Evolutionary Conservation of Tissue-specific Lymphocyte–Endothelial Cell Recognition Mechanisms Involved in Lymphocyte Homing

Nora W. Wu,*‡ Sirpa Jalkanen,* Philip R. Streeter,* and Eugene C. Butcher*

*Laboratory of Experimental Oncology, Departments of *Pathology and ‡Pediatrics, Stanford University School of Medicine, Stanford, California 94305; and the *Veterans Administration Medical Center, Palo Alto, California 94304

Abstract. Tissue-specific interactions with specialized high endothelial venules (HEV) direct the homing of lymphocytes from the blood into peripheral lymph nodes, mucosal lymphoid organs, and tissue sites of chronic inflammation. These interactions have been demonstrated in all mammalian species examined and thus appear highly conserved. To assess the degree of evolutionary divergence in lymphocyte–HEV recognition mechanisms, we have studied the ability of lymphocytes to interact with HEV across species barriers. By using an in vitro assay of lymphocyte binding to HEV in frozen sections of lymphoid tissues, we confirm that mouse, guinea pig, and human lymphocytes bind to xenogeneic as well as homologous HEV. In addition, we show that mouse and human lymphoid cell lines that bind selectively to either peripheral lymph node or mucosal vessels (Peyer's patches, appendix) in homologous lymphoid tissues exhibit the same organ specificity in binding to xenogeneic HEV. Furthermore, monoclonal antibodies that recognize lymphocyte "homing receptors" and block homologous lymphocyte binding to peripheral lymph node or to mucosal HEV, also inhibit lymphocyte interactions with xenogeneic HEV in a tissue-specific fashion. Similarly, anti-HEV antibodies against organ-specific mouse high endothelial cell "addressins" involved in lymphocyte homing to peripheral lymph node or mucosal lymphoid organs, not only block the adhesion of mouse lymphocytes but also of human lymphocytes to target mouse HEV. The results illustrate a remarkable degree of functional conservation of elements mediating these cell–cell recognition events involved in organ-specific lymphocyte homing.

MANY developmentally important cell–cell interactions appear highly conserved during evolution. Examples include neuronal targetting and other morphogenetic cell movements during embryogenesis, spermatozoan binding to ova, and specific cellular interactions during immune responses. The occurrence of identical cell–cell interactions in different species does not preclude, however, the possibility of evolutionary change in the molecular mechanisms involved. In fact, comparisons of glycoprotein receptors (e.g., CD4, CD8; references 25, 26, 38) and enzymes (e.g., cytochrome c; 14) from different species indicate that substantial modifications, including numerous amino acid substitutions, occur in most proteins during the evolutionary divergence of species. That recent evolutionary changes have also occurred in a number of well-characterized cell adhesion molecules (including neural and liver cell adhesion molecules [N-CAM and L-CAM; 16], fibronectin receptors and related integrins [19, 29], the LFA-1/Mac-1 family [reviewed in 31] and lymphocyte homing receptors [reviewed in 5]) is indicated by the existence of serologically defined differences in these molecules in different mammalian species. The evolutionary drift implied could permit significant divergence in the functional characteristics and compatibility of adhesion molecules in differing species, as well, although such divergence in functional properties is not necessary if evolutionary alterations effect only noncritical protein domains.

To assess evolutionary change in functional aspects of structures mediating conserved cellular interactions requires determination of the degree to which such interactions are possible between cells of different species. We previously applied this approach to the analysis of a highly conserved cell–cell interaction, the specific binding of lymphocytes to specialized high endothelial venules (HEV)[1] (7). The ability of lymphocytes from several vertebrate species to bind to mouse lymph node HEV was shown to decline exponentially with increasing evolutionary distance of the lymphocyte donor from the mouse. The results were interpreted as suggest-

Sirpa Jalkanen's present address is the Department of Medical Microbiology, University of Turku, Turku 52, Finland.

1. *Abbreviations used in this paper*: HEV, high endothelial venules; PBL, peripheral blood lymphocytes; PLN, peripheral lymph nodes; PP, Peyer's patches; RAR, relative adherence ratios.

ing a progressive exponential decline in the compatibility of lymphocyte–HEV recognition systems in diverging species, presumably as a result of continuous random changes in the interacting lymphocyte and endothelial cell surface structures involved.

A corollary of this hypothesis, not examined in earlier studies because in vitro assays using nonmurine HEV had not been developed, was that HEV from any species should bind homologous lymphocytes best with progressive decline in the efficiency of interaction with evolutionary distance separating the HEV and lymphocyte donors. We have now tested this prediction by assessing the interaction of mouse, guinea pig, and human lymphocytes with HEV in homologous and xenogeneic combinations. We have assessed lymphocyte binding to both peripheral lymph node (PLN) and to mucosal (Peyer's patch or appendix) HEV from each of the species studied because lymphocytes use distinct recognition systems to interact with HEV in these different lymphoid organs (9). We employed a simple in vitro assay system in which lymphocytes bind to HEV in frozen sections of these lymphoid tissues (8, 20, 32). In previous studies, it has been shown that lymphocyte binding to HEV in this frozen section assay closely predicts xenogeneic or homologous lymphocyte interactions with mouse HEV under more physiologic conditions either during perfusion through the systemic vasculature or in short-term in vivo localization experiments (6, 7).

In contradiction of our previous hypothesis, the results indicate that the functional interactions and, by implication from mAb inhibition studies, the molecular mechanisms of lymphocyte–HEV recognition are highly conserved among mammalian species, occurring with similar tissue specificity and antibody inhibition characteristics even in xenogeneic combinations (mouse/human) in which lymphocyte and endothelial cell donors are separated by >180 million years of evolution (30). The findings imply a remarkable degree of conservation in the function and specificity of mechanisms involved in lymphocyte–endothelial cell recognition during the divergence of mammalian species.

