*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 11436–11440, December 1992
Immunology

# Selective endothelial binding of interleukin-2-dependent human T-cell lines derived from different tissues

(adhesion/homing receptors)

MARKO SALMI*†, KAISA GRANFORS*, MARJATTA LEIRISALO-REPO‡, MARTTI HÄMÄLÄINEN§, RICHARD MACDERMOTT¶, RAULI LEINO‖, TAPANI HAVIA**, AND SIRPA JALKANEN*

*National Public Health Institute and Department of Medical Microbiology, Turku University, Kiinamyllynkatu 13, SF-20520 Turku, Finland; ‡Department of Medicine, Helsinki University Central Hospital, SF-00290 Helsinki, Finland; §Rheumatism Foundation Hospital, SF-18120 Heinola, Finland; ¶Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104; and Departments of ‖Medicine and **Surgery, Turku University Central Hospital, SF-20520 Turku, Finland

*Communicated by Irving L. Weissman, July 13, 1992*

**ABSTRACT** **The ability of lymphocytes to recognize and bind to high endothelial venules (HEVs) is essential for lymphocyte migration from the blood into lymphoid tissues and into sites of inflammation. Endothelial cell binding capacity also critically determines the clinical usefulness of T-cell lines and clones in immunotherapy. In the present study, interleukin-2-dependent T-cell lines were derived from the blood, lamina propria of the gut, inflamed synovium, synovial fluid, and peripheral lymph nodes. After 3–8 weeks of culture, the expression of homing-associated molecules and binding to mucosal, synovial, and peripheral lymph node HEVs were analyzed. Cell lines derived from the blood and mucosal sites bound significantly better to mucosal and synovial HEVs than to peripheral lymph node HEVs. Three out of seven synovial T-cell lines showed preferential binding to synovial HEVs, whereas the rest bound almost equally well to synovial and mucosal HEVs. T-cell lines from peripheral lymph nodes bound preferentially to lymph node HEVs despite the lack of L-selectin (the peripheral lymph node homing receptor). Expression of the known homing-associated molecules did not predict the HEV-binding specificity of these lines. Importantly, two cell lines bound well to synovial venules, but poorly, if at all, to mucosal or peripheral lymph node HEVs, supporting the concept that synovial-specific HEV recognition mechanisms exist. In conclusion, the tissue origin of T-cell lines critically determines their selectivity for endothelial cell recognition, and besides the known "homing receptors," other molecules may also mediate tissue-specific HEV-binding of interleukin-2-activated T cells.**

Most virgin lymphocytes continuously recirculate between the blood and lymphoid tissues. Lymphocytes extravasate from the blood mainly through a specialized endothelium of postcapillary high endothelial venules (HEVs, refs. 1 and 2). At least four functionally distinct lymphocyte–endothelial cell recognition systems exist that control lymphocyte traffic to peripheral lymph nodes, to mucosal sites, to inflamed synovium, and to inflamed skin (3). The first step in lymphocyte extravasation, binding of lymphocytes to endothelial cells, is critically controlled by interactions between homing-associated molecules on the lymphocyte surface and their ligands on HEVs (3–9). These molecules that confer the specificity and stability of the adhesive interactions include members of several distinct adhesion receptor families. For example, on lymphocytes L-selectin mediates lymphocyte binding to vascular endothelium in peripheral lymph nodes and an integrin, very late activation antigen 4 (VLA-4, $\alpha 4/\beta 1$ and $\alpha 4/\beta 7$), participates in homing at mucosal lymphatic sites. CD44, a multifunctional proteoglycan, and lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18), a $\beta 2$-integrin, serve as general adhesion molecules strengthening lymphocyte–HEV interaction in a non-organ-specific fashion.

Peripheral blood lymphocytes (PBLs) or tumor-infiltrating lymphocytes (TILs) stimulated and expanded *in vitro* with interleukin 2 (IL-2) are being used in clinical trials [e.g., in treatment of certain malignancies (10)]. Entrance of the activated T cells into the tumor tissue after adoptive transfer is an obvious prerequisite for the efficacy of this kind of therapy. However, expression of the homing-associated molecules and endothelial cell binding properties of human T-cell lines after long-term activation are poorly known and, therefore, the aim of this study was to analyze these characteristics.

