Possible Role for Cell-surface Carbohydrate-binding Molecules in Lymphocyte Recirculation

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ABSTRACT We are investigating the hypothesis that carbohydrate-binding molecules on the cell surface are involved in the recirculation of lymphocytes from the bloodstream into lymphoid organs. This phenomenon requires the specific attachment of circulating lymphocytes to the endothelial cells of postcapillary venules. Using an in vitro assay to measure the adhesive interaction between lymphocytes and postcapillary venules, we have found that L-fucose, D-mannose, and the L-fucose-rich, sulfated polysaccharide fucoidin specifically inhibit this binding interaction. L-fucose shows stereo-selective inhibitory activity at concentrations >18 mM while fucoidin produces 50% inhibition at ~1–5 x 10^-8 M. Fucoidin appears to interact with the lymphocyte, and not the postcapillary venule, to inhibit binding. These data suggest that cell surface carbohydrates (fucoselike) and carbohydrate-binding molecules (cell surface lectins) may contribute to the specific attachment of lymphocytes to postcapillary venules.

Normal lymphocytes circulate repeatedly through the bloodstream, tissues and lymphatic system (1, 2). This phenomenon has been termed “recirculation” and is thought to be critical to the normal function of the immune system, perhaps ensuring the widest possible distribution and exposure to antigen of an individual's repertoire of lymphocyte clones. The portal of entry for bloodstream lymphocytes into tissues appears to be the postcapillary venules. In rodents and humans, the postcapillary venules of lymph nodes and Peyer's patches have an unusual histologic appearance characterized by a single layer of prominent, cuboidal endothelial cells resting on a basement membrane (termed high endothelial venules, HEV).

Histologic studies have documented an intimate association of intravascular lymphocytes with HEV (2-4). Electron microscopic studies suggest that circulating lymphocytes first attach to the luminal surfaces of HEV, then pass through their interendothelial junctions and basement membrane into the surrounding nodal parenchyma (4). Thus, the first step in lymphocyte recirculation is the attachment of blood-borne cells to specialized postcapillary venules.

A more detailed examination of this adhesive interaction was made possible by the development of an in vitro assay using lymphocyte suspensions and frozen tissue sections. Stamper and Woodruff and Butcher et al. were able to show that fresh lymphocytes bound avidly and specifically to the HEV of either glutaraldehyde-fixed (5, 6) or air-dried (7, 8) frozen sections of lymph nodes. This binding was found to correlate with the flux of blood-borne lymphocytes into lymph nodes in vivo. For example, thymocytes, which have a diminished capacity to enter peripheral lymphoid organs when compared to cells derived from spleen, lymph nodes, or peripheral blood, show correspondingly less binding to HEV in frozen sections (5, 7). The most convincing demonstration of the correlation between attachment to HEV in vitro and entry into lymph nodes in vivo is in the work of Butcher et al. (8). These investigators quantified the binding of lymphocytes from different species to the HEV of mouse lymph nodes in vitro, and compared this measurement to the flux of these cells into lymph nodes in vivo. The binding in vitro and the flux in vivo diminished in parallel as the evolutionary distance between the mouse and the source of the lymphocytes increased. These data suggest that the binding of lymphocytes to HEV in frozen sections reflects a physiologically significant adhesive interaction.

We have employed a modification of the in vitro assay described above to study the nature of the adhesive molecules on the surface of lymphocytes and HEV. In this study we report evidence suggesting that carbohydrate-binding molecules on the cell surface participate in this interaction.

MATERIALS AND METHODS

Chemicals: Monosaccharides were obtained from Sigma Chemical Co. (St. Louis, MO). Fucoidin was purchased from K & K Labs, Plainview, NY and...
used without further purification except as indicated. Heparin (H1253), chondroitin sulfate (C5234), dextran sulfate (D7151), hyaluronic acid (H1751), dermatan sulfate (C4259), thyroglobulin (T1126), horseradish peroxidase (P3250), invertase (I4753), glycogen (G9251), mannran (M7504), and bovine albumin (A4378) were obtained from Sigma Chemical Co. Heparan sulfate was a kind gift of Dr. A. Linker (Veterans Administration Hospital, Salt Lake City, UT).

