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Non-Hodgkin's Lymphoma in the First Two Decades

Morphologic and Immunocytochemical Study

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Summary. The histopathologic and anatomoclinical features of 211 cases of non-Hodgkin's lymphoma (NHL) were reviewed and each case reclassified according to Lukes-Collins, Kiel and Rappaport criteria. Immunologic determination of cell phenotype in 63 cases as well as assessments of immunoglobulin and lysozyme by immunoperoxidase in 48 cases, permitted a precise definition of cell lineage on a functional basis and showed a high degree of predictability of immunologic phenotype of lymphoma cells by conventional morphology. The results of immunologic cell typing and immunoperoxidase studies were consistent with functional schema of Lukes-Collins and Kiel. "True" histiocytic proliferations, (9 cases) showed biologically different behavior from malignant lymphoma (ML), by a high incidence of extralymphatic (skin and soft tissue) presentation, rapid course, and frequent conversion to histiocytic leukemia. Fifty-two percent of studied ML cases were categorized morphologically as B-cell line proliferations, 36.7% as T-cell line and 6.9% as undefined group (ML "U" type). The ratio of T-cell to B-cell malignancies was 1:1.4. The convoluted type lymphoma was characterized by a high incidence (70%) of anterior mediastinal presentation, high incidence (over 60%) of hematologic and CNS involvement, and a high probability of testicular relapse, especially late. In contrast to malignancies of the T-cell line, B-cell proliferations tended to be localized below

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the diaphragm, and the most common anatomic location of lymphoma growth appeared to be the gastrointestinal tract, where small and large noncleaved follicular center cell types predominated. The nodular lymphoid hyperplasia in the vicinity of intestinal lymphoma and lack of secretory component within enterocytes were other substantial findings in abdominal B-cell malignancies. Among immunoblastic proliferations, the B-cell type was found to predominate over T-cell and was characterized as highly aggressive disease with a relatively common marrow infiltration. A low anatomic stage of disease and a favorable outcome were closely related to small cleaved type of follicular center cell lymphoma, which comprised only 1.5% of the patients studied. The less defined and morphologically heterogeneous group formed ML "U", which in over 95% of patients converted to acute lymphocytic leukemia. This joint analysis of "primary leukemic" and "non-leukemic" NHL patients disclosed striking differences in anatomic presentation and natural history of disease between T, B and null cell proliferations versus related cytologic types.

Key words: Non-Hodgkin lymphoma – Children – Immunoperoxidase.

Introduction

The impetus given to pathology by recent advances in basic immunology has resulted in several new proposals concerning functional classification of non-Hodgkin's lymphoma (NHL). Immunologic as well as immunopathologic studies in NHL reported during the last five years have provided strong support for the original classification concepts proposed by Lukes-Collins and Lennert and colleagues (Gérard-Marchant et al. 1974; Lennert et al. 1975; Lukes and Collins 1975; Lennert and Mohri 1978; Lennert and Stein 1978; Lukes 1978) and have affected considerable modifications of the older Rappaport terms (Rappaport 1966; Nathwani et al. 1974; Berard et al. 1977; Byrne 1977). It also has become evident that the traditional separation between acute lymphocytic leukemia and NHL in children is mostly artificial, being based only upon the degree of bone marrow infiltration by tumor cells (Barcos and Lukes 1975; Lukes et al. 1978b; Murphy 1978; Williams et al. 1978). Interrelationships between different histologic types of NHL and acute lymphocytic leukemia (ALL) evolving from them have not been fully studied, owing to the fact that from most large reported series of NHL in children, leukemic cases have been excluded (Hutter et al. 1975; Lemerle et al. 1975; Murphy et al. 1975; Pinkel et al. 1975; Traggis et al. 1975; Watanabe et al. 1975; Hausner et al. 1977).

Several studies of NHL in children using either Rappaport's original or modified classification failed to demonstrate any appreciable value of histology in predicting therapeutic results, the likelihood of relapse or length of survival. This was probably because more than 95% of pediatric NHL are of the diffuse type and actually belong to high malignancy groups (Hutter et al. 1975; Lennert 1977; Garwicz et al. 1978; Lennert and Stein 1978; Frizzera and Murphy 1979). The only prognostically valuable indicators identified by these studies were

location of the primary tumor and its extension (Berry and Keeling 1970; Lemerle et al. 1975; Murphy et al. 1975; Pinkel et al. 1975; Byrne 1977; Hausner et al. 1977).

Cell-surface marker studies and histoimmunologic techniques now allow the lineage of neoplastic cells to be determined, even in formalin-fixed, paraffin-embedded tissue (Taylor and Burns 1974; Knowles II et al. 1977; Lennert and Mohri 1978; Lukes et al. 1978b; Stein et al. 1978; Taylor 1978; Mann et al. 1979). Lysozyme (muramidase) is a highly specific protein (enzyme) for the phagocytic-mononuclear cell series and their precursors, but is absent from lymphoid T or B cell lines at any level of modulation (transformation) (Schmalzl and Braunsteiner 1970; Pinkus and Said 1977; Lukes et al. 1978; Kokoshis and DiLuzio 1979). Conversely, intracytoplasmic immunoglobulin, even single chain varieties, can be demonstrated relatively easily in lymphoid B cell lines and can be used to determine the clonality of lymphoid cells (Lukes 1978; Lukes et al. 1978b; Pinkus and Said 1978; Stein et al. 1978, 1979; Taylor 1978; Mann et al. 1979).

The present retrospective study was undertaken (i) to redefine the morphologic subtypes of NHL in children in light of recent classifications; (ii) to compare the results of morphologic cell typing with those obtained by cell-surface analysis; (iii) to determine the presence or absence of intracytoplasmic immunoglobulin and lysozyme in large cell lymphomas; (iv) to evaluate the presence of a secretory component in enterocytes in gastrointestinal lymphoma cases; and (v) to compare the anatomic presentation of disease with the histologic type.

Materials and Methods

Three-hundred and eight cases of "lymphosarcoma", "lymphoblastosarcoma", "reticulum cell sarcoma" and "malignant lymphoma" indexed in the Department of Pathology of St. Jude Children's Research Hospital during the period 1962–1978 were reviewed. The material consisted of biopsies that were either performed at St. Jude Children's Research Hospital, performed elsewhere and referred with patients for further evaluation or sent for consultation only. Of the 308 cases, 211 were selected for further morphologic evaluation, for full and adequate histopathologic documentation and for determination of the anatomic extension of disease at diagnosis. The remaining 97 cases were excluded from the study because histopathologic material was judged as inadequate or of poor technical quality or because proliferation represented malignant histiocytosis or because the age of patients exceeded 20 years. These 211 cases of previously untreated NHL were reviewed and classified without prior knowledge of anatomic presentation, stage of disease at the time of diagnosis, or presence or absence of bone marrow involvement at diagnosis or during the course of disease. Clinical records were then reviewed to verify these parameters. Due to the long period over which patients were accrued, and the resulting disparity among treatment protocols, no attempt was made to evaluate the effects of therapy according to particular cytologic type.

The staging system presented in this report is currently being used at St. Jude Children's Hospital (Murphy 1978). It was applied retrospectively regardless of the percentage of blast cells present in the bone marrow at the time of admission or before the patient entered any leukemia and/or lymphoma treatment protocol being used in that institution during the period 1962–1978.

Morphologic Studies. Three classifications were used: the modified Rappaport (Nathwani et al. 1974; Berard et al. 1977; Byrne 1977), the Lukes-Collins (Lukes and Collins 1975; Lukes 1978) and the modified Kiel (Gérard-Marchant et al. 1974; Lennert et al. 1975; Lennert and Mohri 1978;

Lennert and Stein 1978; Stein et al. 1978). Final diagnosis was based upon the results of a battery of standard histologic stains including hematoxylin-eosin, PAS, methyl-green pyronine, reticulin and Giemsa. In most cases, Wright stained imprints were also available. In some of the large-cell lymphoma cases, imprints stained for specific (naphthol-ASD-chloracetate) and nonspecific (α-naphthylacetate) esterase were also available. All cases were classified twice by two observers independently (WTD, MG-D).The classification of five cases could not be agreed upon; these were reviewed jointly and a final consensus reached. A sample of 30 cases was classified by a third observers (R.F. Dorfman, M.D., R.A. Warnke, M.D., J. S. Burke, M.D. – Stanford University) as a gauge of consistency. No discrepancies were noted.

Cell-Markers Studies. Cell surface markers were determined in 63 cases. The results of these studies were not known until after morphologic typing was accomplished. Technical details and methods of evaluations have been described elsewhere (Murphy et al. 1978).

Immunoperoxidase Studies. Forty-eight cases classified as malignant lymphoma (ML), follicular center cell (FCC), large non-cleaved; ML immunoblastic B; ML immunoblastic T; or "true" histiocytic proliferation were evaluated for the presence of intracytoplasmic immunoglobulin and lysozyme. The standard immunoperoxidase peroxidase-antiperoxidase (PAP) technique (Taylor and Burnes 1974; Taylor 1978) with rabbit antihuman lysozyme and antihuman monospecific sera against immunoglobulin (lambda, and kappa, μ, γ and α chains) (obtained from DAKO Accurate Chemicals, Hicksville, New York) was used. In some cases both the PAP and direct immunoperoxidase techniques (KnowlesII et al. 1977) using peroxidase-conjugated F(ab)2 fragments of antisera against μ, γ and α heavy chains (purchased from Litton Bionetitics Inc., Kensington, MA.) were done. Immunologically proven myeloma cases were used as a control for immunoglobulin staining, intestinal biopsies (Paneth cells), salivary gland or normal bone marrow myeloid cells served as positive controls for lysozyme staining.

Secretory component (SC) was identified by the PAP method using the anti-SC of IgA serum (obtained from DAKO Accurate Chemicals, Hicksville, New York). Biopsies of normal intestinal mucosa served as a positive control.

Definitions of Terms. The term malignant lymphoma (ML) was used exclusively to designate malignant proliferation of a lymphoid cell line. Cases of malignant, "true" histiocytic proliferation (muramidase positive, immunoglobulin negative) were exclude from clinicopathologic description, but were included in Rappaport's classification under the term ML histiocytic (Table 3).

ML, convoluted type, was diagnosed when a few of the cells displayed nuclear convolutions (or subdivisions) as seen in otherwise typical ML convoluted type. We did not follow the recommendation of Nathwani et al (1974) that 10% of the cells in lymphoblastic proliferation must be convoluted to allow diagnosis.

ML of Burkitt's type (ML undifferentiated, Burkitt's according to Rappaport and ML lymphoblastic, Burkitt type according to Kiel) was diagnosed only when the cells in all available sections showed uniform round-to-oval nuclei that were smaller or equal to histiocyte nuclei. Cases with even slight pleomorphism were considered to be non-Burkitt tumors. The "starry-sky" pattern was not used as the principal diagnostic argument. Other histologic parameters were adopted as recommended by the World Health Organization (Berard et al. 1969).

The term *monoclonal* was applied to any large cell lymphoma in which 50% or more of the malignant cells revealed positivity with class monospecific antiserum and no positive reactions were found using other class-specific immunoglobulin sera. The term *bitypic* was used when large cell proliferation displayed positivity with one class specific serum and more than 50% of the cells reacted with anti-lambda and anti-kappa sera.

By the term extralymphatic lymphoma, we imply that the presenting site of disease is a tissue where no lymphoid tissue is normally present. Intestinal as well as thymic tumors were not considered extralymphatic.

Results

The complete case population is listed by diagnosis in Table 1 according to the Lukes-Collins classification and immunologic cell type. Summarized in Ta-

Table 1. Distribution of patients by diagnosis: Lukes-Collins classification and related immunologic cell type in NHL

Morphologic type	No. of pts. (% of total)	Age range (median)	Male/female ratio	Immunologic type[a]		
				T	B	Null
B cell line						
Small lymphocyte	–	–	–	–	–	–
Plasmocytoid lymphocyte	1 (0.5)	4	1:0	ND	ND	ND
Follicular center cell						
Small cleaved	3 (1.5)	4, 5, 17	3:0	ND	ND	ND
Large cleaved	3 (1.5)	4, 5, 7	1:2	ND	ND	ND
Small non-cleaved	65 (32.2)	2–19 (9.1)	4.2:1	0	27	0
Large non-cleaved	10 (4.9)	2–18 (8.6)	9.0:1	0	5	0
Immunoblastic B	23 (11.4)	4–18 (12.3)	1.3:1	0	4	0
Hairy cell leukemia	–	–	–	–	–	–
T cell line						
Small lymphocyte	–	–	–	–	–	–
Convoluted lymphocyte	69 (34.2)	2–20 (11.3)	2.9:1	19	0	5
Cerebriform lymphocyte	–	–	–	–	–	–
Immunoblastic T	5 (2.5)	4–20 (14.2)	1:5	1	0	0
Lymphoepithelioid cell	–	–	–	–	–	–
U cell group	14 (6.9)	4–16 (8.2)	2.5:1	0	0	1
Unclassifiable	9 (4.4)	–	–	0	1	0
Total	202 (100)	–	3:1	20	37	6

ND = Not done

[a] Number of patients with surface markers

bles 2 and 3 are cases as defined using the Kiel and Rappaport classification criteria respectively. Nine cases of true histiocytic proliferation included in the Rappaport scheme were facultatively excluded from the Lukes-Collins and Kiel tabulations. As seen in Table 1, 52% of cases belong morphologically to the B-cell line, 36.7% to T cell proliferation and 6.9% to undefined "U-cell" group. The ratio of B-cell to T cell malignancies is 1.4:1. The age distribution of 202 cases is shown schematically in Fig. 1. The sex ratio is 3:1 (151 males and 51 females).

Clinical data concerning anatomic presentation of malignancy at diagnosis were available for all patients, whereas follow-up observations were available for all but 32 cases, for which we provided histologic consultations only. The overall survival rate was 41%; in 7 patients the time of observation was less than 12 months.

