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

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Abstract. The tissue-specific homing of lymphocytes is directed by specialized high endothelial venules (HEV). At least three functionally independent lymphocyte/HEV recognition systems exist, controlling the extravasation of circulating lymphocytes into peripheral lymph nodes, mucosal lymphoid tissues (Peyer’s patches or appendix), and the synovium of inflamed joints. We report here that antibodies capable of inhibiting human lymphocyte binding to one or more HEV types recognize a common 85–95-kD lymphocyte surface glycoprotein antigen, defined by the non-blocking monoclonal antibody, Hermes-1. We demonstrate that MEL-14, a monoclonal antibody against putative lymph node “homing receptors” in the mouse, functionally inhibits human lymphocyte binding to lymph node HEV but not to mucosal or synovial HEV, and cross-reacts with the 85–95-kD Hermes-1 antigen. Furthermore, we show that Hermes-3, a novel antibody produced by immunization with Hermes-1 antigen isolated from a mucosal HEV-specific cell line, selectively blocks lymphocyte binding to mucosal HEV. Such tissue specificity of inhibition suggests that MEL-14 and Hermes-3 block the function of specific lymphocyte recognition elements for lymph node and mucosal HEV, respectively. Recognition of synovial HEV also involves the 85–95-kD Hermes-1 antigen, in that a polyclonal antiserum produced against the isolated antigen blocks all three classes of lymphocyte-HEV interaction. From these studies, it is likely that the Hermes-1–defined 85–95-kD glycoprotein class either comprises a family of related but functionally independent receptors for HEV, or associates both physically and functionally with such receptors. The findings imply that related molecular mechanisms are involved in several functionally independent cell–cell recognition events that direct lymphocyte traffic.

SPECIFIC heterotypic cell–cell associations are an essential feature in the development and function of complex biological systems. In general, however, the molecular basis of such interactions remains a mystery. Models based on cell type–specific recognition elements or receptor/ligand systems, such as the chemoaffinity hypothesis for the development of neural connections (Sperry, 1965), have recently been replaced in part by the concept that cellular connections may be determined by the temporal and spatial regulation of relatively nonspecific cell adhesion molecules (Edelman, 1983). One system in which cell type–specific recognition does operate is the interaction of lymphocytes with high endothelial venules (HEV,1 venules specialized to support the extravasation of circulating lymphocytes from the blood). Lymphocyte–HEV interactions exhibit a remarkable tissue specificity: Functional in vivo and in vitro studies have revealed the existence of at least three independent lymphocyte–endothelial cell recognition systems, controlling lymphocyte extravasation in peripheral lymph nodes, in the mucosal lymphoid organs (Peyer’s patches or appendix), and in inflamed synovium (Butcher et al., 1980; Stevens et al., 1982; Chin et al., 1984; Jalkanen et al., 1986c). As assessed in an in vitro assay of lymphocyte binding to HEV in frozen sections (Stamper and Woodruff, 1976; Butcher et al., 1979; Jalkanen and Butter, 1985), many normal small B and T lymphocytes are apparently able to interact with all three HEV classes (Stevens et al., 1982), but certain transformed or neoplastic lymphoid lines as well as some differentiated effector cell populations display exquisite specificity for particular organ HEV (reviewed in Jalkanen et al., 1986b; Butcher, 1986; Gallatin et al., 1986). These organ-specific lymphocyte–HEV interactions control the migration and tissue distribution of lymphocyte subsets, and are thought to play a key role in determining the characteristics of local immune responses.

Our laboratory has undertaken to identify and characterize molecules involved in lymphocyte recognition of lymph

1. Abbreviations used in this paper: ALS, antilymphocyte serum; HEV, high endothelial venule; PBL, peripheral blood lymphocyte.
node, mucosal, and synovial HEV in the human. We have previously isolated a monoclonal antibody, Hermes-1, that defines 85-95-kD human lymphocyte surface glycoproteins involved in lymphocyte binding to lymph node HEV (Jalkanen et al., 1986a). Because Hermes-1 does not interfere directly with the binding event, the importance of the Hermes-1 antigen in lymphocyte adherence to lymph node HEV was inferred from three principal observations: (a) expression of the antigen on normal and transformed human lymphocyte populations is correlated with their HEV-binding ability; (b) fluorescence-activated cell sorter-selected Hermes-1 variants of a cloned cell line display increased HEV-binding ability; and (c) the affinity-isolated Hermes-1 antigen absorbs antibodies that are capable of blocking lymphocyte-lymph node HEV binding from heterologous rat anti-human lymphocyte sera. These studies provided convincing evidence of the involvement of the Hermes-1 antigen in lymphocyte binding to lymph node HEV, but they did not address the role of this antigen in the organ specificity of HEV recognition. Nor did they address the molecular mechanisms of lymphocyte binding to mucosal or synovial HEV.