Preparation of Frozen Sections: Superficial or deep cervical lymph nodes were dissected from ether-anesthetized rats (Sprague-Dawley, 180-200 g, obtained from Monson Laboratories, Gilroy, CA), snap frozen at -160°C (in 2-methyl-butanate cooled in liquid nitrogen), and immediately placed in a cryostat for sectioning (EYC Cryostat at -20°C). 10-μm sections were prepared and transferred onto glass slides (Clay Adams Laboratory Systems #A1460, Becton-Dickinson & Co., Rutherford, NJ; or Carlson Scientific Inc., Petone, IL; #00301) kept at room temperature. Sections must be relatively free of corregations and defects for optimal results. We found that the moving the tissue sections from the cutting edge to a highly polished, flat portion of the blade before transfer onto the glass slide increased the yield of such sections. Sections were dried onto the slides at room temperature for 1-3 h, then stored at 4°C until use (in tightly closed slide boxes). Sections prepared and stored in this fashion maintained binding activity for 1-2 wk (storage at room temperature resulted in loss of activity usually within 2-3 d). Before use, the slides were warmed to room temperature in a desiccator (to minimize the formation of condensation on the tissue section). Once removed from 4°C storage, sections were used within 3 h.

Binding Assay: We adapted our method from that of Butcher et al. (6, 7). Lymphocytes were teased from the superficial and deep cervical lymph nodes of two to four Sprague-Dawley rats (8 wk old, 180-200 g), and suspended in buffer at 4°C (Hanks’ balanced salt solution containing 1 mg/ml of bovine serum albumin (BSA), pH 7.4, or minimal essential medium in Earle’s salts (without sodium bicarbonate) containing 1 mg/ml BSA and buffered with 40 mM Tricine, pH 7.4). Both the Hanks’ balanced salt buffers (HBS+) and the Earle’s minimal essential medium (MEM+) were isotonic and isotonic relative to normal rat serum (9). The cells were dispersed by rapid, in-out pipetting (~100 cycles), and the cell clumps dispersed by passage through several layers of cheese cloth. The resultant single cell suspension was washed two to three times in 30-ml aliquots of buffer, suspended at a final concentration of 4 × 10⁷ cells/ml, and kept on ice until use. Cell suspensions prepared in this manner were relatively free of phagocytic cells (<10% as judged by latex particle ingestion) and were ~80% viable by trypan blue exclusion.

Potential inhibitors were added to lymphocyte suspensions (1-2 × 10⁷ cells/ml at 4°C) 10-30 min before the start of the binding assay and, unless otherwise stated, were present throughout the subsequent binding incubation. The binding incubations were conducted on glass slides in 1.4 or 2 cm diameter wax or epoxy circles (wax circles were drawn onto slides at room temperature immediately before the start of a binding assay; prewaxed slides, with 1.4-cm diameter holes cut into a thin epoxy film, were obtained from Carlson Scientific Inc.).

The frozen sections were used in the order in which they were cut. Aliquots of uninfected lymphocytes (controls) and suspensions containing potential inhibitors were layered on the sections in an arbitrary sequence such that the control and each test substance were represented once in a series. The series was repeated until the desired number of replicates had been generated. This procedure was designed to minimize bias arising from the possible heterogeneity in the binding characteristics of HEV from different regions of the lymph node. In view of the marked quantitative difference in binding that we and others (6) have observed from one lymph node to the next, only sections prepared from a single node were used in any one experiment.

The assay was initiated by laying the 4°C cell suspensions onto the sections (at room temperature). The slides were then placed on a metal tray, supported on packed ice, and agitated on a gyratory shaker at 80 or 100 rpm (G-24, New Brunswick Scientific Co., Inc., Edison, NJ; 4-in radius of gyration). The temperature of the slides equilibrated at 7-10°C. After a 30-min incubation, the cell suspensions were decanted and the sections fixed in 3% glutaraldehyde (20 min at 4°C). The slides were then placed in racks, washed in Dulbecco’s phosphate-buffered saline (five, one-second immersions), stained with 0.5% toluidine-blue in a 20% ethanol solution (one, 15-60 second immersion), washed in 95% EtOH (one, two-second immersion), and mounted in glycerol. Sections processed in this manner were stored at 4°C until analysed.

