive interaction in vitro. Control of intercellular adhesion through modulation of receptor-associated sialic acid has been proposed for N-CAM based on the correlation between neural cell adhesion and polysialation of N-CAM in brain tissues. Whether a similar mechanism regulates the lymphocyte–HEVs interaction in vivo remains to be determined.

The precise relationship of the lymphocyte lectin to adhesive structures such as the Hermes 1 antigen and LFA-1 remains to be determined. The Hermes 1 monoclonal appears to recognize an epitope common to the binding sites on lymphocytes for the HEVs of both peripheral lymph nodes and GALTs. The PN-homing receptor precipitated by
Hermes 1 crossreacts with MEL-14 and consists of a single, 90-kd polypeptide, suggesting structural homology between the human and murine structures. In light of the relationship between gp 90 Mel-14 and the murine lectin, we are currently examining the possibility that the Hermes 1 antigen is similarly linked to the human carbohydrate-binding receptor.

LFA-1 participates in a variety of adhesive interactions involving lymphocytes including T cell-mediated cytotoxicity, NK-mediated cytotoxicity, and binding to human umbilical vein endothelium. This latter observation has led to speculation that LFA-1 contributes to lymphocyte recirculation and/or migration into chronic inflammatory lesions. Several lines of evidence indicate that LFA-1 is distinct from the cell-surface lectin involved in binding to HEVs. First, binding of lymphocytes to HEVs in vitro is most efficient at 8°C; LFA-1-mediated adhesion is markedly reduced at low temperature. Second, the binding of lymphocytes to PN-HEVs requires calcium, whereas LFA-1-mediated processes are magnesium dependent. Third, most murine lymphocytes express LFA-1 but neither express the cell-surface lectin nor bind to HEVs. Finally, in S49 murine lymphoma lines, LFA-1 is expressed equally on cell lines with high and low HEV-binding activities. Only the expression of PPME-bead binding sites and Mel-14 antigen correlate with affinity for PN-HEVs. Thus, LFA-1 appears to be distinct from the family of carbohydrate-binding molecules we have described in rodents and humans. Concerted action of the PN-homing receptor and LFA-1 in situ, however, remains an intriguing possibility.

The interactions of PBMCs with PPME-beads and HEVs require Ca and show a relatively modest difference in the inhibitory potencies of M6P > M1P. These observations contrast with the characteristics of both the high and low molecular weight phosphomannosyl receptors (PMRs) that have been implicated in the intracellular transport of acid hydrolases to the lysosome. Recently, both PMRs have been detected in human lymphoid cells and may be expressed at the cell surface. The relationship between these receptors and the PN-homing receptor is currently under study.

The nonspecific inhibition by the glucose phosphates in the HEV-binding assay has been observed with rodent cells and reflects the role of ionic forces in the adhesive interaction. In rodents, increasing the ionic strength above physiologic levels resulted in progressive inhibition of lymphocyte attachment whether the ionic species was sodium chloride or anionic carbohydrates. Furthermore, desulfation of fucoidin and dephosphorylation of PPME eliminated inhibitory activity (L.M. Stoolman and S.D. Rosen, unpublished observations). Inhibition by PPME, fucoidin, M6P, and F1P consistently exceeded that expected on a charge basis alone, however. Thus, biologic activity requires more than the mere presence of negatively charged groups. Perhaps, as has been suggested for the cell-binding domain of fibronectin, the spatial orientation of such groups will prove to be a key factor.

The relationship between the exogenous carbohydrate inhibitors and the endogenous HEV attachment site remains to be defined. Investigation of the murine HEVs indicates that terminal sialic acid residues are critical to the activity of the PN-HEVs attachment site. A more detailed analysis of the PN-HEVs attachment site will be required to determine whether it shares key structural features with either PPME or fucoidin. Such an analysis will also help determine whether the carbohydrate-binding receptor on the lymphocyte attaches directly to the HEVs or, as suggested for ligatin, acts as a membrane attachment site for a ligand that forms an intercellular bridge.

The present study amplifies the work of Butcher et al., who proposed that evolutionary mutations in the interacting
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adhesive molecules on lymphocytes and HEVs resulted in progressive interspecies incompatibility. They also observed a baseline level of compatibility between widely separated species, however, thus suggesting the persistence of certain essential structural features in the adhesive interaction. The current study indicates that a carbohydrate-binding receptor at the lymphocyte surface is a conserved essential determinant for recognition of PN-HEVs.

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Homing receptors on human and rodent lymphocytes--evidence for a conserved carbohydrate-binding specificity

LM Stoolman, TA Yednock and SD Rosen