Roughly one-third of the NHL patients (67 cases) entered in the period 1962–1978 treatment protocols for acute lymphocytic or non-lymphocytic leukemias for the presence of more than 25% blasts in the bone marrow at diagnosis of lymphoma or converted to leukemia within 6 months. Consequently different diagnostic approaches (lymphoma versus leukemia) as well as changing treat-

Table 2. Kiel classification[a]. Relation of morphologic to immunologic cell type in NHL

Low grade malignancy	No of pts. (% of total)	Immunologic[b] type			High grade malignancy	No of pts. (% of total)	Immunologic[b] type		
		T	B	Null			T	B	Null
ML Lymphocytic	–				ML Centroblastic				
B-CLL	–				-primary	10 (4.9)	0	5	0
T-CLL	–				-secondary	–			
Prolymphotic (T or B)	–								
Hairy cell leukemia	–				ML lymphoblastic				
Mycosis fungoides (and Sezary Syndrome)	–				B-lymphoblastic	65 (32.2)	0	27	0
T-zone	–				T-lymphoblastic (convoluted)	69 (34.2)	19	0	5
					Unclassified	14 (6.9)	0	0	1
ML lymphoplasmocytic (-cytoid), Immunocytoma	1 (0.5)	ND	ND	ND	ML Immunoblastic				
					B-type	23 (11.4)	0	4	0
ML plasmocytic	–				T-type	5 (2.5)	1	0	0
					Unqualified	–			
ML centrocytic	3 (1.5)	ND	ND	ND					
ML centroblastic-centrocytic:									
follicular	–								
follicular and diffuse	3 (1.5)	ND	ND	ND					
diffuse	–								
Total	7 (3.5)					186 (92.1)	20	36	6

ND = Not done

[a] "True" histiocytic proliferation (9 cases) and unclassifiable (9 cases) not included

[b] Number of patients with surface markers

ment protocols from 1962 through 1978 prevented any precise correlation of histologic type with prognosis.

Malignant Lymphoma of T-Cell Line

Convoluted Lymphocytes (ML Convoluted). Of the 69 patients classified as ML convoluted, 51 were males and 18 females (Table 1) (male to female ratio 2.9:1.0). Their ages ranged from 2 to 20 years (median 11.3 years). Table 4 summarizes the anatomic distribution of the primary sites of tumor involvement at the time of diagnosis. An anterior superior mediastinal mass with or without generalized lymphadenopathy was observed in 70% of the cases. In 28 patients uni- or bilateral pleural effusion was observed, with 8 presenting with a superior vena cava syndrome.

Bone marrow and CNS involvement (Table 5) occurred mostly in patients presenting with a mediastinal mass. Nearly 60% of ML convoluted patients have experienced at least one hematologic and/or CNS relapse (or both). Malig-

Table 3. Rappaport's (modified) classification, relation of morphologic type to immunological cell type in HNL

Morphologic type	No of pts. (% of total)	Immunologic cell type		
		T	B	Null
ML undifferentiated				
Burkitt	4 (1.9)	0	2	0
Non-Burkitt	74 (35.1)[a]	0	28	0
ML lymphoblastic				
Convoluted	60 (28.4)	14	0	3
Non-convoluted	23 (10.9)	5	0	3
ML lymphocytic				
Well differentiated	1 (1.5)	ND	ND	ND
Poorly differentiated	1 (0.5)	ND	ND	ND
ML "histiocytic"	44 (20.8)[b]	1	7	0
ML "mixed"	4 (1.9)	ND	ND	ND
Total	211 (100.0)	20	37	6

ND = Not done

[a] Include 9 cases of unclassifiable according to Lukes – Collins and Kiel classifications

[b] ML "histiocytic" consist of large cell lymphoma including "trou" histiocytic proliferations (9 cases)

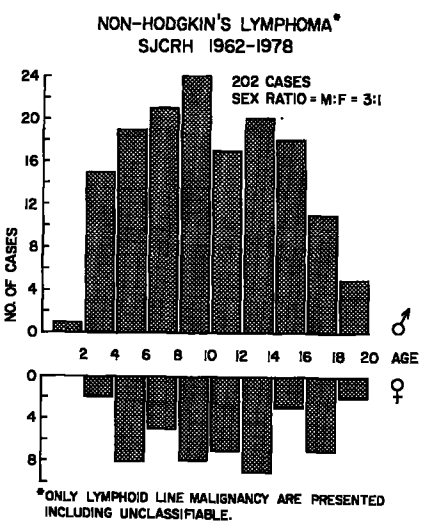

Fig. 1. Age and sex distribution of 202 children with NHL

nant lymphoma was present in spinal fluid without marrow invasion in 2 cases only.

Testicular involvement was seen in 5 patients at the time of lymphoma diagnosis and was observed in another 16 during observation and treatment. These data, however, concern only those patients in whom an attempt was made to confirm testicular involvement by biopsy.

Table 4. Malignant lymphoma of convoluted lymphocyte (69 cases). Rappaport: ML lymphoblastic convoluted (60 cases), ML lymphoblastic non-convoluted (9 cases). Kiel: ML lymphoblastic convoluted type. Anatomic distribution of primary sites of tumor involvement

Primary site	No.[a] of pts. (%)	Comment
Anterior-superior mediastinal mass (MM)	50 (70%)	In 10 cases MM associated with GL 28 cases with pleural effusion 8 cases with superior vena cava syndrome
Generalized lymphadenopathy (GL)	19 (27.3%)	10 cases with MM 2 cases with bone involvement 1 case with retroabdominal mass
Nasopharynx (NPh)	4 (5.5%)	2 cases with GL 1 case with mastoid bone destruction and hearing loss
Isolated regional Lymphadenopathy (IL)	4 (5.5%)	All in cervical and/or supraclavicular region 2 cases with BM involvement
Bone destruction	6 (8.6%)	3 cases with GL 1 case with MM 1 case with primary navicular bone destruction with subsequent GL 1 case with iliac bone involvement with retroabdominal tumor
Other	1 (1.4%)	Scalp – soft tissue tumor – subsequently GL and BM involvement

[a] Total number does not equal total number of ML convoluted because of simultaneous involvement of more than one anatomic site

Table 5. Malignant lymphoma convoluted (69 cases). Bone marrow and CNS involvement at Admission and during observation

Bone marrow and CNS involvement[a]	No. of pts. (%)	Presentation at admission			
		MM	GL	NPh	IL
Bone marrow involvement at admission	16				
Bone marrow involvement during observation					
– within 1 month	5	4	–	–	1
– within 6 months	14	12	3[b]	–	1
– within 12 months	2	1	1	–	–
– more than 12 months	3	2	1	–	–
Total bone marrow involvement	40 (57.8)				
CNS involvement at admission	5				
CNS involvement during observation					
– within 1 month	7	5	1	–	1
– within 6 months	17	14	–	1	2
– within 12 months	8	6	1	–	1
– more than 12 months	5	5	–	–	–
Total CNS involvement	42 (60.6)				

[a] Autopsy CNS and bone marrow involvement not included
[b] In two cases simultaneous GL with MM

Table 6. Malignant lymphoma convoluted type (69 cases), stage and survival

Stage	Alive	Deceased	Not known	Total
I	2	–	–	2
II	2	3[a]	–	5
III	18[b]	21	5	45
IV	4[c]	14	–	18
Total	26	38	5	69

[a] All 3 cases with subsequent bone marrow involvement
[b] In 5 cases there was testicular relapse
[c] In 2 cases CNS or testicular relapse was present

Only 7 patients (Table 6) with ML convoluted presented with anatomically localized disease (Stages I and II), and in 3 subsequent bone marrow leukemic conversion was observed. Twenty-six patients are alive, with the time of observation ranging from 13 months to 10 years (median 4.2 year).

Pathology. In 62 cases a diagnosis of ML convoluted was based on lymph node biopsy, in 1 case on testicular biopsy and in 6 cases upon the biopsy from anterior mediastinal mass, presumably representing thymic tumor.

In all cases ML convoluted displayed a diffuse proliferation. When satisfactory whole lymph node sections were available, a characteristic lobulation (but not nodularity) was apparent under scanning magnification. This delicate subdivision of the lymph node architecture was never complete in terms of nodule formation. At the periphery of the lymph node, one could often observe semilunar in shape, thin, connective tissue bands extending from the capsule into the medulla. This architectural pattern resembling thymic lobulations was never seen in other cytological types of NHL.

A "starry-sky" phenomenon appeared to be an almost constant feature of ML convoluted and seen even in tissue other than lymph node. However, in distinction from ML small noncleaved type, the distribution of "starry-sky" macrophages was extremely irregular, being present in one part of the lymph node and inconspicuous in others. It was not unusual to see, in the same case clusters of histiocytes containing or not containing cellular debris. Leukemic capsule infiltration with peripheral sinus obliteration was another constant feature in most of the cases. However, capsule destruction as disclosed by the reticulin stain was rarely observed. In nearly one-third of ML convoluted lymph nodes, residual islands of non-neoplastic lymphocytes were seen, some with germinal centers containing nondividing cleaved cells.

Under medium magnification, the proliferating cells appeared as a monotonous noncohesive growth, in some areas resembling a blood smear. Postcapillary venules often displayed a "hobnail"-shaped endothelial lining. Slight pleomorphism was seen under oil magnification (Fig. 2). The cells ranged in size, from that of medium-sized lymphocyte to as large as a reactive histiocyte. Small rims of clear cytoplasm, more visible in large cells, occasionally exhibited slight pyroninophilia. The morphology of the nucleus was that of a "primitive" lymphoblast with clear, dusty, dispersed chromatin without condensation along the nuclear envelope; sometimes the nuclear outline was extremely delicate.

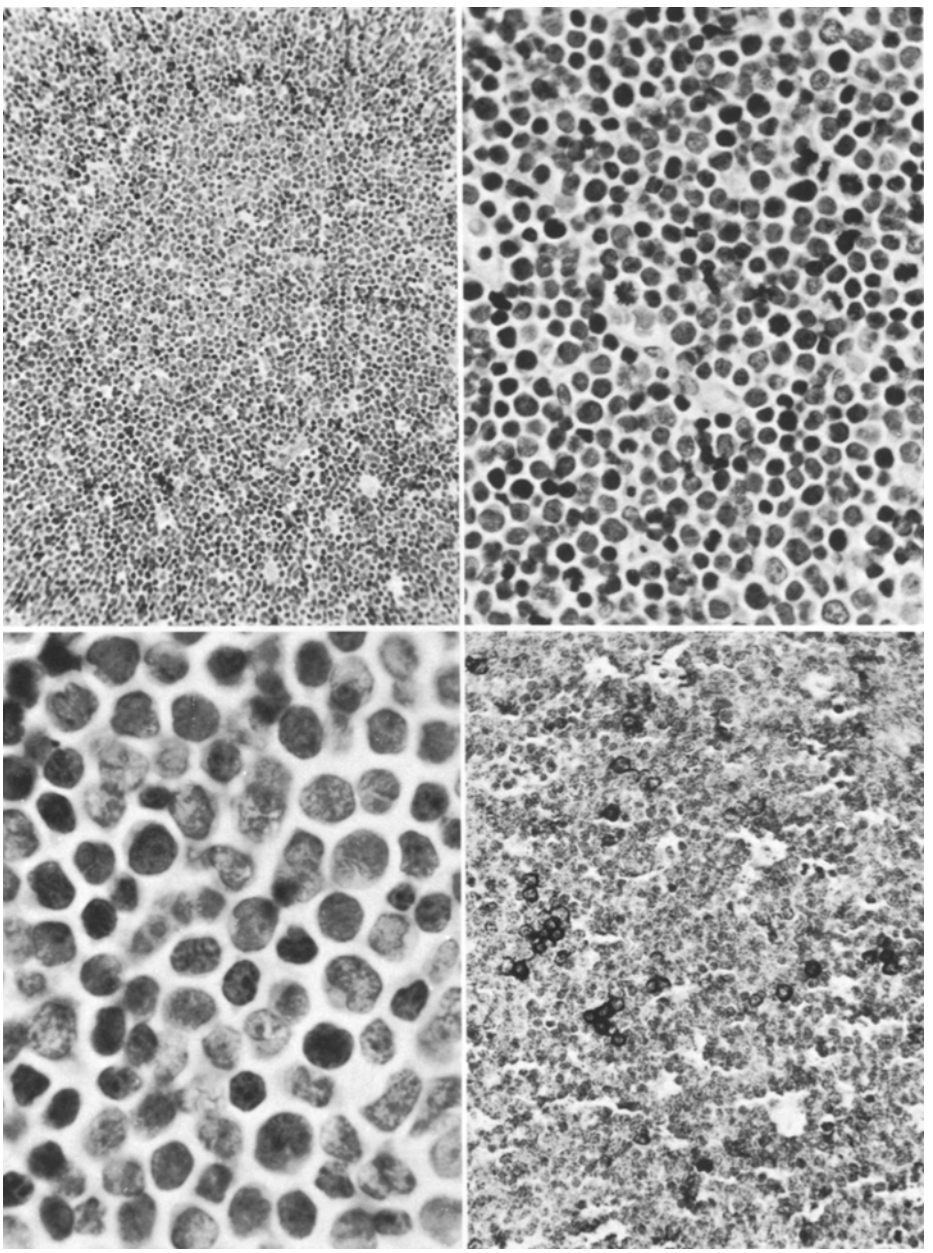

Fig. 2. ML of convoluted lymphocyte. Top: (*Left*) Diffuse proliferation of lymphocytes with scant "starry-sky" pattern and (*left*) high mitotic activity. Note differences in cell size and staining patterns. Bottom: (*Left*) Convoluted or even lobulated lymphocytes with "dusty" chromatin and inconspicuous nucleoli. (*Right*) Same case disclosing clusters of PAS positive cells. Top: (*Left*) H and E, ×160; (*right*) H and E, ×500. Bottom: (*Left*) H and E, ×1,134; (*right*) PAS-hematoxyline, ×315

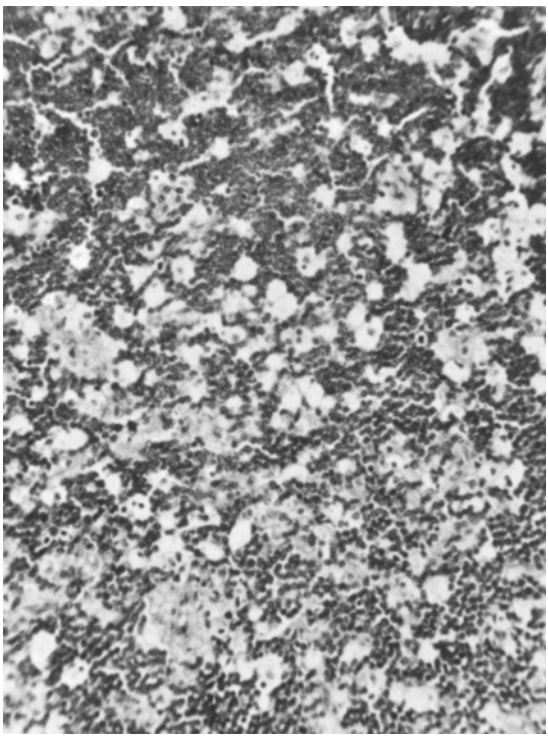

Fig. 3. ML of convoluted lymphocyte – biopsy from anterior, superior mediastinal mass. Note prominent "starry-sky" pattern with an increase in number of epithelioid venules. H and E, ×315

The number of mitotic figure was high to very high. Convolutions of the nuclei were seen with variable frequency. In 9 cases, classified as ML convoluted according to Lukes-Collins, only a few cells displayed linear subdivision of nuclei with delicate irregularities of the nuclear membrane. Those cases were put into the nonconvoluted category in Rappaport's classification. Convolutions were more discernible in PAS-stained sections. Nucleoli were inconspicuous, small, solitary, and usually eccentrically located. In some cases in which the lymph node had been fixed in Zenker's fixative, the nuclear chromatin appeared to be more clumped with less visible nucleoli.