General Characteristics and Quantitation of Lymphocyte Binding in Vitro: In the absence of inhibitors, lymphocytes attach, primarily, to the HEV and to the glass surrounding the tissue section on the slide. Lymphocytes also adhere to the nodal parenchyma away from the HEV; however, the density of binding is at least 50-fold greater at the HEV than at these other sites. The binding to the HEV was easily distinguished from binding elsewhere by virtue of the characteristic picture of tightly packed, darkly stained lymphocytes strongly adherent to the HEV available in any particular frozen section; therefore, this finding suggests that the adhesive bond between lymphocytes and HEV may fluctuate in strength from one lymph node to the next. We used lymph nodes that vary markedly in the nature and degree of their recent immunologic activity. If antigenic stimulation alters the strength of the HEV-lymphocyte binding interaction, then the
fluctuations in the density of lymphocyte binding in vivo may reflect the varied immunologic histories of the lymph nodes and lymphocyte suspensions used in different experiments. This apparent variation in the strength of the lymphocyte-HEV binding interaction did not affect our data since individual experiments were carried out using sections from a single node and single pools of freshly prepared lymphocytes.

Protein Synthesis: The rate of protein synthesis in lymphocytes was determined by measuring the incorporation of \(^{3}H\)leucine (ICN Pharmaceuticals, Inc., Irvine, CA; Cat. #20032, 120 Ci/mM) into TCA-precipitable counts. Lymphocytes prepared as described above were suspended at 5 × 10^6 cells per ml in leucine-free buffer containing penicillin and streptomycin (100 U/ml and 100 \(\mu\)g/ml, respectively). Incubations were begun by adding 1 \(\mu\)Ci of \(^{3}H\)leucine to 1-ml aliquots of cells containing the various test substances. After varying periods of incubation at 37°C, the reactions were stopped with cold trichloroacetic acid (final concentration of 10%), and the precipitates were collected by filtration onto GFC filters (Whatman Inc., Paper Div., Clifton, N.J.). The filters were washed in TCA and absolute ethanol, added to scintillation vials with 10 ml of Hydrofluor (National Diagnostics, Inc., Advanced Applications Institute Inc., Somerville, NJ), and counted. Nonspecific binding was determined by using zero-time blanks.

The rate of \(^{3}H\)leucine incorporation was found to be linear over at least 2 h. Levels of incorporation were compared after 90 min of incubation (the approximate time of exposure of lymphocytes to the inhibitors in the binding assays).

RESULTS

Effect of Monosaccharides

We examined the effects of a series of neutral monosaccharides on lymphocyte attachment to HEV in vitro (Fig. 2). This panel included sugars that are commonly found in cell surface glycoconjugates and that are known ligands for endogenous mammalian lectins (10). At a concentration of 150 mM, d-

![](image)

**FIGURE 2** Lymphocyte attachment in the presence of monosaccharides (150 mM). 0.2 ml of lymphocyte suspensions, containing various test substances, were incubated over sections in 2-cm circles and gyrated at 80 rpm as specified in Materials and Methods (7-10°C; 1.2×10^7 cells/ml in HBS+). **CONT.** (no-added-sugar control); **L-FUC** (l-fucose); **D-FUC** (d-fucose); **MANN** (d-mannose); **NAcGLU** (N-acetyl-d-glucosamine); **D-GAL** (d-galactose). RELATIVE BINDING = Binding in presence of inhibitor + binding in control × 100. Data pooled from multiple independent experiments, total number of replicates (frozen sections)—control (15), L- and d-fucose (19), d-mannose (11), N-acetyl-d-glucosamine (8), d-galactose (10). Differences between l-fucose and d-fucose, d-mannose and d-fucose, statistically significant (by t test) with probabilities less than 0.0005 and 0.005 respectively. d-glucose (data not shown) produced no significant inhibition relative to d-fucose.

fucose, d-glucose, d-galactose, and N-acetyl-d-glucosamine enhanced lymphocyte binding relative to the no-added-sugar control, whereas l-fucose and d-mannose had no significant effect. However, lymphocyte attachment in the presence of l-fucose and d-mannose was 50% lower than that in the presence of the other sugars examined.

