Non-Hodgkin's Lymphoma. Immunohistochemical and Electron Microscopical Findings in Relation to Lightmicroscopy

A Study of 74 Cases

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From 74 patients with non-Hodgkin's lymphoma (NHL), fresh biopsy material from involved tissue was investigated histochemically and by light and electron microscopy. The results were compared with the light microscopical conclusions based on three currently utilized classifications, namely of Rappaport, Lukes, and Lennert. The separate groups of low-grade NHL appeared to consist of homogeneous cell populations, both in immunohistological as well as in enzymehistochemical and electron microscopical sense. On the contrary, high-grade NHL constituted a heterogeneous group in which supplementary (immunohistochemical, enzymehistochemical, and electron microscopical) investigation is very useful. All NHLs reacted with anti-human lymphocyte serum, including the so-called histiocytic NHLs according to the Rappaport classification, which proves their lymphocytic origin. Consequently the prefix "histiocytic" should be altered in a morphogenetically correct way. Without exception the B-cell NHLs were characterized by the presence of monoclonal immunoglobulin on the cell membrane and/or intracytoplasmically. In a part of the immunologically non-T-/non-B-/("null cell") NHLs, a B-cell origin was indicated by the presence of ATPase and/or 5′ nucleotidase and a T-cell origin by the presence of dot-like acid phosphatase.

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NON-HODGKIN'S LYMPHOMAS (NHLs) are much debated tumors.[9,10,20,29,40,45] This is mainly due to the various criteria (morphologic, immunologic, and others) on which the several classifications are based.[1,10,13,21,25,28,36] Immunologic and enzymecytochemical properties of neoplastic lymphoid cells appear to correlate with clinical behavior, especially in combination with morphologic characteristics.[3,4,14,16,21,24,35] This correlation was the main reason we decided to examine the lymphoma tissue as extensively as possible.

A study of immunologic markers of NHL cells in suspension was described recently by our lymphoma group.[5] We now want to report on the investigation of tissue sections with several techniques (electron microscopy, immunofluorescence, and enzymehistochemistry) that provide information about architecture and interrelationships[17] between cells.

Another advantage of tissue investigation is the possibility of examining material on which it is difficult to prepare a suspension, *e.g.*, lymphomas rich in collagen fibers.

NHL pathogenesis is largely unknown and it is therefore worthwhile to study as many parameters as possible, although many of them will later appear to be superfluous. These parameters should then be correlated with clinical behavior and prognosis.

Materials and Methods

Fresh biopsy specimens—mainly lymph nodes (or spleens)—from NHL patients before treatment were

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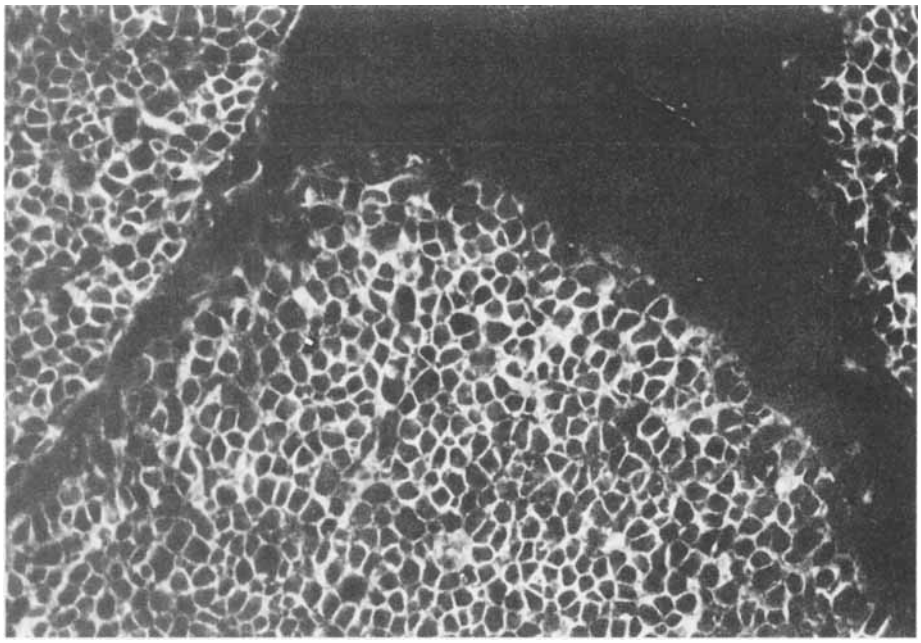

FIG. 1. Cortical area of normal human infantile thymus. Section incubated with anti-T antigen immunoglobulin—TRITC. Membrane fluorescence of cortical thymocytes is shown (×400).

examined in a multidisciplinary way: light microscopy, electron microscopy, immunofluorescence of tissue sections, and enzymehistochemistry.

Light Microscopy

After fixation in buffered formalin (*p*H 7) and embedding in paraplast, 4-μm sections were routinely stained with H and E, reticulin, PAS, Giemsa, and methyl green pyronin. In each case imprint smears —routinely stained with Giemsa and when indicated, with other stains—were compared with the histological slides.

The non-Hodgkin's lymphomas were named according to three classifications: Rappaport,[36] Lukes and Collins,[25] and Lennert.[20]

Electron Microscopy

For electron microscopy an approximately 1-mm thick slide (cross section) was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (*p*H 7.3) for 1–2 hours at 4 C. The slides were rinsed overnight in cacodylate buffer and cut into wedge-shaped blocks. After postfixation in 1% OsO_4 and dehydration in graded ethanol solutions, the blocks were embedded in flat molds in a mixture of Epon and Araldite.

