

Lymphoepithelioid Lymphoma (Lennert's Lymphoma) Is a Monoclonal Proliferation of Helper/Inducer T Cells

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Lymphoepithelioid lymphoma (Lennert's lymphoma) was first described as a special variant of Hodgkin's disease. This lesion is characterized by a high percentage of epithelioid and T cells and rarely contains the classical Hodgkin's/Reed-Sternberg cells. Cytogenetic abnormalities indicate that Lennert's lymphoma is of T cell origin. In the present study, immunohistochemical investigation of four cases of Lennert's lymphoma revealed two major cell populations of T cells that predominantly express the helper-inducer phenotype and Ki-M6- and Ki-M8-positive macrophages and epithelioid cells. Double-staining experiments for the

detection of cell surface antigens and the proliferation-associated antigen Ki67 showed that only the CD4-positive cells (helper-inducer T cells) were proliferating. Examination of the DNA of these Lennert's lymphoma samples also indicated that monoclonal rearrangement of the T cell receptor β -chain genes has occurred, whereas the immunoglobulin heavy- and κ -chain genes remained in germline configuration. Our results strongly suggest that Lennert's lymphoma is a CD4-positive T cell lymphoma.
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ALTHOUGH THE MAJORITY of malignant lymphomas can be classified as being derived from either the T or B cell lineage, the lineage of certain lesions such as Hodgkin's disease, lymphoepithelioid lymphoma (Lennert's lymphoma), and lymphogranulomatosis X/angioblastic lymphadenopathy is uncertain. Lymphoepithelioid lymphoma (LEL) was first described by Lennert¹ and later by Lennert and Mestdagh² as a special variant of Hodgkin's disease characterized by a high percentage of epithelioid cells and rarely containing the Reed-Sternberg cells characteristic of classical Hodgkin's disease. This disease was called Lennert's lymphoma by Dorfman and Warnke³ and Lukes and Tindle,⁴ and as a result of findings that pointed to a T cell origin for the diseases, the term was changed from lymphoepithelioid cellular lymphogranulomatosis to lymphoepithelioid cell lymphoma. Although cytogenetic studies show chromosomal abnormalities that are in accordance with the designation of a T cell-derived malignant lymphoma and immunohistochemical investigations show a high percentage of T cells⁵⁻⁷ in this lymphoma, it is not known whether they represent the clonal population responsible for neoplastic proliferation. In this study, we report the results of immunohistochemical analysis of LEL, using double staining with conjugated monoclonal antibodies in an effort to identify the cell surface phenotype of the proliferating neoplastic cell. We have also characterized T cell receptor β -chain and immunoglobulin heavy-chain gene rearrangements in these cells to substantiate the immunohistochemical findings. Our results strongly support the hypothesis that LEL is a T cell lymphoma, probably of the T helper/inducer class.

MATERIALS AND METHODS

Five lymph nodes from four patients with LEL were examined by conventional light microscopy in hematoxylin and eosin (H&E) and Giemsa-stained paraffin sections and classified according to the criteria described by Lennert et al.^{8,9} A second biopsy specimen (no. 4b) from one patient was taken 2 years after the onset of the disease. One third of each biopsy sample was used for light microscopy, one third for immunohistochemistry, and one third for DNA hybridization.

Immunohistochemistry. Fresh tissue specimens were snap-frozen in liquid nitrogen and stored at -90°C . From these samples 7- μm frozen sections were prepared and fixed for ten minutes in acetone. Then, immunohistochemical staining was performed as described by Stein et al.¹⁰ Three nonlymphocytic monoclonal anti-

bodies, KiM1,¹¹ KiM6,¹² and KiM8¹³ were used. Using double-labeling techniques, we stained for both cell surface antigens and Ki67, a nuclear antigen expressed only in proliferating cells.^{14,15} Immunoperoxidase staining was performed first to visualize the nuclear antigen (Ki67), and after developing with diaminobenzidine, a second monoclonal antibody was applied to the sections and developed using alkaline phosphatase. Other monoclonal antibodies used in this study are given in Table 1.