The mechanism underlying the enhancement of lymphocyte binding to HEV in the presence of a variety of structurally dissimilar monosaccharides is unknown. The effect appeared to be relatively selective since neither the nonspecific binding of lymphocytes to tissue sections away from the HEV, nor the adhesion of lymphocytes to one another in suspension increased under these conditions. It is conceivable that alterations in the membrane structure of either the lymphocyte or the HEV may be involved. Scanning electron micrographs of lymphocytes in suspension have shown an increase in the number and prominence of membrane microvilli in hypertonic solutions (11). Since circulating lymphocytes anchor to the HEV at the tips of their microvilli (4), one might expect that changes induced in these structures by hypertonic sugar solutions might enhance lymphocyte binding in vitro. Regardless of the mechanism, this enhancement suggested that the most appropriate control for judging the inhibitory potency of a particular sugar was an equimolar concentration of other sugars. When viewed in this manner, l-fucose and d-mannose appeared to produce a significant depression in the affinity of lymphocytes for HEV.

In an effort to enhance the suspected inhibitory activities of the simple sugars, we examined the effect of high ionic strength buffers on the attachment of lymphocytes to HEV. Fig. 3 shows that increasing the ionic strength of the incubation buffer, by adding NaCl, results in a dose-dependent reduction in lymphocyte binding. A 40% increase in ionic strength relative to

![](image)

**FIGURE 3** Lymphocyte attachment in the presence of NaCl. 0.12 ml of the lymphocyte suspensions, containing various test substances, were incubated over sections in 1.4-cm diameter circles and gyrated at 80 rpm as specified in Materials and Methods (7-10°C; 2×10^7 cells/ml in MEM+). RELATIVE BINDING (ordinate) = (binding in presence of NaCl) + (binding in control) × 100. Means and SEMs based on four replicates. Degree of inhibition by NaCl varied between experiments and may reflect heterogeneity in the strength of the binding interaction with different lymphocyte suspensions and lymph nodes. The difference between the control (no-added-salt) and 60 mM added NaCl is statistically significant (by t test) with a probability of <0.025.
D-fucose, o-galactose, and GIcNAc (in which ionic strengths
containing neither added salt nor sugar). The addition of 150 mM
L-fucose. In this experiment, 60 mM additional NaCI depressed
attachment (Fig. 2).

relative to the no-added-sugar control. D-fucose, solid line; L-fucose, dashed line. (b) Inhibi-
tion of L-fucose relative to control. RELATIVE BINDING (ordinate) = (binding in the presence of fucose) / (binding in the
presence of D-fucose) x 100.

normal rat serum (produced by adding 60 mM NaCI) resulted
in a 60% decrease in lymphocyte attachment to HEV. Ionic
strength rather than osmolarity appeared to be the critical
factor in reducing adhesiveness, since hypertonic solutions of
D-fucose, D-galactose, and GIcNAc (in which ionic strengths
were unchanged) enhanced rather than inhibited lymphocyte
attachment (Fig. 2).

