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Expression of Lymphocyte Homing Receptor as a Mechanism of Dissemination in Non-Hodgkin's Lymphoma

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To investigate whether the lymphocyte homing receptor (LHR), an adhesion molecule believed to play an important role in the control of normal lymphocyte circulation, influences the spread of non-Hodgkin's lymphoma (NHL), expression of LHR was examined in 107 NHL of various histologic and immunophenotypic subclasses. This analysis revealed that whereas NHL with a putative derivation from recirculating mature T and B lymphocytes almost invariably express high levels of LHR, those akin to sessile mature or

AN IMPORTANT feature of mature lymphocytes is their ability to recirculate continuously between the lymphoid organs. This dynamic process is an essential component of the functional immune system, providing a means for constant surveillance of the organism's tissues by immunocompetent cells.¹⁻³ A central step in the physiology of lymphocyte recirculation is the migration of lymphocytes from the blood to the surrounding tissues through the wall of high endothelial venules (HEV). These specialized venules have a cuboid endothelial morphology and are present in the T-cell areas of lymph nodes and mucosa-associated lymphoid tissues (MALT).¹⁻³ Moreover, they can arise in a variety of chronic inflammatory conditions, including autoimmune thyroiditis, rheumatoid arthritis, and chronic inflammatory bowel disease.⁴⁻⁶

It is now clear that migration of lymphocytes through HEV involves specific recognition of and adhesion to their endothelial lining by lymphocyte homing receptors (LHR) expressed on the lymphocyte cell surface.^{3,5,7,8} In the mouse^{3,9} and in man^{3,10} antibodies against these LHR define a related set of 90-Kd glycoproteins. The expression of LHR and, in parallel, the capacity of various lymphocyte populations to adhere to and migrate through HEV, depends on their state of maturation and activation.^{3,9,10,11} Thus, mature resting T and B lymphocytes, which belong to the recirculating lymphocyte pool, express high levels of LHR. In contrast, cortical thymocytes and germinal-center B cells are sessile cells and generally are either LHR negative or low.^{3,9,10}

In view of the concept that NHL present the malignant counterparts of normal lymphocytes "frozen" at a certain stage of maturation/activation, it is conceivable that the same mechanisms regulating normal lymphocyte traffic are, at least to a certain extent, operating in lymphoid malignancies. If so, the "homing phenotype" of NHLs would be expected to influence the spread of these neoplasms. This possibility is supported by the finding that homing receptor expression in transplantable murine lymphomas correlates with their capacity to give rise to generalized lymph node dissemination on passage to syngeneic recipients.¹² In the present paper the authors have investigated the expression of LHR by human NHL of various types and have correlated these findings to the clinical stage.

MATERIALS AND METHODS

Case selection and classification. A panel of NHL of various histologic and immunophenotypic subclasses was selected from the material collected at the Department of Pathology, Free University

immature lymphocytes tend to express lower levels of LHR. Furthermore, in a survey among diffuse large-cell lymphomas of the B-lineage, the tumors of 11 of 13 patients with stage III/IV disease expressed moderate to high levels of LHR, whereas only two of 17 patients with stage I/II disease had tumors that did so. These findings suggest that LHR is involved in the dissemination of NHL.

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Hospital, Amsterdam from 1985 to 1988 and tested for LHR-antigen expression employing the monoclonal antibodies (MoAbs) NKI-P1 and NKI-P2. Histologic subclassification of NHL was based on the International Working Formulation.¹³ By their staining patterns with pan-T and pan-B MoAbs the NHL were divided into B-lineage and T-lineage. A tumor was considered B-lineage if expression of one or more pan-B antigens was detected in the absence of T antigens and vice versa. Because of homogeneity for the features the authors examined, cases of small lymphocytic lymphoma with or without plasmacytoid differentiation were considered together in the category of small lymphocytic lymphoma. For similar reasons cases of follicular and follicular and diffuse small-cleaved, mixed, and large-cell lymphoma were considered together in the category of follicular lymphoma; diffuse large-cleaved cell, large noncleaved cell, and large cell with immunoblastic features (of the B-lineage) were considered together in the category of diffuse large-cell lymphoma.