## MATERIALS AND METHODS

**Generation of Human T-Cell Lines.** Human PBLs and synovial fluid lymphocytes (the latter drawn from arthritic patients) were isolated by Ficoll/Hypaque gradient centrifugation (Pharmacia). Lamina propria lymphocytes were isolated from surgically resected bowel specimens and from gut biopsies. Nine specimens were from histologically normal and six were from inflamed areas of the bowel. Surgically resected tissues were treated as described (11). Briefly, mucosa was dissected and treated with 0.5 mM EDTA to remove epithelial cells. Lamina propria lymphocytes were then released by overnight stirring at 37°C in Hanks' solution containing collagenase type II (20 units/ml) and isolated by Ficoll/Hypaque gradient centrifugation. Ileal biopsy specimens were extensively washed and then cut to $\approx$1-mm$^3$ pieces. When incubated in the culture medium (see below) in 96-well microtiter plates, lymphocytes grew out from the biopsy specimens within a few days, and then the stromal remnants were removed from the wells. Eventually, all the contaminating cell types died and pure lymphocyte cultures were obtained. Peripheral lymph node lymphocytes were isolated from surgically resected nodes, later determined to be histopathologically normal, and synovial membrane lymphocytes were isolated from inflamed synovectomy specimens. Normal human appendices were obtained from explorative laparotomies.

Lymphocytes isolated from the blood, synovial fluid, synovium, gut, and peripheral lymph nodes were cultured in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HEV, high endothelial venule; IL-2, interleukin 2; PBL, peripheral blood lymphocyte; VLA-4, very late activation antigen 4; LFA-1, lymphocyte function-associated antigen 1; TIL, tumor-infiltrating lymphocyte; mAb, monoclonal antibody; RAR, relative adherence ratio.

†To whom reprint requests should be addressed.

RPMI 1640 medium supplemented with 1 mM glutamine, 10 mM Hepes, penicillin (100 units/ml), streptomycin (0.1 mg/ml), 20% (vol/vol) human AB serum, phytohemagglutinin (Sigma) (1 $\mu$g/ml), and human recombinant IL-2 (500 units/ml; kindly provided by P. Karnani, Lääke-Farmos, Turku, Finland). From 10 to 100 cells per sample started to proliferate, and after 3–4 weeks the total cell yield was 20–50 $\times$ $10^6$ cells per sample.

**Monoclonal Antibodies (mAbs).** mAbs against CD3 (CRL 8001), CD4 (CRL 8002), and CD8 (CRL 8014) were from American Type Culture Collection; mAbs against CD11b (My-4) and CD20 (B1) were from Coulter; mAbs against L-selectin (Leu-8) and the $\alpha/\beta$ T-cell receptor (TCR-1 $\alpha/\beta$) were from Becton Dickinson; and mAb against the $\gamma/\delta$ T-cell receptor (TCR $\delta$1) was from T Cell Sciences (Cambridge, MA). Anti-CD18 (TS1/18) mAb against the $\beta$2 chain and anti-CD11a (TS1/22) mAb against the LFA-1 $\alpha$ chain were gifts from C. Clayberger (Stanford University). Anti-VLA-4$\alpha$ mAbs (HP2/1 and B-5G10) against two different epitopes of the molecule were gifts from F. Sanchez-Madrid (University of Madrid) and M. Hemler (Dana–Farber Institute, Boston), respectively. The anti-$\beta$1 integrin mAb was purchased from Locus (Helsinki). Production of an anti-CD44 mAb (Hermes-3) has been described (12). 3G6, a mAb against chicken T cells, was used as a negative control. Fluorescein isothiocyanate-conjugated sheep anti-mouse IgG was obtained from Sigma.

**Immunofluorescence Staining and Flow Cytometric Analyses.** The staining protocol was essentially the same as described (11). Briefly, the cells were incubated with unconjugated primary mAbs and then with fluorescein isothiocyanate-conjugated anti-mouse IgG. Thereafter, the cells were fixed in phosphate-buffered saline containing 1% formaldehyde. Freshly isolated PBLs stained with the same antibodies were always included as controls. Samples were analyzed using a FACScan cytometer (Becton Dickinson) and data were collected from 10,000 cells.

***In Vitro* HEV-Binding Assay.** Details of this technique have been described (13). Briefly, lymphocytes in suspension were incubated with mild rotation for 30 min at 7°C on freshly cut frozen sections of human and mouse (specific-pathogen-free BALB/c mice) peripheral lymph nodes, human appendix, mouse Peyer's patches, and human synovia. Adherent cells were fixed in 1% glutaraldehyde. The number of lymphocytes bound to HEVs was counted single-blind under dark-field illumination. Each sample was analyzed on four to six sections per tissue type, and an average 110 HEVs were counted. To facilitate direct comparison among sample populations and among different experiments, the relative adherence ratios (RARs) were determined. The RAR is the calculated number of sample cells bound to a HEV normalized to the number of reference lymphocytes bound under the same conditions. Freshly isolated PBLs were included in each experiment as such a reference population.