In the studies reported here, we describe monoclonal antibodies that selectively inhibit lymphocyte binding to lymph node or to mucosal HEV, and demonstrate that these blocking antibodies recognize the Hermes-1-defined 85-95-kD glycoprotein class ("gp90"). Furthermore, we show that an anti-gp90 serum blocks lymphocyte binding to lymph node, mucosal, and synovial HEV. The results suggest that the Hermes-1-defined gp90 either comprises a family of antigenically and structurally related receptors for HEV, or is associated both physically and functionally with lymphocyte surface molecules responsible for HEV recognition.

Material and Methods

Cells
Human peripheral blood lymphocytes (PBL) were isolated using Ficoll-Hypaque (Histoaque-1077, Sigma Chemical Co., St. Louis, MO). Cell lines were a gift of E. Engleman (IBW4, KCA), and Dr. H. Kaplan (LB25) of Stanford University.

In Vitro HEV Assay
The in vitro assay of lymphocyte binding to HEV in frozen sections has been described (Jalkanen and Butcher, 1985). Briefly, sample lymphocytes in RPMI 1640 containing 5% fetal calf serum and 20 mM Hepes, pH 7.4, were incubated with mild rotation for 30 min at 7°C on freshly cut frozen sections of human peripheral lymph nodes, appendix, or inflamed synovium, or mouse peripheral nodes or Peyer's patches. (Similar results are obtained on mouse and human HEV (Wu, N., S. Jalkanen, and E. C. Butcher, manuscript in preparation.) Synovium, from patients with either rheumatoid arthritis or the inflammatory arthritis associated with Lyme disease, was generously provided by A. Steere and R. Fox. Assays were carried out at a cell density (2 x 10^6/ml)-well below that required for saturation of HEV (i.e., at a density known to yield linear increases in HEV-bound cells with increases in cell number (Jalkanen and Butcher, 1985)). After incubation, adherent cells were fixed in cold PBS containing 1% glutaraldehyde, and cell binding was quantitated microscopically as described in Jalkanen and Butcher (1985) and in the figure legends. Results are presented as relative adherence ratios, the calculated number of sample cells bound to HEV per reference (control antibody-treated or untreated) cell binding under identical conditions (see figure legends). Standard errors were determined by the delta method (Rao, 1965).

Labeling and Immunoprecipitation
Human PBL were iodinated with 125I (New England Nuclear, Boston, MA) by the lactoperoxidase method as described (Jalkanen et al., 1986a). Iodinated cells were lysed with 2 ml of 0.1% saponin, 0.1% SDS, 0.1% deoxycholate, 0.5% desoxycholate, and 0.1% proteinase K (pH 7.4), and the lysate was clarified by centrifugation at 10,000 x g for 15 min. For the analyses of the ME1-14 antigen presented in Fig. 2, 500-μl aliquots were preincubated two times for 4 h at 4°C with 25 μl of cyanogen bromide-Sepharose 4B beads coupled with normal rat serum, and immunoprecipitation was carried out with Sepharose 4B beads conjugated with Hermes-1, ME1-14, or the control antibody 3G12. All antigens were conjugated at 5 mg/ml of packed Sepharose beads. To determine whether the ME1-14 antigen on PBL was also recognized by Hermes-1, one aliquot was preincubated with Hermes-1 two times with 12 μl of Hermes-1-Sepharose beads for 5-12 h at 4°C before immunoprecipitation with ME1-14.

