HIGH ENDOTHELIAL VENULE BINDING AS A PREDICTOR OF THE DISSEMINATION OF PASSAGED MURINE LYMPHOMAS

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Lymphoid neoplasms display a wide spectrum of growth patterns in vivo, from presentation as a solitary extralymphoid tumor, to disseminated involvement of all lymphoid organs. Since normal lymphocytes are uniquely mobile cells throughout much of their life cycle, it is reasonable to propose that the dissemination of malignant lymphocytes may reflect their expression of normal lymphocyte homing mechanisms. The migration and traffic of normal lymphocytes to lymph nodes is controlled in large part by the regulated expression of surface receptors for high endothelial venules (HEV), specialized venules that mediate the extravasation of circulating lymphocytes from the blood into lymphoid organs and sites of chronic inflammation (reviewed in reference 1). By using a quantitative in vitro assay of lymphocyte binding to HEV in frozen sections of lymphoid tissues (2–3), it has been shown that at least two functionally distinct and independently regulated lymphocyte homing receptor/HEV ligand recognition systems exist in the mouse, one controlling lymphocyte interactions with HEV in the mucosal lymphoid organs, Peyer's patches, and the other directing lymphocyte traffic to peripheral lymph nodes (4, reviewed in reference 1).

Both functional in vitro assays and immunologic studies with mAbs to putative homing receptors have confirmed that lymphomas can also express receptors for HEV (1, 4). The present studies were undertaken to examine whether the expression of functional homing receptors for HEV might play an important role in the in vivo behavior of neoplastic lymphocytes. We have compared the patterns of growth of several HEV-binding and nonbinding murine lymphomas after passage into syngeneic recipients. The results indicate that the ability to bind to HEV either regulates the hematogenous dissemination of malignant lymphocytes to HEV-bearing organs, or is coregulated with factors determining this metastatic behavior.

Materials and Methods

Lymphomas. The lymphomas used in these studies are as follows: L1-2, an Abelson virus–induced C57L lymphoma; RAW112, an Abelson virus–induced BALB/c lymphoma. This work was supported by National Institutes of Health grant AI-19957, and an award from the Veterans Administration to E. C. Butcher, NIH fellowship CA-07879 to N. W. Wu, and NIH grant AI-09072 to I. L. Weissman. E. C. Butcher is an Established Investigator of the American Heart Association.
abnormal lymphoid architecture. Only two of the HEV-binding lymphomas, BK37 and ABE 8.1/2 (American Type Culture Collection, Rockville, MD), an Abelson-induced BALB/c lymphoma; EL-4, a chemical carcinogen-induced C57BL lymphoma; BW5147 (American Type Culture Collection), an AKR T cell lymphoma; TK1, TK2, TK5, TK52, TK23, TK40, spontaneous AKR/Cum T cell lymphomas; TKJ43, an AKR/J lymphoma; and BK37, an AKR/Cum B cell lymphoma.

In Vitro Assay of Lymphocyte Binding to HEV. The ability of lymphoma cells and normal BALB/c mesenteric node lymphocytes (whose binding defines a relative adherence ratio [RAR] of unity) to adhere to HEV was determined in vitro as described previously (2, 4). Lymphoid tissues for sections and control lymphocytes were from 6–8-wk-old BALB/c mice.

Passage of Lymphomas. Lymphomas were passaged by injection of $10^6$ to $10^7$ cells into the right thigh muscle of syngeneic mice. To improve the frequency of successful passage, most recipients were given 400 rad whole body irradiation 18–24 h before transfer of cells. Animals were killed 7–28 d later, and the indicated organs were removed and weighed. Blood, anticoagulated with heparin, was taken for white cell counts. White cell counts were performed by hemocytometer. Large blasts, infrequent in control blood, constituted a minimum of 50% and in most cases >80% of the circulating leukocytes in mice bearing lymphomas.

Histologic Examination of Tissues. Peyer's patches, spleens, femurs, and selected lymph nodes from representative recipients of all lymphomas except TK2 and TK52 were examined histologically to assess involvement by lymphoma. Tissues were fixed in Telye-zhinskii's fixative and processed routinely for sectioning and staining with hematoxylin and eosin.