Materials and Methods

Cell suspensions

Mouse and guniea pig lymphocytes were obtained by pressing minced mesenteric lymph nodes through a 200-guage stainless steel screen, using cell suspension medium (RPMI 1640 containing 5% FCS and 10 mM Hepes, pH 7.2–7.3, hereafter referred to as medium) to rinse. Lymphocytes were passed through a nylon monofilament mesh (nitex; Sullivan, Inc., San Francisco, CA), to produce a single cell suspension. Human peripheral blood lymphocytes (PBL) from normal healthy adults (ages 20–40 yr) were isolated using Ficoll-Hypaque (Histopaque-1077; Sigma Chemical Co., St. Louis, MO).

Animals

BALB/c and C3H/eb mice were purchased from the Institute for Medical Research (San Jose, CA) and were maintained in our colony. Hartley Albino Guinea pigs (EZH Caviary, WIlliams, CA) were used.

Tissues

Human PLN and appendix were from surgical or biopsy specimens, determined to be pathologically normal. PLNs (axillary, brachial and cervical lymph nodes) and Peyer's patches (PP) of 6-wk-old BALB/c mice and of 10-wk-old guinea pigs were used.

Cell Lines

The mouse tumor cell lines used were 38C13, a chemical carcinogen-induced C3H/eb lymphoma (3, 18); P815, a chemical carcinogen-induced DBA/2 mastocytoma (17); and RAW112, an Abelson virus induced BALB/c lymphoma (kind gift from R. Coffman, DNAX Corp., Palo Alto, CA; 27). Human B cell lymphoblastoid and tumor cell lines were gifts from E. Engleman (KCA), the late H. Kaplan (LB25), and S. Smith (KW, a B cell lymphoma cell line derived from a pediatric patient) all from Stanford University School of Medicine, Stanford, CA.

mAbs

MEL-14 (17), a rat IgG2a; Hermes-3 (21), a mouse IgG2a; and MECA-367 (35), a rat IgG2a; have been described. 30G12 (24), specific for the mouse common leukocyte antigen (gift from J. Ledbetter, Oncogen, Seattle, WA) was used as a rat IgG2a control for MEL-14. Mouse IgG2a control antibodies for Hermes-3 included H2/33, which defines a distinct epitope on the human lymphocyte Hermes antigen (21, 22) and does not block lymphocyte binding to HEV; and UPC 10 (purified myeloma protein, Sigma Chemical Co., St. Louis, MO). MECA-20 (15), a rat IgG2a anti-mouse endothelial cell antibody was used as a control for MECA-367. MECA-79 (kindly provided by P. R. Streeter), a rat IgM, is an anti-addressin antibody that inhibits the binding of mouse lymphocytes to peripheral node HEV in vivo and in vitro. The class matched control for MECA-79 was OZ-42, a rat IgM mAb that recognizes a determinant restricted to developing mouse brain (a generous gift from L. B. Pickford and R. Rouse, Stanford, CA).

In Vitro Assay of Lymphocyte Binding to HEV

Preparation of frozen sections: human, mouse, and guinea pig lymphoid tissues were embedded in Tissue Tek II OCT compound (Miles Scientific, Naperville, IL) and frozen on dry ice. Mounted tissue was stored at −70°C until used. Freshly cut 10-μm frozen sections were placed on clean glass microscope slides. Wax pen circles (Mark-tex, Tech Pen; and Mark-Tex Corp., Englewood, NJ) 1.8 cm in internal diameter were drawn around the lymphoid tissue. To avoid the reported lymphotoxicity of OCT compound (4), the OCT was removed from the slides using fine forceps. Lymphocytes decanted from the slides after completion of the HEV assay were viable, as assessed by trypan blue or propidium iodide exclusion.

In vitro HEV assay conditions: modifications of the original Stamper and Woodruff assay (32) have recently been adapted to several species (4, 20, 34). The mouse and human in vitro HEV assays as performed by us have been described in detail (8, 20, 33). Briefly, sample lymphocytes in 100-μl medium were incubated for 30 min at 7°C with gentle agitation of freshly cut, frozen sections of mouse, guinea pig, or human lymphoid tissues. Lymphocytes were applied at a concentration of 3×10^7/ml, well within the range in which the number of cells binding per unit area of HEV varies linearly with the input cell concentration (8, 20). After incubation, the cell suspensions were gently removed and the slides with adherent cells were fixed for at least 1 h at 4°C in 1.1× PBS containing 1% glutaraldehyde (diluted from 49% stock, EM Science, Cherry Hill, NJ).

To adapt the assay to guinea pig lymphoid sections, we assessed the effects of temperature, speed of rotation, duration of incubation, and cell concentration on binding of guinea pig and mouse lymphocytes to guinea pig lymph node HEV. We found the same conditions described above to be optimal (data not presented).

To facilitate quantitative comparisons between experiments, an internal standard population of FITC-labeled lymphocytes (10) was mixed with each sample population before assay. The fixed tissue sections were examined under a microscope with darkfield illumination. Cell binding to HEV was counted single blind. In each experiment, a minimum of six sections for each sample population were examined so that >100 HEV could be counted per sample. Data reduction and analyses were as described (8, 20, 33).

Results are presented as relative adherence ratios (RAR) ± standard error (9, 20). The RAR is the calculated number of sample cells bound to HEV per reference cell binding under identical conditions (see figure legends). The reference populations employed in each experiment are described in the figure legends.