Gene rearrangement. Single-cell suspensions were prepared from the snap-frozen lymph node biopsy specimens, and DNA was extracted as described by Minden et al.¹⁶ Southern blot analysis was performed using germline DNA from cultured skin fibroblasts of normal volunteers as a control and DNA from lymphoma cells after overnight digestion with ten units of restriction enzyme (*EcoRI*, *BamHI*, or *HindIII*) per microgram of DNA. Digested DNA was electrophoresed through 0.6% to 0.8% agarose and transferred to nitrocellulose filters as described by Southern.¹⁷ Filter-bound DNA fragments were then hybridized to nick-translated ³²P-labeled gene probes and visualized on autoradiographs.¹⁶ Two probes were used in this study: the constant region of the T cell antigen receptor β -chain characterized by Yoshikai et al.¹⁸ and the joining region of the immunoglobulin heavy-chain gene, a gift from P. Leder.¹⁹

RESULTS

Four biopsy specimens taken at the onset of the patients' disease, which present identical histologic pictures characteristic of LEL, are described in Fig 1. The biopsy sample from patient 4 (4b) showed a low number of epithelioid cells and a high percentage of large, basophilic cells, which were determined by light microscopy to be atypical T cells (Fig 2).

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Table 1. Monoclonal Antibodies Used in This Study With Their Specificity and Source of Reference

Monoclonal Antibody	Cluster	Specificity	Source/Reference
Leu-4	CD3	All T cells	BD
Lyt-3	CD2	Sheep erythrocyte receptor	New England Nuclear, Boston
Leu-1	CD5	T cells, some B cells	BD
Leu-3a	CD4	Helper/inducer T cells	BD
Leu-2a	CD8	Suppressor/cytotoxic T cells	BD
To15	CD22	All B cells	Stein et al ¹⁰
Ki67		Proliferating cells	Gerdes et al ¹⁵

Abbreviation: BD, Becton Dickinson.

Immunohistochemistry. Immunohistochemically investigated biopsy specimens 1 to 4a were phenotypically identical (Table 2). More than 90% of the T cells expressed the T cell antigens CD3, CD5, and CD2, and the majority of lymphoid cells (>80%) expressed CD4, whereas three of the four samples were composed of less than 10% CD8-positive cells (case 3 contained 20% CD8-positive lymphocytes). All samples contained a high percentage of Ki-M1+, Ki-M6+, and Ki-M8+ epithelioid cells. B cells (CD22+), which were diffusely distributed or found only in small clusters, made up about 5% of all lymphoid cells. The number of proliferating cells (Ki67+) ranged from 10% to 30% in the early biopsy specimens (1 to 4a), but in the last biopsy sample (4b) demonstrated an elevated rate of proliferation that was as high as 60%. Lymphoid cells that were still expressing CD3 and CD2 did not express the CD5 or CD4 antigen of helper/inducer T cells. Double-labeling experiments with Ki67, which labels proliferating cells, and monoclonal antibodies recognizing cell surface antigens clearly showed that proliferating cells expressed T cell antigens CD3, CD5, and CD2 (Fig 3A). As shown in Table 3, less than 3% of CD8-positive lymphocytes in each biopsy sample were positive for Ki67 (Fig 3B), whereas a high proportion of lymphoid cells (30% to 70%) expressed Ki67 and the CD4 antigen simultaneously. Epithelioid cells Ki-M1+, Ki-M6+,

or Ki-M8+ were Ki67-negative. The same B cells reacted with anti-IgM or the pan-B cell antibody.