**Statistical Analyses.** Paired Student's *t* test, Student's *t* test, and correlation analysis (BMDP Statistical Software, Los Angeles) were used to analyze the statistical significance.

## RESULTS

**Phenotype of the T-Cell Lines.** IL-2-activated T-cell lines were generated from 20 PBL, 7 synovial fluid, 2 synovial membrane, 4 peripheral lymph node, and 15 gut specimens. Every line from each tissue type was from a different individual. After culturing, the proportion of activated cells belonging to distinct T-cell subclasses differed significantly depending on the tissue origin (Table 1). More CD8-positive cells were obtained from the blood ($P$, 0.038) and from the gut ($P$, 0.002) than from the peripheral lymph node lines. Moreover, more $\gamma/\delta$-positive T-cell lines arose from synovium ($P$, 0.046 when compared to gut; $P$, 0.002 when compared to blood) and from peripheral lymph node ($P$, 0.005 when compared to gut; $P$, 0.008 when compared to blood) samples. No B cells, macrophages, or granulocytes were detectable.

**Homing-Associated Molecules on T-Cell Lines.** All lines were homogeneously LFA-1 (CD11a/CD18)-positive. VLA-4$\alpha$ was present on >90% of cells in all but one line, and most lines expressed this antigen at a markedly higher level than did fresh PBLs. Both anti-VLA-4$\alpha$ antibodies gave identical staining results. Anti-$\beta$1 mAb against the $\beta$ chain of the VLA heterodimers stained all but one cell line tested. In contrast to LFA-1 and VLA-4$\alpha$, L-selectin was practically absent from most lines (Fig. 1*A*). Even though two PBL lines still contained up to 40% weakly L-selectin-positive cells, neither the staining intensity nor the number of positive cells in any sample was even nearly comparable to those of fresh PBLs. CD44 expression on cultured cell lines was characteristically heterogeneous (Fig. 1*B*). Most cell lines (32/48) were at least as strongly CD44-positive as freshly isolated PBLs, whereas 14/48 showed a bimodal expression of this antigen with variable proportions of strongly CD44-positive and CD44-negative/weakly CD44-positive cells. Moreover, two cell lines derived from the gut were almost CD44-negative. In general, the set of homing-associated molecules expressed was not dependent on the tissue from which the line was derived. However, mucosal T-cell lines did express somewhat less CD44 antigen (71% on an average) than other lines [$P$, 0.01 when compared to PBLs (89% CD44 positive), 0.04 when compared to peripheral lymph node lines (98% CD44 positive), and 0.02 when compared to synovial cell lines (93% CD44 positive)].

**Expression of Adhesion Molecules as a Function of Culture Time.** Because there were marked differences in the phenotype of fresh PBLs and T-cell lines derived from PBLs (see above), one PBL specimen was chosen for further analysis to study the effect of culture time on the expression of adhesion molecules (Fig. 2). Samples at the different times were stained and analyzed under identical conditions. Intensity of

Table 1. Expression of T-cell-specific antigens on T-cell lines derived from various tissues of the body as determined by flow cytometry analyses

| Tissue origin | CD4/CD8* | No. of lines† | | % positive cells‡ | | Lines expressing $\gamma/\delta$, no.§ | | |
|---|---|---|---|---|---|---|---|---|
| | | CD4 predom | CD8 predom | $\alpha/\beta$ | $\gamma/\delta$ | <5% | 6–30% | 31–65% |
| Blood | 0.5 | 3 | 16 | 81 | 1 | 13 | 0 | 0 |
| Gut | 0.2 | 2 | 13 | 93 | 4 | 7 | 3 | 0 |
| Syn | 0.7 | 4 | 5 | 80 | 14 | 3 | 4 | 2 |
| PLN | 1.8 | 3 | 1 | 68 | 24 | 2 | 0 | 2 |

PLN, peripheral lymph node; Syn, synovium.
*Mean ratio of cells expressing CD4 to cells expressing CD8.
†Number of predominantly CD4- or CD8-positive lines.
‡Mean percentage of positive cells.
§$\gamma/\delta$ analysis was not done on all lines.