PAS-positive, diastase-resistant granules were a prominent feature in paraffin sections of three cases of ML convoluted and a "dusty" intracytoplasmic material in another five cases (Fig. 2).

Imprints from lymph nodes bone marrow smears, and pleural effusion available from some patients were similar in cytologic appearance. One often got the impression that cytocentrifuge preparations, as opposed to smears, enhanced the linear subdivision of nuclei.

In 6 cases, surgical material was obtained from an anterior superior mediastinal mass. Cytologically, the proliferation did not differ from that seen in

lymph node, although some differences are worthy of mention. In three cases extraordinarily intensive "starry-sky" phenomenon was observed and in some areas histiocytes predominanted (Fig. 3). Large, bizzare, convoluted and sometimes lobulated cells were observed in 5 patients which we interpreted as immunoblasts. Nucleoli and amphophilic cytoplasm were often observed. Hassall's corpuscles were seen in 5 cases, most being mineralized. Contrary to the peripheral lymph node biopsy, ML convoluted tumor obtained from the thymic mass showed increased numbers of capillary venules with prominent epithelioid appearing endothelium. Necrosis was not an uncommon feature..

In both lymph node and mediastinal tumor tissues, sparse plasma cells were observed. Their presence was not related to residual lymphoid follicles because they could also be found as well within otherwise pure lymphoblastic proliferation. In several cases of ML convoluted, we observed a considerable number of eosinophils which in some oil magnification areas were almost equal in number to lymphoma cells. This peculiar pattern was not consistent and in a large number of ML convoluted cases eosinophils were difficult to find. Mast cells were also observed.

Immunological cell typing was performed in 24 cases of ML convoluted, in 19 patients tumor cells formed E rosettes and did not have detectable surface immunoglobulin. In 5 cases, neither E rosettes nor surface immunoglobulin were found and the lymphoblastic cells were described as "null cells". Two of these patients had huge mediastinal masses at admission, another two presented with generalized lymphadenopathy and one presented with isolated cervical involvement and leukemic marrow.

Immunoblastic T (IBS-T). Of 5 patients interpreted as IBS-T (4 cases correspond in Rappaport's classification to ML histiocytic and one case to ML mixed type) 4 were female and 1 was male. In two patients, a mediastinal mass was present associated with generalized lymphadenopathy, although cervical lymph node enlargement predominated in the clinical picture. Two other patients presented with a retroperitoneal mass with slight peripheral lymph node enlargement; in one L3-L4 vertebral bone destruction was observed with a cauda equina syndrome. One patient presented with localized cervico-supraclavicular lymphadenopathy of 10 years duration. Three previous biopsies did not show evidence of malignancy.

At the time of diagnosis, the bone marrow was not involved in any of these patients. However, in two, malignant cells described as malignant histiocytes were found in marrow smears after one month and six months of observation. Three patients died within 12 months with disseminated disease. Two others are well after observation, one for more than 9 months and the other for more than 33 months.

Pathology. In all 5 patients, malignant proliferation was located in the deep cortex and in the interfollicular areas (Fig. 4). That architectural growth pattern coupled with the constellation of cytologic appearances of the proliferating cells were of greatest value in establishing the diagnosis of IBS-T. Residual non-neoplastic lymphoid islands of non-modulating cleaved lymphocytes (cen-

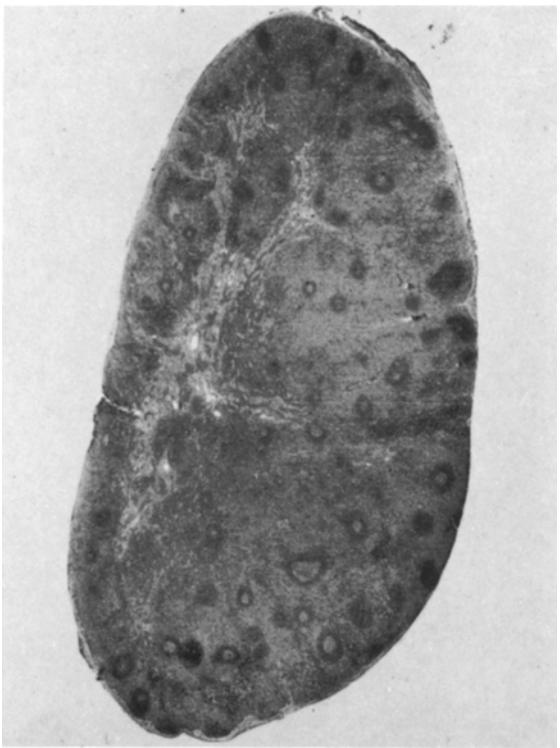

Fig. 4. ML Immunoblastic of T cell. Malignant proliferation situated in the interfollicular areas (*T*—dependent zone) with residual non-neoplastic follicules (*B*—dependent zone). H and E, ×12

trocytes) were present in all cases. The malignant proliferation exhibited a slight pleiomorphism and differences in cell sizes (Fig. 5). Abundant, very clear cytoplasm without conspicuous granulations was apparent in large cells. One of the outstanding features of proliferating immunoblasts was extremely well defined ("pencil-drawn") cytoplasmic borders between the cells, which were more discernible in PAS-stained sections. Nuclei, mostly vesicular in appearance, displayed variable configurations of the nuclear envelope. In most instances, however, only slight irregularities were seen. Clear subdivisions or lobulations comparable to these seen in ML convoluted were not observed. Distinct and prominant nucleoli of different size, from one to three in number, were consistently present. Nuclear chromatin in smaller cells resembled "dusty" patterns and in larger ones tended to clump along nuclear membrane. Mitotic activity differed from field to field, but mitotic figures were always easily found. The "starry-sky" phenomenon was not observed in any case. In 3 cases however, a considerable number of histiocytes were recognized because of their less distinct cellular border, lack of cohesiveness, oval or kidney-shaped nucleus, lack of prominent nucleoli and slightly granular cytoplasm. This population of non-neoplastic cells was confirmed to be histiocytic in origin by demonstration

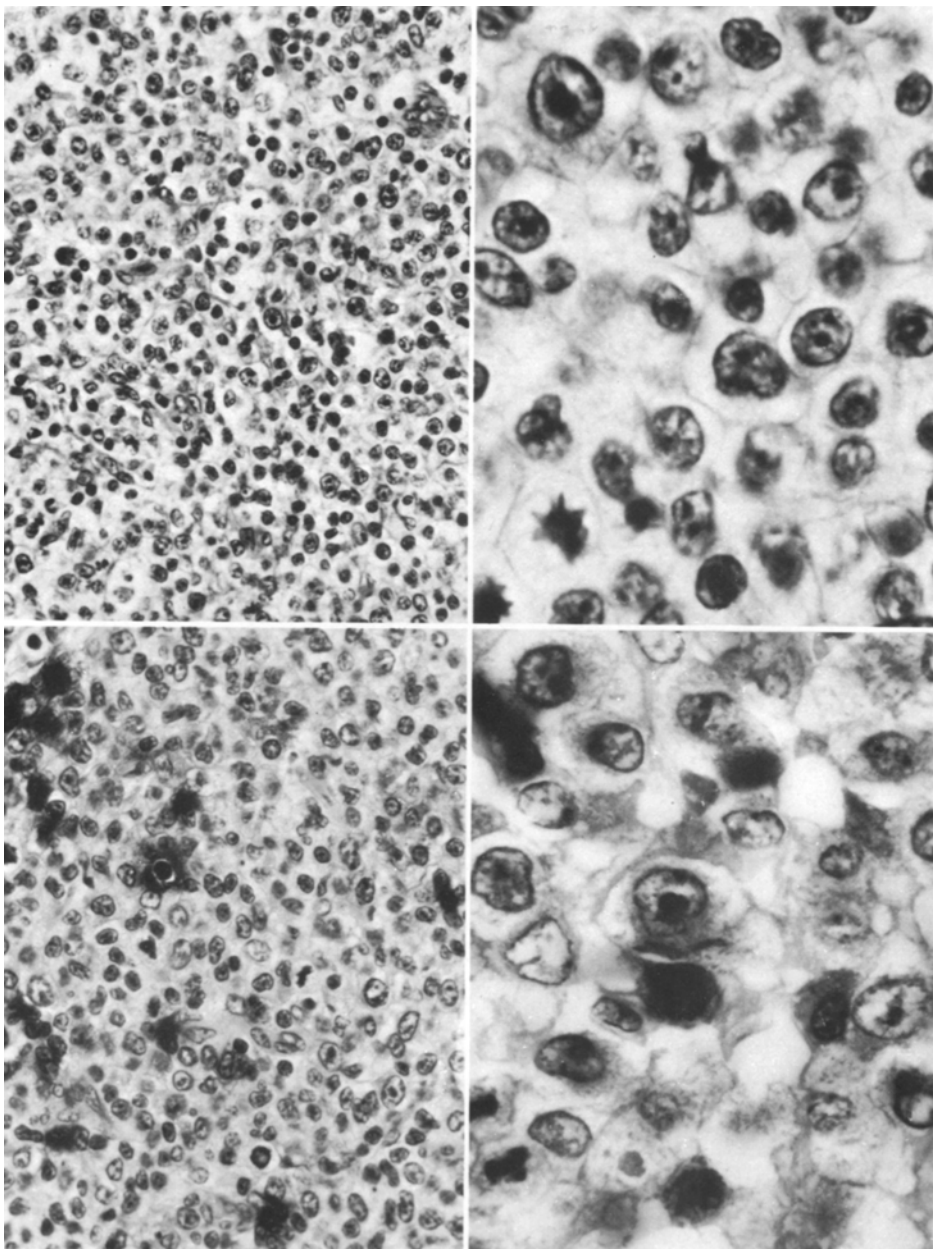

Fig. 5. ML Immunoblastic T. Top: (*Left*) Predominantly large cell proliferation with clear cytoplasm showing slight compartmentalization by postcapillary venules. (*Right*) Cytologic details of immunoblasts T showing prominent cytoplasmic borders, clear, abundant cytoplasm and variations in nuclear shape. Bottom (*left*) PAP reaction with antimuramidase (lysozyme) and (*right*) PAP with anti-kappa seras remain negative in large cells compartment. Note the positive (dark) plasma cell. Top: *Left* H and E, ×260; *right* H and E, ×1,452. Bottom: *Left* PAP – with anti muramidase – hematoxylin, ×572, *right* PAP – with anti lambda-kappa hematoxylin, ×1,512

of intracytoplasmic lysozyme (muramidase) (Fig. 5). In two other cases, histiocytes were seen as 2–5 cell clusters near postcapillary venules. Again, as in ML convoluted, eosinophils were a constant finding in all confirmed IBS-T.

Malignant proliferating immunoblasts showed no evidence of cytoplasmic immunoglobulin. The apparently mature plasma cells intermingled with the T-immunoblasts were clearly positive, disclosing a polyclonal pattern. In two cases plasma cells were numerous and located near postcapillary venules. In only one case was cell surface typing performed, and the malignant immunoblasts were positive for E rosette formation.

Malignant Lymphoma of B Cell Line

As seen in Table 1, 106 cases (58%) were classified as B cell proliferation. Follicular center cell lymphoma of the small non-cleaved type, diagnosed in 65 patients, was the most frequent cytologic type. Lymphocyte surface markers were studied in 36 patients with morphologically designated B-cell lymphoma, and surface immunoglobulin was identified in each instance. No discrepancy was noted between immunologic and morphologic cell typing (Tables 1 and 2).

Plasmacytoid Lymphocyte (ML PL). Only one validated ML PL was encountered. Morphologically, this proliferation corresponds to ML well differentiated with plasmacytoid features in Rappaport's classification (Table 3) and to ML lymphoplasmocytic (immunocytoma) in the Kiel classification (Table 2). Ileocecal tumor was discovered and incompletely resected in a 9-year-old boy with an acute abdomen. The child was considered to have Stage III disease without bone marrow or CNS involvement but with clinical signs of questionable malabsorption, with weight loss and dysproteinemia manifested by elevated IgA. He remains alive 9 months after diagnosis.

The intestinal lesion was described as an anular proliferation involving approximately 7 cm of terminal ileum and 10 cm of ascending colon including the ileocecal junction. Cytologically, ML-PL consisted of small lymphocytes (which predominated), immunoblasts, plasmacytoid lymphocytes and plasma cells (Fig. 6). PAS staining showed diastase-resistant intracytoplasmic globules that were present in plasma cells forming Russel's bodies. Immunoperoxidase (PAP) showed that PAS-positive inclusions corresponded to IgA-kappa type immunoglobulin. The same class of immunoglobulin was found within large malignants cells. Several lymph nodes adjacent to the ileocecal mass displayed essentially the same histopathologic and immunopathologic patterns. intestinal mucosa adjacent to ML-PL revealed an abundant plasma cell infiltrate containing the same heavy and light chain as cells in the tumor mass. Duodenal mucosa, obtained by Cosby capsule biopsy, did not show villous abnormalities. A few plasma cells were noted in lamina propria and most of them shared the same IgA-kappa characteristics as ileocecal ML-PL.

Evaluation for secretory piece of IgA, which is normally produced by enterocytes, disclosed a lack of normal production in the epithelial cells covering the tumor mass and in the vicinity of tumor. Conversely, enterocytes in the duodenal biopsy reacted strongly with anti-secretory component serum.