For the analyses with Hermes-3 and with the anti-gp90 serum presented in Figs. 4 and 6, 200-μl aliquots of lysate, prepared as above, were preincubated four times with 12 μl of protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) bearing roughly 5 μg of rabbit anti-mouse (Sigma Chemical Co.) or anti-rat (Calbiochem Behring Corp., San Diego, CA) Ig, and saturating levels of either normal mouse serum or the control monoclonal antibodies indicated in the figure legends. Specific immunoprecipitation was carried out with 12 μl of protein A-Sepharose beads, armed with anti-mouse Ig and Hermes-3 or anti-gp90, or with anti-rat Ig and Hermes-1.

For SDS-PAGE, the beads were washed four times in lysis buffer, the radiolabeled antigen was eluted by boiling 3 min in 50 μl of Laemmli sample buffer (Laemmli, 1970), and the immunoprecipitate was analyzed by SDS-PAGE (9% polyacrylamide) under reducing conditions. Molecular weight standards were myosin, 205,000; β-galactosidase, 166,000; phosphorylase B, 94,000; bovine serum albumin, 66,000; egg albumin, 45,000; and carbonic anhydrase, 29,000.

Monoclonal Antibodies

Production and characterization of Hermes-1 (Jalkanen et al., 1986a) and ME1-14 (Gallatin et al., 1985) have been described. Both are rat IgG2 antibodies.

Hermes-3, a mouse IgG2a, was produced by immunization of BALB/c mice with purified Hermes-1 antigen from KCA, a human B lymphoblastoid line that binds preferentially to mucosal HEV. 7 x 10^9 KCA cells were lysed in 3.5 liters of lysis buffer (2% Trition X-100, 0.15 M NaCl, 0.01 M Tris, 1.5 mM MgCl2, 1 mM PMSF, and 1% aprotinin), and the clarified lysate was adjusted to pH 5.8 with HCl and applied to a DEAE-Sepharose-6B column (Pharmacia Fine Chemicals). The bound acidic proteins were eluted with 0.8 M NaCl at pH 5.2. The effluent was diluted to isotonicity with deionized water, adjusted to pH 7, and applied sequentially to three Sepharose 4B columns, derivatized respectively with normal rat serum, with an irrelevant monoclonal antibody (L3B12), and with Hermes-1 (5 mg/ml column volume). The material bound to the Hermes-1 column was eluted with 50 mM triethanolamine and typholyzed. The affinity binding and subsequent steps were repeated twice. The resulting antigen preparation contained Hermes-1 antigen (roughly 80% as assessed by SDS-PAGE) admixed with lesser amounts of contaminating antibody from the affinity column. BALB/c mice were immunized three times during a 6-8-wk period with the affinity-purified Hermes-1 antigen in complete Freund's adjuvant (+/−) or incomplete Freund's adjuvant (−/−) intraperitoneally, followed by a final intrasplenic injection of the purified antigen in saline. The spleen cells were fused (Kohler and Milstein, 1979) 3 days later with Sp2/0 (Schulman et al., 1978) mouse myeloma cells. Hybrid clones were selected in hypoxanthine-aminopterin-thymidine medium, and supernatants that stained PBL in an immunofluorescence assay were screened by analyzing their blocking activity in the in vitro HEV assay. The Hermes-3-secreting hybrid was subcloned two times by limiting dilution. Rat IgG2a control antibodies included 3G12, specific for the mouse lymphocyte antigen T200, a generous gift of J. Ledbetter (Ledbetter and Herzenberg, 1979); and 281-2, against murine epithelial cell surface proteoglycan, a gift of M. Jalkanen (Jalkanen et al., 1985).

Antisera
A polyclonal mouse antiserum against the Hermes-1 antigen (anti-gp90) was obtained from mice hyperimmunized with the purified antigen, isolated and prepared as described above under the discussion of Hermes-3. A hyperimmune rat antiserum against human lymphocytes (antilymphocyte serum, ALS) was produced by repeated immunization with whole human peripheral blood and tonsillar lymphocytes, as described (Jalkanen et al., etc.)
In experiments designed to exclude a nonspecific effect of surface-bound polyclonal antibodies on PBL binding to HEV (see Fig. 7), the ALS was absorbed with isolated Hermes-1 antigen to remove antibodies reactive with the gp90 class; 400-μl aliquots were incubated with 200 μl of Hermes-1-conjugated Sepharose beads bearing adsorbed gp90 (Hermes-1 antigen) from the lysate of ~2 × 10⁶ tonsil cells.