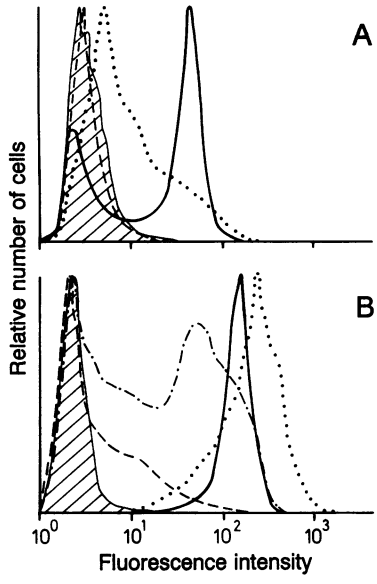


FIG. 1. Heterogeneous expression of adhesion molecules on T-cell lines. Flow cytometry profiles of IL-2-activated T-cell lines are presented to show the extremes of L-selectin and CD44 expression. (*A*) L-selectin: ---, negative cell line; ····, positive cell line. (*B*) CD44: ---, negative/weakly positive cell line; ····, strongly positive cell line; -·-·-, line with bimodal expression. Staining of freshly isolated PBLs (solid line) is shown for comparison and hatched histograms represent background staining (3G6).

CD11a and CD18 staining increased after the first days and only a single homogeneously positive population became apparent. Activated T cells rapidly lost L-selectin. After 1 day of culture the intensity of L-selectin staining was considerably lower than that of fresh PBLs. Gradually, the number of L-selectin-positive cells further diminished and after 2 weeks of culture almost no L-selectin-positive cells were detectable. VLA-4$\alpha$ was up-regulated within 5 days. After prolonged culture for 3 weeks, however, the intensity of VLA-$\alpha$4 staining again decreased. During the culture, a subpopulation of CD44-negative/weakly CD44-positive cells arose from the uniformly strongly CD44-positive starting

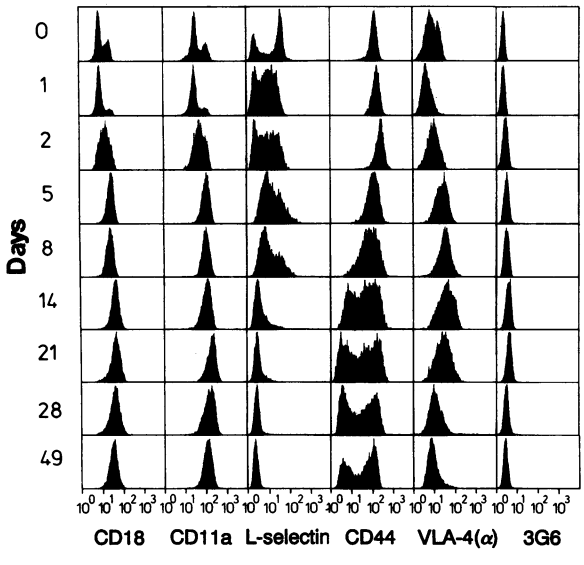


FIG. 2. Time-dependent changes in the expression of homing-associated molecules on a PBL cell line shown by flow cytometry. Fluorescence staining in the logarithmic scale is shown on the *x* axis and the relative number of cells is shown on the *y* axis.

population, and after 3–4 weeks, the number of CD44-negative cells reached its maximum (Fig. 2).

**HEV-Binding Properties of the Activated T Cells.** An *in vitro* frozen section assay was used to determine the HEV-binding capacity of the T-cell lines generated from different tissues. Binding of three T-cell lines derived from each different tissue type was tested on both human and mouse (mucosal and peripheral lymph node) HEVs and found to be independent of the species donating the target tissue (data not shown). These data are in concordance with the previous report (14) describing preservation of HEV-binding specificity in xenogeneic systems. Therefore, mouse peripheral lymph nodes, mouse Peyer's patches, and human synovia were routinely used. Culturing time of T cells (3 vs. 8 weeks) did not affect the selectivity or efficiency of HEV binding that was tested by analyzing twice the HEV-binding properties of 11 cell lines at 2–3 week intervals (after reaching the phenotypic steady state at 3–4 weeks of culture).