Follicular Center Cell, Small Non-Cleaved (ML(FCC)SNC). ML(FCC)SNC was the most common cytologic type of B cell line proliferation, being diagnosed

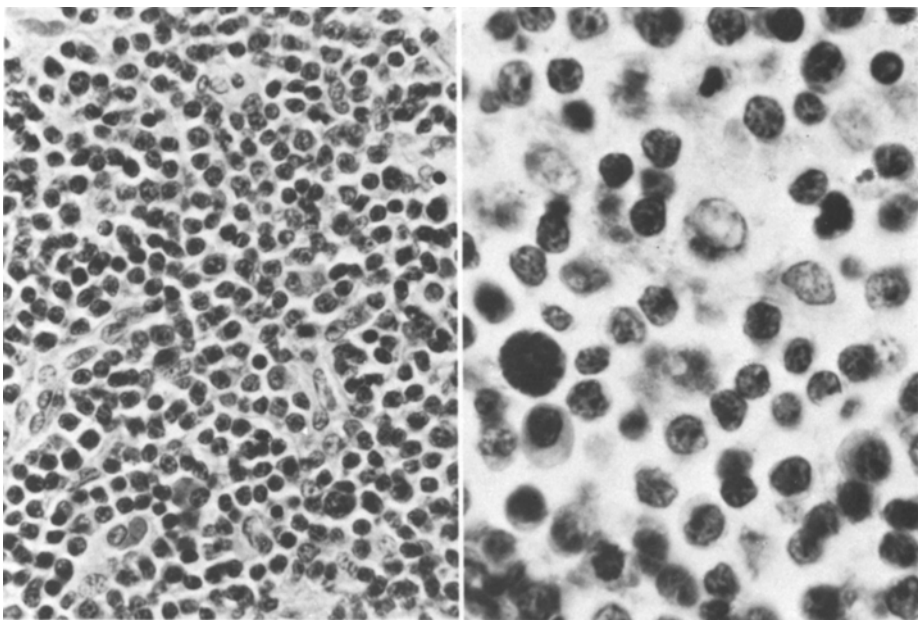

Fig. 6. ML of plasmacytoid lymphocyte (or ML lymphocytic well differentiated with plasmacytoid features (Rappaport) or Immunocytoma (Kiel) (*left*) Ileocecal tumor demonstrating a mixed proliferation of lymphocytes, non-cleaved transformed lymphocytes, plasmocytoid lymphocytes and plasma cells which (*right*) show intracytoplasmic, PAS positive inclusions which are immunoglobulins (IgA-kappa). (*Left*) H and E, ×280; (*right*) PAS – hematoxylin, ×1,134

in 65 cases. ML(FCC)SNC corresponds to ML undifferentiated by Rappaport's criteria. In 4 patients, Burkitt's type lymphoma was diagnosed, with the remaining 61 fitting the category of undifferentiated non-Burkitt lymphoma. According to the Kiel classification, 61 cases showed the features of ML lymphoblastic B cell type and 4 the ML lymphoblastic Burkitt's type. In 27 of these cases, determination of cell surface markers confirmed the B cell origin of the tumor cells, which demonstrated surface immunoglobulin and a lack of E rosette formation.

Among 65 cases of ML(FCC)SNC, 52 were males and 13 female (Table 1) with male-to-female ratio 4.2:1. Four children were black and 61 white. The ages of the patients ranged from 23 months to 18.7 years (median 9.1). The primary site of disease was the gastrointestinal tract in roughly 60% of the cases (Table 7). All proliferations classified as Burkitt tumor presented as ileocecal region lymphoma.

Bone marrow involvement was present at the time of diagnosis in 9 patients, each with unresectable gastrointestinal lymphoma, all of whom died within 12 months. In 12 other patients, bone marrow was the site of relapse. In 30 patients considered to have stage III disease (Table 8), 26 had gastrointestinal lymphoma with bone marrow involvement observed in 19 (or 78%). CNS involvement in ML(FCC)SNC was observed in 17 patients within 6 months of

Table 7. Malignant lymphoma (FCC) small non-cleaved (65 cases). Rappaport: ML undifferentiated: Burkitt 4; ML undifferentiated: non-Burkitt 61. Kiel: ML lymphoblastic B type 61; ML lymphoblastic Burkitt type 4. Anatomic presentation at the beginning of observation

Anatomical site pts.	No.[a] of (%)	Comment
Mediastinal anterior superior mass (MM)	1 (1.5)	
Isolated regional lymphadenopathy (IL) (cervical-supracl.)	10 (15.3)	1 with BM involvement at admission, 2 with nasopharyngeal tumor coexist.
Generalized lymphadenopathy (GL)	14 (21.5)	2 with GIT tumor, 1 with epidural tumor
Gastrointestinal tract (GIT)	–	
– stomach	1	
– terminal ileum	19	1 with GL in 2 with epidural tumor
– ileocecal region	4	1 with GL
– colon	1	
– other NOS	8	
Abdomen (NOS)	5	
	38 (58.57)	
Nasopharynx (including oral cavity) (NPh)	5 (7.6)	1 with primary gingival presentation, 2 with GL

[a] Number does not equal number of ML(FCC)SNC cases because of simultaneous involvement of more than one anatomical site

Table 8. Malignant lymphoma (FCC) small non-cleaved stage of disease and survival

Stage	Alive	Deceased	NOS	Total
I	7	–	–	7
II	12	3[c]	1	16
III	7[b]	15	8	30
IV	3	8	1	12
Total	29	26	10	65

[a] 1 Case with present CNS relapse
[b] 1 Case with present CNS relapse
[c] 1 Case died from *Pneumocystis carinii* pneumonia – in complete remission

diagnosis (Table 9). Ten presented at admission with unresectable gastrointestinal disease. In 5 patients, an epidural mass was the predominant anatomic location of relapse. This particular growth behavior was not observed in other cytologic types of NHL in our study. Twenty-nine patients are alive, with the time of observation ranging from 10 months to 7 years (median 4.8 years).

Pathology. In only one case was minimal nodularity noted. The remaining cases showed a diffuse growth pattern. The "starry-sky" pattern was a constant feature and majority of ML(FCC)SNC bore at least a superficial resemblance to classic Burkitt's tumor. However, variations in nuclear size, slight irregularities in the shape of the nuclear envelope, and variation in the size of the cytoplasmic

Table 9. Malignant lymphoma (FCC) small non-cleaved bone marrow and CNS involvement at admission and during observation[a]

Bone marrow or CNS involvement	No. of pts. (%)	Comment
Bone marrow involvement at admission	9 (13.8)	1 case with simultaneous CNS involvement
Bone marrow involvement during observation		
– within 1 month	6 (9.2)	All III°, 5 patients with GIT lymphoma and 1 with GL at presentation
– within 6 months	4 (6.1)	All III°, 4 patients with GIT lymphoma, 1 with abdominal NOS disease
– within 12 months	1	II°, nasopharynx
– above 12 months	1	III°, GIT
Total bone marrow involvement	21 (32.3)	
CNS involvement at admission	1	With BM simultaneously, 1 case with epidural tumor
CNS involvement during observation		
– within 1 month	10 (15.3)	2 with II° cervical and NPh location, 4 with III° all GIT, 4 with II°, 2 GIT, 2 others with GL at admission, in 2 epidural mass
– within 6 months	6 (9.2)	5 with III°, 4 with GIT lymphoma and 1 with GL, 1 with II° and GIT
– within 12 months or above	0	
Total CNS involvement	17 (26.5)	

[a] Multiple relapses in same patients as well as BM and CNS involvement at autopsy is not presented

rim were the main reasons why only 4 cases were classified as Burkitt's type lymphoma. The basic cell proliferating in ML(FCC)SNC was a medium-sized lymphocyte; however, after a careful search, it was not unusual to find some cells that were the size of the nucleus of reactive histiocytes. In a few instances, mainly in the gastrointestinal lymphoma group, clusters of such cells constituted 20–25% of the ML(FCC)SNC cell compartment and there was some question as to whether these cases should be classified as small non-cleaved or as large non-cleaved lymphomas in Lukes' terminology, as a primary centroblastic lymphoma in Kiel terminology, or as a histiocytic type by Rappaport's criteria. The slightly basophilic, small rim of cytoplasm characteristically displayed vacuolations in paraffin sections, these were better seen in imprints. No PAS-positive intracytoplasmic granules were noted in any of the ML(FCC)SNC cases studied. A prominent nuclear membrane enveloped a rather condensed, clumped chromatin, containing one to three small, but distinct nucleoli. Degener-

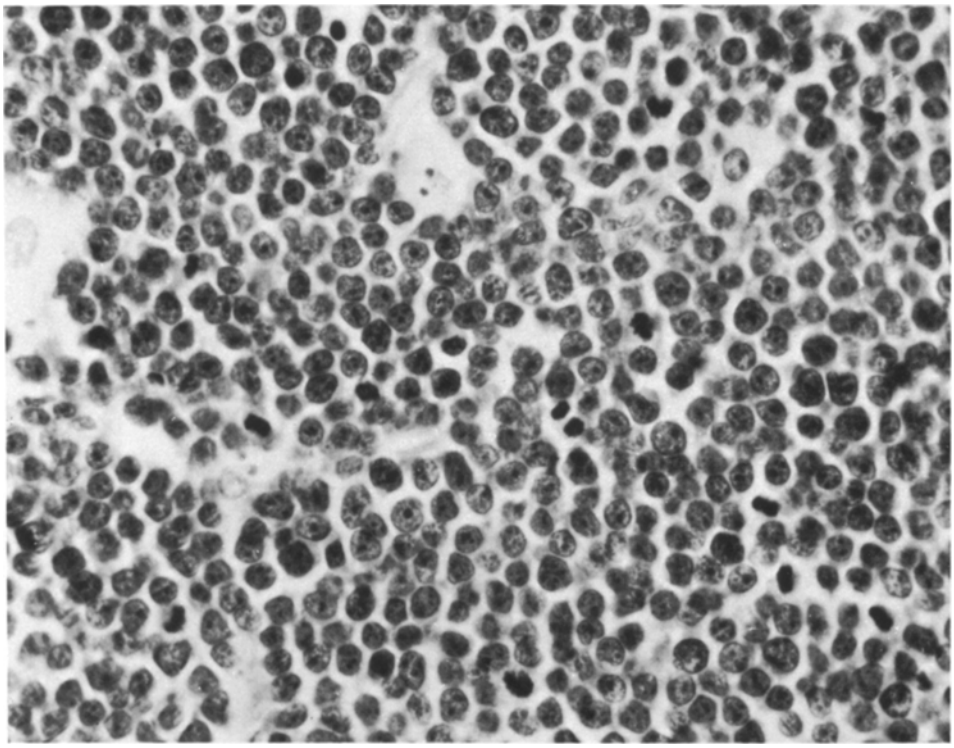

Fig. 7. ML (FCC) Small non-cleaved cell presented as intestinal tumor and subsequently converted to ALL. This tumor corresponds to ML lymphoblastic of B cell (Kiel) or to ML undifferentiated non-Burkitt (Rappaport). H and E, ×1,200

ative changes in individual cells were often seen, but not as often and with such intensity as in ML(FCC) large non-cleaved. High mitotic activity was a constant feature of all ML(FCC)SNC (Fig. 7). In only two cases was an admixture of cleaved cells noted.

There was no difference in cellular morphology between the intestinal lymphomas and tumor in adjacent lymph nodes. Subsequent biopsies in distant areas disclosed a similar histology. In 6 cases, however, postmortem examination indicated evolution of small noncleaved lymphoma to large noncleaved type.

Eosinophils were a constant component of intestinal ML(FCC)SNC but were rarely noted in lymph node distant from the abdominal cavity. Plasma cells were present in most intestinal ML(FCC)SNC and were interpreted as a non-neoplastic inflammatory and/or reactive infiltrate. In most instances, plasma cells were seen at the advancing edge of lymphoma or within intestinal lamina propria covering the tumor mass, sometimes being admixed with lymphoma cells. We did not observe any transitional cells between small noncleaved lymphoma cells and plasma cells, such as immunoblasts or lymphoplasmacytoid cells, as were seen in the intestinal ML-PL. Non-neoplastic plasma cell infiltrate was constantly observed especially in cases where the intestinal mucosa showed a lymphoid follicular hyperplasia adjacent to the tumor mass (Fig. 8). This particular morphologic finding was observed in 18 cases of intestinal ML(FCC)SNC. In the 20 remaining patients with intestinal small noncleaved proliferations, intestinal mucosa adjacent to tumor was

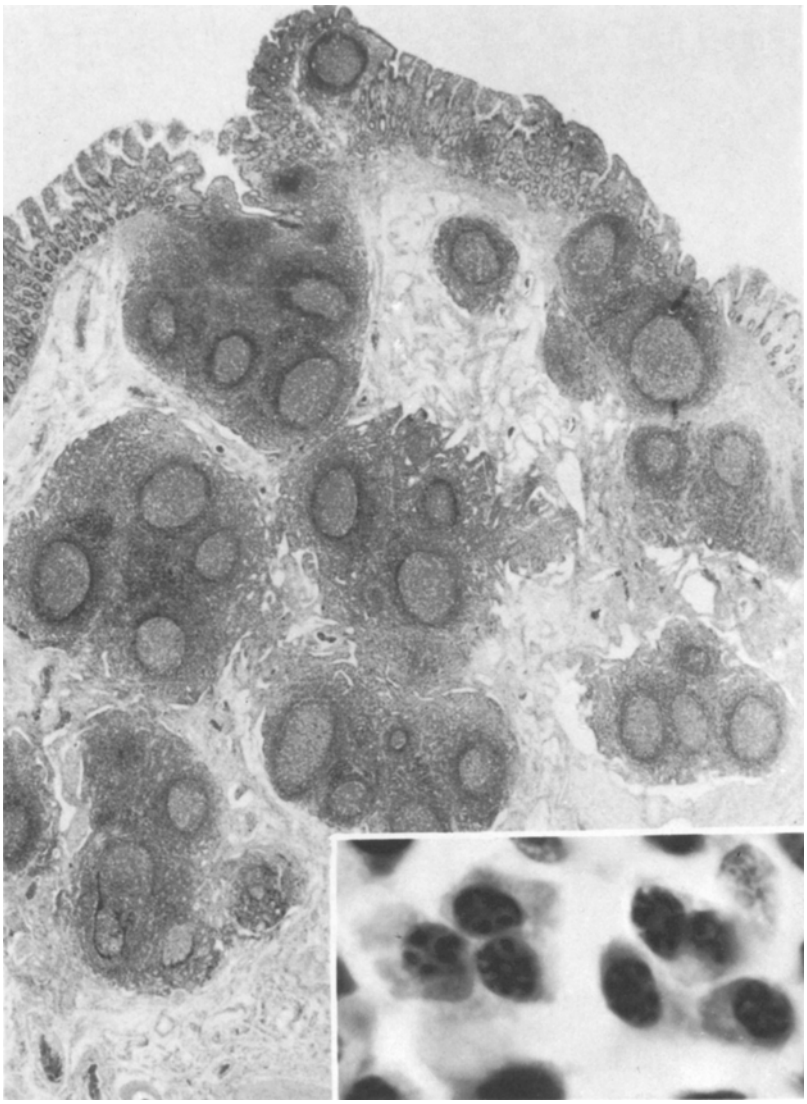

Fig. 8. Nodular lymphoid hyperplasia of the jejunum in the vicinity of a ML(FCC)SNC. Well-defined hyperplastic follicules merge with a lamina propria infiltrated by mature plasma cells (*inset*). H and E, ×12, *inset* ×1,404

not available for adequate assessment. Immunoperoxidase (PAP) immunoglobulin investigation disclosed that the dominant heavy chain being produced by large cells of hyperplastic nodules was IgM. The plasma cell infiltrate showed a polyclonal pattern, with IgM as a predominating heavy chain.