Determination of somatic rearrangement. DNA from these biopsy specimens was examined for rearrangement of the T cell receptor β -chain (TcR β) and immunoglobulin heavy-chain (IgH) genes. DNA samples were digested with each of the restriction enzymes *EcoRI*, *HindIII*, and *BamHI* and analyzed by Southern gel analysis. The TcR β genes had undergone rearrangement in all five DNA samples. Following digestion with *EcoRI*, rearranged bands were detected in samples 2 (patient 1), 3 (patient 2), 4b (patient 4), and 5 (patient 3) at 8.2 kb, 9.4 kb, 10.3 kb, and 8.0 kb, respectively (Fig 4). Except for sample 4 (patient 4), the germline bands at 10.5 kb and 3.7 kb were detected either as a result of the presence of nonlymphoid cells in the lymph node biopsy specimen or of rearrangement of only one allele in the clonal cell population.

In one case, two biopsy specimens were obtained from one patient. Sample 1 (patient 4, biopsy a) contained the DNA from the first biopsy specimen; sample 4 (patient 4, biopsy b) represented DNA from the second lymphoma biopsy 2 years later. The rearrangement pattern in these two samples was different. No rearrangement could be seen in sample 1 following DNA digestion with *EcoRI*, whereas in sample 4, a deletion of the 10.5-kb germline band and a faint rearranged band at 10.3 kb were seen, indicating that, in addition to the

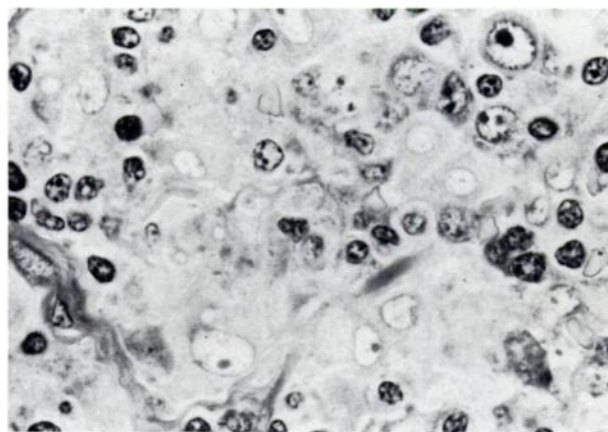


Fig 1. Lymph node biopsy specimen from a patient with Lennert's Lymphoma (patient 4, specimen a). Small lymphoid cells show slight nuclear irregularity. In the lower left is a large cell resembling a Hodgkin's cell. Some epithelioid cells have light nuclei (6- μ m paraffin section, Giemsa staining; original magnification \times 1024; current magnification \times 686).

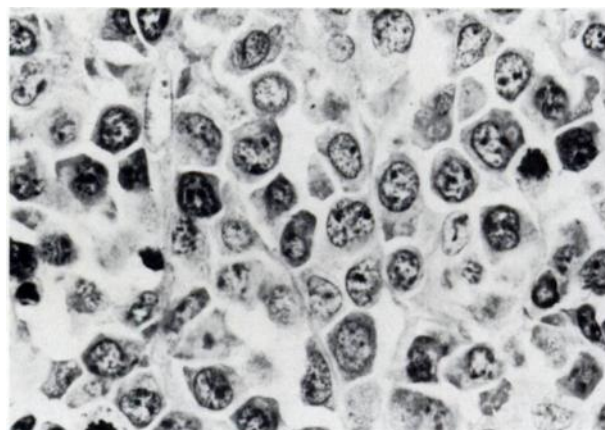


Fig 2. Second biopsy sample from patient 4 (4b). The lymphoma consists of large blastic cells with prominent, slightly basophilic cytoplasm. Epithelioid cells are missing (6- μ m paraffin section, Giemsa staining; original magnification \times 1024; current magnification \times 686).