Results and Discussion

Lymphomas were selected for the study because they displayed one or the other extreme in homing receptor expression (Table I): either failing to bind detectably to HEV (relative adherence generally <5% of that of control lymph node cells), or binding extremely well to lymph node HEV, to mucosal HEV in Peyer's patches, or to both HEV classes (adherence nearly equivalent to or exceeding that of control mesenteric lymph node lymphocytes on one or both HEV types).

In Exp. 1, when mice receiving the highest cell dose of syngeneic lymphoma cells developed obvious lymphoma (large palpable lymph nodes and/or significant local mass in the thigh), all animals with detectable local masses or lymph node enlargement were killed and tumor involvement at the site of injection and in various lymphoid organs was assessed grossly by weight. Animals without detectable disease at that time were allowed to progress further before they were killed. In Exp. 2, animals receiving various tumor cell doses were killed only when gross involvement of lymph nodes or massive involvement at the local site developed.

The results, presented in Table I and in Figs. 1 and 2, demonstrate a clear correlation between the expression of functional HEV-binding ability and the in vivo metastatic patterns of the transferred lymphomas. HEV-binding lymphomas uniformly metastasized by the blood to give gross, symmetric enlargement of all lymph nodes. Peyer's patches were also observed to be enlarged by visual inspection. Histologically, lymph nodes were replaced by lymphoma cells, and involvement of Peyer's patches by each of the HEV-binding lymphomas except BK37 was extensive, with total effacement of the lymphoid architecture. BK37, a B lineage lymphoma that binds preferentially to peripheral lymph node HEV, involved Peyer's patches focally leaving much of the architecture of normal lymphocyte domains intact.
| Table I: In Vitro HEV-binding and In Vivo Growth Characteristics of Passed Morine Lymphomas |
|---------------------------------------------------------------|
| **Cells and type** | **Binding to HEV** | **Exp.** | **N** | **Day assayed** | **White count** | **Local mass** | **Lymph node weights** | **Spleen** |
| | (RAR ± SE)* | | | | | | | | |
| | Peripheral LN | Peyer’s patches | | | | | | | |
| **Nonbinding lymphomas** | | | | | | | | |
| L1-2 Pre-B | 0 | 0 | 1 | 3 | 15-18 | — | 5-5 | — | 11-20 | — | 15-17 | 210-220 |
| EL-4 T cell | 0 | 0 | 1 | 2 | 20 | — | 2-5 | — | 16-17 | — | 29 | 540 |
| TK2 T cell | 0 | 0 | 1 | 2 | 15-26 | — | 2-3 | — | 5-15 | — | 10-30 | 100-280 |
| TK52 T cell | 0 | 0 | 1 | 1 | 10 | — | 3 | — | 10 | — | 30 | 440 |
| TK5 T cell | 0 | 0 | 1 | 2 | 15-18 | — | 0.1-2 | — | 5-7 | — | 9 | 280-290 |
| ABE 8.1/2 Pre-B | 0.1 ± 0.03 | 2 | 2 | 2 | 16-19 | 5-15 | 3-13 | 1-3 | 7-15 | 4-11 | 15-18 | 442-586 |
| BW5147 T cell | 0 | 0 | 2 | 5 | 15-20 | 5-19 | 6-15 | 3-5 | 7-24 | 7-14 | 50-31 | 90-187 |
| RAW112 Pre-B | 0 | 0 | 2 | 5 | 15-20 | 14-25 | 5-14 | 2-6 | 12-17 | 2-4 | 7-31 | 190-195 |
| **HEV-binding lymphomas** | | | | | | | | | |
| BK37 B cell | 1.7 ± 0.20 | 0 | 1 | 1 | 22 | — | 1 | — | 170 | — | 100 | 400 |
| TK1 T cell | 0.15 ± 0.03 | 2 | 5 | 17-24 | 54-97 | 4-1 | 29-42 | 51-83 | 55-179 | 157-185 | 213-416 |
| TK23 T cell | 0.55 ± 0.07 | 2 | 5 | 18-20 | 17-46 | 6 | 27-36 | 59-75 | 67-100 | 545-397 | 321-471 |
| TK40 T cell | 0.15 ± 0.01 | 1 | 1 | 7 | — | 2 | 45-122 | — | 80-130 | 280-1100 |
| TK43 T cell | 0.34 ± 0.10 | 2 | 3 | 12 | 11-44 | 14-50 | 28-68 | 36-141 | 315-500 | 429-486 |
| **Controls** | | | | | | | | | |
| C57BL/6 LN cells | Unity** | Unity | 1 | 3 | 14 | — | 4-6 | — | 26-30 | — | 69-107 |
| AKR/J LN cells | Unity | Unity | 1 | 2 | 14 | — | 4-7 | — | 4-14 | — | 40-50 |
| BALB/c LN cells | Unity | Unity | 1 | 2 | 14 | — | 4-7 | — | 4-14 | — | 40-50 |