Antibody Inhibition of Lymphocyte–HEV Binding

Sample lymphocytes (either mouse or human PBL) were incubated on ice for 1 h with the anti-homing receptor antibodies (MEL-14 or Hermes-3) or isotype-matched controls (α-T200 or H2/33) at a concentration of 100 μg/3×10^7 cells. The antibodies were included during the HEV assay. For

MEL-14 inhibition studies, the same number of MEL-14–treated or control antibody–treated lymphocytes were placed in parallel on serial frozen sections of mouse PLN, mouse PP, human PLN, and appendix tissue. The number of MEL-14–treated cells bound per HEV was determined single blind by microscope, and the average was divided by the average number of control antibody cells per HEV (20) to yield a RAR. For Hermes-3 blocking studies, a mouse FITC internal standard lymphocyte population was used and the RAR was calculated in the usual manner as above (9). In each experiment, a control population of lymphocytes incubated with medium alone was also included. Results for each sample population were expressed as a percent of control (untreated, medium alone) lymphocyte binding ± standard error.

The anti-vascular addressin antibodies, MECA-367 and MECA-79, or isotype-matched controls, MECA-20 and OZ-42, respectively, were preincubated on freshly cut serial frozen tissue sections at 100 μg/ml for 30 min at 7°C. The antibodies were then gently decanted off and equal numbers of sample cells were added to the sections for the HEV assay. The number of cells binding per HEV were counted single blind under the microscope. Control treatments with medium alone were included in each experiment. Results were expressed as a percent of lymphocyte binding to control HEV.

Results

It has been shown that lymphocytes use functionally distinct and independently regulated systems to interact with HEV in different tissue sites, including PLN (9, 11, 17, 21), the mucosal lymphoid organs (PP or appendix; 9, 11, 17, 21, 35), inflamed synovium (21, 23), and perhaps other tissues. Molecular mechanisms involved in lymphocyte recognition of PLN and mucosal HEV have been characterized and shown to involve gp90-kD lymphocyte surface "homing receptors" (12, 17, 21, reviewed in 5), and tissue-specific endothelial cell antigens termed "vascular addressins" (35).

To examine the evolutionary conservation of the functional recognition events involved, we have assessed the binding of homologous and xenogeneic lymphocytes to both PLN and to mucosal HEV from mice, guinea pigs, and humans. As shown in Fig. 1 *a*, mouse lymphocytes bind significantly better than guinea pig or human lymphocytes to mouse HEV, and guinea pig lymphocytes bind slightly better than human PBL. The relative binding is similar on PLN and on PP HEV. The overall results are comparable to those described previously in studies examining binding to mesenteric lymph node HEV (7), which express both peripheral lymph node and mucosal determinants for lymphocyte recognition (9). The binding of human PBL to mouse PLN HEV is illustrated in Fig. 2.

The same relative binding efficiency (mouse lymphocytes > guinea pig > human) is observed in lymphocyte interactions with guinea pig and human HEV, as well (Fig. 1, *b* and *c*), although the difference in binding between mouse and guinea pig or human lymphocytes is somewhat less than on mouse HEV. This similarity in relative binding, regardless of the HEV donor, suggests that the mechanisms mediating lymphocyte recognition of both PLN and mucosal HEV have been conserved to a much greater extent than hypothesized previously.

Binding of Organ-specific Cell Lines

Most normal lymphocytes, however, are thought to express homing receptors of several specificities (17, 33, reviewed in 5), and therefore could potentially interact with xenogeneic HEV via more than one receptor class. To examine the operation of PLN vs. mucosal HEV recognition systems separately, we employed well-characterized human and mouse

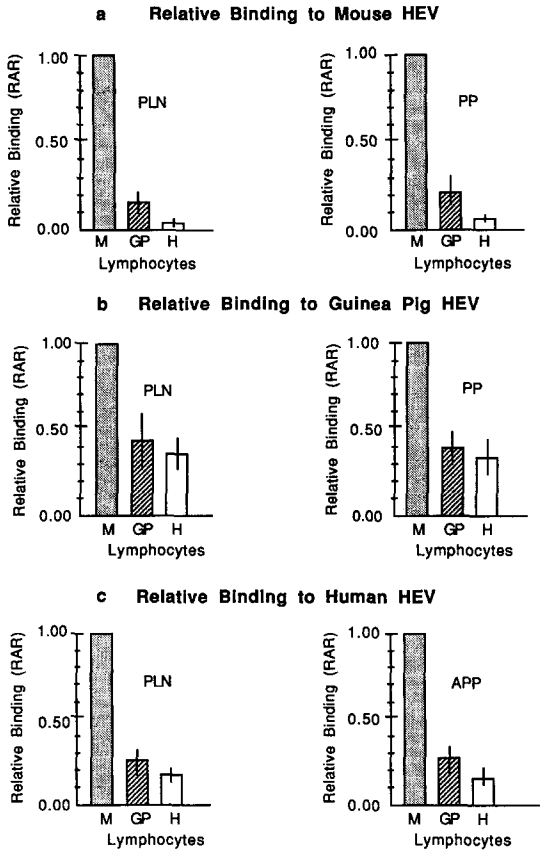

Figure 1. Relative binding of mouse (*M*), guinea pig (*GP*), and human (*H*) lymphocytes on homologous and xenogeneic HEV. (*a*) Relative binding to mouse HEV; peripheral lymph nodes (*PLN*) and mucosal associated Peyer's patches (*PP*). (*b*) Relative binding to guinea pig HEV; *PLN* and *PP*. (*c*) Relative binding to human HEV; *PLN* and appendix (*APP*). Mouse lymphocytes (*M*) were arbitrarily chosen as the reference population, defining an RAR of one. The RAR represent the calculated number of sample lymphocytes binding to HEV per mouse lymphocyte adhering under the same conditions. The mean ± SEM is presented, based on at least five experiments (n = 5–12). The RAR of mouse lymphocytes is one by definition.

cell lines that express unique homing receptors capable of binding exclusively to (homologous) PLN or mucosal HEV.