T-cell lines usually bound best to HEVs of the same tissue type from which they were derived (Fig. 3). Cells from the gut bound well to mucosal HEVs but bound negligibly to lymph node HEVs (Fig. 3*A*). Binding to synovium revealed two distinct patterns. Two mucosal cell lines showed practically no binding to synovial HEVs, whereas the rest bound to various extents (Fig. 3*A*). The efficient synovial HEV binding of one mucosal T-cell line is shown in Fig. 4. The binding properties of lines derived from normal and inflamed gut did not differ from each other. Synovial T cells bound well to synovial HEVs but bound less efficiently to mucosal HEVs, and usually only occasional lymphocytes were encountered on peripheral lymph node sections (Fig. 3*B*). On average, PBL lines bound almost equally well to mucosal and synovial HEVs but binding to peripheral lymph node HEVs was weaker (Fig. 3*C*). T-cell lines derived from peripheral lymph nodes adhered best to lymph node HEVs, while binding to synovial and mucosal HEV was only about one-third as efficient (Fig. 3*D*). As can be seen in Fig. 3, majority of cultured lines showed weaker adherence than fresh control PBLs (RAR, 1.0 by definition) to all types of tissues tested, but few cell lines bound extremely well.

**Two T-Cell Lines Bind Selectively to Synovial HEV.** A distinctive HEV-binding pattern for two IL-2-activated T-cell lines was observed. One line derived from synovial fluid (Fig. 3*B*, line 6) bound very efficiently to synovial HEVs (RAR, 1.7) but did not bind significantly to mucosal (RAR, 0.02) or lymph node (RAR, 0.06) HEVs. Interestingly, also one PBL line (Fig. 3*C*, line 2) bound almost exclusively to synovial HEVs (RARs, 2.7 for synovial, 0.06 for mucosal, and 0.2 for lymph node HEVs).

**Lymphocyte Adhesion Molecules and the HEV-Binding Capacity.** Correlation analyses revealed that expression of the known homing-associated molecules on activated T-cell lines did not directly predict the HEV-binding specificity. For example, loss of L-selectin did not prevent certain cell lines from binding to peripheral lymph node HEVs well [Figs. 3*D* (all four peripheral lymph node lines were practically L-selectin-negative) and 5]. Furthermore, most cell lines derived from different types of tissues were close to 100% VLA-4$\alpha$-positive and the intensity of staining was often superior to that of fresh PBLs. Yet, many cell lines derived from PBLs, peripheral lymph node, or synovium did not bind mucosal HEVs well. However, cell lines derived from the gut that expressed similar levels of VLA-4$\alpha$ preferentially adhered to mucosal HEVs. The lack of correlation between $\alpha$4 expression and mucosal HEV binding may be due to the absence of the alternative form of VLA-4 ($\alpha$4/$\beta$7) that is the principal mucosal homing receptor in mouse (15) and may play a similar role in humans (since human $\beta$7 was not characterized when the experiments of the present study were performed, we were unable to determine its expression on activated T