An investigation for the secretory component of IgA was performed in 16 ML(FCC)SNC cases in which viable intestinal mucosa covering the tumor

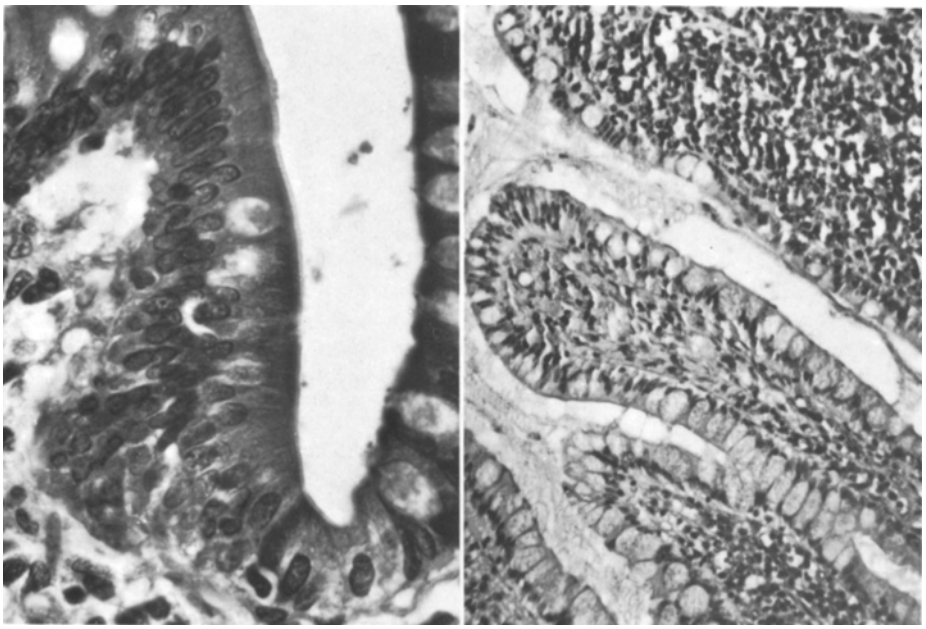

Fig. 9. (*Left*) Secretory component (SC) within enterocytes in normal intestinal mucosa visualized by anti-secretory component serum. (*Right*) Intestinal epithelium infiltrated by ML(FCC)SNC. Note absence of reaction in covering epithelium for SC. (*Left*): PAP with anti-SC, hematoxylin, ×612; (*right*): PAP with anti-SC, hematoxylin, ×520

and adjacent area was available. The component could not be demonstrated in enterocytes covering the tumor mass, but was evident, although with a low reaction intensity, in non-involved adjacent intestinal mucosa (Fig. 9). We were able to demonstrate that the intensity of the reaction in epithelial cells gradually diminished in the adjacent, normal mucosa moving toward the viable mucosa covering the lymphomatous mass.

Three of 24ML(FCC)SNC cases studied postmortem showed multifocal lymphoma along the gastrointestinal tract, forming isolated fungoid masses ranging from 3 mm to 3 cm in diameter. In two of these, the primary malignant lymphoma had presented as resectable disease. The third patient presented with a retroperitoneal tumor that histologically was considered to be a Burkitt's type lymphoma. In none of these cases was the primary tumor area involved at the time of autopsy.

Follicular Center Cell, Large Non-Cleaved (ML(FCC)LNC). Ten cases were classified as ML(FCC)LNC. Four belonged to the ML "histiocytic" category, 6 to ML undifferentiated and 1 to ML "mixed" in Rappaport's classification. All of them are ML centroblastic by Kiel classification criteria.

Nine patients were male and one was female (Table 1). Predominant (in 7 patients) anatomic localization of ML(FCC)LNC were the distal jejunum and the ileocecal region. Bone marrow

Table 10. Malignant lymphoma (FCC) large non-cleaved

Stage	Alive	Deceased	NOS	Total
I	1	–	–	1
II	3[a]	–	1	4
III	–	4	1	5
IV	–	–	–	–
Total	4	4	2	10

[a] Nodular and diffuse in 1 case

and CNS were not involved at admission in any case, however, marrow involvement was observed within 2 months after diagnosis in 2 patients and within 6 months in another. All of these presented as gastrointestinal lymphoma. CNS involvement occurred in 2 patients within 6 months and both initially had unresectable intestinal lymphoma. Four patients died within 6 months of diagnosis; all were unresectable gastrointestinal tumors (Table 10). Four patients, all of whom presented with localized disease, are alive from 14 to 74 months after diagnosis (median 31 months).

Pathology. One case disclosed vague nodularity of the lymph node architecture being predominantly diffuse (Fig. 10). The remaining 9 cases revealed a diffuse proliferation of medium to large cells whose sizes approximated that of a histiocyte. The nuclear size in some cases was similar to that observed in small non-cleaved type, but there was abundant cytoplasm which showed extensive pyroninophilia (or giemsophilia). Cell borders were easily recognizable. Small intracytoplasmic vacuoles, similar to those seen in small non-cleaved type were observed, but not as frequently. The "starry-sky" pattern was present in 8 cases and the histiocytes responsible for that phenomenon were positive with anti-lysozyme sera. Round-to-oval nuclei with prominent nuclear membrane gave a vesicular appearance to some of the nuclei. There were 2 to 4 small distinct nucleoli usually opposed to the nuclear membrane. High mitotic activity was a constant feature. Necrotic areas were not seen, however, individual cells necrosis was common. An admixture of small non-cleaved cells was perceptible in a few instances, but cleaved cells were not observed. A unique partly follicular ML(FCC)LNC displayed a mixture of small non-cleaved and large non-cleaved cells with a predomination (over 50%) of the last ones. For that reason, this particular case has been classified as a mixed-type lymphoma in Rappaport's scheme.

Eosinophils and plasma cells were observed frequently in gastrointestinal ML(FCC)LNC cases. In four cases a considerable number of plasma cells were seen in adjacent to the tumor edge intestinal mucosa as well as in lamina propria covering the tumor mass. With the immunoperoxidase technique, the plasma cell infiltrate was found to have a polyclonal pattern. The main heavy chains present in the plasma cells were IgM and IgA. Mucosal ulceration was evident in 2 cases. Focal follicular lymphoid hyperplasia, as seen in small noncleaved lymphoma, was demonstrable in only one case because in others the quantity of intestinal wall adjacent to main tumor mass was inadequate for examination. In one instance, lymphoid follicles were trapped within the substance of lymphoma. The evaluation for the secretory component of IgA again failed to disclose this protein within epithelial cells covering the tumor mass. Immunological cell typing was done in only five patients and confirmed appurtenance of proliferation to the B cell line. Immunoperoxidase (PAP) immunoglobulin estimation with monospecific sera demonstrated that kappa light chains

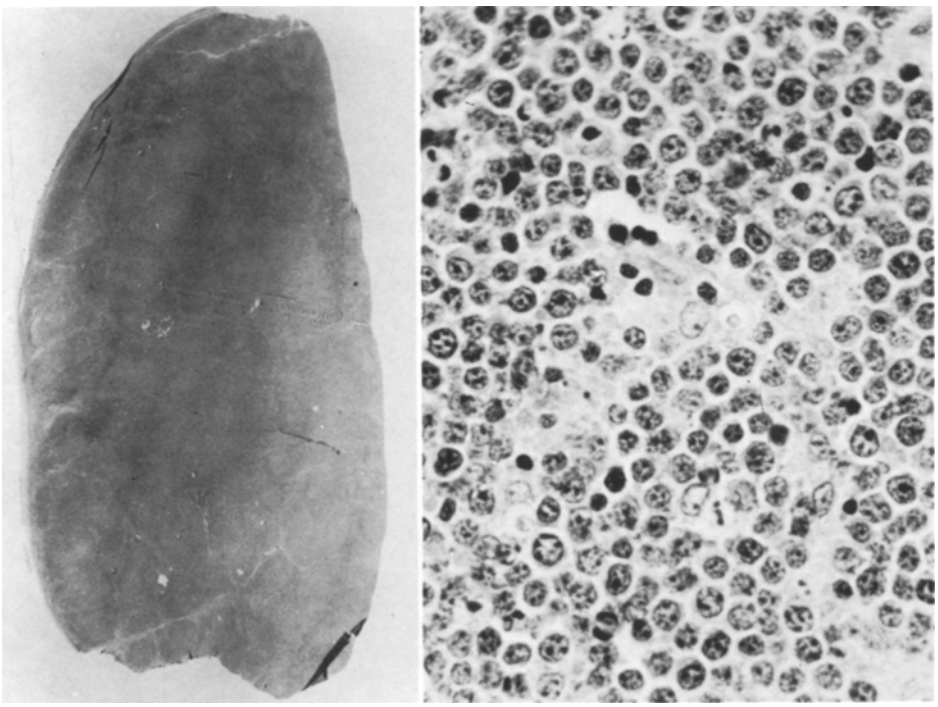

Fig. 10. ML (FCC) Large Non-Cleaved. (*Left*) Nodular and (predominantly) diffuse growth pattern within a lymph node. (*Right*) Large non-cleaved cells (or centroblasts using Kiel criteria) with prominent nuclear membrane, and 1–3 nucleoli opposed to nuclear envelop. *Left*: H and E, ×13; *right*: H and E, ×500

and μ heavy chain were the predominant class of immunoglobulin. No lysozyme (muramidase) positive cells were noted within malignant lymphoid proliferation – contrary to eosinophils and "starry-sky" histiocytes, which remained strongly positive.

Autopsy records and histology were available in three cases. Two of these displayed a cytologically pleomorphic pattern, which in one case was considered a possible evolution to immunosarcoma of B cell. In both cases histiocytic leukemia had been postulated as a consequence of "histiocytic" lymphoma and/or reticulum cell sarcoma generalization.

Immunoblastic B (IBS-B). IBS-B accounted for 23 cases, being the second most frequent of the B cell line proliferations. Thirteen patients were male and 10 female (sex ratio M:F=1.3 to 1.) (Table 1). The youngest patient was 4 years old, the oldest 18 years old. The main anatomic regions involved by IBS-B are summarized in the Table 11. In 14 patients IBS-B presented at admission as regional, localized disease. A unique extralymphatic non-Hodgkin's lymphoma was the IBS-B that presented as a soft tissue foot lesion. In two patients, IBS-B was observed as a second malignancy.

Unequivocal bone marrow involvement at the time of diagnosis was found

Table 11. Malignant lymphoma immunoblastic B (23 cases). Rappaport: ML "histiocytic", Kiel: ML immunosarcoma B. Anatomical sites involved at the time of diagnosis

Anatomical site involved	No. of pts.	Comment
Isolated lymph node involvement (IL)	8	One patient with ALL in remission diagnosed 7 years before IBS-B
Generalized lymphadenopathy (GL)	6	One patient with eosinophilic granuloma of femur diagnosed 2 years before development of IBS-B
Gastrointestinal tract lymphoma (GIT)	7	Two patients presented GL; five with unresectable
Nasopharynx (NPh)	1	
Extranodal (foot lesion)	1	Subsequently presented retroabdominal mass
Bone marrow (BM) involvement at diagnosis	2	
Bone marrow involvement during observation	3	In all during first month of observation – all presented GL at admission
CNS involvement at admission	2	In one intracranial tumor growth with GL and BM involvement; in other primary site localized in distal ileum with GL – tumor cells only in CSF
CNS involvement during observation	2	One after 26 months

Table 12. ML immunoblastic B – stage and follow-up

Anatomical stage	Alive	Deceased	NOS	Total
I	3	1	–	4
II	2	2	1	5
III	2	2	6[b]	10
IV	1	2	0	3
Total	8	7	7	22[a]

[a] One case with extranodal primary site (foot lesion) not included
[b] One with questionable BM involvement

in one patient and was probable in one other. In three other patients, marrow involvement occurred within one month of observation. All 5 patients presented a generalized disease upon admission. The central nervous system was involved in 2 patients at admission. One presented with intracranial (subdural) solid tumor growth with simultaneous marrow involvement. In two others CNS involvement was reported as a late site of relapse after 12 and 26 months of treatment.

Eight patients are reported to be alive from 9 to 41 months (median 30 months); in 7 follow-up observations were not available (Table 12).

Pathology. Morphologic identification of IBS-B were based solely on histology. In all cases a diffuse proliferation was observed – with some degree of trabecular

sclerosis in 3 instances. The basic cell proliferation consisted of large cells (25 to 35 μm in diameter) with abundant, pale-to-amphophilic but pyroninophilic cytoplasm with an oval-to-round vesicular nucleus containing one or two, large, prominent, usually centrally located nucleoli (Fig. 11). Despite superficial monomorphism, IBS-B usually comprised some bizzare bi- or multinucleated cells resembling Reed-Sternberg cells. A "starry-sky" pattern was seen in four cases. The histiocytic origin of those cells was confirmed by a positive immunoperoxidase reaction with antilysozyme.

The intensity of plasma cell modulation differed even within a single section. Multinucleated plasma cells were rarely observed. Plasmacytoid cells were seen frequently, however, not in all cases. Large, noncleaved cells were observed as the predominant cell in two cases, although islands of immunoblasts (consisting of 20–25 cells) and diffuse immunoblastic modulation in several areas inclined us to favor IBS-B.

Immunomorphologic assessment of IBS-B is summarized in the Table 13 and shown in Fig. 12. Lysozyme (muramidase) was not identified within the immunoblast's cytoplasm in any case. The only lysozyme-positive cells were granulocytes, macrophages and probably dendritic reticular cells. In 15 cases immunoblastic proliferation expressed monoclonality kappa and 8 cases were monoclonal with lambda chain. In 3 cases immunoblasts showed bitypic staining features.

Follicular Center Cell, Small Cleaved [ML(FCC)SC]. Three cases have been classified as ML(FCC)SC type and all disclosed a follicular (nodular) growth pattern (Fig. 13). Two were classified according to Rappaport as ML "mixed" type nodular and diffuse, one as ML poorly differentiated, and all as ML centroblastic-centrocytic according to Kiel criteria.