For good orientation and appropriate selection for electron microscopic study, 1-μm toluidine-blue-stained sections of the longest side of the wedge-shaped blocks were compared with 4-μm H and E-stained histologic sections. Thin sections of selected parts, stained with uranyl acetate and lead hydroxide were examined in a Philips EM 301 electron microscope.

Immunofluorescence

Small, fresh tissue blocks of maximally 0.5 × 0.5 × 0.5 cm were quick frozen in liquid nitrogen immediately after removal of the specimen and stored in liquid nitrogen until needed. The 4-μm sections were prepared in a cryostat and air-dried. These were fixed for 10 minutes in acetone at room temperature, rinsed in phosphate-buffered saline (*p*H 7.2) (PBS) for 2 × 2 minutes and subsequently incubated with the antiserum at room temperature for 60 minutes in the direct method and 30 minutes in the indirect method, respectively. Finally the sections were rinsed and mounted in glycerin-PBS mixture and examined under a Leitz orthoplan microscope equipped with epi-illumination.

Antisera. Rabbit anti-immunoglobulin A,M,G heavy chain sera were purchased from Dakopatts (Denmark); they were labeled with fluorescein isothiocyanate (FITC) and used in a dilution of 1:100, 1:50 or 1:25 (for double staining preparations), and 1:200, respectively. Rabbit anti-kappa and lambda light chain sera labeled with FITC were also purchased from Dakopatts. The working dilution of these sera was 1:75 and 1:100.

The sheep anti-immunoglobulin E was the same as had been used in a study on mast cells and atopy.[12] It was used in a dilution of 1:20. Wherever necessary, serial dilutions up to 1:320 were made to estimate the endpoint titer of the IgE mastcell membrane fluorescence. The horse anti-human T lymphocyte antigen globulin (HTLA) was prepared in the Slotervaart Hospital, Pathology Department, by purification and

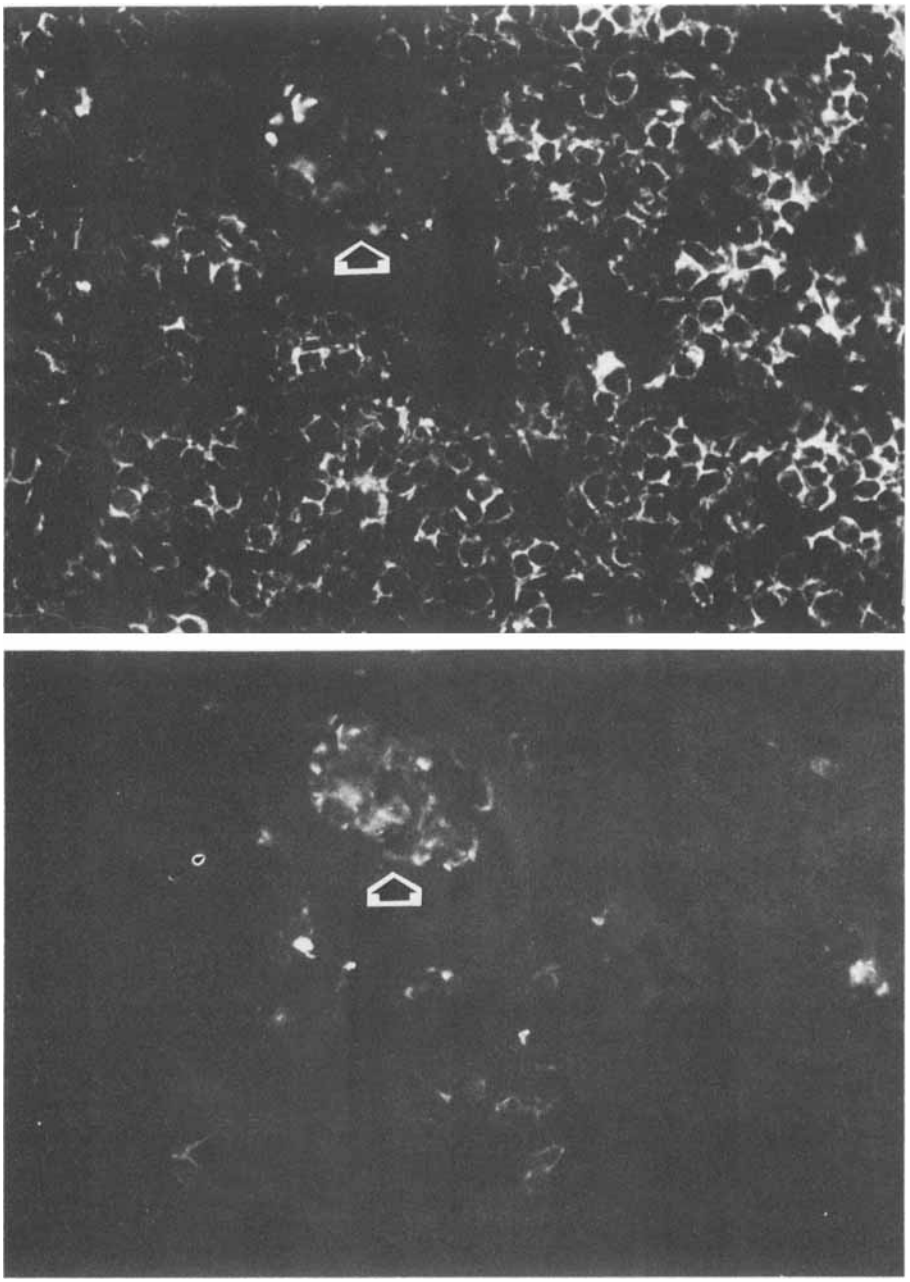

FIG. 2. Medullary area of normal human infantile thymus. (*top*): Section incubated with anti-T antigen immunoglobulin—TRITC. (*bottom*): Same section incubated with anti-IgM-FITC. Shown clearly is that the Hassall's corpuscle is negative and most small lymphoid cells are T-antigen positive. Only some scattered cells are B cells (positive for surface IgM). Nonspecific staining of interior of Hassall's corpuscle (arrow) (×400).

labeling of remnants of horse antithymocyte preparations with TRITC.