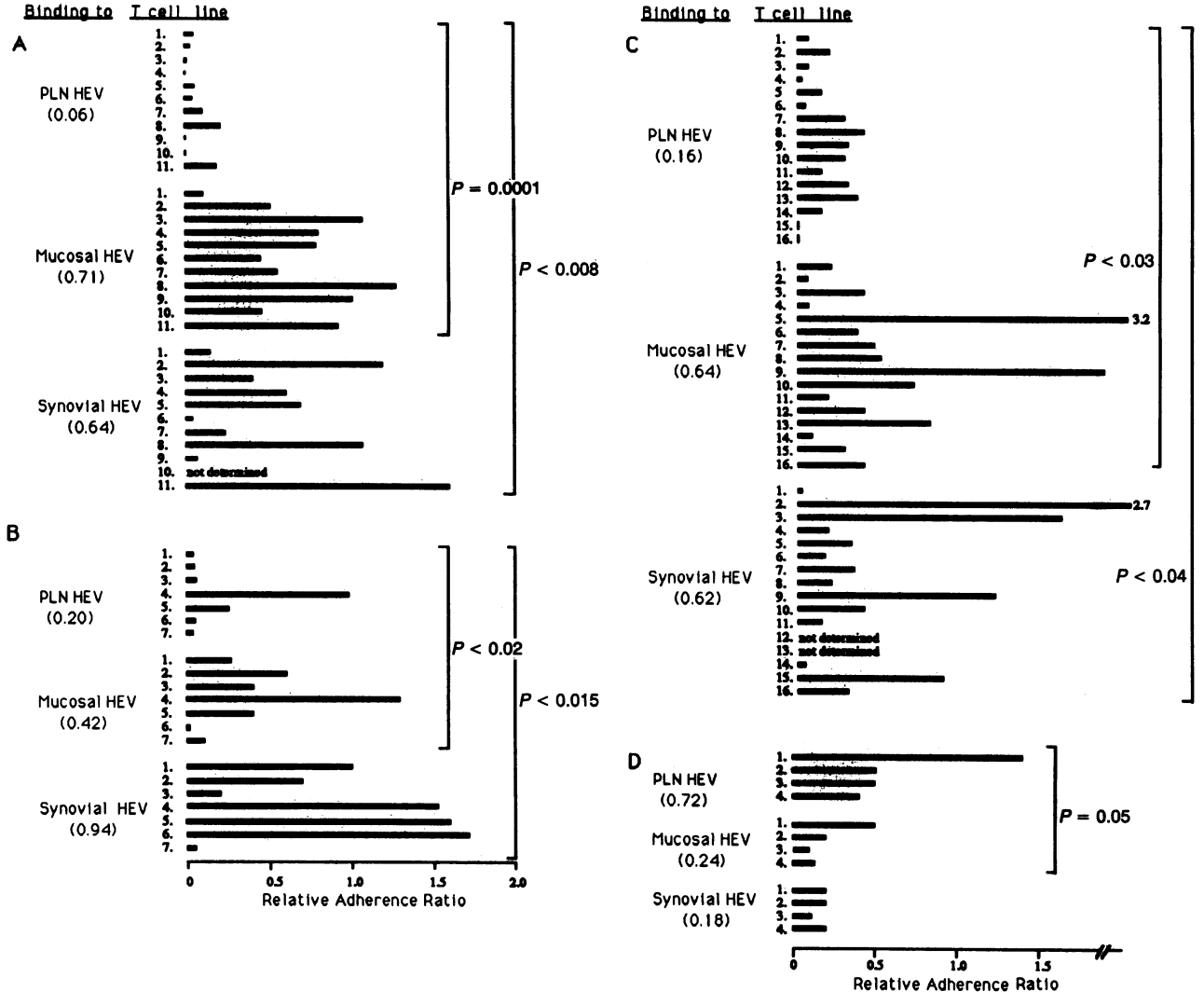


FIG. 3. HEV-binding properties of T-cell lines derived from various tissues. (*A*) Gut (11 lines). (*B*) Synovium (7 lines). (*C*) PBLs (16 lines). (*D*) Peripheral lymph nodes (4 lines). Binding of cells to peripheral lymph node (PLN), mucosal, and synovial HEVs is shown. Each bar represents an individual cell line. Results are shown as RARs, and the numbers in parentheses are the mean RAR for each target tissue. The *P* values were determined by using a paired Student's *t* test.

cells). Finally, many of the cell lines that were practically 100% positive when stained for LFA-1 or CD44 did not show efficient binding to HEVs. Thus, the expression of these two non-organ-specific adhesion molecules was not sufficient for lymphocyte binding to HEVs in different target tissues.

## DISCUSSION

This study was performed to assess the capacity of lymphocytes to bind to HEVs after long-term *in vitro* culture. The main finding was that the tissue origin most critically determined the HEV-binding properties of IL-2-driven lymphocytes. Stimulated T-cell lines usually bound most efficiently to the same type of tissue from which they were derived: lamina propria lymphocytes adhered best to mucosal HEVs, synovial lymphocytes adhered best to synovial HEVs, and peripheral lymph node lymphocytes adhered best to lymph node HEVs. Lines derived from PBLs bound relatively well both to mucosal and synovial HEVs. Hence, *in vitro*-activated lymphocytes retain the same interdependence between the tissue origin and binding specificity as freshly isolated immunoblasts. Earlier *in vivo* animal studies have shown that immunoblasts from the gut, peripheral lymph nodes, and skin preferentially home to the same type of tissue from which they were isolated (1, 16–19), and HEV-binding properties of freshly isolated human lamina propria immunoblasts are strikingly similar to those of mucosal T-cell lines (M.S., K.G., R.M., and S.J., unpublished data). Similar behavior of freshly isolated immunoblasts and T-cell lines may reflect the fact that our culture system predominantly expands preactivated lymphocytes.