In the mouse system, 38C13, a B cell lymphoma, binds exclusively to HEV in peripheral lymph nodes. P815, a mastocytoma, binds preferentially to mucosal (PP) HEV. These specificities, reported previously (2, 17), are illustrated in the top panels of Fig. 3, *a* and *c* (data from current experiments). As shown in Fig. 3, *b* and *d* (*top*), 38C13 and P815 cells also bind human HEV in a tissue-specific manner, interacting selectively with PLN and appendix HEV, respectively. Furthermore, RAW112, a mouse pre-B cell lymphoma that lacks functional homing receptors and does not bind to either PLN or PP HEV in the mouse (2, 17; see Fig. 3, *a* and *c*, current data), also fails to bind to human HEV (Fig. 3, *b* and *d*).

Parallel results were obtained with human cell lines: LB25, a peripheral lymph node–specific B lymphoblastoid line; KCA, a mucosal HEV-specific B lymphoblastoid line; and a nonbinding B lymphoma, KW. As illustrated in Fig.

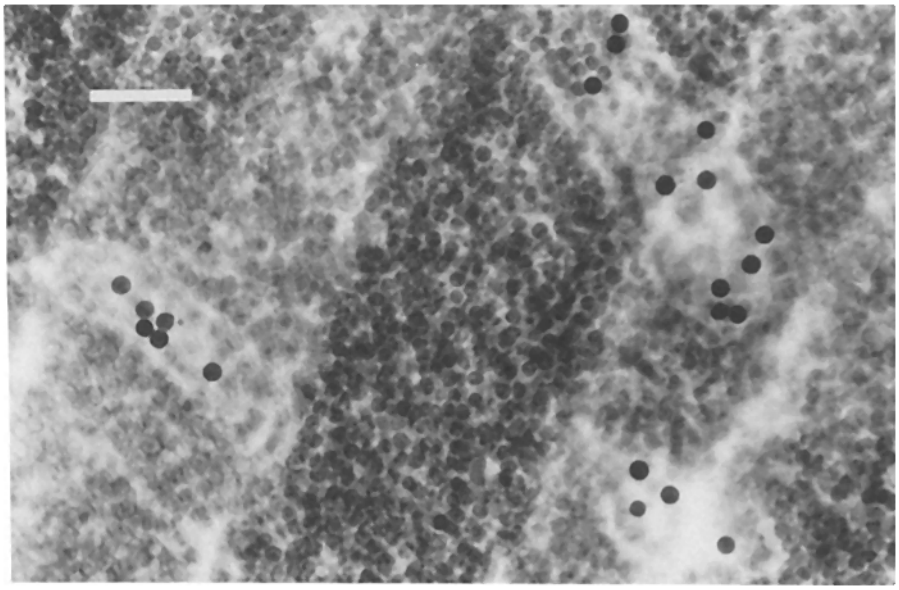

Figure 2. Human PBL bound to HEV in a frozen section of mouse peripheral lymph node. The PBL are the round dark circles lying above the plane of tissue. The outline of the HEV is delineated by a distinct, poorly stained basement membrane. Thionine stain. Bar, 50 μm.

3, *a–d* (*bottom*), these lines exhibit comparable tissue-specific binding to mouse and to human HEV. Not only is the qualitative organ-specificity conserved in xenogeneic interactions, but the relative binding of the cell lines (vs. the PBL reference population) is quantitatively similar as well on human vs. mouse HEV. These findings support a high degree of functional conservation in tissue-specific lymphocyte–endothelial cell recognition systems.

Inhibition of Xenogeneic Interactions by mAbs against Lymphocyte Homing Receptors

mAbs have been described that interfere with lymphocyte surface molecules involved in HEV recognition (12, 17, 21, 28). MEL-14 recognizes a gp90 putative mouse "homing receptor" and blocks mouse lymphocyte binding to PLN but not PP HEV (17). MEL-14 also cross reacts with putative hu-