Before labeling with TRITC, the preparation was dialysed in order to free it from glycine and possibly other small molecules and was absorbed to acetone-dried human liver powder. Finally it was extensively absorbed with human ABO red cells and with spleen tissue removed at autopsy from a patient with a chronic lymphocytic leukemia (CLL) typed as M,D, kappa surface immunoglobulin (SIg) positive. This serum was used in a dilution of 1:75 and proved to be specific for thymus lymphocytes and lymphocytes in the thymus-dependent areas in spleen and lymph nodes. There were also some scattered T antigen positive cells lying in the outer zone of the germinal centers and corona of lymph follicles (see Figs. 1–5). It did not stain CLL cells of B-cell origin, nor hairy cells, monocytes, or SIg-positive normal B-cells in cell suspensions. Controls of the anti-T antigen immunoglobulin consisted of absorption of the antiserum with human thymocytes—prior to incubation of sections of thymus, spleen and lymph node—and, in the indirect technique, normal horse serum with labeled anti-horse serum as second layer. Both controls were ab-

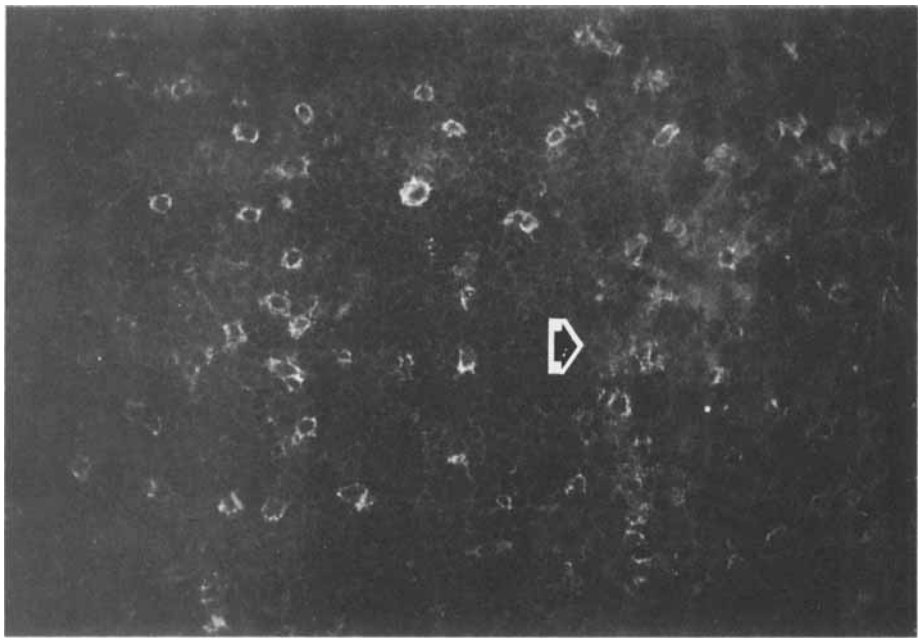

FIG. 3. Cortical area in normal human lymph node. Incubation with anti-T antigen immunoglobulin reveals that very few T cells are present in the corona and reaction center of a lymph follicle. The reaction center is seen at the right side (arrow) (×250).

solutely negative. Double staining of all tissue samples was carried out, by subsequent incubation of one section with this TRITC-labeled horse anti-T antigen (HTLA) and FITC-labeled rabbit anti-human IgM. Similarly double staining for the presence of IgD and IgM in one section was performed on all tissue samples by incubation with FITC labeled goat anti-human IgD (1:40) (Nordic, the Netherlands) followed by incubation with rabbit anti-human IgM labeled with TRITC. A horse anti-human lymphocyte serum (ALS) absorbed with human serum and liver powder was applied with a fluorescent second layer. The fluorescence procedure was as described before.[37]

Enzyme Histochemistry

In liquid nitrogen-frozen 8-μm sections corresponding to the slides prepared for fluorescence were used for enzyme histochemistry. Prior to incubation the sections were fixed in formadex. The fixation time (f.t.), the incubation time (i.t.) and the temperature (t.) for the enzyme histochemical reactions are mentioned with the methods.