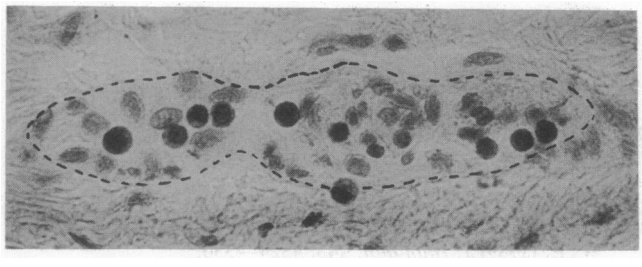

FIG. 4. T-cell lines of intestinal origin have capacity to bind to synovial HEVs. Several activated T cells (dark round cells) are bound to a large HEV. The dashed line outlines the basement membrane of the HEV. (Diff-Quick staining; ×260.)

The capacity of lymphocytes to bind to HEVs *in vitro* has been shown to parallel their presumed migratory status *in vivo* (2). Therefore, preservation of the HEV-binding capacity of most T-cell lines after a long-term *in vitro* activation

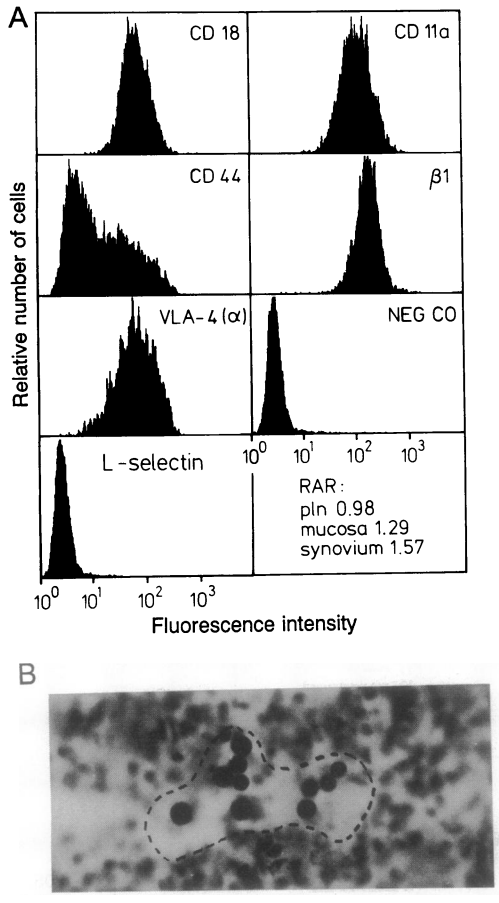


FIG. 5. Discrepancy between the homing receptor expression and the HEV-binding capacity. (*A*) Expression of homing-associated molecules on a line derived from the synovium is shown in the histograms. (*B*) Specific binding of T cells from the same line to a peripheral lymph node HEV in the *in vitro* binding assay is shown. (Diff-Quick staining; ×280.) Note that despite efficient binding to peripheral lymph node HEVs, the cells lack L-selectin. NEG CO, negative control (3G6); pln, peripheral lymph node.

may predict proper homing of *in vitro*-expanded TILs *in vivo*. Furthermore, the importance of the tissue origin to the HEV-binding properties of IL-2-activated T cells suggests that when infused back to the patient, circulating TILs would preferentially home to the tissue type from which they were isolated. Actually, indium-labeled TILs have been observed to localize preferentially to tumor sites *in vivo* (20). However, because TILs derived from skin biopsies of melanoma patients homed both to the primary tumor site and into metastases, other factors besides the tissue origin, such as expression of vascular adhesion molecules in the tumor, must influence on the final distribution of TILs *in vivo*.

Most cell lines were L-selectin-negative at the end of the culture but a few of them still specifically and efficiently adhered to peripheral lymph node HEVs. Binding was not due to "general stickiness" of these cell lines to all types of HEVs, since, e.g., the peripheral lymph node-derived lines did not adhere well to synovial and mucosal HEVs. A discrepancy between homing receptor expression and HEV binding has also been reported in a few other studies showing that L-selectin-negative lymphokine-activated killer cells were able to bind to peripheral lymph node HEVs *in vitro* (21) and that expression of L-selectin was not an absolute requirement for dissemination of murine T-lymphocyte clones to lymph nodes after adoptive transfer (22). Thus, these results together with our present data suggest that L-selectin may not play an essential role in binding of activated cells to peripheral lymph node HEVs.

