Human T-Cell Malignancies

Correlative Clinical, Histopathologic, Immunologic, and Cytochemical Analysis of 23 Cases

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Twenty-three T-cell neoplasms were investigated for their reactivity with the OKT monoclonal antibodies and expression of certain cytochemical markers. Fourteen neoplasms with diverse histopathologic features, T-cell chronic lymphocytic leukemia, mycosis fungoides, the Sézary syndrome, T-immunoblastic sarcoma, and a pleomorphic large-cell lymphoma, expressed the T helper cell phenotype, $OKT3^{+}T4^{+}$. Nine other neoplasms displayed marked inter- and intratumor heterogeneity. Seven of these cases, lymphoblastic lymphoma, T-cell acute lymphoblastic leukemia, and tumors with features of T-immunoblastic sarcoma or the multilobated lymphoma of Pinkus, expressed intrathymic phenotypes. The other 2 cases, a lymphoblastic lymphoma and a so-called Lennert's lymphoma, expressed the previously undescribed $OKT3^{+}T10^{+}$ phenotype. These studies demonstrate that the T-cell malignancies are divisible into phenotypes corresponding to normal maturational stages of T-cell differentiation and functionally distinct T-cell subsets. Such studies should provide a basis for understanding the biologic heterogeneity, clinical diversity, and significance of the variable cytomorphologic characteristics of T-cell malignant tumors and assist in the further delineation of normal human T-cell heterogeneity. (Am J Pathol 1982, 106:187–203)

THE T-CELL MALIGNANCIES, which constitute approximately 20% of the human lymphoproliferative malignancies,[1] display markedly heterogeneous clinical behavior[2,3] and diverse cytomorphologic features.[4] Indeed, their peculiar cerebriform, convoluted, and multilobated nuclear configurations have been employed in efforts to subdivide them into distinct clinicopathologic entities.[1,4–10] The T-cell malignancies also show variability of function *in vitro*[11,12] and variability of expression of phenotypic markers such as heat-stable sheep erythrocyte rosette formation (E^{37}),[13] terminal deoxynucleotidyl transferase (TdT),[14] expression of Fc receptors for IgM and IgG,[15] and reactivity with anti-TH_2.[3,16] This marked clinical, histopathologic, and immunologic diversity suggests that the heterogeneity of the T-cell malignancies is related to their origin in phenotypically and functionally distinct T-cell subsets.

The recently developed OKT series of hybridoma

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monoclonal antibodies, which preferentially react with human T cells at varying stages of differentiation and with distinct functional properties, have been especially useful in a more precise characterization of normal human T cells.[17-19] Briefly, OKT3 reacts with mature, postthymic peripheral T cells and medullary thymocytes, while OKT6 reacts with most cortical thymocytes, and OKT10 reacts with virtually all thymocytes. OKT6-reactive lymphocytes are absent from normal peripheral blood and lymph nodes.[20] $OKT3^+T4^+T8^-$ and $OKT3^+T4^-T8^+$ cells comprise reciprocal peripheral T-cell subsets, analogous to the TH_2^- and the TH_2^+ subsets,[21] which are commonly associated with helper and suppressor/cytotoxic function, respectively. OKT5 reacts with an $OKT3^+T4^-T8^+$ subset. Medullary thymocytes express the phenotypes associated with the helper and suppressor subsets but retain reactivity with OKT10. The majority of thymocytes are reactive with OKT4, T5, T6, T8 and T10. OKT9 reacts with a small population of early thymocytes but also detects an activation-specific antigen.[22]

Lymphocytes have also been characterized according to the presence or absence of certain hydrolytic enzymes. Acid phosphatase (AP) has been shown to be acquired by fetal thymocytes by the 12th week of gestation[23] and then to be retained throughout T-cell differentiation and peripheralization.[24] β-Glucuronidase (BG) is acquired later in fetal thymic development and is expressed by the majority of, but not all, postgestational thymocytes and mature peripheral T cells.[25] Acid α-naphthyl acetate esterase (ANAE) is the last of the three enzymes to be acquired and is expressed by the majority of mature, resting peripheral T cells[26,27] and by medullary but not cortical thymocytes.[28] These results have led to the proposal of a differentiation scheme wherein maturing T cells progress from $AP^+BG^-ANAE^-$ to $AP^+BG^+ANAE^-$ and finally to $AP^+BG^+ANAE^+$.[24]

In the present study we used the OKT hybridoma monoclonal antibodies and cytochemical markers to delineate the distinctive phenotypes of 23 T-cell malignancies and relate them to their normal benign counterparts at equivalent stages of differentiation and of corresponding subsets. In addition, the clinical and cytomorphologic features of these 23 T-cell neoplasms were studied, and the interrelationships between the clinically, histopathologically, and immunologically defined categories were examined. In this way we hoped to provide a basis for understanding the biologic heterogeneity, clinical diversity, and significance of the variable cytomorphologic characteristics of human T-cell malignancies.

Materials and Methods

Mononuclear Cell Isolation

Representative portions of 14 surgical biopsy specimens were obtained, under sterile conditions, from 12 patients undergoing diagnostic biopsy evaluation for possible malignant lymphoma. We prepared cell suspensions by teasing apart the tissue in tissue culture medium RPMI 1640 until the cells were separated from the connective tissue stroma.[29] The viability of the initial cell suspensions ranged from 75% to 95%. Thirteen samples of heparinized venous blood were collected from 12 patients with leukemic involvement, either at the time of diagnosis or during the course of therapy. A mononuclear cell suspension of greater than 95% viability and free from contaminating erythrocytes was prepared from each tissue and peripheral blood specimen by Ficoll-Hypaque density gradient centrifugation.

In some instances, neoplastic cells were cryopreserved in liquid nitrogen in the presence of dimethylsulfoxide with the use of the Cryomed programmed freezing system. In these cases the viability of the cryopreserved cells after thawing was greater than 85%, and the cells' surface membrane proteins and cytoplasmic enzymatic activities were shown to be intact by comparative analysis both before and after cryopreservation.

Cell Marker Analysis

We demonstrated surface immunoglobulin (SIg) and Ia antigens by direct immunofluorescence, utilizing fluorochrome-conjugated $F(ab')_2$ fragments of rabbit anti-human immunoglobulin antiserums and Ia heteroantiserums.[30] Spontaneous sheep erythrocyte (E) rosette formation was assayed with sheep erythrocytes treated with Vibrio cholerae neuraminadase (VCN Type V, Sigma Chemical Co., St. Louis, Mo) at 4 C and non-VCN-treated sheep erythrocytes at 37 C.[31] A Wright-Giemsa-stained cytocentrifuge smear of each E rosette suspension was prepared, and the cytomorphologic features of the E-rosetting and the non-E-rosetting cells were examined and compared with those viewed in the standard histopathologic sections. TdT activity was assayed biochemically as previously described.[32]

Reactivity With the OKT Hybridoma Monoclonal Antibodies

The OKT series of anti-T-cell hybridoma monoclonal antibodies was a gift from Dr. Patrick Kung and Dr. Gideon Goldstein. Their preparation, characterization, and pattern of distribution have been previously described in detail.[17-19]

Cell surface membrane determinants reactive with the OKT hybridoma monoclonal antibodies were demonstrated by indirect immunofluorescence employing rhodamine conjugated $F(ab^1)_2$ antibody fragments of affinity-purified goat anti-mouse IgG as the secondary antiserum. An appropriate ascites control was used in each experiment. Briefly, 5×10^5 mononuclear cells, resuspended in phosphate-buffered saline (PBS) with 2% bovine serum albumin (BSA) and 0.1% azide (PBS-BSA-azide), were incubated for 30 minutes at 4 C in 10×75-mm plastic tubes (Falcon 2038) with 0.025 ml of the appropriately diluted monoclonal antibody. Following incubation, the cells were washed three times with PBS-BSA-azide at 4 C, the supernatant was removed, 0.025 ml of the appropriately diluted fluorochrome-conjugated $F(ab^1)_2$ fragments of the goat anti-mouse IgG was added to the plastic tubes, and the cells were reincubated for 30 minutes at 4 C. Following incubation, the cells were washed three times with PBS-BSA-azide at 4 C, the supernatant was removed and 0.025 ml of the resultant cell suspension was placed on a glass slide with a Pasteur pipette, coverslipped, and sealed with a high-quality clear nail polish.