The ages of the patients were 4, 5, and 17 years. All three patients had a 3- to 4-month history of chronic lymphadenopathy (cervical and/or supraclavicular) and in two, the first lymph node biopsy was interpreted elsewhere as a nonspecific lymphadenitis. Bone marrow involvement was observed in one patient. All are alive from 7 to 10 years.

Pathology. The lymph node architecture was entirely replaced by neoplastic cells in one case; in two others residual, compressed lymphoid tissue was evident at the periphery of the lymph node. In all cases, the lymphoma was composed of nodules with well-defined borders and an expansive type of growth. Internodular spaces showed prominent vascularity due to displacement of capillaries by enlarging tumor follicles. Neither sclerosis nor capsule invasion was observed in any ML(FCC)SC. Small cleaved cells within the nodules predominated in all patients. Large cleaved and large noncleaved cells did not exceed 20% of the nodule cells component. The mitotic rate was low and if mitosis was observed, it occurred in large cells.

Follicular Center Cell, Large Cleaved [ML(FCC)LC]. Three cases were classified as ML(FCC)LC. All belonged to the category of ML "histiocytic" type by Rappaport's criteria and ML centrocytic (large cell) by the Kiel classification.

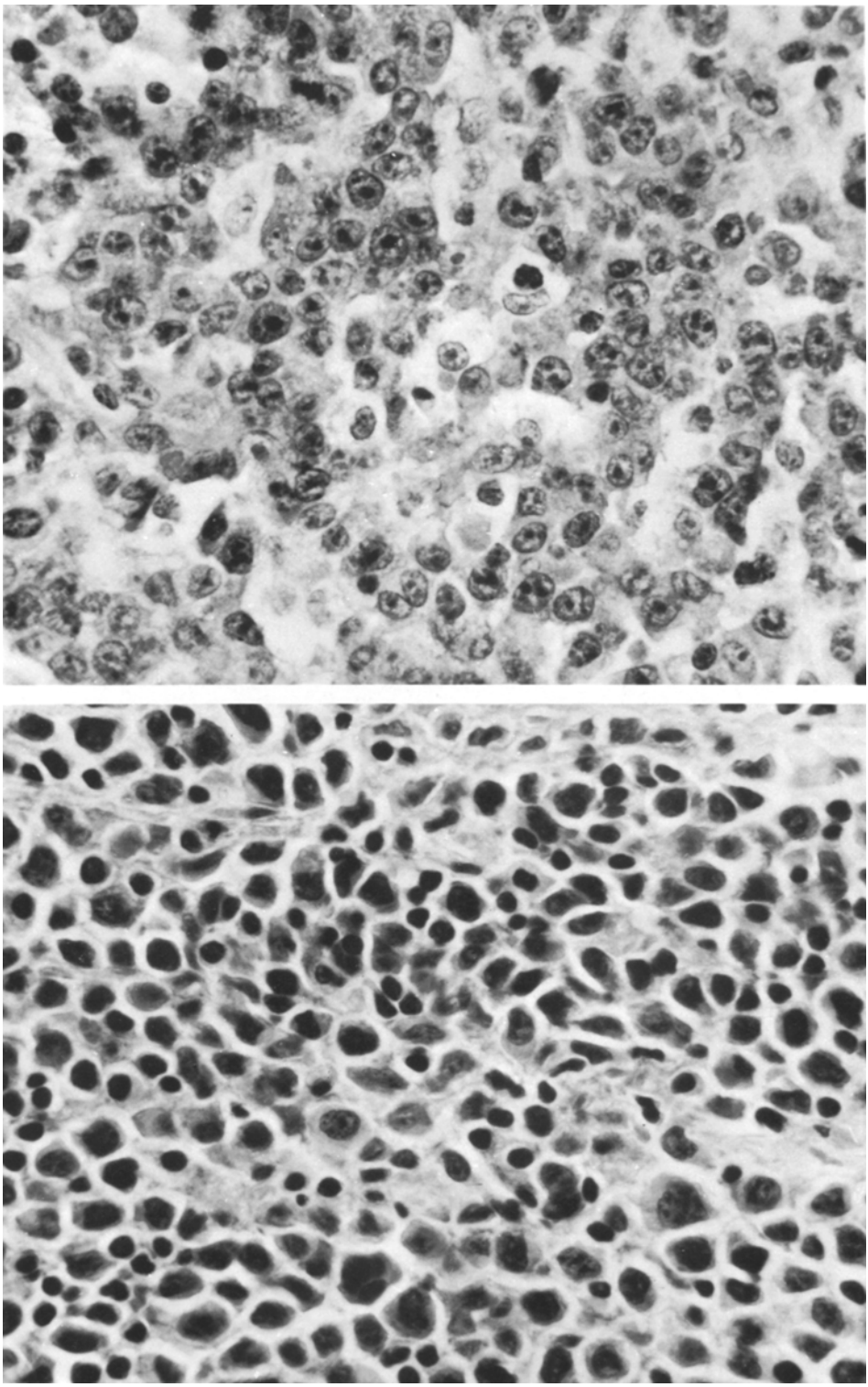

Fig. 11. ML Immunoblastic B cell. Top: Diffuse proliferation of large ("histiocytic" according to Rappaport) cells with abundant cytoplasm and prominent nucleoli. Bottom: ML Immunoblastic B presented as a food lesion. Note the pleiomorphism of the large cells and somewhat plasmocytoid features. Top: H and E, ×1,092; Bottom: H and E, ×1,092

Table 13. Large cell proliferations: cell surface and immunopathologic evaluation

Diagnosis (Lukes-Collins classification) (n=number of cases)	No. of pts. with immuno-pathologic studies	No. of pts. with cell surface markers			Immunopathology (No. of pts.)					
		T	B	Null	Muram.	Kappa	Lambda	IgA	IgG	IgM
ML (FCC), large non-cleaved n=10	9[a]	–	2	–	H+Gr	7	4	2	–	7
ML immunoblastic T n=5	5	1	–	–	H+Gr	PC	PC	–	PC	–
ML immunoblastic B n=23	22[b]	–	4	–	H+Gr	15	8	–	17	3
Histiocytic (true) proliferation n=9	9	–	–	–	9	PC	PC	–	–	–
Total n=48	45	1	6	–	9	22	12	2	17	10

PC=plasma cells; GR=eosinophilic granulocytes; H=histiocytes

[a] In 1 case only light chains estimation

[b] In 2 cases only light chains estimation; in 3 bitypic

The ages of patients were 4, 5, and 7 years, respectively. All presented with generalized disease at the time of lymph node biopsy – Stage III in 2 cases and IV in one. Bone marrow infiltration was observed subsequently in one of them. All patients died within 2 years from diagnosis.

Pathology. Morphologically, they presented with a diffuse lymphoma growth pattern totally effacing the lymph node architecture. Capsule and perinodal fat tissue was infiltrated in two of them. Cytologically, the proliferation was characterized by a monomorphic, cohesive growth of histiocyte-sized cells with extraordinary cleavage of nuclear contour, with finely dispersed chromatin and inconspicuous nucleoli, scanty, ill-defined cytoplasmic limits (Fig. 14). The mitotic rate was moderate, much lower than in ML convoluted type. Small cleaved cells were observed in small numbers, not exceeding 10% of the cleaved cell's compartment. Noncleaved cells or immunoblasts were not identified.

In 2 patients with ML(FCC)LC studied postmortem, tumor cells showed cytologically predominantly a noncleaved pattern, suggesting the possibility of lymphoma cell evolution toward noncleaved type.

Cell surface markers were not studied in any case of cleaved cell lymphoma. Immunoperoxidase study disclosed that only a small population of large cleaved cells were positive with antikappa serum. Evaluation of heavy chains was very difficult because of the very scanty rim of cytoplasm. In addition, a few plasma

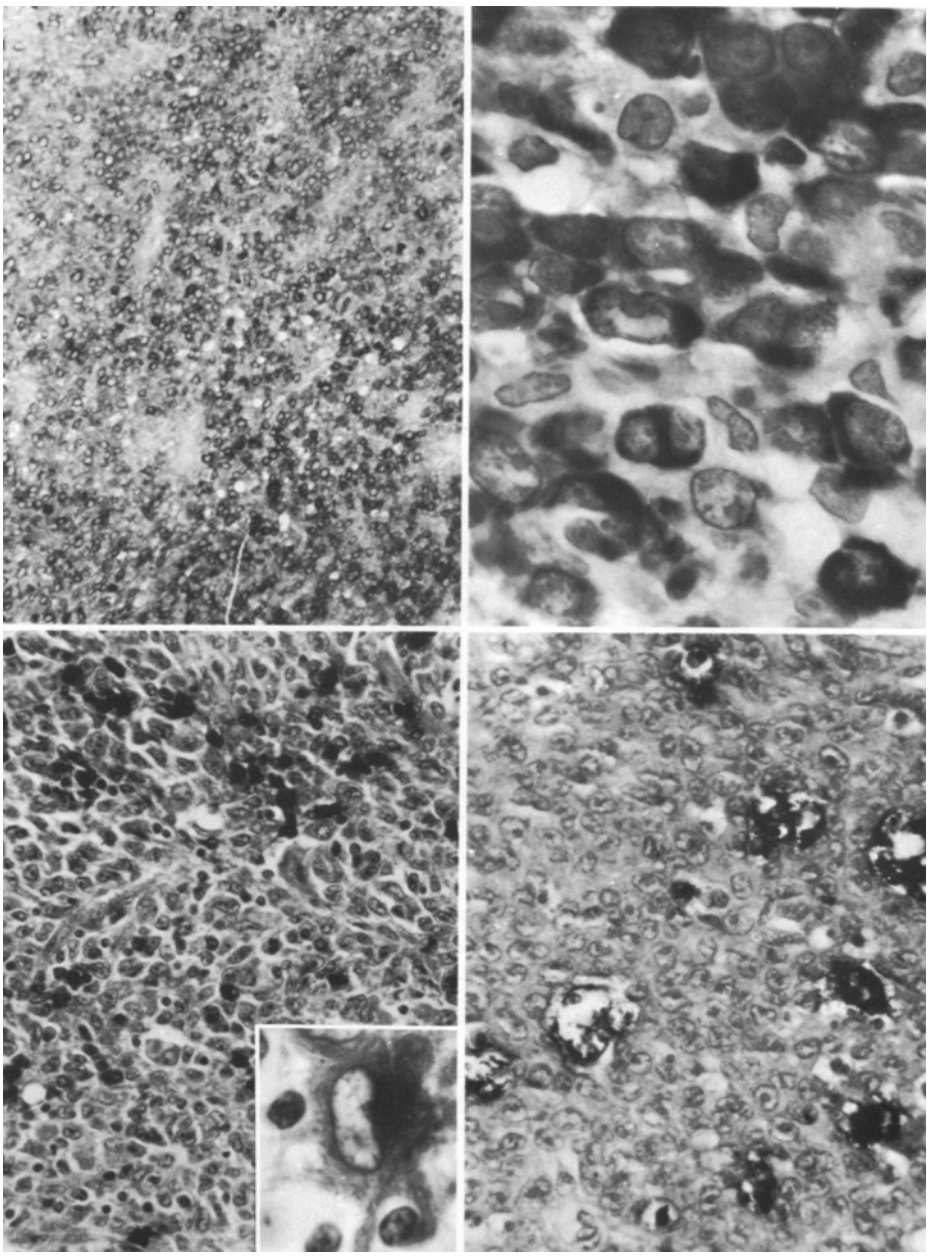

Fig. 12. ML Immunoblastic B stained with anti-kappa serum (PAP method). Top: (*Left*) Immunoglobulin containing immunoblasts show a dark intracytoplasmic reaction product. Endothelial cells as well as macrophages remain negative. (*Right*) Cytological details of immunoglobulin containing immunoblasts (kappa chain). Bottom: (*Left*) lysozyme (muramidase) present within eosinophils (dark cells) and in the reticular cells, probably dendritic (*inset*), or within the "starry-sky" macrophages (*right*). Immunoblasts remain negative. Top: PAP with anti-kappa serum, hematoxylin, *left*: ×100; *right*: ×1,053

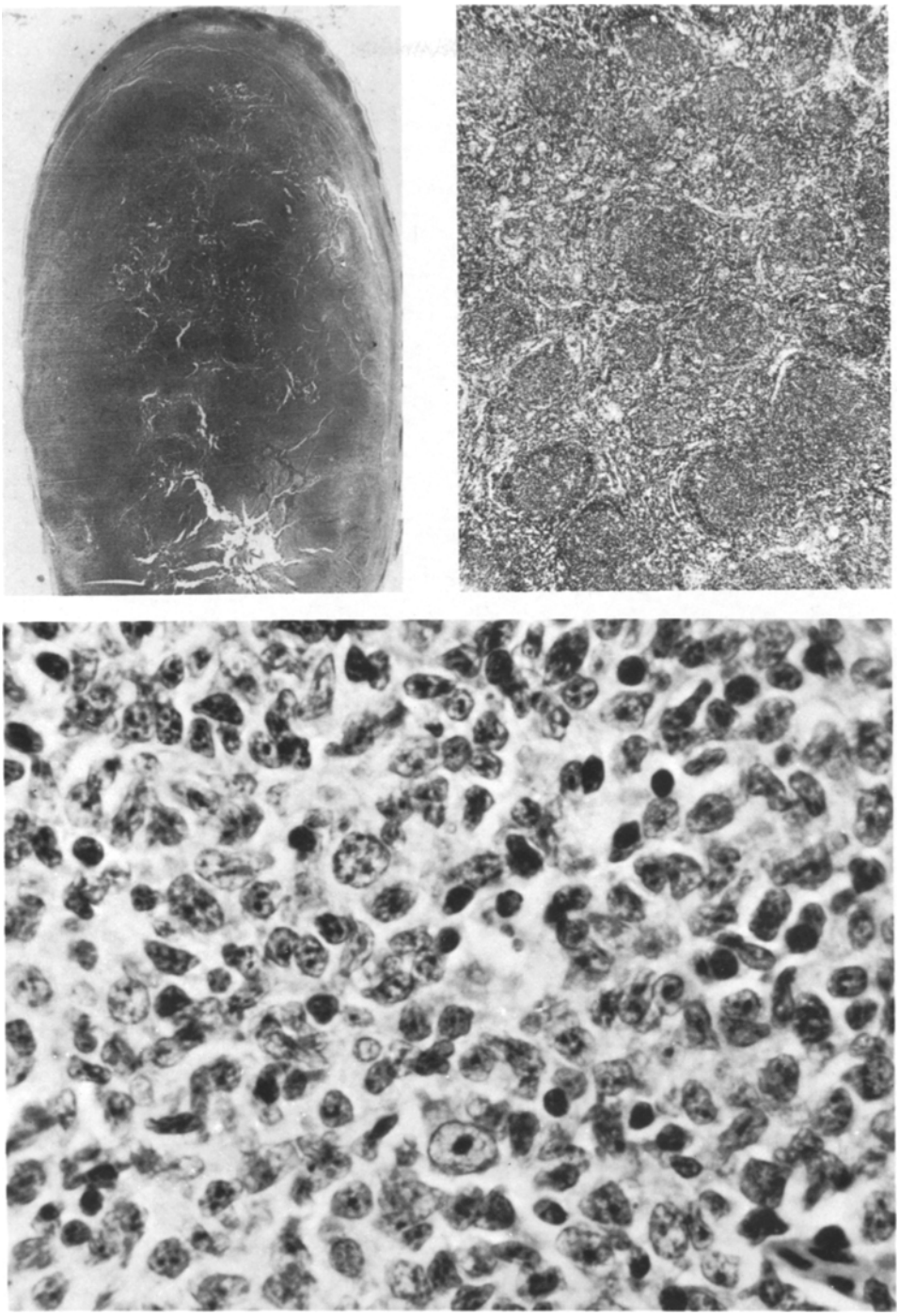

Fig. 13. ML(FCC) small cleaved with nodular and diffuse architecture. Top: (*Left*) Partial involvement of the lymph node with a semilunar area of residual lymphoid tissue beneath the capsule. (*Right*) Neoplastic nodules, relatively uniform in size and shape, showing an expansive growth, displacing and crowding the vessels in the interfollicular areas. Bottom: Cytologic appearance of ML(FCC)SC in predominantly diffuse area. The mixed population of large and small cells in the neoplastic nodules resulted in classification of ML "mixed" (Rappaport) and ML centroblastic – centrocytic (Kiel). Top: (*Left*) H and E, ×12; (*right*) H and E, ×36; Bottom: H and E, ×1,120