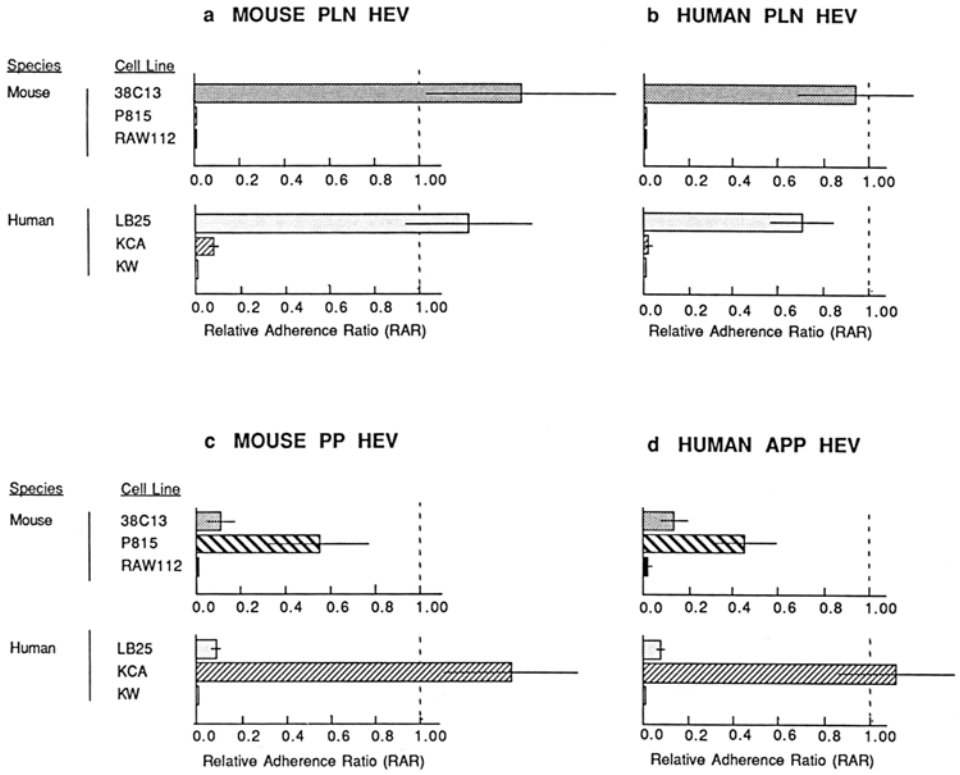

Figure 3. Organ specificity of binding of mouse and human cell lines to homologous and xenogeneic HEV. Binding (*RAR*) of the murine cell lines, 38C13, P815, and RAW112, is expressed relative to control mouse lymphocytes. Binding of human cell lines LB25, KCA, and KW is presented relative to human PBL. The *dotted lines* represent the RAR of the control lymphocyte population in each panel. (*a*) Binding to mouse PLN HEV. (*b*) Human PLN HEV. (*c*) Mouse PP HEV. (*d*) Human appendix (*APP*) HEV. The data represent the mean ± SEM of RAR from multiple experiments (n = 4–9).

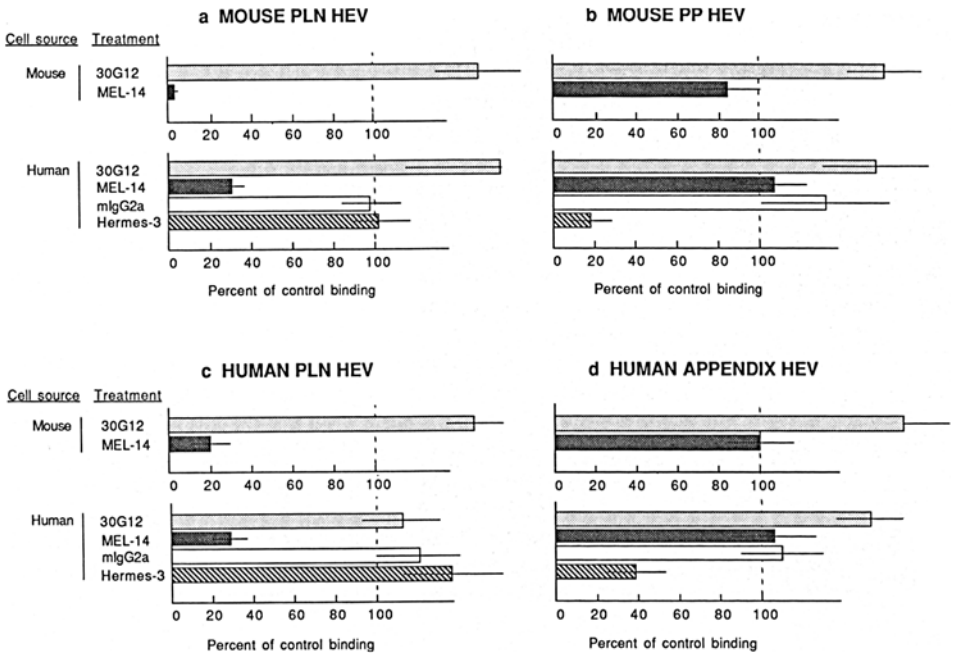

Figure 4. Inhibition of lymphocyte binding to homologous and xenogeneic HEV by mAbs against mouse and human lymphocyte "homing receptors." Sample cells (mouse and human lymphocytes) were pre-incubated 1 h in medium containing the indicated mAbs and then were submitted (with antibody) for HEV assay. No difference in HEV binding ability was noted between control cells incubated with medium alone (*dotted line*) and cells treated with isotype-matched control antibodies 30G12 or H2/33 and UPC10 (mIgG2a) (see Fig. 4). Data are expressed as the percent of control binding and represent the means ± SEM of three to five experiments per antibody treatment.

man lymphocyte homing receptors (the gp90-kD Hermes-1 antigen) and inhibits human lymphocyte binding to human PLN HEV (21). mAb Hermes-3, which sees a distinct epitope on the human Hermes-1 antigen, selectively blocks human lymphocyte binding to human mucosal HEV (21). To further address the conservation of tissue-specific recognition mechanisms, we asked whether these anti-lymphocyte homing receptor antibodies could interfere with lymphocyte recognition of xenogeneic as well as homologous HEV.

As shown in Fig. 4 (*top* in *a–d*), MEL-14 inhibits mouse lymphocyte binding to both mouse and human PLN HEV, but not to HEV in mouse PP or human appendix. Similarly, MEL-14 inhibits human PBL binding to both mouse and human HEV in a tissue-specific manner (Fig. 4, *a–d*, *bottom*). Finally, Hermes-3 blocks human lymphocyte binding to both mouse and human mucosal HEV, but not to PLN HEV of either species (Fig. 4, *a–d*, *bottom*). The results suggest that lymphocytes use the same or similar molecular mechanisms in binding to homologous and xenogeneic HEV.