Cytochemical Markers

Cytocentrifuge smears were prepared by spinning 0.025 ml of each mononuclear cell suspension ($2-5 \times 10^6$ cells/ml) onto glass microscope slides by cytocentrifugation at 500 rpm for 5 minutes (Shandon Elliot Cytocentrifuge). ANAE, BG, and AP activity were demonstrated cytochemically, using, respectively, α-naphthyl acetate,[27] naphthol AS-BI-B-D-glucuronide,[25] and naphthol AS-BI phosphoric acid[33] as substrates coupled to hexazonium pararosaniline.

Microscopic Examination of Slides

The cytochemical slide preparations were examined by conventional light microscopy with an American Optical microscope equipped with a high-resolution oil-immersion objective. For each cytochemical marker, and in each case studied, attention was paid to the percentage of positive cells, the staining pattern, and the cytomorphologic features of the positive and negative cell populations. The immunofluorescent slide preparations were examined with a Leitz Dialux microscope equipped with alternating phase optics, incident fluorescent illumination, and a filter system appropriate for fluorochrome-stained preparations.

Results

Clinical

The 23 patients included in this study (Table 1) represent unselected, consecutive patients admitted to or seen at the Columbia Presbyterian Medical Center in whom phenotypic analysis of a representative peripheral blood and/or tissue specimen demonstrated the presence of a T-cell malignancy.

Patient 1 had T-cell chronic lymphocytic leukemia (T-CLL) and developed cutaneous involvement terminally. Patients 2–13 had various forms of cutaneous T-cell lymphoma (CTCL), either limited to the skin or extending into the peripheral blood and/or lymph nodes. Patient 14 presented with a nasopharyngeal lymphoma with epidermotropic features analogous to CTCL and subsequently developed nodal disease. In none of these 14 patients did thymic involvement develop, regardless of the extent of their disease.

Patients 15 and 19–22 shared many of the clinical features usually associated with T-cell lymphoblastic lymphoma or T-cell acute lymphoblastic leukemia (T-ALL). These included youth, male sex, a mediastinal mass, extensive adenopathy, eventual leukemia and central nervous system (CNS) involvement, and a generally downhill and rapidly fatal course. None of these patients developed cutaneous involvement during the course of their disease.

Patients 16–18 and 23 had node-based T-cell lymphomas. In Patient 18 a leukemic phase developed, and in Patient 23 CNS involvement developed. However, none of these 4 patients developed cutaneous disease or thymic involvement.

Histopathology

The lymph node biopsy from Patient 1, T-CLL, showed effacement by a monotonous population of neoplastic lymphoid cells, approximately equal in size to normal lymphocytes and with scanty cyto-

Table 1—Clinical Characteristics of 23 Patients With T-Cell Malignancies

Patient No.	Age	Sex	History	Clinical presentation	Organ involvement	Diagnosis	Course	Follow-up
1	60	M		Lymphadenopathy, hepatosplenomegaly; WBC 270,000.	PBL, BM, LN, liver	T-CLL	WBC to 750,000, anemia ↑ adenopathy, ↑ hepatosplenomegaly, cutaneous involvement, treated with repeated leukophoresis.	Died 9 months. Widely disseminated disease.
2	75	F	Seven years multiple cutaneous nodules treated elsewhere with irradiation.	1980: Cutaneous nodules of cheek, abdomen, thigh.	Skin	MF	Lesions flattened after therapy; WBC normal, inguinal LN and BM biopsies negative.	Alive with cutaneous disease 8 years after initial skin lesions.
3	75	F	1970: Generalized erythema, pruritis, treated with steroids. 1977: SS diagnosed, treated with electron beam irradiation elsewhere.	1979: Ulcerated nodules of scalp, face, arm; WBC 23,000 (67% lymphocytes); axillary and inguinal adenopathy.	Skin, PBL ? LN	MF, SS	Enlarging facial mass and facial nerve palsy, treated by irradiation; WBC 18,000–23,000; no LN biopsy.	Alive with cutaneous and PBL involvement 11 years after initial symptoms.
4	65	M		1 year generalized erythema, pruritis, WBC 40,000 (85% lymphocytes).	Skin, PBL	SS	Persistent elevated WBC.	Alive with cutaneous and PBL involvement 3 years after initial symptoms.
5	58	M	1978: MF diagnosed, treated with electron beam irradiation elsewhere.	1980: Hepatosplenomegaly, WBC 12,000 (62% lymphocytes).	Skin, PBL	MF, SS	Developed multiple cutaneous plaques; WBC 23,000; no adenopathy.	Alive with cutaneous and PBL involvement 3 years after diagnosis.
6	79	M	1978: WBC 18,000 (86% lymphocytes), erythematous rash treated with Cytoxan.	1981: Generalized erythematous rash; persistent elevated WBC.	Skin, PBL	SS	Persistent elevated WBC 18,000–23,000.	Alive with cutaneous and PBL involvement 3 years after diagnosis.
7	91	M		1 year generalized erythema, pruritis, hepatosplenomegaly, axillary adenopathy; WBC 17,000 (65% lymphocytes).	Skin, PBL, ? LN	CTCL	Persistent elevated WBC 17,000–42,000.	Alive with cutaneous and PBL involvement 1.5 years after presentation.
8	53	M	7 years slowly progressive pruritic, erythematous rash.	1980: Generalized erythema, generalized lymphadenopathy; WBC normal.	Skin, LN	CTCL	1980 staging: liver and generalized nodal involvement; BM negative; Persistent ↑ WBC.	Alive with disseminated disease 1 year after diagnosis, 8 years after presentation.
9	47	M		10 months: Solitary enlarging 4-cm scalp mass; WBC normal	Skin	CTCL, LU	3 months later developed right cervical LN involvement; WBC normal.	Alive 1 year after presentation.
10	73	M	1975: Generalized erythema and pruritis diagnosed as CTCL with lymph node involvement elsewhere; no response to multiple therapeutic regimens.	1980: Pruritis, diffuse erythema, inguinal and axillary adenopathy; WBC 14,300 (53% lymphocytes).	Skin, PBL, LN.	CTCL	Inguinal LN; persistent elevated WBC; further systemic evaluation negative.	Alive with cutaneous, PBL and LN disease 6 years after presentation.
11	68	M	8 years progressive psoriasis.	1980: Generalized erythroderma, large groin plaques; inguinal adenopathy; WBC normal.	Skin, LN	CTCL	Inguinal LN involved; WBC normal; serum IgG monoclonal spike; further systemic evaluation negative.	Alive with cutaneous and LN disease 6 months after diagnosis of CTCL and 8½ years after initial symptoms.