Due to the complex nature of lymphocyte–endothelial interactions, the discrepancy between the surface expression of adhesion molecules and the actual HEV-binding capacity may have several reasons. Expression of one homing receptor may not be enough for efficient binding, and on the other hand, down-regulation of one homing-associated molecule may be compensated with increased expression of others. For example, in the present study loss of L-selectin is associated with up-regulation of other homing-associated molecules such as LFA-1. Furthermore, the functional activity of adhesion molecules cannot be measured with the mAbs that were used. Finally, there still may be additional tissue-specific homing molecules to be found. For example, in the present study two IL-2-activated T-cell lines were discovered that almost exclusively bound to synovial HEVs (14–85 times better than to other types of HEVs). Since the synovium represents a distinct HEV-binding specificity (23), these data provide further indirect evidence that synovium-specific adhesion molecule(s) may exist and these two cell lines should prove useful in search of such molecules.

We thank Drs. C. Clayberger, M. Hemler, and F. Sanchez-Madrid for generously providing mAbs, P. Karnani for IL-2, and Dr. E. Eerola for help with statistical analyses. This work was supported by the Finnish Academy, the Finnish Cancer Foundation, the Sigrid Juselius Foundation, the Rheumatism Research Foundation, and the Finnish Life and Pension Insurance Companies.

1. Gowans, J. L. & Knight, E. J. (1964) *Proc. R. Soc. London Ser. B* **159**, 257–282.
2. Butcher, E. C. & Weissman, I. L. (1989) in *Fundamental Immunology*, ed. Paul, W. E. (Raven, New York) pp. 117–137.
3. Picker, L. J. & Butcher, E. C. (1992) *Annu. Rev. Immunol.* **10**, 561–591.
4. Stoolman, L. M. (1989) *Cell* **56**, 907–910.
5. Shimizu, Y., Newman, W., Tanaka, Y. & Shaw, S. (1992) *Immunol. Today* **13**, 106–112.
6. Yednock, T. A. & Rosen, S. D. (1989) *Adv. Immunol.* **44**, 313–378.
7. Springer, T. A. (1990) *Nature (London)* **346**, 425–434.
8. Butcher, E. C. (1991) *Cell* **67**, 1033–1036.
9. Larson, R. S. & Springer, T. A. (1990) *Immunol. Rev.* **114**, 181–217.
10. Rosenberg, S. A., Packard, B. S., Aebersold, P. M., Solomon, D., Topalian, S. L., Toy, S. T., Simon, P., Lotze, M. T., Yang, J. C., Seipp, C. A., Simpson, R. N., Carter, C., Bock, S., Schwartzentruber, D., Wei, J. P. & White, D. E. (1988) *N. Engl. J. Med.* **319**, 1676–1680.
11. Jalkanen, S., Nash, G. S., de los Toyos, J., MacDermott, R. P. & Butcher, E. C. (1989) *Eur. J. Immunol.* **19**, 63–68.
12. Jalkanen, S. T., Bargatze, R. F., de los Toyos, J. & Butcher, E. C. (1987) *J. Cell Biol.* **105**, 983–990.
13. Jalkanen, S. T. & Butcher, E. C. (1985) *Blood* **66**, 577–582.
14. Wu, N., Jalkanen, S., Streeter, P. & Butcher, E. C. (1988) *J. Cell Biol.* **107**, 1845–1852.
15. Holzmann, B. & Weissman, I. L. (1989) *Immunol. Rev.* **108**, 45–61.
16. Criscelli, C., Vasalli, P. & McCluskey, R. T. (1969) *J. Exp. Med.* **130**, 1427–1442.
17. Parrot, D. M. V. & Ferguson, A. (1974) *Immunology* **26**, 571–588.
18. Rose, M. L., Parrot, D. M. V. & Bruce, R. G. (1976) *Cell. Immunol.* **27**, 36–46.
19. Issekutz, T. B., Chin, W. & Hay, J. B. (1982) *Immunology* **46**, 59–66.
20. Griffith, K. D., Read, E. J., Carrasquillo, J. A., Carter, C. S., Yang, J. C., Fisher, B., Aebersold, P., Packard, B. S., Yu, M. Y. & Rosenberg, S. A. (1989) *J. Natl. Cancer Inst.* **81**, 1709–1716.
21. Steen, P. D., McGregor, J. R., Lehman, C. M. & Samlowski, W. E. (1989) *J. Immunol.* **143**, 4324–4330.
22. Bookman, M. A., Groves, E. S. & Matis, L. A. (1986) *J. Immunol.* **137**, 2107–2114.
23. Jalkanen, S., Steere, A. C., Fox, R. I. & Butcher, E. C. (1986) *Science* **233**, 556–558.