**Immunofluorescence Cell Staining**

Single-color indirect immunofluorescence was carried out as described (Jalkanen et al., 1986a) using saturating levels of first-stage antibodies followed by the appropriate second-stage reagent, either FITC-goat anti-rat IgG (Sigma Chemical Co.) or FITC-sheep anti-mouse IgG (Fab2 fragments, Sigma Chemical Co.). Negative control staining was carried out with a mixture of the two second stages.

**Results**

**A gp90 Involved in Lymphocyte Recognition of Lymph Node HEV**

Lymphocyte interactions with HEV can be studied in a simple in vitro system in which lymphocytes adhere selectively to HEV when incubated on fresh frozen sections of lymph nodes or other tissues (Stamper and Woodruff, 1976; Butcher et al., 1979; Jalkanen and Butcher, 1985). By using this assay system, we have now observed that monoclonal antibody MEL-14, originally selected as recognizing putative mouse lymph node homing receptors (Gallatin et al., 1983), cross-reacts in a functionally significant manner with the human Hermes-1 antigen. As reported previously (Jalkanen et al., 1986c) and confirmed in additional experiments here, MEL-14 inhibits the binding of human PBL to lymph node HEV, but not to mucosal or to synovial HEV (see Fig. 1, shaded bars). The interpretation that this inhibition is due to blockade of a specific lymphocyte receptor (and not to nonspecific toxic effects or membrane alterations) assumes that many or all individual lymphocytes express receptors for all HEV types. Although this assumption is supported by murine studies (Stevens et al., 1982), it remained formally possible that MEL-14 was simply toxic to cells bearing its cognate antigen, and that most or all PBL binding to mucosal and synovial HEV were MEL-14- and therefore unaffected. MEL-14 stains human lymphocytes only weakly in immunofluorescence assays, presumably because its cross-reactivity with the human antigen represents a relatively low avidity interaction; thus it has not been technically feasible to assess directly the expression of the MEL-14 antigen by PBL bound to mucosal or synovial HEV. Therefore, we tested the effect of MEL-14 on a cloned, homogeneous human cell line, IBW4, that is capable of binding to both lymph node and to
mucosal HEV (Jalkanen et al., 1986a). As shown in Fig. 1 (hatched bars), MEL-14 also blocked the binding of IBW4 in an organ-specific fashion. This specificity of inhibition suggests, as argued in the mouse studies (Gallatin et al., 1983), that the effect of MEL-14 cannot be due to nonspecific alterations in lymphocyte function, but rather that the MEL-14 antigen either functions as a specific receptor for lymph node HEV, or enjoys a close physical association with the actual recognition elements.

As shown in Fig. 2, MEL-14 immunoprecipitates a 90-kD band from lysates of surface iodinated human PBL, and this MEL-14 antigen is precleared by absorption with Hermes-1. Thus both the Hermes-1- and MEL-14-defined epitopes are present on human lymphocyte surface glycoproteins (gp90) involved in recognition and binding to lymph node HEV.

**Recognition of Mucosal HEV Also Involves the Hermes-1-defined gp90**

We next wished to identify elements involved in recognition of mucosal HEV. Our approach was based on the observation that antibody Hermes-1 binds in high levels not only to lymph node HEV-specific cells, but also to the B lymphoblastoid line KCA, which interacts preferentially with mucosal HEV. (KCA cells bind 0.6-1.0 times as well as PBL to appendix or Peyer's patch HEV, but essentially at background levels, ≤0.05 times as well as PBL, to HEV in lymph nodes or in inflamed synovium [Jalkanen et al., 1986a;c].) One possible explanation for the staining of KCA with Hermes-1 was that molecules involved in recognition of mucosal HEV also bear the Hermes-1 epitope. With this possibility in mind, the Hermes-1 antigen was purified by ion exchange and affinity chromatography from lysates of KCA, and the isolated antigen was used to immunize mice for the production of monoclonal antibodies. Antibodies were selected initially for their ability to stain PBL in indirect immunofluorescence studies, and were subsequently tested for inhibition of lymphocyte-HEV binding in the in vitro assay.