Table 1—*Continued*

Patient No.	Age	Sex	History	Clinical presentation	Organ involvement	Diagnosis	Course	Follow-up
12	69	M	1968: Progressive erythroderma. 1976: SS diagnosed, treated with Price-Hill regimen elsewhere.	1977: Generalized erythroderma, axillary adenopathy; WBC 22,100 (82% lymphocytes).	Skin, LN, PBL	CTCL, T-IMB	Symptomatic response and ↓ WBC with chemotherapy. 1979: Generalized massive adenopathy, splenomegaly; BM involved.	Died 3 years after diagnosis. Autopsy: widely disseminated disease; pulmonary candidiasis, bronchopneumonia.
13	72	M	1973: Erythematous rash, cervical adenopathy, IgM spike diagnosed Waldenstrom's, treated with Chlorambucil; responded with ↓ spike, ↓ adenopathy.	1980: Abdominal pain, weight loss, cervical adenopathy, splenomegaly.	LN	CTCL	Extensive retroperitoneal adenopathy radiologically.	Alive with extensive LN involvement 8 years after presentation.
14	54	F	1979: Nasopharyngeal mass diagnosed as carcinoma.	1980: enlarging groin mass.	Nasopharynx, LN	CTCL-like, T-IMB	Nasopharynx biopsy reviewed, diagnosis revised to lymphoma; splenomegaly; WBC normal.	Alive 14 months after presentation.
15	36	M		Anorexia, nausea, abdominal pain, 10-cm mediastinal mass; WBC 43,700 (82% blasts).	Mediastinum, PBL, BM	LLB	Recurrent mediastinal mass with negative BM after completing maintenance therapy.	Died 3 months; no autopsy.
16	71	M	1970: Inguinal and axillary NPDL lymphoma treated by radiation and chemotherapy. 1978: Cervical NPDL lymphoma.	1979: Weight loss, anemia, pathologic fracture of left humerus; axillary and cervical adenopathy.	LN, bone	Lennert's lymphoma	Skeletal survey shows multiple foci of involvement; BM and WBC normal.	Alive with disease 2 years after diagnosis of second lymphoma.
17	52	F	3 years multiple episodes of fever, swollen joints, vasculitis.	1 week abdominal pain, abdominal mass, leukemoid reaction.	Retroperitoneal and mesenteric LNs	T-IMB	Laparotomy: mesenteric and retroperitoneal adenopathy; liver, spleen, BM, and WBC normal; no mediastinal mass or cutaneous disease.	Alive 5 months after presentation.
18	24	M		Weakness, weight loss, generalized adenopathy, hepatosplenomegaly; WBC 14,700 (49% lymphocytes).	LN, PBL, BM, liver, spleen	ML	Staging demonstrated systemic disease; WBC 33,000; enlarging lymph nodes; thrombocytopenia; neutropenia; *E coli* sepsis.	Died 4 months. Autopsy: subarachnoid and GI hemorrhages; *E coli* sepsis.
19	32	M		Ecchymoses, headaches, anemia; WBC 170,000.	BM, PBL, CSF	T-ALL	Remission 4 months; then blast crisis with anemia, platelets; WBC 300,000, continuous BM and CSF relapses.	Died 10 months. Autopsy: disseminated disease, no mediastinal mass.
20	6	M		6-cm cervical mass, large anterior mediastinal mass, hepatosplenomegaly; WBC 24,500 (80% blasts).	Mediastinum, LN, BM, PBL	T-ALL/ LLB	CSF normal; remission.	Alive in remission 4 months.

Table 1—*Continued*

Patient No.	Age	Sex	History	Clinical presentation	Organ involvement	Diagnosis	Course	Follow-up
21	14	F		Malaise, mediastinal mass, pleural effusion, cervical adenopathy; WBC normal.	Mediastinum, LN	LLB	Remission 2 years; then CNS relapse; remission-2 more years, then jaundice, thrombocytopenia, liver and BM involved.	Died 4 years; no autopsy.
22	18	M		Weakness, generalized adenopathy, mediastinal mass causing airway obstruction.	LN, mediastinum	LLB	Remission 4 months; then WBC 30,000, platelets, bleeding; BM involvement and CNS relapse; terminal *S aureus* and pneumococcal sepsis; no cutaneous disease.	Died 15 months; no autopsy.
23	63	F		Cervical adenopathy; WBC normal.	LN	ML	Initial regression of adenopathy; then rapidly enlarging adenopathy, CNS relapse, diffuse skeletal involvement; no cutaneous disease.	Died 13 months; no autopsy.

Abbreviations: T-CLL = T-cell chronic lymphocytic leukemia; MF = mycosis fungoides; SS = Sézary syndrome; CTCL = cutaneous T-cell lymphoma; T-IMB = T-immunoblastic sarcoma; ML = multilobated T-cell lymphoma of Pinkus; LLB = lymphoblastic lymphoma; LU = large cell lymphoma, undifferentiated; NPDL = nodular, poorly differentiated lymphocytic lymphoma; ALL = acute lymphoblastic leukemia; LN = lymph node; PBL = peripheral blood; BM = bone marrow; WBC = white blood cells; CSF = cerebral spinal fluid.

plasm, clumped nuclear chromatin, and inconspicuous nucleoli. In contrast to the round, regular nuclei of B-CLL, these T-CLL nuclei were generally notched and occasionally even slightly cerebriform, although never to the degree seen in CTCL. Numerous mitoses were present, also in sharp contrast to the usual B-CLL, which generally lacks readily observable mitotic activity.

The cutaneous biopsies from Patients 2 and 3 showed the typical epidermotropic infiltrate of mycosis fungoides (MF), including focal epidermal exocytosis (Pautrier abscesses).[34] The neoplastic cell population was polymorphous and contained numerous cerebriform cells and occasional large, hyperchromatic MF cells. The neoplastic lymphoid cells isolated from the peripheral blood of Patient 3 displayed the classic cerebriform nuclei of Sézary cells.[5]

The cutaneous biopsy specimen from Patient 5 showed an epidermotropic infiltrate of cerebriform neoplastic lymphoid cells, but Pautrier abscesses were absent. The cutaneous biopsies from Patients 4 and 6 were not available for review. However, most of the peripheral blood lymphoid cells isolated from these 3 patients showed the cerebriform nuclei of Sézary cells and were considered typical of peripheral blood involvement by CTCL.

The lymph node biopsies in Patients 7, 8, 10, 11, and 13 showed similar histopathologic features (Figure 1). In each case the neoplastic lymphoid cells preferentially infiltrated the paracortical and interfollicular (T-cell) zones with at least partial preservation of germinal centers and sinuses. The neoplastic cells were larger than normal lymphocytes, had modest amounts of cytoplasm, and had variably indented, grooved, and cerebriform nuclei with finely dispersed chromatin, characteristic of lymph node involvement by CTCL.[34]

A cutaneous biopsy from Patient 9 showed dermal infiltration by neoplastic lymphoid cells with grooved, folded, and cerebriform nuclei with finely dispersed chromatin, characteristic of CTCL. A lymph node biopsy, performed several months later (Figure 2), showed total effacement by a pleomorphic cell population with high mitotic activity, focal necrosis, and marked karyorrhexis. The large neoplastic cells contained abundant acidophilic cytoplasm and had large round to polygonal nuclei that often contained 1–3 nucleoli. The small cerebriform cells seen in the cutaneous biopsy were absent. This case shares histopathologic features with a previously reported case of CTCL that transformed into a pleomorphic large-cell lymphoma but retained helper function *in vitro*.[35]