MoAbs. The development and specificity of the hybridomas NKI-P1 and NKI-P2 will be described elsewhere (S.T. Pals, manuscript in preparation). In brief, the MoAbs secreted by these hybridomas immunoprecipitate a major product with a mol wt of 90 Kd from 125I-labeled human monocytes. This product was shown to be identical to the human LHR described by Jalkanen et al.¹⁰ Functional studies using the MoAbs NKI-P1 and NKI-P2 showed both MoAbs to inhibit the binding of human peripheral blood lymphocytes to HEV in vitro, indicating that they are directed against a functional epitope on the LHR. Immunoperoxidase staining was performed on acetone-fixed cryostat section by an indirect immunoperoxidase procedure, as described previously.⁶ NKI-P1 and NKI-P2 were employed as supernatant at concentrations to give optimal staining results. The second stage antibody, horseradish peroxidase-conjugated rabbit antimouse IgG (DAKO, Denmark) contained 5% normal human serum. Sections were routinely counterstained with haematoxylin. Since cross-blocking studies indicated

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that NKI-P1 and NKI-P2 recognize overlapping epitopes and since the staining results obtained with these MoAbs were virtually identical, they are considered together. Staining intensity was scored semiquantitatively on a scale of 0 to 3 (0, negative; 1, weak; 2, moderate; 3, strong).

Clinical staging procedures. Expression of LHR antigen, as defined by staining with NKI-P MoAbs, was correlated to clinical stage in 30 patients with diffuse large-cell lymphoma of the B-lineage who had undergone a complete staging procedure within 6 weeks following the initial biopsy. The staging procedure routinely included thorough physical examination, blood profile and buffycoat examination, rhinolaryngoscopy, chest and abdominal X-rays, a variable combination of abdominal/pelvic computerized tomography (CT), lymphangiography and echography, and bone marrow smears and biopsies. Results were classified according to the Ann Arbor staging system.¹⁴ For statistical evaluation of staging results the chi-square test was used.

RESULTS

LHR expression in non-Hodgkin's lymphoma. In accordance with other reports describing the distribution of LHR in normal and reactive lymphoid tissues,^{3,9,10} the authors observed a high expression of the antigens recognized by NKI-P1/2 on most cells of both the B-lineage and T-lineage as well as on macrophages/dendritic cells. B lymphocytes in primary follicles, mantle zones, and interfollicular areas and T lymphocytes in the interfollicular and paracortical areas of the lymphoid tissues were strongly NKI-P1/2 positive (2 to 3+). Plasma cells were also positive. In contrast, germinal center B cells were weak to negative (0 to 1+). Similarly the vast majority of cortical thymocytes lacked LHR antigen expression (0).

The various subtypes of NHL were heterogeneous with respect to LHR antigen expression (Table 1). Diffuse small-cell lymphomas/leukemias of the B-lineage were all strongly NKI-P1/2 positive (2 to 3+). The intensity of staining in these lymphomas was similar to that observed in normal primary follicles and mantle zones. The plasma cell tumors also showed uniform LHR antigen expression. Similarly, all

peripheral T-cell lymphomas, regardless of their histologic type, stained intensively with the NKI-P antibodies and in this respect were comparable to normal mature T cells (2 to 3+).

In contrast to the strong LHR antigen expression in the above-mentioned NHL, those subtypes of NHL with characteristics of germinal center B cells, including follicular lymphoma and diffuse large-cell lymphoma, showed a highly variable LHR antigen expression. In follicular lymphoma LHR antigen-expressing tumor cells were either not detectable (50% of cases) or presented a subpopulation of tumor cells with a relatively high-staining intensity that tended to be localized at the periphery of the malignant follicles. By their staining pattern these lymphomas thus resembled reactive germinal centers. Similarly, approximately half of the diffuse large-cell lymphomas of the B-lineage either failed to express detectable LHR antigen or showed only weak expression. Interestingly, the other half of the lymphomas in this category showed a homogeneous expression of moderate-to-high levels of LHR antigen (2 to 3+). These differences in expression were not related to the histologic subtype of diffuse large-cell lymphoma (data not shown).