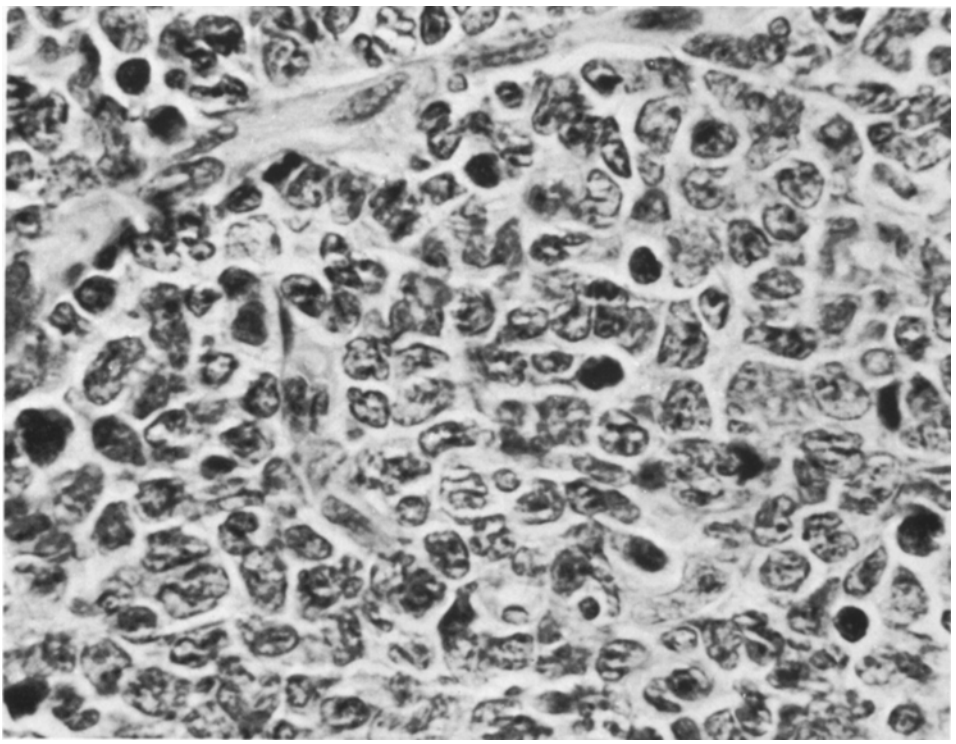

Fig. 14. ML(FCC) Large cleaved cell type which could be classified as "histiocytic" type (Rappaport) or centrocytic (large cell) type (Kiel). H and E, ×1,200

cells, when present, showed a definite polyclonal pattern and these were considered as a non-neoplastic tumor component.

Malignant Lymphoma of "U" Cell Group (ML"U")

Malignant lymphoma of the "U" cell group (or ML lymphoblastic undefined in the Kiel classification, or mainly ML nonconvoluted in Rappaport's) accounted for 14 cases and exhibited both clinically and morphologically a heterogenous group.

A preponderance of males was obvious (M:F=2.5:1) (Table 1). Ten patients presented at the time of diagnosis with generalized lymphadenopathy, with leukemic marrow. Localized lymphadenopathy was observed in 3 patients – two of whom showed bone marrow involvement. A mediastinal mass was present in 4 patients and was usually associated with peripheral lymphadenopathy. All but one child with ML "U" entered a treatment protocol for ALL. Four patients are alive with the time of observation ranging from 20 months to 10 years (median 5.8 years) after diagnosis. Nine of those patients fulfilled criteria of ML lymphoblastic nonconvoluted, mainly on the basis of high mitotic

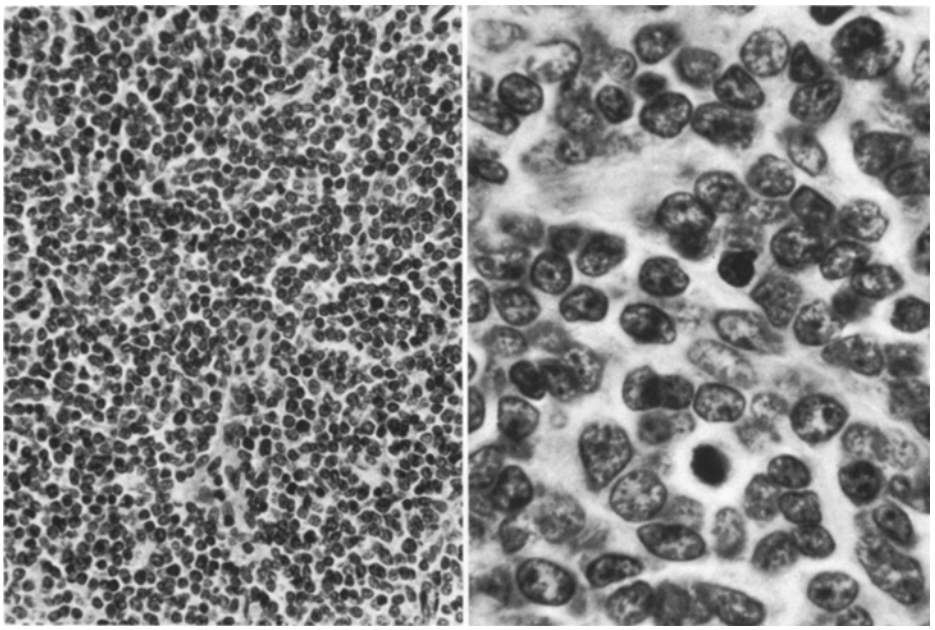

Fig. 15. ML "U" type, classified as ML lymphoblastic non-convoluted (Rappaport) and ML lymphoblastic "others" (Kiel). (*Left*) Diffuse monomorphic proliferation with high mitotic ratio. (*Right*) Cytologic details showing a relatively prominent nuclear membrane as compared to a convoluted cell nucleus, regular outline and visible nucleoli. (*Left*) H and E, ×315; (*right*) H and E, ×1,134

activity, primitive "dusty" chromatin, inconspicuous nuclei and a nonconvoluted nuclear outline. Another 5 cases resembled the small noncleaved cell type to some extent, but in contrast disclosed: (i) larger than ML(FCC)SNC nuclear size, (ii) less evident or hardly appreciable cytoplasm without vacuolation, (iii) a less prominent nuclear envelope and (iv) "nondusty", but somewhat clumped chromatin with usually recognizable small, eccentrically located nucleoli (Fig. 15). High mitotic activity was a consistent feature, although unlike in ML convoluted and ML(FCC)SNC, "starry-sky" macrophages were not seen and again as opposed to MF(FCC)SNC and LNC, individual cell necrosis was not observed. Cell surface analysis was performed in one case only, disclosing a lack of E-rosettes formation and a lack of surface immunoglobulin.

Discussion

It has been postulated that the evaluation, characterization and categorization of NHL in children should include all forms of lymphocyte neoplasms without regard to whether they are confined to the lymph nodes, thymus, gastrointestinal tract on one hand, or whether they extend to bone marrow, peripheral blood and/or arachnoid tissue (Pinkel et al. 1975). We made no attempt to exclude patients with "primary leukemia" from this study, being aware that it represents

an important element of the biologic-pathologic picture of lymphoma evolution and seemed scientifically unwarranted. This approach is reflected in both the Lukes-Collins and Kiel classifications. Rappaport's modified classification attempts a compromise by introducing the ML lymphoblastic convoluted and nonconvoluted designations.

ML of convoluted lymphocytes is a well recognized entity representing pre-T cell malignancy (Barcos and Lukes 1975; Lukes and Collins 1975; Murphy et al. 1975; Stein et al. 1976, 1979; Byrne 1977; Hausner et al. '977; Lennert 1977; Lennert and Mohri 1978; Lukes 1978; Williams et al. 1978; Mann et al. 1979). Numerous investigators have documented that in the majority of cases, tumor cells have T-cell markers including receptors for sheep erytrocytes, reactivity with HTLA antisera, terminal deoxynucleotydyl transferase activity and, what for some is sine qua non for diagnosis, acid phosphatase activity.

The term convoluted lymphoma-leukemia is preferred by some authors (Lukes et al. 1978b; Williams et al 1978) reflecting the close relationship between that particular type of lymphoma and leukemia. In a retrospective study of 56 pediatric lymphomas of the convoluted lymphocyte, Williams et al. (1978) found that 42 (75%) presented with mediastinal involvement. Nearly 60% of our patients with ML convoluted had bone marrow or CNS involvement at admission or developed it during the first 12 months of observation, and 50 of 69 patients presented with an anterior superior mediastinal mass. By considering ML convoluted as a single entity, whether localized or leukemic, one finds associations that might be missed. For instance, ML convoluted in our study was characterized by a high incidence of testicular involvement, especially as a site of late relapse. Unfortunately, that particular finding is absent from several published studies of non-Hodgkin's lymphoma, owing to the exclusion of "primary" leukemic NHL (Hutter et al. 1975; Lemerle et al. 1975; Murphy et al. 1975; Pinkel et al. 1975; Traggis et al. 1975; Watanabe et al. 1976; Hausner et al. 1977). Hustu and Aur (1978), following patient with ALL were able to demonstrate that extramedullary (testicular) leukemia had usually been associated with a mediastinal mass at the time of diagnosis. Among 22 patients with ML convoluted type who were examined postmortem, 70% had testicular involvement, with nine considered to be in clinical and hematological remission.

A perfect correlation between T-cell markers and ML convoluted has not been established. Pangalis et al (1979), who studied not lymph nodes but peripheral blood and marrow smears from ALL patients were unable to correlate nuclear convolutions with the presence of a mediastinal mass. An attempt to correlate nuclear convolutions with cell surface markers in our study disclosed 5 cases of ML convoluted lymphoma in which tumor cells failed to form E-rosettes, despite the fact that 3 patients presented clinically with a mediastinal mass and evolution to ALL. Lennert et al. (1978) has also reported T lymphoblastic lymphoma without nuclear convolution, but simultaneously disclosing acid phosphatase activity. Clinical evidence of a mediastinal mass and cytological evidence of acid phosphatase activity, however, seems to be nonspecific for convoluted lymphoma/leukemia of thymic origin because it may also be observed, although far less frequently, in ALL cases in which the cells do not possess immunological T or B markers.

We observed plasma cells in nearly 50% of cases of ML convoluted type,

even in biopsy specimens taken directly from anterior mediastinal masses. The significance of this finding is controversial, although some authors regard such plasma cells as a special type (i.e., T-associated plasma cells) (Lennert et al. 1978). In any ML convoluted studied with immunoperoxidase plasma cells disclose intracytoplasmic immunoglobulin.

The second most frequent type of NHL in our study formed B cell proliferation of small noncleaved lymphoma/leukemia, which affected more than one third of studied patients. All of the ML(FCC)SNC and some of the ML(FCC)LNC fit the category of ML undifferentiated (Burkitt or non-Burkitt) in Rappaport's classification (Byrne 1977). Both endemic and nonendemic Burkitt tumors have been reported to show a monoclonal pattern of surface immunoglobulin predominantly of the IgM class. It seems to be accepted that "undifferentiated" lymphoma is in fact follicular center-derived malignancy, although a follicular (nodular) phase is rarely observed because of its rapid proliferation rate (Lukes and Collins 1975; Berard et al. 1977; Lennert and Mohri 1978; Lewin et al. 1978; Lukes et al. 1978a; Williams et al. 1978; Mann et al. 1979; Murphy et al. 1979). Our study essentially confirmed previous observations that all cases classified as ML(FCC)SNC (ML lymphoblastic Burkitt type or ML lymphoblastic B-type according to Kiel terms) demonstrated surface immunoglobulins.

There was no clear-cut distinction in anatomic presentation or behavior between the small non-cleaved and the large non-cleaved type of lymphoma. Both were characterized in three-fourths of patients by primary gastrointestinal involvement similar to non-endemic Burkitt tumor (Lewin et al.), and by comparable frequencies of bone marrow and CNS involvement. One-third of patients with ML(FCC)SNC in our study had bone marrow or CNS involvement (or both) at the time of lymphoma diagnosis or developed it during the course of disease. We regard both leukemic and "primary" non-leukemic small noncleaved type proliferations as a single entity: ML(FCC)SNC that constitutes histopathologic substrate for B-cell ALL or acute leukemia with Burkitt cells (Seligmann et al. 1977; Mann et al. 1979). It was not unusual to encounter patients with ML(FCC)SNC or ML(FCC)LNC who presented with a regional (localized) disease and leukemic marrow. Meningeal involvement, finally, was another characteristic feature of ML(FCC)SNC, quite often appearing as a solid growth in epidural spaces. This observation confirms previous reports related to ML undifferentiated (Watanabe et al. 1976).