The neoplasms in Patients 15 and 19–22 showed

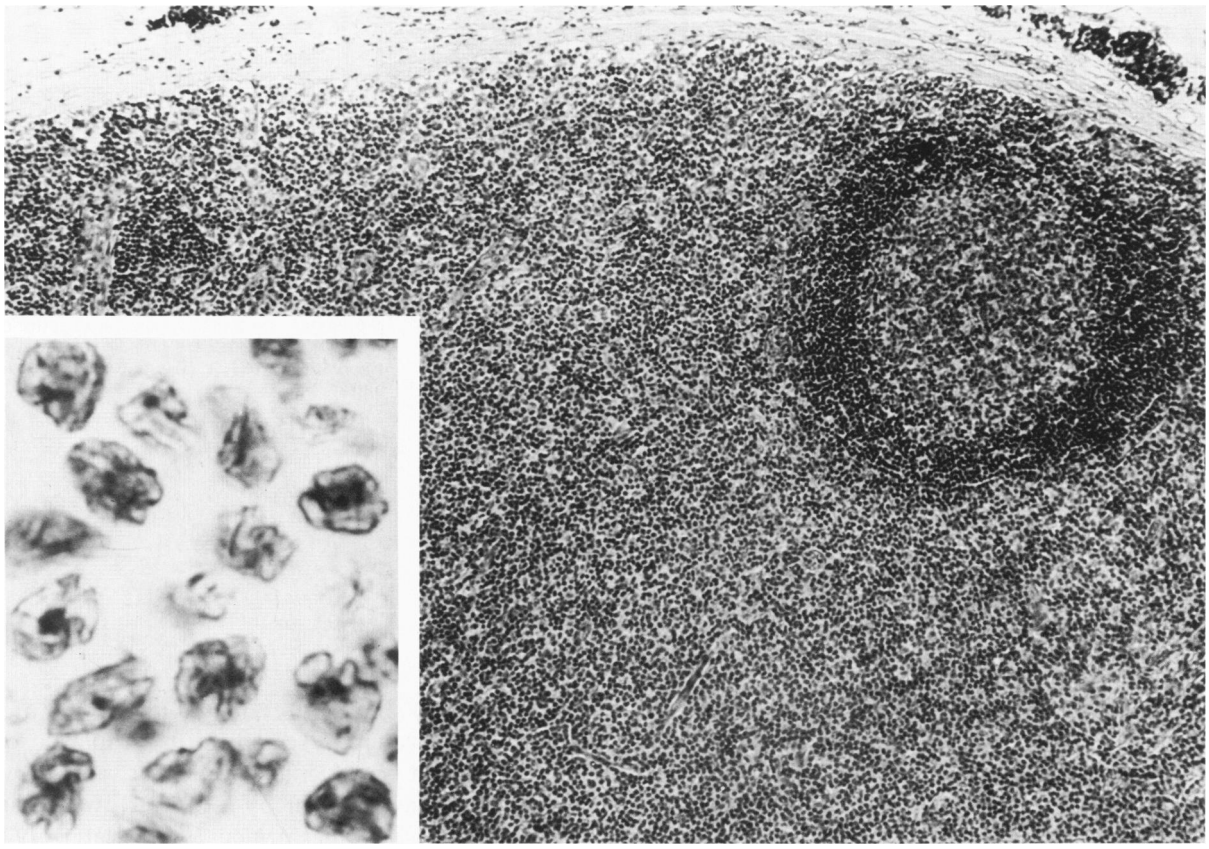

Figure 1—This lymph node (Patient 11; cutaneous T-cell lymphoma) shows preferential infiltration of the paracortex by small neoplastic cells with cerebriform nuclei, characteristic of Sézary cells. A residual germinal center remains. These neoplastic cells were $OKT3^+T4^+$. (Hematoxylin-phloxine-safran, × 82; **Inset**, × 1400)

similar cytomorphologic features (Figure 3). In each case the neoplastic cells were larger than normal lymphoid cells, possessed scanty cytoplasm, and had large, often convoluted nuclei with finely dispersed, dustlike chromatin and inconspicuous nucleoli, characteristic of lymphoblastic lymphoma or acute lymphoblastic leukemia of the convoluted nuclear type.[7]

Previous lymph node biopsy specimens (1970 and 1978) from Patient 16 showed a follicular, small cleaved-cell lymphoma (nodular, poorly differentiated lymphocytic lymphoma of Rappaport).[36] However, the most recent lymph node biopsy (1980) (Figure 4) showed replacement by large cells with abundant clear cytoplasm, distinct cell boundaries, and large round to slightly polygonal nuclei containing finely dispersed chromatin and occasional nucleoli. Occasional large binucleate cells with prominent nucleoli, mimicking Reed-Sternberg cells, were seen; but true Reed-Sternberg cells were absent. Small cerebriform cells, also believed to be malignant, were admixed with the predominant large-cell population. Also present were large numbers of benign-appearing epithelioid histiocytes, which were arranged in clusters and in nests and individually. These histologic features were similar to those described for so-called Lennert's lymphoma or malignant lymphoma with a high content of epithelioid histiocytes.[10]

The lymph node biopsies in several patients showed histologic features that made them difficult to classify precisely. The lymph node biopsy specimens in Patient 23 (Figure 5) showed total architectural effacement by neoplastic lymphoid cells with scant amounts of cytoplasm and variability of nuclear size and shape. The predominant cell population had large, irregularly indented, cerebriform, convoluted, and even multilobar nuclei with finely dispersed chromatin and occasional nucleoli. The lymph nodes obtained from Patient 18 showed preferential infiltration of the paracortical and interfollicular (T-cell) zones by large neoplastic cells with

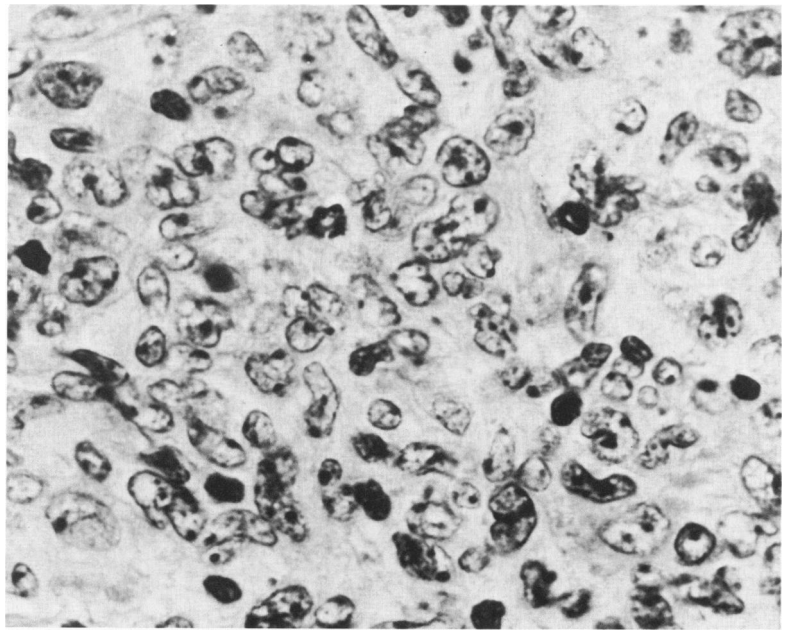

Figure 2—This lymph node (Patient 9) shows obliteration by a pleomorphic neoplastic cell population containing abundant cytoplasm and large round to polygonal nuclei with prominent nucleoli. These neoplastic cells were OKT3$^+$T4$^+$ and are presumably related to the patient's CTCL. (Hematoxylin-phloxine-safran, ×600)

multilobated and occasionally cerebriform nuclei which contained 1–3 prominent, acidophilic nucleoli. The cytomorphologic characteristics of the neoplastic cells in these 2 patients were distinguishable from the cerebriform nuclei of CTCL and the convoluted nuclei of lymphoblastic lymphoma and shared characteristics with the multilobated T-cell lymphoma of Pinkus.[9]

The original lymph node biopsy obtained from patient 12 (1977) showed preferential infiltration of the T-dependent zones by cerebriform neoplastic cells similar to that of patients 7, 8, 10, 11, and 13 with lymph node involvement by CTCL. However, in a lymph node biopsy performed 2 years later (1979), which was investigated in this study (Figure 6), the cerebriform neoplastic cells represented a minority population. Here, the predominant cell population was large, contained moderate amounts of clear cyto-

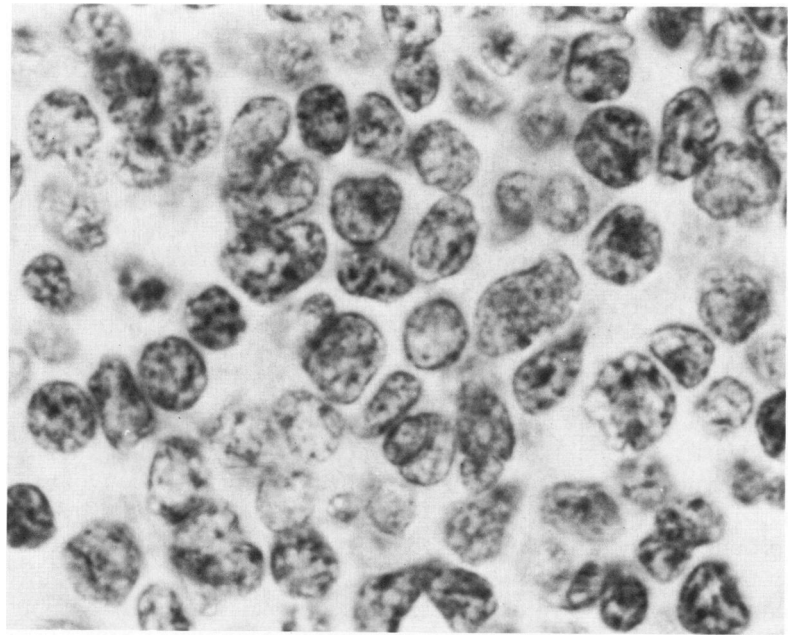

Figure 3—The neoplastic cells from Patient 22 had scanty cytoplasm, large round and occasionally convoluted nuclei with finely dispersed chromatin and inconspicuous nucleoli, characteristic of lymphoblastic lymphoma. These neoplastic cells expressed a phenotype compatible with an intrathymic stage of differentiation. (Hematoxylin-phloxine-safran, ×1250)