Among the limited number of cases of Burkitt's and lymphoblastic lymphoma tested LHR antigen expression was generally low or absent, a finding that is consistent with their immature character.

LHR expression and clinical stage. To test the hypothesis that LHR expression plays a role in the dissemination of NHL, the authors compared the expression of LHR antigen with clinical stage in all patients with diffuse large-cell lymphoma for whom complete staging reports were available. The authors selected this subtype of lymphoma because it represented the largest subgroup in their series and, more importantly, because it was heterogeneous with respect to both LHR expression and clinical stage at presentation (Table 1).

As is shown in Fig 1, LHR expression by diffuse large-cell lymphomas of the B-lineage was clearly correlated to clinical stage: whereas 85% of stage III and IV lymphomas expressed LHR at moderate to high levels (2 to 3+), this relatively high-staining intensity was present in only 12% of the NHL in clinical stage I and II, the majority of lymphomas in the latter groups being 0 to 1+ (Fig 1). This difference between LHR expression in stage I and II v III and IV was highly significant (chi-square = 15.9, $P < .001$).

DISCUSSION

The cellular and molecular interactions involved in metastasis are undoubtedly complex, but the adhesive properties of tumor cells are likely to play a key role in this process.^{15,16} Although, because of their motility, lymphocytes may sometimes be thought of as nonadhesive cells, this is incorrect: Cell-cell adhesion is involved in almost every step during an immune response,¹⁷ and the fact that lymphocytes may recirculate through blood and lymph appears to result from their ability to regulate their adhesive properties, including their LHR receptor expression, continuously.^{3,9,10,11,17,18}

The marked heterogeneity in LHR expression among NHL of various subclasses in the authors' present study

Table 1. Lymphocyte Homing Receptor Expression in Non-Hodgkin's Lymphoma

Subtype	No. NKI-P+ No. tested (%)	Staining Intensity Range*
B-cell lymphoma		
Diffuse small-cell	24/24 (100)	2-3+
small lymphocytic	20/20 (100)	2-3+
small cleaved	4/4 (100)	2-3+
Diffuse large-cell	30/36 (80)	0-3+
Follicular	4/8 (50)†	0-3+
Burkitt	0/3 (0)	0
Myeloma/plasmocytoma	3/3 (100)	2-3+
T-cell lymphoma		
Peripheral	29/29 (100)	2-3+
Mycosis Fungoides (MF)	12/12 (100)	2-3+
Non-MF	17/17 (100)	2-3+
Lymphoblastic	2/4 (50)	0-2+

*0, negative; 1+, weak; 2+, moderate; 3+, strong.

†Important intratumor NKI-P expression heterogeneity with a variable population of strongly positive tumor cells at the periphery of the malignant follicles.

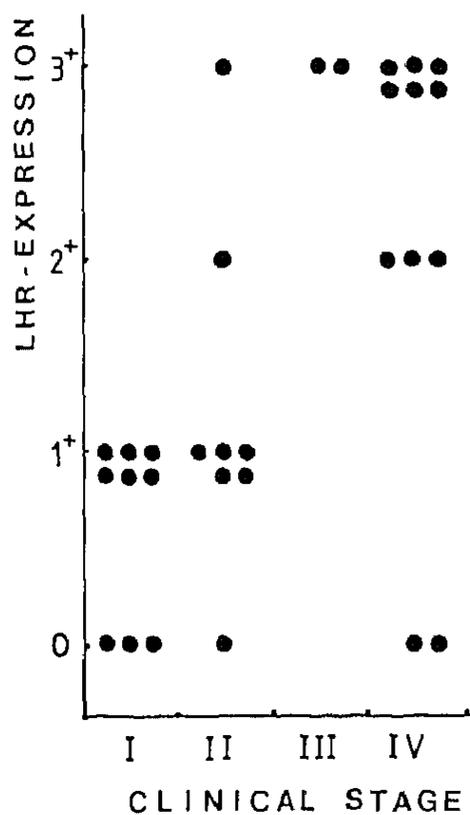


Fig 1. Expression of LHR antigen in diffuse large-cell lymphoma of the B-lineage in relation to clinical stage (Ann Arbor). 85% of stage III and IV lymphomas are LHR 2 to 3+ v only 12% of NHL in stage I and II ($P < .001$).