One antibody, Hermes-3, inhibited the binding of PBL to mucosal HEV (Fig. 3). Furthermore, even though Hermes-3 stained nearly all PBL (and therefore was not interacting exclusively with a mucosal HEV-specific lymphocyte subset; cf. Fig. 5), the antibody was without effect on lymphocyte binding to lymph node or synovial HEV. Thus, as argued above for MEL-14, the inhibition by Hermes-3 cannot be secondary to nonspecific membrane or cellular effects. Immunoprecipitation analyses confirm, as predicted from the immunogen used, that Hermes-3 recognizes an 85-95-kD species. As shown in Fig. 4 (lanes A and B), preclearing experiments demonstrate that the Hermes-3-defined protein bears the Hermes-1 epitope. It is intriguing that Hermes-3 is in fact able to preclear the entire gp90 class recognized by Hermes-1 (Fig. 4, lane D). In addition, as shown in Fig. 5, B and C, Hermes-3 stains intensely not only the mucosal HEV-specific cell line KCA, but also the lymph node HEV-specific line LB25. (LB-25 binds ~70% as well as PBL to lymph node HEV, but essentially at background levels to HEV in mucosal lymphoid tissues or in synovium [Jalkanen et al., 1986a;c].) These findings suggest that Hermes-3 recognizes gp90 involved, directly or indirectly, in recognition both of lymph node and of mucosal HEV, but that antibody binding to the Hermes-3 epitope interferes selectively with the mucosal HEV-binding activity (presumably steri-
A Polyclonal anti-gp90 Blocks All Three Specificities of Lymphocyte–HEV Binding

To determine whether the Hermes-1–defined gp90 might also be involved in recognition to synovial HEV, a serum from mice hyperimmunized with the isolated Hermes-1 antigen from KCA was employed. This polyclonal anti-Hermes-1 antigen (anti-gp90) serum is highly specific, immunoprecipitating a diffuse 85–95-kD band from PBL (Fig. 6, lane A), identical to that precipitated by Hermes-1 (lane E). Furthermore, the anti-gp90 serum displays the same staining pattern as Hermes-1 in immunohistologic studies of human tonsil and thymus (not shown). As shown in Fig. 6, lanes A and B, the 85–95-kd species precipitated by the polyclonal antiserum is precleared by Hermes-1, indicating that Hermes-1 sees the entire set of PBL surface molecules recognized by the polyclonal antiserum. Hermes-3 also preclears the polyclonal-defined molecules (lane C), and the polyclonal antiserum preclears both the Hermes-1 and Hermes-3–defined species (lanes F and H, respectively). Thus both monoclonal antibodies and the polyclonal anti–gp90 serum define the same 85–95-kD glycoprotein class.

As shown in Fig. 7, the polyclonal antiserum effectively blocks lymphocyte binding not only to mucosal, but also to lymph node and to synovial HEV. To exclude a nonspecific effect of surface-bound polyclonal antibodies, additional experiments were carried out with a rat antiserum against human lymphocytes (ALS) produced by hyperimmunization with PBL and tonsillar lymphocytes: the ALS was absorbed with affinity-isolated Hermes-1 antigen to remove anti-gp90 reactivity. The absorbed serum no longer inhibited lymphocyte binding to HEV (Fig. 7) even though it retained extensive reactivity with lymphocytes, staining PBL about 5–10 times as brightly as the specific anti–gp90 serum by flow cytometry (both antisera used at a 1:200 dilution). These findings demonstrate involvement of the gp90 in lymphocyte recognition of synovial as well as lymph node and mucosal HEV.

Discussion

We have used serologic approaches to define lymphocyte surface molecules involved in specific recognition of lymph node, mucosal, and synovial HEV in the human. We have shown (a) that an 85–95-kD glycoprotein antigen, defined by monoclonal antibody Hermes-1, is involved in each of these functionally independent adhesion events; (b) that organ–specific receptor activities are discriminated functionally by two monoclonal antibodies that recognize this gp90 class, MEL-14, which specifically inhibits human lymphocyte binding to lymph node HEV, and Hermes-3, which blocks only binding to mucosal HEV; and (c) that a polyclonal anti–gp90 serum, produced against highly purified Hermes-1 antigen from a mucosal HEV-specific cell line, inhibits all three classes of lymphocyte–HEV recognition. These findings demonstrate that a common molecular mechanism, associated with the gp90 Hermes-1 antigen, is involved in at least three functionally independent recognition systems that direct lymphocyte homing.