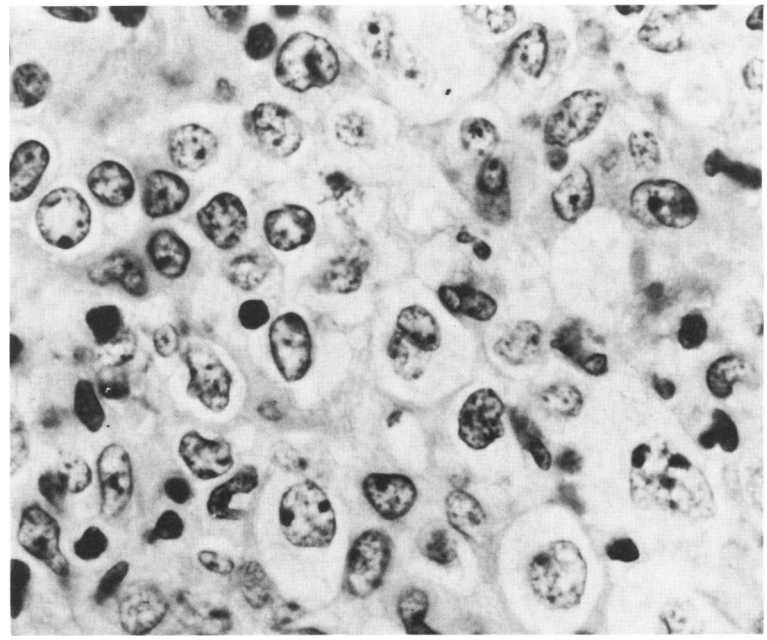

Figure 4—This lymph node (Case 16) was replaced by large neoplastic cells with abundant clear cytoplasm, distinct cell boundaries, and large round to slightly polygonal nuclei. Epithelioid histiocyte clusters were also present. These histopathologic features are similar to those described for so-called Lennert's lymphoma. (Hematoxylin-phloxine-safran, × 600)

plasm, and had large round to slightly polygonal nuclei with occasional nucleoli, similar to that of the T-immunoblastic sarcoma of Lukes and Collins.[37] Thus, in this patient with CTCL, sequential lymph node biopsies showed an evolution of the histopathologic characteristics of cerebriform cell lymphoma to T-immunoblastic sarcoma.

The lymph node biopsy specimens in Patients 14 and 17 shared some histopathologic features with those of Patient 12. A previous nasopharyngeal biopsy in Patient 14 had shown an "epidermotropic" infiltrate of cerebriform lymphoma cells analogous to CTCL. A follow-up lymph node biopsy 2 years later showed effacement by a polymorphous infiltrate of small cerebriform cells and large immunoblast-like cells, similar to the T-immunoblastic sarcoma of Lukes and Collins.[37] The lymph node biopsy in Patient 17 showed a lymphoma composed of predomi-

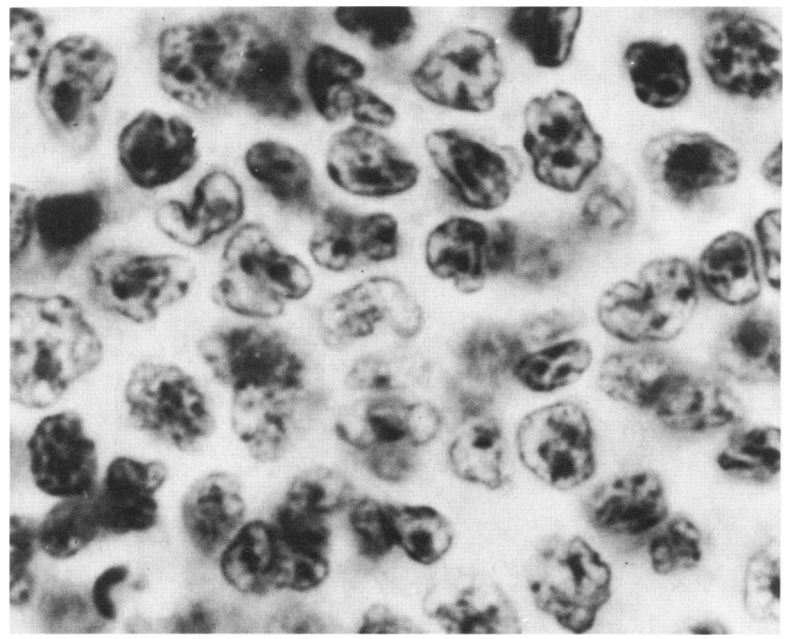

Figure 5—The neoplastic cells in Patient 23 expressed an early thymic phenotype, OKT6$^+$T10$^+$. They were large, contained scanty cytoplasm, and showed variability of nuclear size and shape. Occasional nuclei were multilobated, sharing cytomorphologic features with the multilobated T-cell lymphoma of Pinkus. (Hematoxylin-phloxine-safran, × 1400)

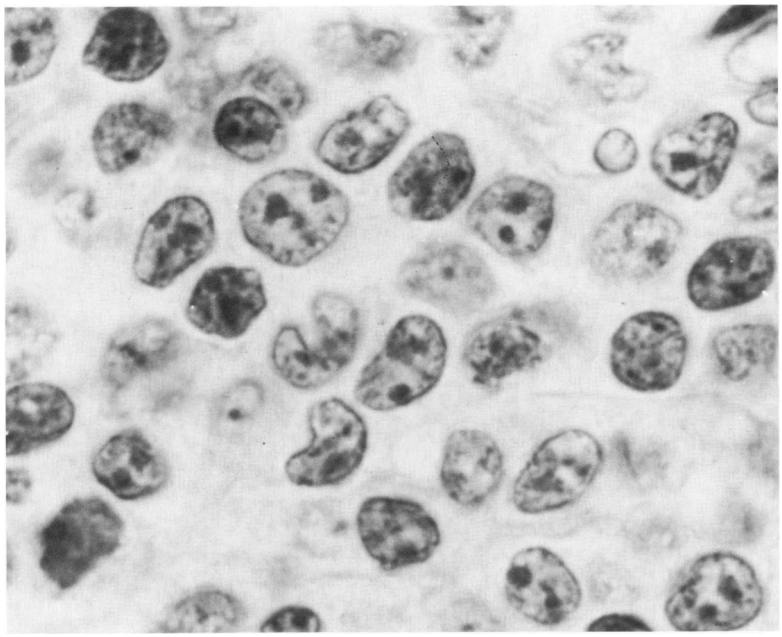

Figure 6—Patient 12 had a CTCL with nodal involvement by small neoplastic cerebriform cells. Repeat lymph node biopsy 2 years later showed replacement by a predominantly large-cell population similar to that described by Lukes and Collins as a T-immunoblastic sarcoma. These large neoplastic cells were $OKT3^{+}T4^{+}$. (Hematoxylin-phloxine-safran, × 1150)

nantly large cells with moderate amounts of clear cytoplasm and round, regular nuclei with clumped chromatin. Occasional large bizarre Reed-Sternberg-like cells and scattered plasma cells were also present. The latter case shared histopathologic features with Lennert's T-zone lymphoma[4] and Lukes and Collins' T-immunoblastic sarcoma.[37]

Immunology and Cytochemistry

The predominant cell population isolated from each of the 27 peripheral blood and tissue specimens investigated in this study was identified as malignant by cytologic criteria under phase microscopy and with the aid of Wright-Giemsa-stained cytocen-