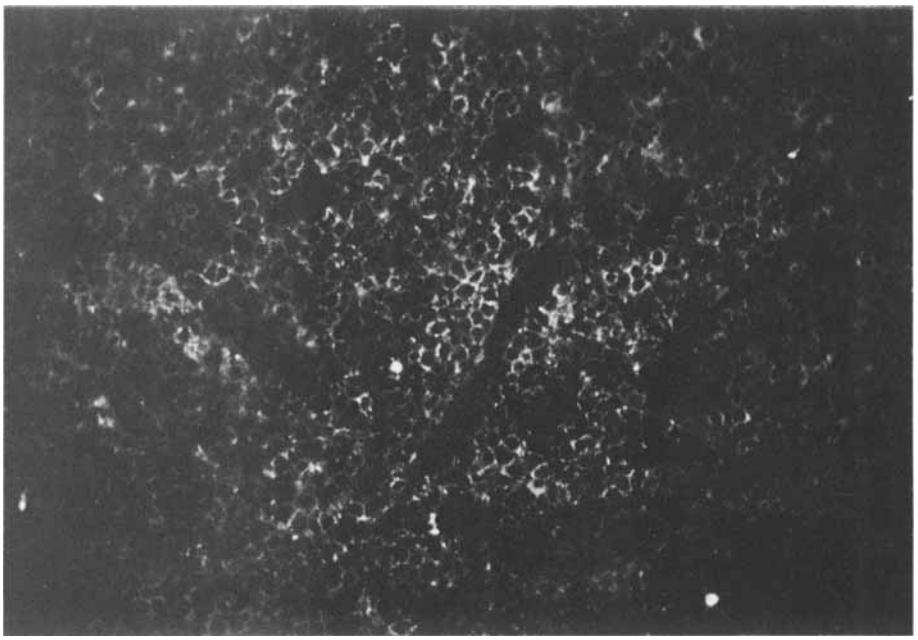

FIG. 4. Paracortical area in normal human lymph node. Incubation with anti-T antigen immunoglobulin. Many T cells are present around postcapillary venules (×250).

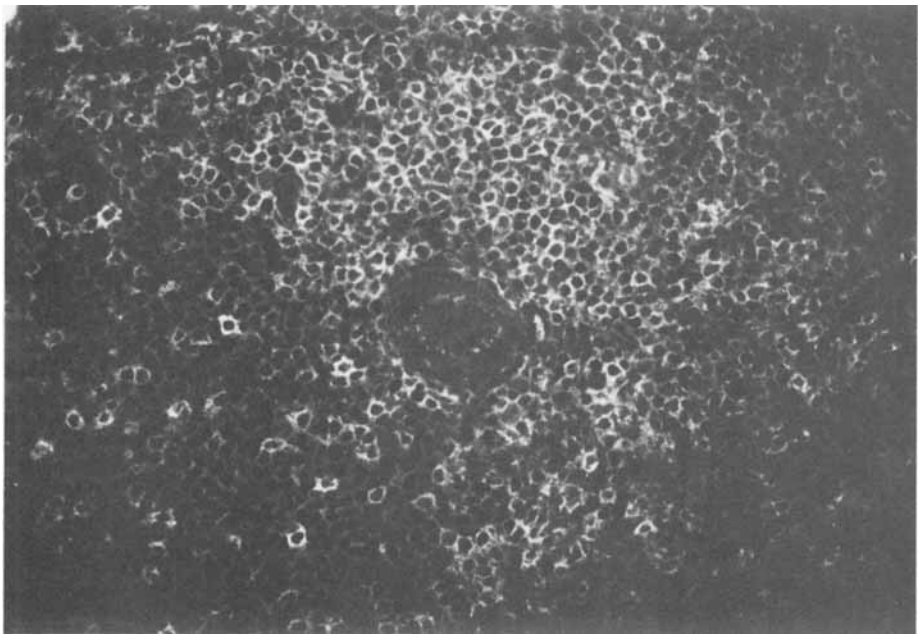

FIG. 5. Periarteriolar lymphocyte sheath in normal human spleen. Incubation with anti-T antigen immunoglobulin. Many T cells are lying around the small arteriole especially at the upper side (×250).

The following enzyme reactions were performed:

Alkaline phosphatase: according to Li *et al.*[22] with hexazotized triamino-tritolyl-methanchloride as coupling salt. Stutte,[44] f.t. 10 minutes, i.t. 30 minutes, t. 37 C.

Acid phosphatase: according to Katayama *et al.*[18] f.t. 10 minutes, i.t. 30 minutes, t. room temperature.

α-naphthylacetate esterase: according to Yam *et al.*[47] f.t. 10 minutes, i.t. 5 minutes, t. room temperature. Horwitz *et al.*[15] f.t. 5 minutes, formalin vapour; t. room temperature.

Nonspecific acid esterase: according to Horwitz *et al.*[15] f.t. 18 hours, i.t. 40 minutes, t. room temperature.

Naphthol AS-D chloro-acetate esterase: according to Leder,[19] f.t. 10 minutes, i.t. 30 minutes, t. room temperature.

5' nucleotidase and adenosine triphosphatase (ATPase): according to Melnick,[30] f.t. 10 minutes, i.t. 90 minutes, t. 37 C.

Imprint smears were subjected to the same enzymatical investigations. The enzyme activity per cell was expressed as negative, ± (moderate activity), + (obvious activity), ++ (high activity), and +++ (very high activity). The degree of enzyme activity of the lymphoma tissue as a whole is indicated in Table 1.

Results

In concordance with our previous study on NHL cells brought into suspension,[5] NHL was divided into the following groups: follicular NHL, diffuse lymphocytic NHL, histiocytic NHL, undifferentiated NHL, and mycosis fungoides. The lymphoma cells all showed positive fluorescence with ALS proving their lymphocytic origin. We used the transformation scheme of the normal lymphocyte as described by Lennert[20] (Fig. 6) to define where the differentiation line was disturbed. The results are put together in Table 2.

In our opinion the tumors originating from genuine histiocytic cells (monocytic/phagocytic system) in fact are not NHLs; results of investigation on these tumors will be published elsewhere.

Follicular NHL

The predominant cell type was the small cleaved cell,[25] Lennert's centrocyte[20] (60–90% of the total cell population), characterized by irregularly outlined or cleaved nuclei, small nucleoli, and pale-staining cytoplasm that showed a weak reaction in the Giemsa and methyl green pyronin stain.