Table 2. Immunophenotypic Characteristics of Infiltrating Cells in Lymph Nodes With Lennert's Lymphoma

Specimen	CD19 To15	CD3 Leu-4	CD2 Lyt-3	CD5 Leu-1	CD4 Leu-3a	CD8 Leu-2a	Ki67	CD4/CD8 Ratio
1	5	>90	>90	>90	85	5	10	>10:1
2	5	>90	>90	>90	80	3	20	>10:1
3	5	>90	>90	>90	80	20	30	4:1
4a	5	>90	>90	>90	80	5	15	>10:1
4b	5	>90	>90	10	5	2	60	—

Specimens 4a and 4b are from two sequential biopsies performed on the same patient in a 2-year interval. Counts are given in percentages.

original rearrangement on one of the alleles, a deletion of $C_{\beta 1}$ took place in the other (Fig 4).²⁰ This was supported by the finding of $C_{\beta 2}$ rearrangement in sample 4 as well as in sample 1 when *Hind*III was used as the restriction enzyme. Figure 5 shows rearranged bands for sample 1 (patient 4, biopsy a) at 6.9 kb (lane 1) and for sample 4 (patient 4, biopsy b) at 6.5 kb and 4.5 kb (lane 2). No immunoglobulin heavy-chain

Table 3. Percentage of Ki67-Positive Cells That Simultaneously Express a Cell Surface Antigen

	Ki67 + CD22	Ki67 + CD3	Ki67 + CD4	Ki67 + CD8	Ki67 + KiM6	Ki67 + KiM8
1	<3	50	40	<3	<3	<3
2	<3	50	30	<3	<3	<3
3	<3	60	50	<3	<3	<3
4	<3	80	70	<3	<3	<3
4b	<3	70	<3	<3	<3	<3

gene rearrangement could be observed in any of the samples (data not shown).

DISCUSSION

Immunohistochemistry has provided a number of tools for the assignment of cells to the B or T cell lineage. With most malignant non-Hodgkin's lymphomas, if one has a clonal expansion of a monomorphous lymph node infiltrate, these

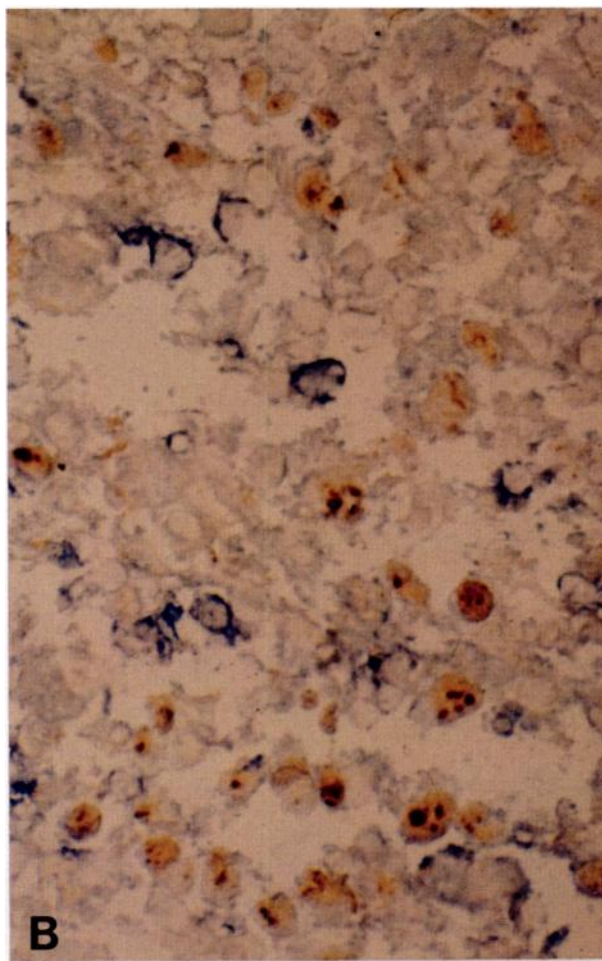
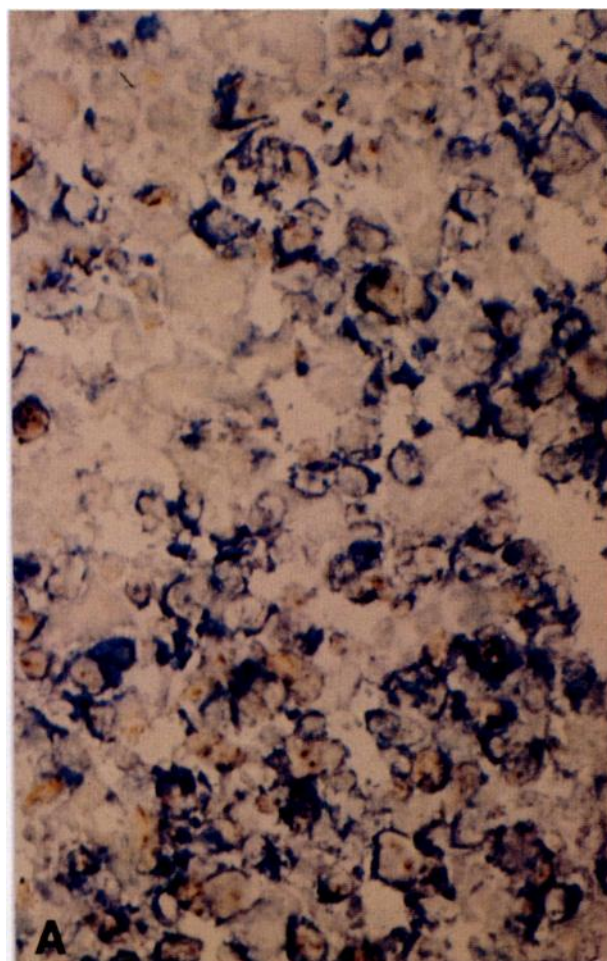