Table 2—Results of Phenotypic Analysis of 14 T-Cell Malignancies Expressing the $OKT3^{+}T4^{+}$ Phenotype

Patient No.	Diagnosis	Tissue	Ia	S	E^{4}	E^{37}	ANAE	BG	AP	T3	T4	T5	T6	T8	T9	T10	TdT
1	CLL	PBL	0	0	89	0	96	94	95	95	95	0	0	1	0	0	–
		LN	0	0	80	4	83	88	87	90	87			4		0	
2	MF	Skin	14	10	94	8		81		76	71						
3	MF, SS	PBL	4	2	97	7	78	87	95	88	90	0	0	1	0	0	–
4	SS	PBL	8	3	84	3	83	81	88	87	85			2		0	
5	MF, SS	PBL	19	2	78	0	81	75	75	84	50	3	0	0	0	0	
6	SS	PBL	9	0	92	2	93	90	94	96	87			5		0	
7	CTCL	PBL	5	2	85		78	36	82	96	20			6		2	
8	CTCL	LN	19	9	55	1	2	61	81	69	63			6		0	
9	CTCL, LU	LN	16	5	79	3	12	44	74	89	67			10		3	
10	CTCL	LN	2	1	90	4	2	52	75	95	95			5		0	
11	CTCL	LN	13	11	75	2	0	91	90	82	74	1	0	4	0	2	–
12	CTCL, T-IMB	LN	3	1	63	2	14	1	90	95	95			3		0	–
13	CTCL	LN	2	0	93				95	95	92			2		0	
14	CTCL-like, T-IMB	LN	15	5	86		13	48	88	55	74	12	0	10	0	0	

Figures represent the percentage of cells expressing each marker.

CLL = chronic lymphocytic leukemia; MF = mycosis fungoides; SS = Sézary syndrome; CTCL = cutaneous T-cell lymphoma; PBL = peripheral blood; LN = lymph node; LU = large cell lymphoma, undifferentiated; T-IMB = T-immunoblastic sarcoma.

Table 3—Results of Phenotypic Analysis of Nine T-Cell Malignancies Expressing OKT3⁺T10⁺ and Intrathymic Phenotypes

Patient No.	Diagnosis	Tissue	Ia	SIg	E^4	E^{37}	ANAE	BG	AP	T3	T4	T5	T6	T8	T9	T10	TdT
15	LLB	PBL	1	1	72	0	5	90	98	75	2	4	0	3	0	43	+
16	Lennert's lymphoma	LN	22	8	88	8	26	57		91	0			8		60	–
		LN	18	13	83	23	38		78	80	1	13		19	0	46	
17	T-IMB	LN	9	8	92		19	38	68	95	87	3	1	3	35	74	
18	ML	PBL	21	3	70		75	81	80	74	7			40		42	+
19	ALL	PBL	0	0	97	44	2	75	100	69	33			40		91	
20	ALL-LLB	PBL	4	1	94	84	2		98	5	96	2	25	95	50	95	
21	LLB	PBL	6	0	92	22	2	59	95	5	77	77	70	66	55	57	+
22	LLB	PBL	3	2	73		16	90	100	3	9			30		78	
		PBL	0	0	76	35	6		98	10	1	0	0	31	45	74	+
23	ML	LN	3	2	58	1	2	4		15	6	3	65	4	61	74	
		LN	2	0	80	0	4	62	83	2	0			0		68	–

Figures represent the percentage of cells expressing each marker.

LLB = lymphoblastic lymphoma; ALL = acute lymphoblastic leukemia; IMB = immunoblastic lymphoma; PBL = peripheral blood; LN = lymph node; ML = multilobated T-cell lymphoma of Pinkus.

trifuge smears and was identical to that viewed in the standard histopathologic sections. In each case the malignant cell population was $Ia^-SIg^-E^+$, ie, expressed the phenotype of the majority of normal T cells.

The largest group in this series, 14 of the 23 patients studied (Table 2, Cases 1–14), expressed the $OKT3^+T4^+$ phenotype, ie, the phenotype commonly associated with the mature, peripheral T helper cell subset. These 14 $OKT3^+T4^+$ malignancies were homogeneous, in that virtually no OKT6-, T9-, or T10-reactive cells were present. The very few $OKT5^+$ and $T8^+$ cells present in these cases were recognizable as small, benign lymphocytes under phase-microscopic examination and were easily distinguishable cytomorphologically from the larger neoplastic-appearing $OKT4^+$ malignant cells. In some instances (Table 2, Cases 8 and 12) these tumors displayed slight heterogeneity with respect to E rosette formation and contained both E^+ and E^- malignant cells. There was also considerable variation in the intensity of fluorescent staining with OKT3 and T4 in these 14 cases, presumably a reflection of variable antigenic density. In some instances the neoplastic cells were brightly $OKT3^+T4^+$, comparable to normal, nonneoplastic T cells, and in other instances they were only weakly $OKT3^+T4^+$. In several instances (Table 2, Cases 5, 7, 9 and 14) these neoplasms showed varying degrees of heterogeneity with respect to the expression of OKT3 and OKT4. For example, Case 5 contained both $OKT3^+T4^+$ and $OKT3^+T4^-$ malignant cells, and Case 14 contained $OKT3^+T4^+$ and $OKT3^-T4^+$ malignant cells. In one instance (Table 2, Case 7) only a small percentage of the neoplastic cells were $OKT3^+T4^+$, the majority being $OKT3^+T4^-$. However, this heterogeneity did not prevent recognition of the $OKT3^+T4^+$ phenotype as the predominant phenotype in any of these cases. Where examined, these $OKT3^+T4^+$ neoplasms formed heat-labile E rosettes and lacked TdT activity.

Most of the neoplastic cells were AP^+ in each of

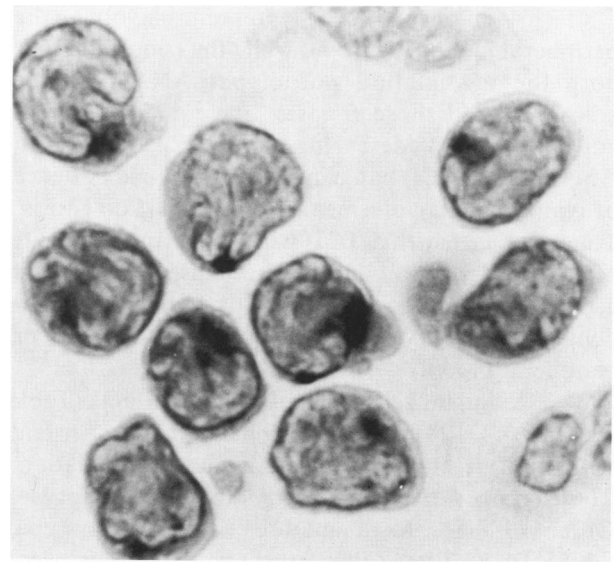

Figure 7—The cells isolated from the peripheral blood of Patient 4 showed the typical cerebriform nuclear features of Sézary cells. These cells were $OKT3^+T4^+$ and displayed focal ANAE positivity. (× 1500)