Under electron microscope, the centrocytic cells

TABLE 1. Degree of Enzyme Activity in the Tumor Cells

Enzyme activity	–	±	+	++	+++
No. of tumor cells (tc) in relation to amount of enzyme activity	No activity of individual tumor cells	<50% tc ± <25% tc +	>50% tc ± 25–75% tc + <25% tc ++	>75% tc + 25–75% tc ++ <25% tc +++	>75% tc ++ >25% tc +++

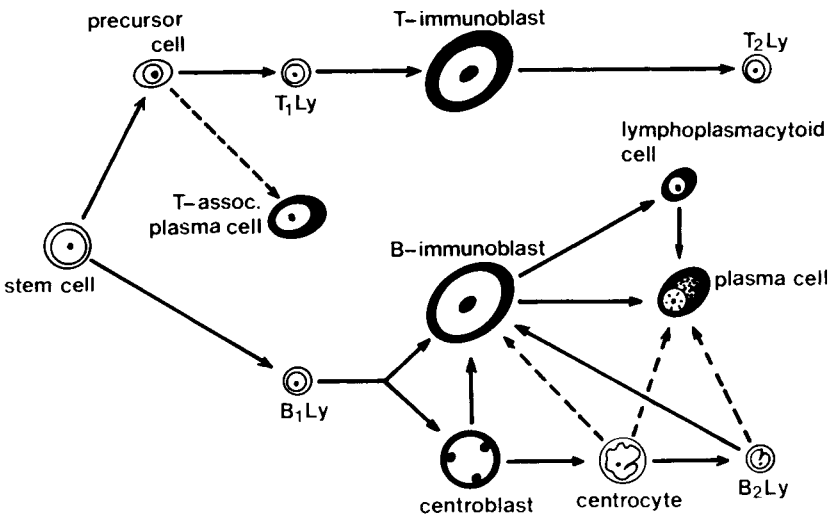

FIG. 6. Transformation scheme of normal lymphocytes (Lennert,[20] by courtesy of Springer Verlag, Heidelberg).

showed irregularly outlined nuclei with moderately condensed chromatin; the cytoplasm contained free ribosomes and variable numbers of polysomes; bristle-coated vesicles were always present. In every case large noncleaved cells—centroblasts according to Lennert—were seen with disperse chromatin, multiple small nucleoli generally localized against the nuclear membrane, and a few strands of rough endoplasmic reticulum in the cytoplasm. The cells were surrounded by a network formed by protrusions both of dendritic reticulum cells and of lymphoid cells. The cell protrusions were often embedded in dense amorphous background material. Dendritic reticulum cells were also observed in the interfollicular area.

All follicular NHL appeared to originate from monoclonal B cells; there was a bright fluorescence of the outer rim of the cells, presumably the cell membrane, positive for IgM mainly together with IgD, or IgG, both in combination with kappa in nearly all cases (see Table 2). In some cases a great number of T cells could be found between the follicular structures and a lesser number also scattered between the lymphoma cells. In all cases a part of the tumor cells showed moderate to strong activity of 5′ nucleotidase; in three cases a number of cells exhibited obvious ATPase activity. Few lysosomal enzymes were demonstrated.

Diffuse Lymphocytic NHL

Four cases fulfilled the criteria of well-differentiated lymphocytic cells (CLL type according to Lukes and Lennert): small round nuclei with condensed chromatin and a small rim of cytoplasm containing free ribosomes and few or small organelles. In two cases a leukemic picture was present. In these cases a high ATPase activity was evident. A monoclonal weak surface fluorescence could be demonstrated. Normal T cells were easily found.

Six cases of diffuse poorly differentiated NHL were investigated, four of which were almost entirely (85–95%) built up of small cleaved cells (centrocytes); one case contained 40% cells with lymphoplasmacytoid features. The cells of these cases showed a weak membrane fluorescence, monoclonal, indicating a B cell character, and a distinct activity of the enzyme ATPase. Fewer T cells were observed than in well-differentiated lymphocytic NHL.

Diffusely involved splenic tissue and blood cells were investigated in five patients with hairy cell leukaemia. The hairy cells showed the characteristic features,[6,33,38] especially in the electron microscope, with many long and slender protrusions on the cell surface, a relatively well-developed Golgi apparatus, and a number of smooth and bristle-coated vesicles. In some hairy cells the protrusions had engulfed erythrocytes completely. The hairy cells appeared to be of monoclonal B cell origin in four cases with a vague fluorescent broad rim of the immunoglobulin obviously caused by the hairy protrusions; none showed cytoplasmic fluorescence. In one case no immunoglobulins could be demonstrated. Histochemically, acid phosphatase activity could be demonstrated and was especially evident in imprint smears and in four cases was tartrate resistant.

Histiocytic NHL

In contrast to the former groups of NHL, this group showed much variety in morphology, immunology, and enzyme histochemistry (see Table 2). Several types of large cells were encountered: large cleaved (centrocytic) cells, large noncleaved (centroblastic) cells, and immunoblastic cells. The latter cell type showed large nucleoli, a rather regular nucleus, and pyro-

TABLE 2. Results of Multidisciplinary Examination of All NHLs, Except for the Cases of Mycosis Fungoides