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in the presence of inhibitory monosaccharides. Therefore,
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remaining in suspension after a binding assay (with gluta-
aldehyde) and determining the number of single cells. The
number of single cells depends on both the number of cells
forming aggregates and the number of cells that attach to the
glass or the tissue section. Since the majority of added lympho-
cytes remain in suspension at the end of a binding assay
(>80%), the number of singles should reflect, primarily, the
degree of cell agglutination. We found that L-fucose did not
produce a significant decrease in the number of single cells
when compared to a no-added-sugar control, D-galactose, or
d-fucose. We obtained identical results when lymphocyte ag-
glutination was assessed under the same conditions in the
absence of tissue sections. Thus, cellular agglutination does not
appear to be a factor in the inhibition of binding produced by
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The dose-response curves for L- and D-fucose under permis-
sive conditions are shown in Fig. 5. Relative to the no-added-
sugar control (Fig. 5 a), D-fucose produced a dose-dependent
increase in lymphocyte attachment that plateaued at a level
50% above the control (p < 0.05 at 37 mM). In contrast, L-
fucose caused little if any increase in lymphocyte attachment
at low concentrations followed by a significant inhibition in
binding at concentrations of 75 and 150 mM (p < 0.05). When
compared to equal concentrations of D-fucose, L-fucose pro-
duced significant inhibition at concentrations of 18 mM (see
figure legend for p values) and above (Fig. 5 b). D-mannose
also produced dose-dependent inhibition but was approxi-
mately twofold less potent than L-fucose. Thus, increasing the
ionic strength of the incubation medium with NaCI enhanced
the potency of L-fucose relative to both no-added-sugar con-
tral controls containing equal concentrations of D-fucose.

In light of NaCI's synergistic affect on inhibitory monosac-
charides, we re-examined the behavior of a series of neutral
sugars in high salt (Fig. 6). At a concentration of 150 mM, only
L-fucose, D-mannose, and a-methyl-D-mannoside depressed
lymphocyte attachment relative to the no-added-sugar control.
D-galactose, N-acetyl-D-glucosamine, L-rhamnose, D-fucose,
d-glucose, D-ribose, and N-acetyl-D-galactosamine either en-
hanced binding or had no significant effect relative to the
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specific monosaccharides.
In light of the known inhibitory effect of D-mannose on cell division (12) and L-fucose on cell division and DNA synthesis (13) in cultured cells, we examined rat lymphocytes for evidence of sugar-induced toxicity under "permissive" conditions. Pre- and post-assay measurements showed 75-80% of cells capable of excluding trypan blue regardless of the nature of the added sugar. Furthermore, the rate of protein synthesis, as judged by [3H]leucine incorporation, was depressed to the same extent for the added sugar. Moreover, the rate of protein synthesis was depressed to the same extent for the added sugar. In addition, hypertonicity with or without the various monosaccharides resulted in equivalent degrees of inhibition suggests that hypertonicity may have had a nonspecific effect on lymphocyte metabolism. Such a phenomenon has been described in other cells (14).

**Effect of Glycoconjugates**

Our sugar inhibition results suggested that a carbohydrate-binding molecule, with fucose/mannose specificity, might be involved in the adhesive interaction between lymphocytes and HEV. Frequently, the affinity of lectins for polysaccharides greatly exceeds their affinity for monosaccharides. Such enhanced binding is due either to a valency effect, in which multiple sugar residues in the polysaccharide can bind to multiple sites on the lectin (15, 16), or to the presence of specific oligosaccharide structures in the polysaccharide with high affinity for the lectin (17).

We, therefore, examined the effects of a variety of complex polysaccharides, of defined composition, on the attachment of lymphocytes to HEV (Fig. 7). Fucoidin, a 100,000-dalton, sulfated polysaccharide consisting predominantly of α1,3-linked L-fucose (18), proved to be a potent inhibitor (at 5 μg/ml). A series of structurally dissimilar, charged polysaccharides (19) with equal or greater charge densities (heparin, dermatan sulfate, dextran sulfate), and equal or greater size (hyaluronic acid) showed no significant inhibitory activity at fivefold higher concentrations. In addition, thyroglobulin, yeast mannan, glycogen, horseradish peroxidase, and yeast invertase were inactive at 1 mg/ml.

Fucoidin was significantly more potent than the active monosaccharides with a one-half maximal inhibitory activity, depending on the experiment, of 1-5 × 10⁻⁶ M (Fig. 8). Gel filtration analysis (Sephacryl 300, Pharmacia Inc., Piscataway, NJ) of the commercial fucoidin preparation revealed a minor (<10% by weight) protein component. However, the protein-free polysaccharide fractions were potently inhibitors of the lymphocyte-HEV binding interaction, indicating that the protein was not essential for inhibitory activity (data not shown).