Fig 3. Immunohistochemical double labeling of a lymph node infiltrated by lymphoepithelioid lymphoma. (A) T cells are stained by a CD3 antibody (blue surface staining). Ki67 cells (proliferating cells) show a brown nuclear staining. The majority of Ki67+ cells are additionally positive for CD3 antibody. (B) T suppressor/cytotoxic cells are stained by CD5 antibody (blue surface staining). Ki67+ shows a brown nuclear staining. Not a single cell shows double labeling for both antigens (7- μ m cryostat sections, double-staining technique with immunoperoxidase reaction (nuclear antigen Ki67) and immunoalkaline phosphatase reaction for cell surface antigens, no nuclear counterstaining; original magnification \times 960).

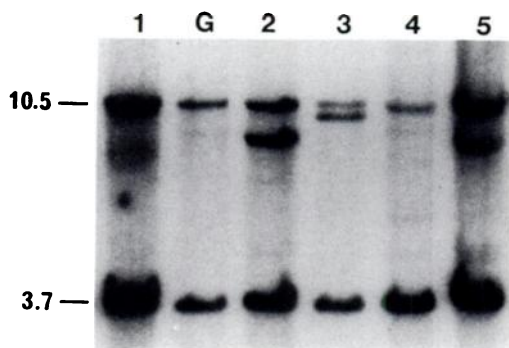


Fig 4. Southern analyses were performed on DNA from lymphoma cells from patients with lymphoepithelioid lymphoma. DNA was restricted with one of the enzymes *EcoRI*, *BamHI*, or *HindIII*. Filters were hybridized to nick-translated probes of the constant region of the $TcR\beta$ and the joining region of the IgH. DNA prepared from cultured skin fibroblasts of healthy volunteers was used as germline control (G). DNA from tissue samples was digested with *EcoRI* and hybridized with the $TcR\beta$ probe. Lane 1 (specimen 4a) and lane 4 (specimen 4b) contain samples derived from subsequent lymphoma biopsies from the same patient. Lane 2 is from patient 1; lane 3, patient 2; and lane 5, patient 3. Numbers at the left side denote sizes of the germline fragments in kilobases.