Pat. no.	Sex	Age (yrs)	Source	Light- and electron microscopical diagnosis according to			Immunology				Histochemistry					
				Rappaport	Lukes	Lennert	SIg	CIg	HTLA	ALS	5 NT	ATP	AlP	AcP	tart.	ANAE
							Follicular									
29	F	30	LN	LPD	sm.cl.	CB/CC	− κ	−	−	+	+	±	−	−		−
31	M	55	LN	LPD	sm.cl.	CB/CC	M κ	−	−	+	++	++	+	−		−
33	F	43	LN	LPD	sm.cl.	CB/CC	M D κ	−	−	+	+	−	+	−		−
34	F	75	LN	LPD	sm.cl.	CB/CC	M D κ	−	−	+	+	+	+	±		−
68	F	27	LN,SPL	LPD	sm.cl.	CB/CC	G κ	−	−	+	+	−	−	−		−
97	F	35	SPL	LPD	sm.cl.	CB/CC	M D κ	−	−	+	+	−	−	−		−
155	M	63	LN	LPD	sm.cl.	CB/CC	G κ	−	−	+	+	−	−	−		−
166	F	76	LN	LPD	sm.cl.	CB/CC	G κ	−	−	+	++	++	−	−		−
167	F	74	LN	LPD	sm.cl.	CB/CC	M D λ	−	−	+	±	±	+	−		−
214	M	68	LN	LPD	sm.cl.	CB/CC	G κ	−	−	+	±	±	−	−		−
196	F	73	LN,BM	LPD	sm.cl.	CB/CC	M λ	−	−	+	+	−	−	−		−
							Diffuse									
47	F	75	LN,BL	LWD	L.CLL	L.CLL	M κ	−	−	+	−	++	−	±		−
111	M	60	LN	LWD	L.CLL	L.CLL	M D κ	−	−	+	±	+++	−	−		−
117	M	62	LN	LWD	L.CLL	L.CLL	M D κ	−	−	+	±	++	−	−		−
207	F	65	LN	LWD	L.CLL	L.CLL	M D λ	−	−	+	−	+++	−	−		−
168	M	45	LN	L.macr.	ly.pl.	imm.cyt.	M κ	−	−	+	−	++	−	−		−
211	F	71	LN	L.macr.	ly.pl.	imm.cyt.	− −	M κ	−	±	−	++	−	+	−	+
10	F	74	LN	LPD	sm.cl.	CC	M D κ	−	−	+	++	++	−	−		−
46	M	58	LN	LPD	sm.cl.	CC	M D λ	−	−	+	+	+	−	−		−
76	M	65	LN	LPD	sm.cl.	CB/CC	M κ	−	−	+	−	+	−	−		−
136	F	65	LN	LPD	sm.cl.	CB/CC	M D λ	−	−	+	−	++	−	−		−
202	M	51	LN	LPD	sm.cl.	CC	M λ	−	−	+	±	+++	−	−	−	−
212	M	62	LN	LPD	sm.cl.	CB/CC	M D λ	−	−	+	nt	nt	nt	nt		nt
32	M	43	SPL,BL	HCL	HCL	HCL	M G κ	−	−	+	++	++	−	+	+	−
66	M	67	SPL,BL	HCL	HCL	HCL	M D λ	−	−	+	++	++	−	+	+	−
145	M	53	SPL,BL	HCL	HCL	HCL	M D λ	−	−	+	±	++	−	+	+	−
156	M	48	SPL	HCL	HCL	HCL	G κ	−	−	+	+/++	++	−	+	−	−
217	M	44	SPL,BL	HCL	HCL	HCL	−	−	−	+	−	+	−	+	+	−
70	M	68	SPL	HWD	la.cl.	CC	A κ	−	−	+	+	++	−	−		−
119	M	64	LN	HWD	la.cl.	CC	−	−	−	+	−	−	−	−		−
139	M	50	LN	HWD	la.cl.	CC	−	−	−	+	−	+++	−	+		−
152	F	55	LN	HWD	la.cl.	CC	−	−	−	+	++	+	±	−		−
157	M	24	skin	HWD	la.cl.	CC	−	−	−	+	−	±	−	−		−
165	F	72	LN	HWD	la.cl.	CC	M κ	−	−	+	−	±	−	−		−
174	F	78	LN	HWD	la.cl.	CC	−	M κ	−	+	−	++	−	−		−
183	M	30	LN	HWD	la.cl.	CC	−	−	−	+	−	−	−	−		−
77	M	66	LN	HPD	la.n.cl.	CB	M λ	−	−	+	−	++	−	−		−
79	F	65	skin	HPD	la.n.cl.	CB	−	−	−	+	−	−	−	−		−
120	M	68	LN	HPD	la.n.cl.	CB	M κ	−	−	+	−	+++	−	−		−
148	F	57	tonsil	HPD	la.n.cl.	CB	−	−	−	+	±	++	±	−		−
182	M	30	LN	HPD	la.n.cl.	CB	−	−	−	+	−	++	−	−		−
188	F	68	LN	HPD	la.n.cl.	CB	M κ	−	−	+	−	−	−	−		−
190	F	68	LN	HPD	la.n.cl.	imm.bl.	−	−	−	+	−	−	−	−		−
200	F	72	LN	HPD	la.n.cl.	CB	−	−	−	+	−	+++	−	−		−
69	M	36	LN	HPD	imm.bl.	imm.bl.	−	A κ	−	+	−	++	−	+		−
122	F	62	LN	HPD	imm.bl.	imm.bl.	−	−	−	+	−	+++	−	−		−
134	M	26	LN	HPD	imm.bl.	imm.bl.	−	−	−	+	−	±	−	±		−
153	F	75	LN	HPD	imm.bl.	imm.bl.	−	M λ	−	+	−	+	−	−		−
178	M	62	LN	HPD	imm.bl.	imm.bl.	−	−	−	+	−	+++	−	−		−
179	F	64	rhinoph.	HPD	imm.bl.	imm.bl.	−	−	−	+	−	−	−	−		−
209	F	55	LN	HPD	imm.bl.	imm.bl.	M κ	−	−	+	−	+	−	−		−
210	M	72	nose	HPD	imm.bl.	imm.bl.	M λ	−	−	+	−	+	−	−		−
60	F	3	skin	U	sm.n.cl.	ly.bl.	− −	−	−	+	±	−	−	−		−
87	F	13	LN,BL	U	sm.n.cl.	ly.bl.	−	−	−	+	−	±	−	−		−
186	M	17	LN	U	sm.n.cl.	ly.bl.	M κ	−	−	+	−	−	−	−		−
189	M	21	LN	U	sm.n.cl.	ly.bl.	−	−	−	+	−	−	−	+	−	−
197	M	32	LN	U	sm.n.cl.	ly.bl.	M κ	−	−	+	−	−	−	−		−
65	M	30	LN,BL	U(LPD)	convol.T	ly.bl.T	−	−	+	+	±	−	−	±		−
149	M	22	BL,test.CSF	U(LPD)	convol.T	ly.bl.T	−	−	+	+	nt	nt	nt	+		nt
169	M	29	pl.eff.,CSF	U(LPD)	convol.T	ly.bl.T	−	−	+	+	nt	nt	nt	+		nt
173	M	8	test.,CSF	U(LPD)	convol.T	ly.bl.T	−	−	+	+	−	−	−	++		−