Inhibition with Antibodies against Vascular Addressins

Recently, mAbs have been produced defining "vascular addressins," tissue-specific HEV antigens involved in lymphocyte-HEV binding. mAb MECA-367 defines an antigen selectively expressed by mouse mucosal HEV, blocks mouse lymphocyte binding to such HEV in vitro, and inhibits lymphocyte homing to Peyer's patches in vivo (35). Antibody MECA-79 defines a PLN node addressin, and blocks mouse lymphocyte interactions with homologous peripheral lymph node HEV in vitro and in vivo (36).

As illustrated in Fig. 5, MECA-367 and MECA-79 also inhibit the interaction of human lymphocytes with mouse mucosal and PLN HEV, and they do so in a tissue-specific manner. Thus mouse PLN and mucosal HEV appear to use the same mechanisms to bind homologous and xenogeneic lymphocytes. Together with inhibition of xenogeneic interactions by antihoming receptor antibodies, the results indicate that comparable mechanisms are involved in homologous and xenogeneic lymphocyte–HEV binding, and support the conservation of functional properties of lymphocyte–HEV interaction molecules during mammalian evolution.

Discussion

Guinea pigs diverged from mice roughly 40–50 million years ago (30, 37). These rodents diverged from the primate lineage ~90 million years ago (during the primary mammalian radiation that led to the differentiation of the various orders of mammals), and are therefore separated from humans by 180 million years of evolution (30, 37). The ability of lymphocytes and HEV from these species to interact efficiently and specifically implies a striking conservation in functional lymphocyte–HEV recognition mechanisms. Additional support for such conservation comes from the recent observations of Stoolman et al. (34) demonstrating that mannose-6-phosphate and a phosphomannan from *Hansenula holstii* can inhibit lymphocyte recognition of PLN HEV in both humans and rats. While our quantitative data does not preclude slight functional divergence in the lymphocyte homing receptors and/or endothelial cell addressins involved, the preservation of organ-specific endothelial cell discrimination, even across rather distant species barriers (mouse vs. human), suggests that such functional drift has been minimal during the evolutionary expansion of mammalian species. Perhaps these mechanisms, which regulate the entry of lymphocyte and other leukocyte subsets into diverse tissues in the body, evolved to much their present form well before mammalian speciation. It would be of interest to seek such tissue-specific endothelial cell recognition systems in avian, reptilian, and even more distant species.

The present studies do not address why there are substantial differences, as reported previously (7), in the overall

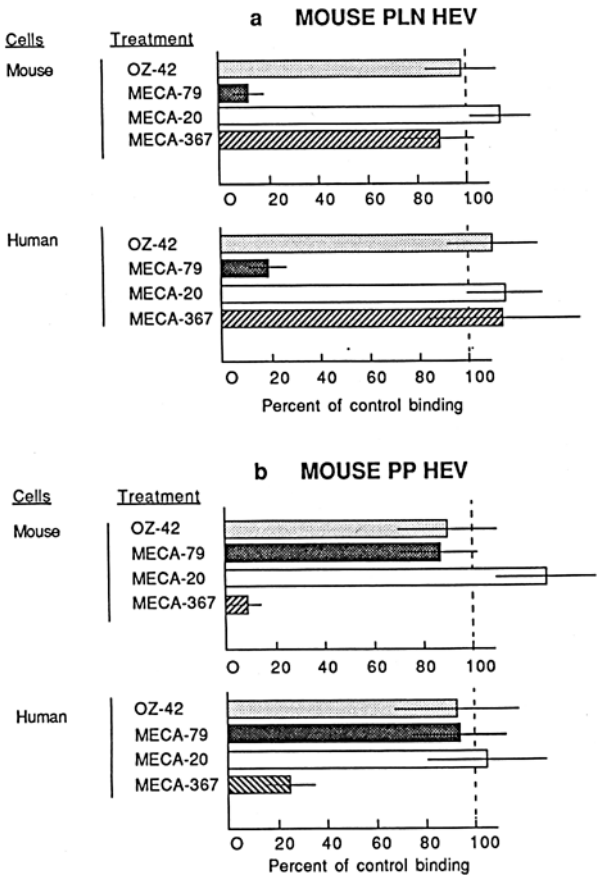

Figure 5. The effect of antibodies against mouse vascular addressins on the HEV binding of mouse and human lymphocytes. (*a*) Binding to mouse PLN HEV. (*b*) Mouse PP HEV. MECA-367 inhibits the binding of both mouse lymphocytes (*top sections*) and human PBL (*bottom sections*) to mouse Peyer's patch (*PP*) HEV. MECA-79 blocks the interaction of mouse and human lymphocytes with mouse peripheral lymph node (*PLN*) HEV. Isotype-matched antibodies had no effect relative to medium control sections (*dotted line*). Results are expressed as the percent of lymphocyte binding on control tissue sections treated with medium alone. Data represent the mean ± SEM of at least three experiments (n = 3–6) per antibody treatment.