tools can be used to characterize and phenotype the neoplastic cell clone. In malignant lymphomas such as Hodgkin's disease, which are composed of a mixture of different cell types, it is difficult or even impossible to recognize selectively the neoplastic cell clone and to determine from which lineage it was derived. One such disease is the special variant of Hodgkin's disease lymphoma described by Lennert¹ and later by Lennert and Mestdagh² as epithelioid cellular lymphogranulomatosis, later distinguished from Hodgkin's disease and designated lymphoepithelioid cell lymphoma.⁹ Recent results indicate that in addition to the high percentage of epithelioid cells there are large numbers of T cells present in this disease^{6,7}; the true nature of this disease is still unclear, however, because it remains to be seen whether the T cells compose a clonal neoplastic cell population or whether they only are cells that interact with the neoplastic cells.²¹⁻²⁴

Histologic and immunohistochemical results show two characteristic cell types in LEL:T cells with the helper-inducer phenotype and epithelioid cells expressing histiocyte antigens. The progression of LEL in patient 4 is remarkable. The first biopsy specimen showed features of LEL, histologically and phenotypically, and the second biopsy specimen, taken 2 years after the onset of the disease, showed features of a high-grade malignant peripheral T cell lymphoma, indicating a shift from a low-grade malignant lymphoma (LEL) to a high-grade malignant lymphoma, a phenomenon known from B cell malignancies. Using a monoclonal antibody that recognized an antigen expressed in all proliferating cells from the late G1 phase to the S phase (Ki67),¹⁵ we tried to define the dominant cell type of the proliferating cells. Double-labeling experiments show that mainly the CD4-positive helper/inducer T cells were proliferating because they were strongly Ki67-positive and the CD8+ lymphocytes and epithelioid cells were Ki67-negative. If, as in other

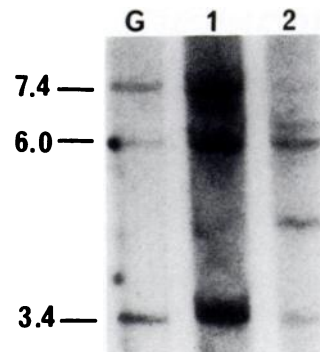


Fig 5. DNA samples from one patient with Lennert's lymphoma digested with *HindIII* and probed with $TcR\beta$. Lane 1 represents the first lymph node biopsy sample (4a) and lane 2 the second one 2 years later (4b).

malignancies, the proliferating cells represent the neoplastic cell clone, these results leave little doubt as to the T cell nature of Lennert's lymphoma and indicate that the neoplastic cells are phenotypically helper/inducer T cells.

The claim is further substantiated by the detection of clonal gene rearrangement of the β -chain of the T cell antigen receptor in each biopsy specimen, whereas the immunoglobulin heavy chain genes remained in germline configuration. The intensity of the rearranged bands observed with the $TcR\beta$ probe was similar to that in cases of peripheral T cell lymphomas. In contrast to this observation, a mixture of cells from Hodgkin's disease with a large number of epithelioid cells showed only faint rearranged bands resulting from a low percentage (about 5%) of monoclonal proliferating T cells,²⁵ thus favoring the view that LEL is a T cell lymphoma and not a Hodgkin's disease variant.

In one of the patients, a second lymphoma biopsy was performed 2 years after the first one. Hybridization of the DNA from this second biopsy specimen with the $TcR\beta$ probe demonstrated a rearrangement pattern that probably involves three alleles and is completely different from that of the first biopsy specimen. By analogy to B cell lymphomas, a diagnosis of a biclonal T cell lymphoma is in order²⁶ because upon comparison with the first and the second biopsy samples, the size differences in $TcR\beta$ -containing fragments indicate that during the development of the lymphoma other subclones have displaced the original clone. This observation is consistent with the proposal of a multistep pathogenesis as described in acute lymphoblastic leukemias by Fialkow.²⁷

In accordance with these findings are the results of cytogenetic studies that have shown numerous chromosomal abnormalities, especially those involving chromosome 3, which is characteristic of T cell lymphomas.^{28,29} The characteristically high levels of epithelioid cells in this lesion raise the possibility that the neoplastic CD4-positive clones are functional and produce cytokines to attract these cells.

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