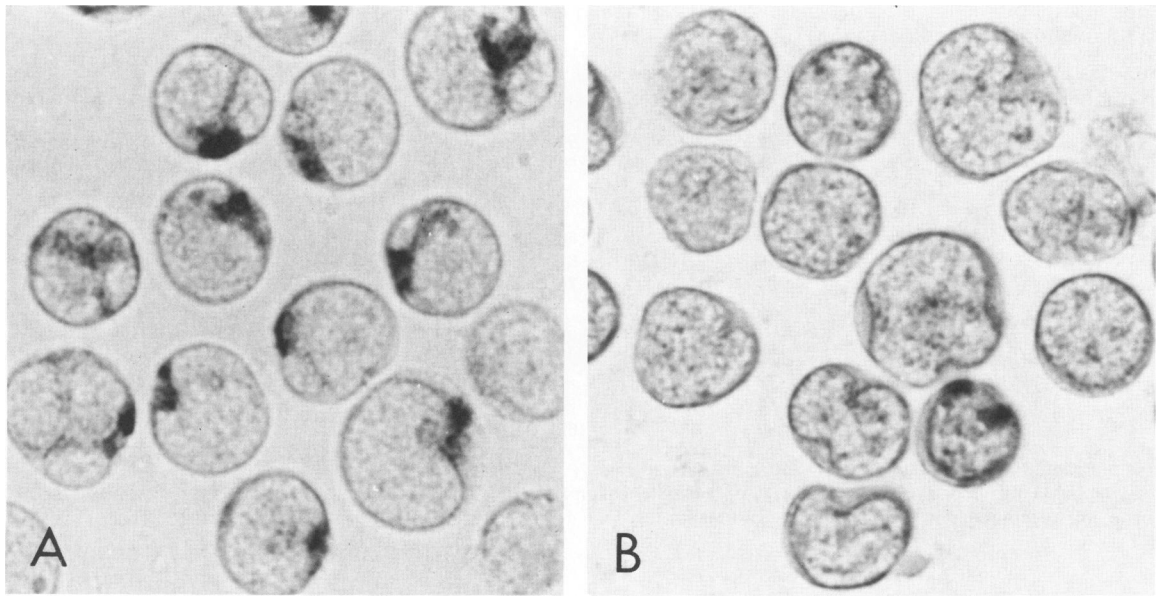

Figure 8—The neoplastic cells from Patient 20 (T-ALL, thymic phenotype) displayed focal acid phosphatase positivity (**A**) but lacked acid α-naphthyl acetate esterase (**B**). The only ANAE$^+$ cells present were small, benign-appearing lymphocytes that were presumably residual normal T cells. (**A**, × 1500; **B**, × 1350)

these 14 OKT3$^+$T4$^+$ malignancies. However, these malignancies were divisible, according to their expression of ANAE and BG activity, into three distinct phenotypes: ANAE$^+$BG$^+$ (7 cases), ANAE$^-$BG$^+$ (5 cases), and ANAE$^-$BG$^-$ (1 case). Particularly interesting in this regard is the observation that the neoplastic cells were ANAE$^+$BG$^+$ in those 7 OKT3$^+$T4$^+$ cutaneous T-cell lymphomas involving the peripheral blood (Figure 7), while the neoplastic cells bore the less mature phenotypes ANAE$^-$BG$^+$ or ANAE$^-$BG$^-$ in those 6 cases where the CTCL involved lymph nodes. None of the patients with ANAE$^+$BG$^+$ CTCL had clinical or histologic evidence of lymph node involvement at the time of diagnosis. Thus, patients with CTCL with nodal involvement, often indicative of a more advanced disease, expressed a phenotype distinct from that of those CTCL patients with disease limited to the skin and/or peripheral blood.

The malignant cells isolated from 7 patients (Table 3, Cases 17–23) expressed phenotypes consistent with various stages of intrathymic differentiation. These ranged from an early thymic phenotype, OKT6$^+$T9$^+$T10$^+$, to a mature thymic phenotype, OKT3$^+$T4$^-$T8$^+$T10$^+$. Five of these neoplasms were TdT$^+$ and/or formed heat-stable E rosettes (E^{37}) where tested.

Differing percentages of neoplastic cells showed variability of reactivity and intensity of staining with the various OKT antibodies in each of these 7 cases. Thus, in contrast to the relatively homogeneous OKT3$^+$T4$^+$ malignant tumors these 7 neoplasms with intrathymic phenotypes displayed considerable inter- and intra-tumor heterogeneity. Case 17, for example, was distinctive in that it contained malignant cells that were still reactive with anti-OKT9, although they had acquired OKT3 reactivity. Case 18 contained a large proportion of OKT3$^+$T10$^+$ cells, a sizable percentage of which were OKT8$^+$, ie, analogous to medullary thymocytes that have diverged along the suppressor/cytotoxic pathway and are acquiring OKT8. Recent functional studies in our laboratory (data not shown) demonstrate that the malignant cells in the latter case are capable of differentiating into cytotoxic cells. Case 19 was similarly heterogeneous, in that it contained a mixture of OKT3$^+$T8$^+$ and OKT3$^+$T8$^-$ malignant cells. Case 20 was heterogeneous with respect to OKT6 and OKT10 and was of interest because it appeared to represent an expansion of the relatively uncommon OKT5$^-$T8$^+$ cell population. Case 21 expressed OKT4 and T8, analogous to the common thymocyte, but only contained a minor proportion of cells reactive with OKT6. Case 22 appeared to be at a transitional stage, the cells losing OKT9 and gaining OKT6. Case 23, which expressed the earliest phenotype in this series (OKT6$^+$T9$^+$T10$^+$), paradoxically was E^{37-}TdT$^-$. Interestingly, the neoplastic T cells in the latter case could be induced to express TdT by exposure to allogeneic epidermal cells.[38]

The vast majority of the neoplastic cells isolated from each of these 7 T-cell malignancies were AP^+ (Figure 8). Five of these 7 malignant tumors, which expressed phenotypes consistent with various stages of cortical thymocyte differentiation, were BG^+ANAE^- (Figure 8), analogous to the majority of normal cortical thymocytes. The malignant cells from Case 18 expressed a mature thymic phenotype $OKT3^+T4^-T8^+T10^+$, and were $ANAE^+$, ie, analogous to the mature medullary thymocyte. A small proportion of the malignant cells in Case 17, $OKT3^+T4^+T9^+T10^+$, also appeared to be $ANAE^+$, perhaps suggesting a transition between the cortical and the medullary stage of thymocyte differentiation.

The neoplastic cells isolated from 2 patients in this series (Table 3, Cases 15 and 16) were distinctly unusual, in that they were primarily $OKT3^+T10^+$. In the case of Patient 15, a TdT^+ lymphoblastic lymphoma with peripheral blood involvement, the malignant cells were clearly reactive only with OKT3 and T10. This represents a previously undescribed phenotype, intermediate between the thymic and postthymic stages of T-cell differentiation and in which the cells do not appear to have diverged along either the OKT4 or the OKT8 pathway, despite acquiring OKT3 reactivity. In the case of Patient 16, a TdT^-, so-called "Lennert's lymphoma"[10] it is unclear whether the small number of $OKT5^+T8^+$ cells represent residual normal T cells or actually belong to the malignant clone which is primarily $OKT3^+T10^+$. We favor the latter possibility in view of the lack of any $OKT4^+$ cells which would be expected from an admixture of residual normal T cells. Formal proof will, however, require positive selection experiments for the $OKT5^+T8^+$ cells and subsequent cytomorphologic and karyotypic analysis. Thus, the neoplastic cells in this case may be differentiating along the suppressor/cytotoxic pathway. Two markers of immature T cells, heat stable E rosette formation (E^{37}) and TdT activity, were dissociated in these 2 malignancies of "intermediate" phenotype. Moreover, the $OKT3^+T10^+TdT^+$ malignant cells in case 15 were clearly $ANAE^-$, while a small percentage of the $OKT3^+T10^+TdT^-$ malignant cells in Case 16, which may be differentiating along the suppressor/cytotoxic pathway, were $ANAE^+$. The clinical, histopathologic and $ANAE^+TdT^-$ features of the latter case are analogous to a case described by Han et al.[39]

Discussion

The studies described here demonstrate that the T-cell malignant tumors are divisible, according to their reactivity with the OKT monoclonal antibodies and their expression of certain enzymatic markers, into phenotypes which correspond to maturational stages of T-cell differentiation and functionally distinct T-cell subsets. These studies further demonstrate the marked inter- and intra-tumor phenotypic heterogeneity of the T-cell malignancies, ie, the heterogeneity which exists between groups of neoplasms with distinctive clinical and histopathologic features, within groups of neoplasms believed to represent distinct clinicopathologic entities, eg, the cutaneous T-cell lymphomas and the lymphoblastic lymphomas, and even within individual neoplasms.