HEV-binding abilities of lymphocytes from different species; i.e., who do normal mouse lymphocytes bind better to any HEV than guinea pig or human lymphocytes? Such species differences in lymphocytes are consistent in multiple experiments, have been seen here on HEV from several donors and species, are displayed by mature lymphocytes regardless of their organ source (spleen, blood, lymph nodes; 7), and are observed even in short-term in vivo homing or perfusion experiments (6, 7). These species distinctions may be due to differences in the functional levels of homing receptors or of accessory adhesion molecules expressed by lymphocytes, possibly reflecting an inherent difference in the physiologic requirement for high efficiency lymphocyte binding in the different species. One hypothesis is that such differences relate to the size and hence to metabolism/blood circulation dynamics of the donating species.

Our quantitative analyses have focussed on comparing the ability of lymphocytes from different species to bind to HEV: binding is always normalized to that of a standard lymphocyte population assayed on the same or serial tissue sections. This approach eliminates variability due to local or individual differences in HEV, which can be substantial. HEV from different individuals and even from different lymph nodes or regions of lymph nodes from a given individual can vary greatly in their binding of lymphocytes (20). Therefore, we have restricted ourselves here to comparisons of the tissue specificity of lymphocyte binding by HEV.

From a practical perspective, the current findings of conserved specificity indicate that HEV from mice can be employed as a convenient substrate for in vitro analyses of the endothelial cell binding characteristics of circulating lymphocytes from diverse species. It remains possible, however, that other leukocyte-endothelial cell interactions may not be as highly preserved as the tissue-specific lymphocyte–HEV recognition studied here; therefore, syngeneic or homologous combinations will always be preferred to confirm analyses involving novel leukocyte or endothelial cell systems.

Few other cellular recognition events have been studied in quantitative assays of xenogeneic cell–cell interactions. It is noteworthy, however, that aggregation of brain membrane vesicles mediated by the homophilic neural cell adhesion molecule, N-CAM, also occurs efficiently across species barriers, and retains its inherent tissue-specificity in xenogeneic as in homologous interactions (16). Thus the functional properties and specificity of N-CAM, like those of lymphocyte homing receptors and vascular addressins, appear highly conserved even though there are significant differences in the antigenicity and primary sequence of N-CAMs in different species (1, 13). A high degree of functional stability in spite of significant evolutionary change in structural features may be common for molecules mediating cellular interactions critical for morphogenesis and cellular positioning.

In conclusion, the present studies demonstrate remarkable conservation in the specificity and function of molecules mediating tissue-specific cell–cell interactions involved in lymphocyte homing in mammals. The ability of lymphocytes to interact with precise specificity with tissue-specific endothelial cell determinants from relatively distant species implies that there has been little functional alteration in the recognition elements involved over at least 180 million years of evolution and suggests that these recognition systems antedate mammalian speciation.

We thank R. Bargatze for his critical review of the manuscript and Susan Grossman for secretarial assistance.

This study was supported by National Institutes of Health grants AI-19957 and GM-37734. N. Wu is supported by National Institutes of Health Fellowship CA-07879, and P. Streeter is a Senior Fellow of the American Cancer Society, California Division No. 5-67-87. E. C. Butcher is an Established Investigator of the American Heart Association. E. C. Butcher acknowledges the prescience of Jonathon Howard and the warnings of Judith Woodruff in this matter.

Received for publication 19 May 1988, and in revised form 22 July 1988.

References

1. Barbas, J. A., J.-C. Chaix, M. Steinmetz, and C. Goridis. 1988. Differential splicing and alternative polyadenylation generates distinct NCAM transcripts and proteins in the mouse. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:625–632.
2. Bargatze, R. F., N. Wu, I. L. Weissman, and E. C. Butcher. 1987. Homing receptor expression as a predictor of the dissemination of passaged murine lymphomas. *J. Exp. Med.* 166:1125–1131.