Tumor cell numbers ranging from $10^5$ to $10^7$ were injected intramuscularly in the lower right thigh of recipients. Except as indicated recipients were irradiated (400 rad, whole body) 18-24 h before injection. Animals were killed 7-20 d later, the indicated organs were removed and weighed, and blood was taken for white cell counts.

* L1-2 and EL-4 were assayed from cell culture. Other lymphomas were harvested for HEV assay directly from the local or distant lymph nodes of adoptive recipients. Data for tumors taken from in vivo passage may include minor errors introduced by the presence of normal host lymphocytes in the tumor cell population.

** A+B, axillary plus brachial lymph nodes.

† Relative adherence ratio (RAR) < 0.05.

‡ Range of values.

§ No detectable local tumor present.

★ Two recipients were unirradiated.

The RAR of control mesenteric lymph node (LN) cells syngeneic to each lymphoma is unity (1) by definition.

# Control animal was unirradiated.
Figure 1. Examples of the in vivo growth patterns of passaged HEV-binding and nonbinding AKR T cell lymphomas. 10^6 tumor cells were injected subcutaneously in the right thigh of 400 rad whole body--irradiated syngeneic recipients that were killed when gross lymphoma involvement occurred (2–4 wk after tumor transfer). (Left) TK-1, an HEV-binding lymphoma, metastasized hematogenously. It involved all lymph node groups symmetrically (axillary, brachial, and inguinal nodes are circled), as well as the spleen (S) and Peyer's patches (squares). The Peyer's patches were several times normal size, as great an enlargement of these organs as is ever seen; they never demonstrate the massive enlargement seen in lymph nodes, even when histologically completely replaced by tumor. The thymus was enlarged, perhaps by invasion of tumor from the parathymic nodes. The mesenteric node (m) was massive. There was no evidence of local growth at the site of injection. (Right) TK5, an HEV nonbinding lymphoma, grew predominantly as a large tumor at the injection site (T), with lymphatic spread to the local inguinal and right axillary nodes (large circles). The thymus, mesenteric node (m), and Peyer's patches (not readily visible in this irradiated recipient), and the distant lymph nodes on the noninjected side (small circles) appear grossly uninvolved.
FIGURE 2. Mean values from Exp. 2, summarizing the marked differences in the behavior of the nonbinding and HEV-binding lymphomas. The peripheral lymph node data (a) represent the added weights of the salivary lymph nodes and the brachial lymph nodes contralateral to the injection site. (b) Combined ileocecal mesenteric lymph nodes. (c) Weight of tumor at the injection site in the right thigh, estimated based on physical measurement of the tumor. (d) Total white cell count.
and in two of six recipients TK40, produced significant local masses at the injection site.

Nonbinding lymphomas, on the other hand, produced little or no apparent change in distant lymph node groups or in Peyer's patches. The absence of involvement of lymph nodes and Peyer's patches was confirmed in histological examination of tissues from randomly selected recipients of each lymphoma (except TK2 and TK52, which were not studied histologically). A microscopic focus of lymphoma was seen in the mesenteric lymph node from a single recipient of RAW 112. The predominant manifestation of these nonbinding lymphomas was massive local growth, with enlargement of local lymph nodes draining the site of tumor cell injection.