The results lend themselves to two possible interpretations. First, although the organ specificity of functional inhibition by monoclonal antibodies MEL-14 and Hermes-3 requires that the antigenic determinants they recognize be intimately associated with the recognition event, it is possible that the gp90 represents an invariant molecule that is coexpressed and physically associated with functionally diverse receptors yet to be identified, or that constitutes one component of a more complex composite receptor system (analogous perhaps to the CD3/T cell receptor complex). In this model, the inhibition of particular functional activities by monoclonal antibodies against distinct epitopes on the Hermes-1 antigen could represent selective interference with
the association of the invariant gp90 with particular structurally distinct receptors; or selective steric hindrance of the function of particular associated receptors or components.

Alternatively, the 85-95-kD molecular class defined by Hermes-I could consist of a set of closely related but functionally distinct receptors for tissue-specific HEV determinants. This hypothesis is more readily reconciled with the ability of MEL-14 and Hermes-3 to discriminate functionally between human lymphocyte binding activities for lymph node, mucosal, and synovial HEV; this functional discrimination clearly requires that the gp90 class includes molecules or molecular domains uniquely associated with each recognition event.

A corollary of this second hypothesis is that the gp90 on lymph node HEV-specific and mucosal HEV-specific cells, because they would be functionally independent members of the class, would necessarily be structurally and hence anti-

genically distinct. Previous studies in murine species are consistent with this corollary, inasmuch as in these animal models putative homing receptors have been identified by receptor subtype-specific monoclonal antibodies. (Indeed, before the current studies, there was no direct evidence that common or related molecular elements were involved in lymphocyte recognition of different HEV.) For example, monoclonal antibody MEL-14, which was useful for the current studies because of its reactivity with human lymphocyte gp90, defines a 90-kD mouse lymphocyte surface glycoprotein involved in recognition of lymph node HEV, but does not stain Peyer's patch HEV-specific cells (Gallatin et al., 1983). Similarly, Chin et al. (1986) have described a monoclonal antibody, IB.2, that defines an 80-kD rat lymphocyte surface molecule involved in binding to mucosal but not lymph node HEV. Like MEL-14 in the mouse, IB.2 appears to discriminate mucosal and lymph node receptor activities, in that a population of rat thoracic duct lymphocytes that are lymph node HEV-specific is IB.2 negative. Thus putative receptors for lymph node and mucosal endothelium are antigenically distinct in murine species. In light of the shared functional properties of the gp80-90 in the murine and human studies, as well as the similarity in their apparent molecular masses and the reactivity of MEL-14 with both the human and mouse glycoproteins, it seems likely that the murine gp80-90 and the Hermes-I-defined glycoprotein class are analogous. Taken together, these considerations offer significant support for the hypothesis that the gp90 defined by Hermes-I represent a family of homing receptors, with distinct members mediating recognition of lymph node, mucosal, and synovial high endothelium. Direct demonstration of organ-specific binding of purified gp90 to high endothelial cells, and/or correlation of specificity with detailed structural or sequence analyses, would be required to confirm this hypothesis. In addition, it will be important to develop antibodies capable of discriminating antigenically between functionally distinct receptors in the human.

It should be mentioned that there are precedents for families of closely related glycoprotein receptors with diverse specificities, the most obvious being the immunoglobulins and the T cell receptors for antigen. Even a single amino acid within the ligand-binding domain of receptors can completely alter ligand specificity. For example, single amino acid substitutions at residue 226 in the influenza virus hemagglutinin result in striking alterations in ligand specificity: hemagglutinins with leucine at this position are specific for NeuAc-alpha 2--6Gal--; substitution of glutamine results in loss of this activity, with specificity instead for NeuAc-alpha 2--3Gal--; substitution of methionine yields a hemagglutinin capable of interacting with both of these structures (Rogers et al., 1983). This example may be particularly relevant because there is now considerable evidence to suggest that the mouse lymph node homing receptor is a carbohydrate-recognizing molecule (Stoolman et al., 1984; Kieda and Monsigny, 1983; Reddick et al., 1987). It will be important to determine whether the Hermes-I-defined human gp90 also behave as mammalian lectins.