probably reflects these regulatory processes, since it parallels the maturation and activation-dependent expression patterns on (putatively) related compartments of the normal lymphoid system. Thus, whereas NHL akin to peripheral T cells or small, resting B cells, like these benign counterparts, were found to express high levels of LHR, NHL related to germinal center cells or immature lymphoid precursors tended to show a much lower LHR expression (Table 1). Importantly, however, this parallel between LHR expression in reactive lymphoid tissues and their malignant counterparts was far from complete. Particularly in the group of diffuse large-cell lymphomas of the B-lineage, consisting largely of neoplasms related to large germinal center cells, the LHR expression was widely variable, ranging from 0 to 3+ (Table 1).

The central finding of this study is the significant correlation ($P < .001$) between LHR expression and clinical stage in diffuse large-cell lymphoma of the B-lineage; thus high expression of LHR was correlated to stage III/IV disease, while lymphomas exhibiting limited spread were generally LHR low or negative. In combination with current data concerning the role of LHR in the biology of lymphocyte traffic, the authors' findings strongly suggest that LHR plays a role in the dissemination of these NHL.

It should be noted, however, that the correlation between LHR expression and disease stage is incomplete, given that the authors observed wide lymphoma dissemination in some cases without LHR expression. This indicates that LHR

expression is not an absolute requirement for tumor spread. Several factors might explain this observation. First, molecules other than LHR are likely to be involved in lymphoma dissemination. Pertinent to this point are observations by the authors and others indicating that in addition to LHR, LFA-1 is also involved in lymphocyte migration through HEV.^{19,20} Second, factors other than adhesiveness are likely to play a role in lymphoma dissemination. These may include changes in cytoskeletal structure influencing migratory capacity, differences in growth character, etc.

The authors' conclusion that LHR is likely to play a role in the dissemination of NHL is corroborated by the characteristic dissemination and LHR expression patterns of most of the other NHL subcategories. For example, diffuse small-cell lymphoma, a NHL category with strong LHR expression, almost invariably presents at clinical stage IV.¹³ Similarly, peripheral T-cell lymphomas, which all express high levels of LHR, tend to disseminate widely.²¹ Follicular lymphomas present an obvious exception to this rule, since they show a low LHR antigen expression but are mostly widely disseminated at the time of diagnosis.¹³ This might be explained, however, by the finding of strongly LHR antigen-positive tumor cells at the periphery of the malignant follicles in a significant proportion of these lymphomas.²² It is conceivable that these cells comprise the malignant counterparts of early recirculating memory B cells developing from sessile germinal center cells and consequently have a large potency to disseminate.

The general distribution pattern of LHR antigen among NHL of various histologic and immunophenotypic subcategories in the authors' present study resembles the distribution pattern recently described by Picker et al.²² These authors, however, could not demonstrate a significant correlation between LHR expression and clinical stage, although a weak nonsignificant association was observed. Although the authors cannot at present explain this discrepancy, it is conceivable that differences in the fine specificity of the MoAbs used in both studies play a role, since the authors' results indicate that NKI-P1/2 recognizes a different domain on the LHR than Hermes-3 (used by Picker et al²²; S.T. Pals, manuscript in preparation¹⁰).

In conclusion, although the findings presented here cannot definitively prove that LHR is involved in the dissemination of NHL, it is tempting to speculate that high LHR expression in large-cell lymphomas, which correlates with wide dissemination, is an important factor in tumor spread.

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