To determine the target of fucoidin’s action, we preincubated either frozen sections or the lymphocyte suspensions with 25 μg/ml of fucoidin, washed them, and then compared them to untreated controls for binding activity. As shown in Fig. 9, only when lymphocytes were pretreated with fucoidin was there residual inhibitory activity. Thus, fucoidin acts on the lymphocytes to reduce their affinity for HEV.

We observed that fucoidin enhanced the clumping of lymphocytes in suspension, in contrast to the monosaccharide inhibitors; however, this phenomenon did not appear to correlate with the degree of inhibitory activity. Specifically, the percentage of agglutinated cells ranged from 20 to 50% at fucoidin concentrations that decreased lymphocyte attachment by 70-100%. Therefore, the random agglutination of a portion of lymphocytes is a second possibility for the observed inhibitory activity of fucoidin.

**Table 1**

| Additive         | % Maximal protein synthesis |
|------------------|----------------------------|
| None (control)§  | 100 ± 5                    |
| L-Fucose 150 mM | 14 ± 1.5                   |
| 75 mM            | 42 ± 3.3                   |
| D-Fucose 150 mM | 16 ± 2.7                   |
| 75 mM            | 42 ± 3.2                   |
| D-Mannose 150 mM| 17 ± 3.3                   |
| 150 mM           | 15 ± 0.04                  |

*All solutions contained leucine-free, MEM (pH 7.4) supplemented with 1 mg/ml BSA, 60 mM additional NaCl, penicillin, and streptomycin (see Materials and Methods)  
†(Counts in presence of additive) + (precipitable counts in control) × 100  
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§(Counts in presence of additive) + (precipitable counts in control) × 100  
| Additive|| | % Maximal protein synthesis‡ |
|---------|---------------------------------|
| None (control)§ | 100 ± 6 |
| Fucoidin | 66 ± 5 |
| Heparin  | 69 ± 5 |
| Dextran sulfate (8 kdaltons) | 62 ± 6 |
| Chondroitin sulfate | 85 ± 5 |

*All solutions contained leucine-free, MEM (pH 7.4) supplemented with 1 mg/ml BSA, penicillin, and streptomycin and 25 μg/ml of the specified polysaccharide (see Materials and Methods)
of the lymphocyte suspension cannot explain fucoidin's effect. However, given the heterogeneity of the lymphocyte suspensions we employ, it is conceivable that fucoidin selectively agglutinated those cells capable of binding to HEV.

Fucoidin does not appear to be selectively toxic for lymphocytes since pre- and post-incubation measurements of trypan blue exclusion were identical regardless of the nature of the added polysaccharide. Furthermore, Table 1B shows that the rate of protein synthesis in the presence of fucoidin did not differ significantly from that in the presence of equal concentrations of several polysaccharides that had no effect on lymphocyte adhesion. Taken together, these data suggest that fucoidin's inhibitory potency cannot be explained simply on the basis of its charge, its molecular size, or a toxic effect. We favor the hypothesis that fucoidin interacts with a fucose-specific lectin on the lymphocyte surface that participates in the attachment of these cells to HEV in vitro.

DISCUSSION

Membrane-associated carbohydrate-binding molecules (membrane lectins) appear to be involved in a variety of physiologically significant adhesive interactions (20, 21, 22). The attachment of certain bacteria and viruses to their host cells appears to involve lectins (23, 24). The symbiotic relationship between legumes and nitrogen-fixing bacteria is facilitated by the binding of bacterial polysaccharides to lectins on the root hairs of the plant host (25). The developmentally regulated, species-specific intercellular adhesion of the cellular slime molds Dictyostelium discoideum and Polysthondylium pallidum appears to involve membrane-bound lectins with affinity for galactose and galactose-containing glycoproteins (26-29). Finally, lectins with specificities for fucans have been implicated in the cohesion of teratocarcinoma stem cells (30, 31), and in the adhesion of sperm to egg in the algae Fucus (32), in sea urchins (33), and in guinea pigs (34).