ML(FCC)SNC may evolve to larger cells (large noncleaved cells), as was observed in one case biopsied for the second time and in 3 cases at autopsy. In turn, ML(FCC)LNC may evolve to immunoblastic sarcoma, which has led to the "second" diagnosis of histiocytic lymphoma according to Rappaport. The possibility of evolution from small noncleaved to large noncleaved type or from large noncleaved to immunoblastic proliferation may account for the "changes" in diagnosis over the course of a patient's disease.

ML immunoblastic of the B cell type accounted for about 10% of pediatric NHL in our study. Lennert (1977) found only 5 valuable ML immunoblastic among 212 pediatric cases, and Garvicz et al. (1978), using the Kiel classification were unable to identify any ML immunoblastic types. Seven of our 23 IBS-B presented as unresectable gastrointestinal disease with subsequent bone marrow involvement what resulted in diagnosis of "histiocytic leukemia".

Lukes and Collins (Lukes and Collins 1975; Lukes 1978; Lukes et al. 1978a, b) as well as Lennert et al. (Lennert et al. 1975; Lennert and Mohri 1978; Lennert and Stein 1978), anticipated a specific relationship between prior immunologic abnormalities and the development of IBS-B. Although unable to demonstrate such a relationship in our series, we considered the possibility that in two of our patients IBS-B developed as a result of immunologic disturbance from chemotherapy of previously diagnosed ALL and histiocytosis – X.

In 20 cases the diagnosis of IBS-B was made on the basis of histologic section for the presence of plasmacytoid or plasma cell modulation. In 3 cases, a B-cell origin was documented by immunoperoxidase evidence of intracytoplasmic immunoglobulin. In one case (ileocecal resectable tumor) we were unable to document convincingly the presence of intracytoplasmic immunoglobulin, despite the presence of plasma cell modulation. In 3 cases, immunoblasts showed a bitypic staining pattern. Bitypicity as an anomalous staining pattern has been reported being regarded as an expression of anaplasticity (Taylor and Burns 1974; Lennert and Mohri 1978; Lukes et al. 1978b; Pinkus and Said 1978; Stein et al. 1978; Taylor 1978). In each case of IBS-B we have observed a variable proportion of immunoblasts that contained no detectable immunoglobulin. Alternatively, those cells could represent a nonsynthetic phase of the cell cycle. Simultaneous evaluation for lysozyme (muramidase) in parallel sections performed in all cases of IBS-B yielded negative results for all tumor cells with a positive reaction noted for macrophages and reticulum cells.

ML immunoblastic T-cell type constitutes a new histiologic and nosologic entity incorporated recently into both the Lukes-Collins and Kiel classifications. Clinical as well as mainly histologic diagnostic critieria are not so well established as to allow unquestionable recognition of IBS-T on routine paraffin sections; therefore, several investigators have preferred to use the term large cell lymphoma with T-cell markers (Mann et al. 1979).

IBS-T was encountered in 5 patients. Analysis of surface markers in 1 case indicated that over 60% of tumor cells from lymph node formed E-rosettes. Three of these patients were classified as ML "mixed" type, according to Rappaport on the basis of a spectrum of cells ranging in size from small-to-medium lymphocytes to large immunoblasts with water-clear cytoplasm. In cytologic recognition of characteristic – almost "pencil-drawn" cellular membrance and intracellular borders – PAS staining was of great assistance. Still controversial is the presence of plasma cells interspersed with immunoblast T, which we have attributed to the residual centers of B-cell modulation scattered throughout the proliferation. Immunoperoxidase polyclonal staining pattern of plasma cells provided additional support for the view that those cells do not constitute a malignant compartment of IBS-T. T-associated plasma cells might be another possibility for consideration. An increased number of thin-walled postcapillary venules was a constant feature in all 5 patients. That particular phenomenon seemed to be a stable morphologic constituent of most T dependent zone proliferation, including T zone lymphoma, Hodgkin's disease, graft versus host disease or even histiocytosis. A close interrelationship between immunocompetent T cell and macrophages (macrophage inhibition factor-MIF) is one of possible explanation for the widespread presence of reactive macrophages in

two of our cases. The presence of macrophages within IBS-T has been previously pointed out by several investigators (Lennert and Mohri 1978; Lukes et al. 1978b; Mann et al. 1978). We wish to stress that a leading diagnostic argument in favor of IBS-T is the localization of proliferation in T-dependent zone of the lymph node and immunoperoxidase negativity of tumor cell for both immunoglobulin and muramidase (lysozyme). Localization of IBS-T in the gastrointestinal tract is not surprising, regarding the fact that large dividing cells of gut-associated lymphoid system belong to T-dependent lymphocytes (Guy-Grand et al. 1978).

The small number of cases of IBS-T do not allow us to make a precise clinicoanatomical assessment. However, it is our belief based on the patients histories, that IBS-T is a less aggressive malignancy than IBS-B cell.

Malignant lymphomas with a follicular (nodular) growth pattern are very rare in pediatric NHL, however, among few large series of childhood NHL the frequency of nodular lymphoma ranges from 0.5% to 20.6% (Lemerle et al. 1975; Murphy et al. 1975; Traggis et al. 1975; Hausner et al. 1977; Pinkel et al. 1977). Eight cases reported and extensively discussed recently by Frizzera and Murphy (1979) (including our 3 patients), formed an incidence of 2.5% of follicular lymphoma seen during the last two decades in four large US institutions.

Lymphomas of follicular cleaved cell are likewise rare in pediatric age, although in the past, ML lymphocytic, poorly differentiated dominated in reports on childhood NHL (Jenkin et al 1969; Fu and Perzin 1972; Hutter et al. 1975; Lemerle et al. 1975; Traggis et al. 1975). Lennert (1977) was unable to identify any centrocytic among 215 cases of pediatric NHL. Garwicz et al. (1978) recorded one case among 35 studied and pointed out that prognosis was not as good as expected from low-grade malignancy. Three of our patients with a designation of ML(FCC)LC (or ML centrocytic large cell) have shown rapid leukemic transformation and died within 15 months. Morphologically, the lymphoma cells represented large centrocytes rather than low-grade malignancy: small centrocytic tumor. Although that particular group is too small to warrant any definite conclusions, we prefer to consider the ML(FCC)LC as high-grade malignant lymphoma.

Malignant lymphoma of plasmacytoid lymphocyte is another example of extremely uncommon proliferation in children or adolescents, which to our knowledge has gone unreported. Morphologically, our case fulfills all necessary criteria for ML-LP (or Immunocytoma lymphoplasmocytic). The histologic picture was dominated by small, "well-differentiated" lymphocytes interspersed with large cells, plasmacytoid cells and mature plasma cells of Marschalko' type. PAS-positive inclusions within plasma cells were obvious and immunoperoxidase reaction disclosed the presence of immunoglobulin of IgA kappa.

Primary plasmocytoma of the lymph node was not observed in our series; however, two cases have been reported in the literature. Recently, Kruse et al. (1978), in a very elegant study reported clinical and immunological findings for a 10-year-old girl with lymph node plasmocytoma who presented with monoclonal gammopathy of IgA kappa type.

ML "U" type seems to cover a broad spectrum of malignant lymphoma

and closely related ALL which from an immunologic standpoint are referred to as non-T, non-B ALL and probably reflect morphologic substrates for common ALL, pre-B ALL, and unclassified ALL (Janossy et al. 1977; Seligmann et al. 1977; Lennert and Mohri 1978; Lennert and Stein 1978; Lukes et al. 1978b; Pinkus and Said 1978; Vogler et al. 1978; Mann et al. 1979; Stein et al. 1979). The original Lukes-Collins (Lukes 1978) designation of ML "U" cell group was provided for "primitive" cells, without distinctive morphologic-cytologic characteristics and currently available technique fail to mark in the uniform way. The contribution of morphology to recognition of ML "U" type as a histologic entity is far from satisfactory and at present, immunologic surface markers and intracellular biochemical markers can provide a much more clearer understanding than histopathologic designation ML "U" type.

Nodular lymphoid hyperplasia (NLH) has been evidenced in 22 of 61 patients with intestinal B cell lymphoma: in 18 it was associated with ML(FCC)SNC, in 1 with ML(FCC)LNC and in 3 with IBS-B. NLH has been reported as commonly associated abnormalities in hypoglobulinemic states and related immunodeficiencies as well in concert with pediatric intestinal lymphoma (Marcuse and Stout 1950; Faulkner and Dockerty 1952; Mestel 1959; Berry and Keeling 1970; Fu and Perzin 1972; Webster et al. 1977; Lewin et al. 1978; Ranchod et al. 1978; Nagura et al. 1979). We have no information concerning hypoglobulinemia states in our patients, and morphologic evidence of numerous plasma cells in lamina propria, observed in all cases of NHL argues against immunoglobulin abnormalities as opposed to the lack of plasma cells in lamina propria in reported NLH cases associated with hypoglobulinemia.

Extremely valuable information was obtained from assessment of secretory component in intestinal epithelial cells in cases of gastrointestinal lymphoma. The secretory component is a glycoprotein synthesized by gastrointestinal epithelial cells and plays an important role in the passage of immunoglobulin – mainly IgA and IgM – from lamina propria into the intestinal lumen (Brown et al. 1976; Brandtzaeg 1978; Nagura et al. 1979). Recent evidence indicates also that secretory component acts as an epithelial receptor for J chain containing IgA and IgM and as a transport protein between basal and luminal poles of enterocytes. In the normal condition after the presentation of adequately processed antigen by M cell (membranous cells within Peyer's patches) causes B cell line modulation to occur and adequate immunoglobulin is being produced by local plasma cells. After conversion from a dimeric the polymeric form by J chain incorporation, both IgM and IgA are transported as complete secretory antibodies by a specific carrier which is secretory component through the enterocyte onto the intestinal surface. Immunocytochemical evidence of the absence of secretory component in enterocytes in constellation with evidence of NLH of the gut-associated lymphoid tissue might indicate that the alteration of enterocytes is responsible for a gradually progressive disturbance of the feedback control limiting B lymphocyte transformation, thus contributing to development of lymphoma.

The absence of a secretory component in epithelial cells covering the tumor could also be interpreted as secondary to ischemic changes due to tumor proliferation. If so, one would expect an alteration not only in enterocytes, but in the Paneth's cells of the crypt as well. This was not observed in our study; indeed, the cells continued to react strongly with antilysozyme (muramidase) serum.

The term malignant lymphoma in this report exclusively encompasses malignancy of lymphoid (T, B, or null) cell line. "True" histiocytic proliferations

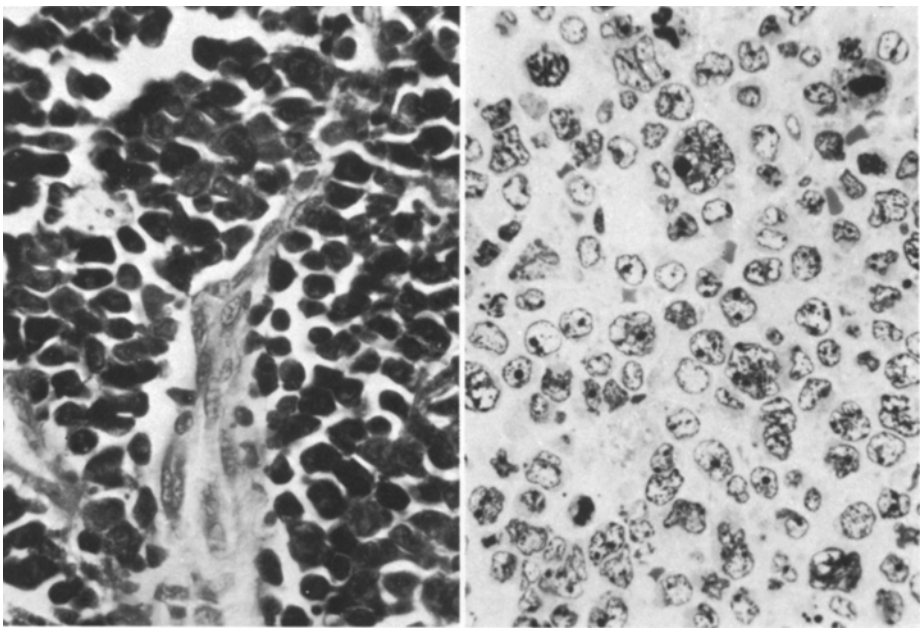

Fig. 16. "True" histiocytic proliferation showing (*left*) strong positivity in all cells with anti-muramidase (lysozyme) serum. The endothelial cells shown in central part remain negative. (*Right*) Same case – note marked variation of nuclear features with the presence of giant bizzare cells showing prominent nucleoli. (*Left*) PAP – with antimuramidase (lysozyme), serum – hematoxyline, ×450; (*right*) Epon section, toluidine blue, ×750

(enclosed in "histiocytic" lymphoma in Rappaport's classification) have been eliminated from the large-cell lymphoma group for substantial differences in both histopathologic pictures and clinical-anatomical presentation. First, "true" histiocytic malignancies demonstrated intracytoplasmic content of lysozyme (muramidase) and did not yield evidence of immunoglobulin (Fig. 16). Second, "true" histiocytic proliferations were characterized by skin and/or soft tissue involvement even without peripheral accessible lymphadenopathy at the time of diagnosis, extreme aggressiveness, poor response to treatment, and, finally, conversion to histiocytic leukemia. Exclusion of histiocytic malignancies resulted in an almost total elimination of primary extralymphatic lymphoma (skin, soft tissue) from our study. This rather peculiar phenomenon (regarding recent concepts of lymphoma development), has been a common finding in studies of pediatric NHL reported in the past decade. Subcutaneous and soft tissue involvement in pediatric lymphoma rarely occur. In our series of 69 patients with ML convoluted type, we observed 8 patients with histologically confirmed subcutaneous or soft tissue masses during the course of disease as a part of widespread malignancy. We have not been able to observe any particular territorial or anatomic predilection of those infiltrations. Mycosis fungoides and Sezary syndrome, which are a part of cutaneous adult lymphoma, were not observed in our study; this together with a review of the literature substantiates that such lymphoma is most probably nonexistant in children.

In conclusion, we have demonstrated that classification of non-Hodgkin's lymphomas by either the Lukes-Collins or Kiel criteria is functionally sound, as judged from simultaneous immunologic cell typing. Moreover, there was a high degree of consistency in lymphoma designation between three independent observers. Joint analysis of "primary leukemic" and "non-leukemic" NHL disclosed a clear difference among T, B and null cell proliferations, both in anatomic presentation and subsequent biologic evolution of tumor. Finally, the immunoperoxidase technique was an extremely valuable tool not only for precise definition of the functional type of large cell proliferation, but also for functional assessment of lymphoid-system-associated tissues, especially intestinal epithelium and reticular cells.

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