Table 2 footnotes appear on page 2217

ninophylic cytoplasm, sometimes containing dilated cisternae of rough ER.

All lymphoma cells appeared to be derived from lymphoid cells indicated by the reactivity with ALS and absence of markers of genuine histiocytic cells (α-naphthyl acetate asterase, acid phosphatase, Fc receptor). In ten cases a monoclonal B-cell origin could be detected whereas the remaining 14 cases were neither positive for surface or cytoplasmic immunoglobulin nor for T-cell antigen. In three cases—two immunoblastic types and one centrocytic type—monoclonal immunoglobulin was found in the cytoplasm. If present, the fluorescence intensity was weak except for case 69 where a strong intracytoplasmic fluorescence was noted; this case contained many swollen cisternae of rough ER filled with moderately electron-dense amorphous material that was condensed into crystalloid tubular structures in some cells. The acid phosphatase activity in nearly all cells of this case was caused by a high number of lysosomes in the Golgi area.

The enzyme pattern of ATPase varied from completely negative in five cases to high activity in 11 cases.

Undifferentiated NHL

Within this group three immunologic subgroups were recognized: T-, B-, and null-cell types as in acute lymphoblastic leukemia. A T-cell origin could be predicted morphologically by the convoluted aspect of the nuclei, present in all our cases (four) of T-cell lymphoma in combination with a mediastinal tumor. In the present series no morphologic difference between B cells and null cells could be found; we had no opportunity to examine a Burkitt lymphoma in our multidisciplinary study.

Under electron microscope, a homogeneously distributed dispersed chromatin was found in all undifferentiated NHL (UNHL). In the T-cell cases the chromatin was quite electron dense and many nuclei showed typical convolutions. In all UNHL the cytoplasm contained polysomes and ribosomes.

Immunologically, all UNHL cells reacted with ALS. Four cases with convoluted morphology appeared to consist of T cells. In two cases B-cell properties were found (monoclonal surface immunoglobulin) and in three cases no immunological T- or B-cell markers could be demonstrated.

Enzymehistochemically, no specific hydrolytic enzyme pattern was revealed. The convoluted T cells showed a dot-like paranuclear accumulation of acid phosphatase; α-naphthylacetate esterase on the other hand was absent. In one immunologically non-T/non-B UNHL focal acid phosphatase was demonstrated (Case 189); morphologically a few convoluted cells with homogeneous electron-dense chromatin were present in this case, which is considered by us an immature T-cell tumor.

Mycosis Fungoides

Skin biopsies and one lymph node showed the characteristic features of mycosis fungoides;[36] particularly in the electron microscope the cerebriform nuclei of mycosis cells were evident. All cases were brightly positive for T-cell antigen, with a greater intensity of fluorescence than in normal thymocytes; the large mycosis cells were less brightly positive for the T-cell antigen than the small mycosis cells. In seven cases a strong IgE mast cell membrane fluorescence was found; in most instances these were preceded by a clinical history of chronic atopic eczema. Enzyme histochemistry did not reveal a specific pattern; in particular there was no dot-like activity of acid phosphatase or α-naphthylacetate esterase.

Table 2 footnotes from page 2216

Ac.P.	: acid phosphatase	la.cl.	: large cleaved
Al.P.	: alkaline phosphatase	L macr.	: lymphocytic with macroglobulinemia
ALS	: human antilymphocyte serum	la.n.cl.	: large noncleaved
ANAE	: α-naphthyl acetate esterase	ly.bl.	: lymphoblastic
ATP	: adenosine triphosphatase	LN	: lymph node
BL	: blood	ly.pl.	: lymphoplasmacytoid
BM	: bone marrow	nt	: not tested
CB	: centroblastic	5 NT	: 5′ nucleotidase
CC	: centrocytic	PD	: poorly differentiated
C Ig	: cytoplasmatic immunoglobulin	pl.eff	: pleural effusion
CLL	: chronic lymphocyte leukemia celltype	rhinoph.	: rhinopharynx
convol.	: convoluted	SIg	: surface immunoglobulin
CSF	: cerebrospinal fluid	SPL	: spleen
H	: histiocytic	sm.cl.	: small cleaved
HCL	: hairy cell leukemia	sm.n.cl.	: small noncleaved
HTLA	: human T lymphocyte antigen	tart.	: tartrate resistant acid phosphatase
imm.bl.	: immunoblastic	test.	: testis
imm.cyt.	: immunocytoma	U	: undifferentiated
L	: lymphocytic	WD	: well-differentiated

Discussion

Multidisciplinary studies of NHL cells are of considerable value in establishing the nature and degree of differentiation of lymphoma cells.[5,20,24,42] These characteristics may be of importance in predicting the clinical course.[3,4,14,16,35] There seems to be a correlation between certain B-cell markers and differentiation.[14,35,42] As it is impractical to measure routinely a large number of markers in each case, a selection should be made.