3. Bergman, Y., and J. Haimovich. 1977. Characterization of a carcinogen-induced murine B lymphocyte cell line of C3H/eB origin. *Eur. J. Immunol.* 7:413–417.
4. Braaten, B. A., G. T. Spangrude, and R. A. Daynes. 1984. Molecular mechanisms of lymphocyte extravasation. II. Studies of *in vitro* lymphocyte adherence to high endothelial venules. *J. Immunol.* 133:117–122.
5. Butcher, E. C. 1986. The regulation of lymphocyte traffic. *Curr. Topics Microbiol. Immunol.* 128:85–122.
6. Butcher, E. C., R. G. Scollay, and I. L. Weissman. 1979. Lymphocyte-high endothelial venule interactions: examination of species specificity. *Adv. Exp. Med. Biol.* 114:65–72.
7. Butcher, E. C., R. G. Scollay, and I. L. Weissman. 1979. Evidence of continuous evolutionary change in structures mediating the adherence of lymphocytes to specialized venules. *Nature (Lond.).* 280:496–498.
8. Butcher, E. C., R. G. Scollay, and I. L. Weissman. 1979. Lymphocyte adherence to high endothelial venules: characterization of a modified in vitro assay, and examination of the binding of syngeneic and allogeneic lymphocyte populations. *J. Immunol.* 123:1996–2003.
9. Butcher, E. C., R. G. Scollay, and I. L. Weissman. 1980. Organ specificity of lymphocyte migration: mediation by highly selective lymphocyte interaction with organ-specific determinants on high endothelial venules. *Eur. J. Immunol.* 10:556–561.
10. Butcher, E. C., R. G Scollay, and I. L. Weissman. 1980. Direct fluorescent labeling of cells with fluorescein or rhodamine isothiocyanate. II. Potential application to studies of lymphocyte migration and maturation. *J. Immunol. Methods.* 37:109–121.
11. Chin, Y. H., R. Rasmussen, A. G. Cakiroglu, J. J. Woodruff. 1984. Lymphocyte recognition of lymph node high endothelium. VI. Evidence of distinct structures mediating binding to high endothelial cells of lymph nodes and Peyer's patches. *J. Immunol.* 133:2961–2965.
12. Chin, Y.-H., R. R. Rasmussen, J. J. Woodruff, and T. G. Easton. 1986. A monoclonal anti-HEBFPP antibody with specificity for lymphocyte surface molecules mediating adhesion to Peyer's patch high endothelium of the rat. *J. Immunol.* 136:2556–2561.
13. Cunningham, B. A., J. J. Hemperly, B. A. Murray, E. A. Prediger, R. Brackenbury, and G. M. Edelman. 1987. neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. *Science (Wash. DC).* 236:799–806.
14. Dickerson, R. E. 1971. The structure of cytochrome c and the rates of molecular evolution. *J. Mol. Evol.* 1:26–45.
15. Duijvestijn, A. M., M. Kerknove, R. F. Bargatze, and E. C. Butcher. 1987. Lymphoid tissue- and inflammation-specific endothelial cell differentiation defined by monoclonal antibodies. *J. Immunol.* 138:713–719.
16. Edelman, G. M. 1987. CAMS and Igs: cell adhesion and the evolutionary origins of immunity. *Immunol. Rev.* 100:11–45.
17. Gallatin, W. M., I. L. Weissman, and E. C. Butcher. 1983. A cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature (Lond.).* 303:30–34.
18. Haran-Ghera, N., and A. Peled. 1973. Thymus and bone marrow derived lymphatic leukemia in mice. *Nature (Lond.).* 241:396–398.
19. Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48: 549–554.
20. Jalkanen, S. T., and E. C. Butcher. 1985. In vitro analysis of the homing properties of human lymphocytes: developmental regulation of functional receptors for high endothelial venules. *Blood.* 66:577–582.
21. Jalkanen, S. T., R. F. Bargatze, J. de los Toyos, and E. C. Butcher. 1987. Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85–95-kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. *J. Cell Biol.* 105:983–990.
22. Jalkanen, S., R. F. Bargatze, L. Herron, and E. C. Butcher. 1986. A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. *Eur. J. Immunol.* 16:1195–1202.
23. Jalkanen, S., A. Steere, R. Fox, and E. C. Butcher. 1986. A distinct endothelial cell recognition system that controls lymphocyte traffic into inflamed synovium. *Science (Wash. DC).* 233:556–558.
24. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47: 63–90.
25. Littman, D. R., and S. N. Gettner. 1987. Unusual intron in the immunoglobulin domain of the newly isolated murine CD4 (L3T4) gene. *Nature (Lond.).* 325:453–455.
26. Nakauchi, H., G. P. Nolan, C. Hsu, H. S. Huang, P. Kavathas, and L. A. Herzenberg. 1985. Molecular cloning of Lyt-2, a membrane glycoprotein marking a subset of mouse T lymphocytes: molecular homology to its human counterpart, Leu-2/T8, and to immunoglobulin variable regions. *Proc. Natl. Acad. Sci. USA.* 82:5126–5130.
27. Ralph, R., I. Nakoinz, and W. C. Raschke. 1974. Lymphosarcoma cell growth is selectively inhibited by B lymphocyte mitogens: LPS, dextran sulfate and PPD. *Biochem. Biophys. Res. Commun.* 61:1268–1275.
28. Rasmussen, R. A., Y.-H. Chin, J. J. Woodruff, and T. G. Easton. 1985. Lymphocyte recognition of lymph node high endothelium. VII. Cell surface proteins involved in adhesion defined by monoclonal anti-HEBFLN (A.11) antibody. *J. Immunol.* 135:19–24.
29. Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion: RGD and integrins. *Science (Wash. DC).* 5:491–497.
30. Simpson, G. G. 1959. The nature and origin of supraspecific taxa. *Cold Spring Harbor Symp. Quant. Biol.* 24:255–271.
31. Springer, T. A., M. L. Dustin, T. K. Kishimoto, and S. D. Marlin. 1987. Lymphocyte function associated (LFA) molecules: cell adhesion receptors of lymphocytes. *Annu. Rev. Immunol.* 5:223–252.
32. Stamper, H. B., Jr., and J. J. Woodruff. 1976. Lymphocyte homing into lymph nodes: in vitro demonstration of the selective affinity of recirculating lymphocytes for high endothelial venules. *J. Exp. Med.* 144:828–833.
33. Stevens, S. K., I. L. Weissman, and E. C. Butcher. 1982. Differences in the migation of B and T lymphocytes: organ-selective localization in vivo and the role of lymphocyte endothelial cell recognition. *J. Immunol.* 128: 844–851.
34. Stoolman, L. M., T. A. Yednock, and S. D. Rosen. 1987. Homing receptors in human and rodent lymphocytes: evidence for a conserved carbohydrate-binding specificity. *Blood.* 70:1842–1850.
35. Streeter, P. R., E. L. Berg, B. N. Rouse, R. F. Bargatze, and E. C. Butcher. 1988. A tissue-specific endothelial cell surface glycoprotein involved in lymphocyte homing. *Nature (Lond.).* 331:41–46.
36. Streeter, P. R., B. T. N. Rouse, and E. C. Butcher. 1988. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol.* 107: 1853–1862.
37. Young, J. Z. 1981. The Life of Vertebrates. Oxford University Press, New York. 435 pp.
38. Zamoyska, R., A. C. Volimer, K. C. Sizer, C. W. Liaw, and J. R. Parnes. 1985. Two Lyt-2 polypeptides arise from a single gene by alternative splicing patterns of mRNA. *Cell.* 43:153–163.