Our present studies indicate that lectins on the lymphocyte surface may be involved in the attachment of lymphocytes to HEV in vitro and thus contribute to lymphocyte recirculation in vivo. Specifically, we find that the sulfated, l-fucose-rich polysaccharide fucoidin and the structurally related monosaccharides l-fucose, d-mannose, and a-methyl-d-mannoside (these monosaccharides have an axial 2-hydroxyl and an equa-
torial 4-hydroxyl) can inhibit the attachment of lymphocytes to HEV in vitro. Furthermore, we have shown that fucoidin inhibits binding by acting on the lymphocyte and not the HEV. Finally, since inhibition occurs at incubation temperatures of 7–10°C (temperatures at which the uptake of sugars and membrane fluidity are, presumably, reduced), we suspect that the inhibitors exert their effects at the cell surface. These findings are compatible with the hypothesis that lectins on the lymphocyte surface interact with complementary receptors on the HEV containing fucose, mannose, or related carbohydrates (most likely as part of protein or lipid-bound oligosaccharides). According to this view, the substances with inhibitory activity would compete with the endogenous receptor for binding sites on the lymphocyte surface, thereby weakening the adhesive interaction between lymphocytes and HEV. Alternatively, the inhibitors might produce alterations in the cell shape or affect intracellular metabolic processes that secondarily result in decreased adhesiveness. Although we have no direct evidence that fucoidin, L-fucose, and D-mannose, compete for the same binding site on the lymphocyte surface, there are several examples of cell-surface carbohydrate-binding molecules with combined L-fucose/D-mannose (16) or L-fucose/fucoidin specificities (34).

High ionic strength buffers both inhibit the attachment of lymphocytes to HEV directly and enhance the potency of carbohydrate inhibitors. The fact that high ionic strength alone inhibits the adhesive interaction does not diminish the likelihood that a lectin-carbohydrate interaction is involved. Salt solutions can elute specifically bound carbohydrates from lectin affinity columns, suggesting that ionic bonds or salt-sensitive tertiary structures contribute to the stability of some lectin-carbohydrate interactions (35). Alternative explanations for the salt effect include: (a) a salt-sensitive adhesive mechanism that is distinct from that involving the putative lectin; (b) metabolic inhibition that secondarily affects adhesion; or (c) the quantitative loss of ionically bound adhesive molecules by salt elution. The synergistic effect between NaCl and the specific monosaccharide inhibitors may indicate that, as the strength of the salt-sensitive bonds diminish, the attraction due to lectin-carbohydrate binding persists and provides the major force holding the lymphocytes in place. Regardless of the precise mechanism, the hyperionic buffers utilized in our studies dramatically and selectively enhance the inhibitory potencies of L-fucose and D-mannose.

The sulfated, L-fucose-rich polysaccharide fucoidin shows far greater potency on a molar basis than the monosaccharide inhibitors. Although its high content of sulfate residues and overall size may contribute to its potency, other structural characteristics must also be important since structurally dissimilar polysaccharides, with comparable charge densities and molecular weights, failed to cause significant inhibition. The synergistic effect of NaCl and L-fucose may indicate that both lectin-carbohydrate and ionic interactions are involved in the attachment of lymphocytes to HEV. If this view is correct, then fucoidin’s potency may reflect its ability to participate in both types of interactions.

Nearly twenty years have passed since Gesner and Ginsberg (36) first suggested that cell–surface carbohydrates play a role in the homing of lymphocytes. Our data are compatible with the hypothesis that a carbohydrate-binding molecule on the lymphocyte surface interacts with a fucose- or mannose-containing receptor on the postcapillary venule. Since the magnitude of lymphocyte attachment in vitro correlates with the flux of circulating cells into lymph nodes in vivo, we propose that a lectin-carbohydrate binding interaction is involved in the first stage of lymphocyte recirculation.

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