The present finding that at least three functionally distinct and independently regulated cell-binding activities are associated with a single apparent molecular class may have important implications for studies of other cell–cell adhesion
molecules: It raises the intriguing possibility that cell adhesion molecules thought to be involved in a relatively general manner in neuron–neuron (Edelman, 1983; Rutishauser, 1984; Faissern et al., 1984), neuron–glial cell (Grumet and Edelman, 1984), or epithelial cell–cell (Edelman, 1983; Yoshida and Takeichi, 1982; Dansky et al., 1983) interactions might instead be composed of multiple functionally independent receptors capable, at least theoretically, of unsuspected fine specificity in vivo. In this context, it may be relevant that several biologically important proteins previously thought to be homogeneous, for example protein kinase C and the GTP-binding protein, have recently been shown to be functionally diverse molecular families (Carpenter et al., 1987; Bourne, 1986).

Interestingly, putative mouse lymphocyte receptors for lymph node HEV appear to be modified by covalent linkage to the polypeptide, ubiquitin (Siegelman et al., 1986). Ubiquitin is an 8.5-kD polypeptide present conjugated to a variety of proteins in the nucleus and cytoplasm of most eukaryotic cells (Hershko, 1983). At least part of the epitope recognized by MEL-14 appears to be determined by the ubiquitin sequence (St. John et al., 1986), and it has been proposed that ubiquitination may play a role in receptor specificity or processing. The cross-reactivity of MEL-14 with the Hermes-1 antigen, as demonstrated here, suggests that the human gp90 may be ubiquitinated as well.

In studies to be reported elsewhere, we have also found that the Hermes-1 antigen bears several N-linked glycans, incorporated sulphate, and considerable endoglycosidase F-resistant sialic acid. The role of these post- or cotranslational modifications in the function and/or specificity of these molecules remains to be determined.

Although it is likely that the gp90 is essential for specific recognition and binding to HEV, other cell surface molecules may also play a role. Rasmussen et al. (1985) have reported a monoclonal antibody, A.11, that inhibits rat lymphocyte binding to lymph node but not to mucosal HEV, and precipitates several molecular species, of M, 40, 63, and 135 kD. Antibodies to certain epitopes of the LFA-1 molecular complex also demonstrate partial inhibition of lymphocyte binding to lymph node and to mucosal HEV both in the mouse (Hamann et al., manuscript submitted for publication) and the human (N. Wu, unpublished data) systems.

Thus several molecular species may operate, presumably in conjunction with the gp90, to effect lymphocyte recognition and adherence to high endothelial cells.

In conclusion, we have demonstrated that antibodies able to interfere with particular organ-specific lymphocyte-HEV recognition events bind to a common 85–95-kD glycoprotein antigen. Further studies must be carried out to determine whether this gp90 represents an invariant component associated with other surface elements conferring recognition specificity, or whether it instead comprises a family of closely related but functionally distinct receptors for HEV. The observation that the gp90 is involved in lymphocyte binding to synovial HEV is of particular interest, in that it raises the possibility of generating monoclonal antibodies capable of specifically interfering with homing to inflamed joints, for example in rheumatoid arthritis, by immunizing with the Hermes-1 antigen from synovial HEV-binding cells. It will be interesting to determine whether, in addition to the three known homing specificities, the gp90 Hermes-1 antigen is involved in directing lymphocyte traffic through many or all different organs in the body, and whether related glycoproteins play a role in other interacting cellular systems.

We thank W. M. Gallatin for observations confirming the cross-reactivity of MEL-14, P. Sallata for technical assistance, P. Verlota for photography, T. Naka for assistance with the FACS, A. Steere and R. Fox for synovial tissue, and D. Lewinsohn, R. Rouse, R. Shiu, and N. Wu for their critical reading of the manuscript.

Dr. Jalkanen was a fellow of the Jane Coffin Childs Memorial Fund for Medical Research; Dr. de los Tojos is Fullbright Scholar; Dr. Butcher is a scholar of the Leukemia Society of America. This study was supported by grant AI0957 from the National Institutes of Health.

Received for publication 2 February 1987, and in revised form 31 March 1987.
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