Our studies confirmed that the non-cutaneous NHLs of low-grade malignancy (follicular NHL and diffuse lymphocytic NHL) exhibited monoclonal B-cell properties in practically all cases[41,46] in contrast with other studies.[27] The intensity of membrane fluorescence varied from moderately bright in follicular NHL to weak in diffuse lymphocytic NHL; 11 of the 23 lymphomas of the lymphocytic group were positive both for IgM as well as for IgD. This might indicate that these malignant cells originate from B cells rather early in their differentiation line.[42] In follicular NHL more T cells were found (mainly in the interfollicular area) than in diffuse NHL in accordance with other studies.[5,20,35]

A combination of the enzymes 5′ nucleotidase and ATPase was frequently seen in the malignant B cells, especially in follicular NHL; the ATPase reaction was usually stronger than in the normal lymphoid counterparts, whereas 5′ nucleotidase showed less activity in tumor cells when compared with their normal counterparts. The tartrate-resistant acid phosphatase reaction in hairy cells was best visible in imprint smears or in blood smears.

In general, enzyme activity is more visible in smears than in frozen tissue sections; this probably explains the very low activity of acid phosphatase in the lymphomas other than hairy cell leukemia and T-lymphoblastic lymphoma in our series, and the varying results in literature.

In comparison with normal lymphoid cells, some enzymes are lacking in lymphoma cells while other enzymes apparently are newly formed. The general assumption that enzyme characterization of malignant cells does not differ from that of corresponding normal cells seems to be incorrect.

The histiocytic lymphomas constituted a heterogeneous group in accordance with other studies,[5,7,17,23,-39,40,43] but all tumors proved to be of lymphocytic origin as indicated by a positive reaction with ALS. In many instances the cell morphology did not permit any prediction about the T-, B-, or null-cell nature of these NHLs; 14 of the 24 cases were null cells neither positive for HTLA nor for SIg or CIg. In nine of the 14 null-cell NHLs of the histiocytic type, histochemical indications for B cells existed, due to the presence of ATPase and/or 5′ nucleotidase.

The undifferentiated lymphomas involved younger patients for the most part. In four cases convoluted cells were present showing a positive reaction for HTLA and a focal accumulation of acid phosphatase. In two undifferentiated NHLs, B-cell properties were demonstrated by monoclonal surface IgM kappa in both lymphomas. In three cases no immunologic markers could be detected so that these cells were designated as null cells. In one of these null-cell cases (Case 189) the presence of acid phosphatase in a dot-like fashion in combination with some convoluted nuclei possibly indicated a T-cell origin. This tumor clinically presented itself as a convoluted lymphocytic lymphoma with early bone marrow and meningeal involvement. As in the management of acute lymphoblastic leukemia[8] the immunologic typing and enzyme pattern of the cells is important. We could find no dot-like enzyme activity of α-naphthylacetate esterase —a marker for T-helper cells—in tumor cells. The demonstration of terminal deoxynucleotidyl transferase in tissue sections was not undertaken in this study because, in our opinion, this method is only reliable in cell suspensions.

In the case of cutaneous lymphoid infiltrates, the multidisciplinary approach gave important information. Fluorescence can detect a polyclonal or monoclonal B-cell or a T-cell population. Mycosis fungoides cells exhibited a characteristic E.M. picture and a strong T-cell antigen positivity that is in accordance with the supposition that mycosis cells originate from T-helper cells. The mastcell IgE binding was high,[12] which indicates that atopic skin disease might be a condition favoring the induction of mycosis fungoides.

The multidisciplinary approach was important in the diagnosis of tumors originating from genuine histiocytic cells,[11] particularly in localized forms where differentiation from a lymphocyte-derived tumor may be very difficult.

Another reason for determining the nature of the lymphoma cells was to examine the validity of the different classifications. In general, a classification of malignant tumors must fulfil the following criteria: a good morphogenetic basis, a good clinical utility, and a good reproducibility. Though the three widely used classifications of Rappaport, Lukes, and Lennert are comparable among themselves, we prefer the classification of Lennert.[20,21] In our viewpoint the Lennert (Kiel[13]) classification approximates the criterion of morphogenetic exactness in the best possible way; the lymphoma cells may be recognized in the transformation line of their normal counterparts. Practically all "histiocytic" lymphomas appear to be of

lymphocytic origin, so the prefix "histiocytic" has to be changed, conforming with the proposals of Lukes, Lennert and others.[43] The results of our studies and those of others[34] contradict the postulation of Lukes and Collins that the T-, B-, or non-T-/non-B-nature of lymphoma cells can be determined morphologically. The name "lymphoblastic cell" appears to be a more justifiable name than "small noncleaved cell" (which is partly medium or large sized), as was also indicated in a recently described modification of the Rappaport classification.[34] Lennert divided them into two groups: low-grade and high-grade malignancy,[13] the determination of which is important for the clinician, for in these two groups a different therapeutic approach must be considered. The reproducibility of the recent classifications must be established as well as correlated with survival; the latter has recently been reported for the Kiel classification.[2,31,32] A study of the importance of our multiparameter analysis for the clinical course is in preparation.

Conclusions

Optimal light microscopy, including imprint cytology and cytochemistry, usually provides sufficient information for diagnosis.[26] Immunohistochemic and electron microscopic investigation may be of great diagnostic aid in cases of large-cell lymphomas, undifferentiated lymphomas, lymphoid infiltrates of the skin, differentiation of pseudolymphomas, and non-lymphomatous tumors (*e.g.*, carcinoma, malignant melanoma) from NHL.

Finally the classification of NHL according to Lennert appears to be the most valid one in the sense of clinical utility and morphogenetic basis.

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