The qualitative patterns of growth observed were characteristic of the particular lymphoma transferred, and did not vary substantially with cell dose or with time (up to 4 wk, the longest period studied). A slight increase in the frequency of contralateral lymph node (combined axillary and brachial) enlargement in animals receiving nonbinding lines was apparent in Exp. 1, particularly with the smallest doses and longest times after injection. In Exp. 2, in which axillary and brachial nodes were assessed separately, it was clear that this effect was limited to the axillary lymph node. It probably represented lymphatic spread of lymphoma cells across the midline, since in no case were the contralateral salivary or brachial lymph nodes involved, and axillary node enlargement was only observed in animals exhibiting extensive and grossly apparent lymphatic dilation with associated edema. In contrast to the general symmetry exhibited by the HEV-binding lymphomas, lymph node involvement by the nonbinding lymphomas was always asymmetrical.

All lymphomas had access to the bloodstream. Both HEV-binding and non-binding lines exhibited significantly elevated levels of white blood cells, the majority of which were blasts (>80% in most cases). More importantly, histologic involvement of the bone marrow was universal, and all lymphomas caused splenic enlargement, at least in some recipients. Thus the expression of functional homing receptors for HEV may correlate with the ability of cells that have successfully entered the blood to extravasate into HEV-bearing organs, rather than primarily with the capacity of lymphomas to enter the bloodstream.

Although some of the lymphomas exhibited a significant preference for binding to peripheral lymph node or to mucosal HEV in vitro, all HEV-binding lymphomas in vivo involved the peripheral lymph nodes, the Peyer's patches, and the mesenteric lymph nodes (which express both peripheral and mucosal binding specificities). This may reflect the fact that binding is never completely specific for one or the other HEV class, or may argue that homing receptor expression is merely one important component of a constellation of co-regulated phenotypic features that determines the localization and growth of lymphomas. It should be pointed out, however, that lymphomas TK1 and TK40, which bind only poorly to peripheral lymph node HEV in vitro, produced a preferential enlargement of the mesenteric lymph nodes compared with the peripheral lymph node-preferential lymphoma BK37 (i.e., the ratio of weights of the mesenteric node, which contains HEV capable of binding mucosa-specific cells, to peripheral nodes was greater in the case of the mucosal HEV-specific lines). Furthermore,
all of the lymphomas that bound well to mucosal HEV caused total replacement
of the Peyer's patch architecture histologically, whereas BK37 displayed only
focal involvement. These observations suggest that receptor specificity may play
a quantitative role in determining organ involvement by adoptively transferred
lymphomas. More detailed studies of the kinetics of homing and growth of
passaged lymphomas, and correlation of the initial presentation of lymphomas
with their HEV-binding phenotype, must be undertaken to understand fully the
role of homing receptors in the natural progression and spread of lymphoid
malignancies.

Summary

It has long been postulated that normal lymphocyte homing mechanisms help
determine the metastatic spread of lymphoid neoplasms. The traffic of normal
lymphocytes is controlled in part by the regulated expression of surface receptors
for high endothelial venules (HEV), specialized venules that mediate the extra-
vasation of circulating lymphocytes from the blood into lymphoid organs and
sites of chronic inflammation. Here we have compared the in vivo growth
patterns of HEV-binding vs. nonbinding murine lymphomas passaged intramus-
cularly into syngeneic recipients. We report that lymphomas that bind well to
HEV (as assessed in a quantitative in vitro assay) disseminate widely via the blood,
involving all lymph node groups symmetrically. Although both HEV-binding
and nonbinding lymphomas gain access to the blood, gross involvement of lymph
nodes by nonbinding lymphomas is limited to nodes draining local tumor at the
site of injection, a prominent feature of these lymphomas; distant lymph nodes
are not enlarged. The results suggest that the expression of functional receptors
for HEV either controls the hematogenous dissemination of malignant lympho-
cyte populations to HEV-bearing organs, or is coregulated with factors deter-
mining this metastatic behavior. The findings support the concept that normal
lymphocyte homing mechanisms are important to the spread of leukemias and
lymphomas.

Received for publication 29 April 1987 and in revised form 10 July 1987.

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