Previously, the T-cell malignancies had been broadly divided, according to their expression of TdT and heat stable E rosette (E^{37}) formation into thymic ($E^{37+}TdT^+$) and postthymic ($E^{37-}TdT^-$) neoplasms.[14] However, the studies described here demonstrate that the latter markers do not always reliably predict the stage of T-cell differentiation, eg, a tumor expressing an early thymocyte phenotype, $OKT6^+T9^+T10^+$, was $E^{37-}TdT^-$. Moreover, analysis with the OKT monoclonal antibodies permitted further subdivision of the lymphoblastic malignant tumors into distinctive phenotypes corresponding to discrete stages of intrathymic differentiation, the identification of a previously undescribed phenotype, $OKT3^+T4^-T8^-T10^+$, and recognition that malignant cells may not be simply frozen at a single stage, as once believed, but may exist at varying developmental stages within an individual neoplasm.

The neoplasm in each of the 12 patients in this series diagnosed by clinical and histologic criteria as CTCL expressed the $OKT3^+T4^+$ phenotype, ie, the phenotype commonly associated with the mature, peripheral helper T-cell subset. This result, which confirms recent similar findings by other investigators,[22,40,41] was anticipated from previous studies which demonstrated that the neoplastic cells in these patients may bear receptors for IgM[42], are unreactive with anti-TH_2[43], and can function as helper T cells *in vitro*.[11] The neoplasms in 2 additional patients, a case of T-CLL with terminal cutaneous involvement (Patient 1), and a nasopharyngeal lymphoma which showed mucosal "epidermotropism" by cerebriform neoplastic cells and hence was analogous to a CTCL (Patient 14), also expressed the $OKT3^+T4^+$ phenotype. It is interesting in this regard that the $OKT3^+T4^+$ (TH_2^-) cell, like the murine Lyl T cell, has been shown to preferentially migrate into the skin, where it is involved in cutaneous delayed hypersensitivity.[44] CTCL would appear to represent a malignant clonal expansion of this epidermotropic T-cell subset.

Despite their unifying clinical features and their comparative phenotypic homogeneity, these 14 $OKT3^+T4^+$ malignant tumors showed the diverse cytomorphologic and histopathologic characteristics of T-CLL (Case 1), mycosis fungoides (Cases 2 and 3), the Sézary syndrome (Cases 4–6), CTCL (Cases 7, 8, 10, 11, 13), T-immunoblastic sarcoma (Cases 12, 14), and a pleomorphic large cell lymphoma (Case 9). Sequential biopsies in Patient 12 demonstrated CTCL, lymph node involvement by CTCL, and finally T-immunoblastic sarcoma. In Case 9 a previous cutaneous biopsy had shown the usual epidermotrophic infiltrate of small, cerebriform neoplastic cells of CTCL, while an involved node showed diffuse replacement by large pleomorphic tumor cells. The latter biopsy probably represents histologic evolution or transformation of the patient's CTCL, analogous to a case described by Lawrence et al.[35] This would appear to represent the T-cell analog of Richter's syndrome,[45] in which a B-large-cell lymphoma of similar surface immunoglobulin isotype supervenes on B-CLL, representing a histologic transformation of the original clonal proliferation and not a second malignancy.

Thus, the neoplastic T cells isolated from neoplasms with diverse cytomorphologic features were united by their expression of an identical phenotype according to their reactivity with the OKT monoclonal antibodies, ie, the $OKT3^+T4^+$ phenotype, commonly associated with the helper T-cell subset. This suggests that certain so-called histopathologic entities, such as T-immunoblastic sarcoma and some pleomorphic large-cell lymphomas may merely represent cell-cycle–associated cytomorphologic expressions of the neoplastic cells derived from a particular T-cell subset.

In contrast to the comparatively homogeneous $OKT3^+T4^+$ cases, the 9 T-cell neoplasms displaying less mature phenotypes demonstrated considerable inter- and intra-tumor heterogeneity. Although this heterogeneity partially reflected differential expression of the cell-cycle–dependent antigens detected by OKT9, there was also heterogeneity of the antigens associated with distinct stages of T-cell development such as OKT6 in Case 20 and OKT4 and T8 in Cases 18 and 19. While a cytofluorograph, rather than conventional immunofluorescence, might indicate that the apparent qualitative differences between "positive" and "negative" cells were actually quantitative, this would not change the conclusion that these neoplasms exhibit marked phenotypic heterogeneity. Indeed, this heterogeneity made it extremely difficult to assign these T-cell malignant tumors to a single stage of intrathymic differentiation proposed by Reinherz et al.[46]

Five of the latter 9 cases (Cases 15 and 19–22) had clinical and cytomorphologic features characteristic of lymphoblastic lymphoma or T-ALL, supposedly a clinicopathologic entity.[47] Several investigators had previously demonstrated the phenotypic heterogeneity of these neoplasms with respect to E and EAC rosette formation,[48,49] and some suggested that this heterogeneity was related to intrathymic differentiation.[23] In this series differential reactivity with the OKT monoclonal antibodies subdivided these 5 clinically and histopathologically similar lymphoblastic lymphomas into 5 distinct phenotypes: three cortical thymic phenotypes, a medullary thymic phenotype with differentiation along the suppressor/cytotoxic pathway ($OKT3^+T4^-T8^+T10^+$), and the previously undescribed $OKT3^+T10^+$ phenotype, which may be intermediate between the thymic and postthymic stages of T-cell differentiation. In fact, whether the $OKT3^+T10^+$ phenotype is representative of a transitional stage of T-cell differentiation or a minor T-cell subset, or represents anomalous antigenic expression due to malignant transformation is presently unclear and awaits analysis of normal T cells for identification and characterization of a naturally occurring $OKT3^+T10^+$ subset.

The extent to which detailed phenotypic characterization of the lymphoblastic malignancies has prognostic significance remains to be determined. However, Nadler et al.[3] have demonstrated that the lymphoblastic malignancies are divisible into clinically distinctive groups according to their reactivity with anti-TH_2, a heteroserum with OKT8-like reactivity.

In man, Ia antigens are preferentially expressed on B lymphocytes, monocytes, and certain hematopoietic precursor cells.[50] However, they are also expressed by a small number of normal, peripheral T cells[30,51] and by certain activated T cells.[52] The neoplastic cells of T-CLL may, in some instances, also express Ia.[43] Whether this is the phenotypic expression of the neoplastic analog of a naturally occurring minor Ia^+ T-cell subset or reflects the activation state of the neoplastic cells has remained unknown. However, all the T-cell neoplasms in this series were Ia^-, despite the presence of several indicators of cell activation, eg, reactivity with OKT9, loss of ANAE, and heat-stable E rosette formation. Thus, it appears more likely that the rare Ia^+ T-cell tumors represent the clonal expansion of a minor T-cell subset.

The 4 patients in this series with node-based T-cell lymphomas, who lacked cutaneous and thymic involvement, displayed certain distinctive features worthy of brief additional comment. Patient 15 had a previously documented follicular, small cleaved cell lymphoma 10 years earlier and subsequently developed a so-called Lennert's lymphoma or malignant lymphoma with a high content of epithelioid histiocytes.[10] This is an uncommonly seen lymphoma that has been shown to be a T-cell-derived neoplasm in several instances in which cell marker studies were performed.[1,39,53] Lennert's lymphoma has also been shown to follow documented follicular lymphoma in at least one instance,[39] raising the possibility that this neoplasm may represent a clonal T-cell proliferation in response to a B-cell neoplasm. This case is, to the best of our knowledge, the first such example of Lennert's lymphoma that has been phenotyped with the OKT monoclonal antibodies. In this case, the neoplastic T cells were primarily $OKT3^+T10^+$, although a small number of $OKT5^+$ and $OKT8^+$ cells were also present, suggesting that this tumor may be differentiating along the $OKT8^+$ or suppressor/cytotoxic pathway. It should be pointed out that T-cell tumors that express the T-cell suppressor/cell phenotype $OKT3^+T4^-T8^+$ (TH_2^+ subset) or that show supppressor function *in vitro* have only rarely been described.[12,40,54,55] Further phenotypic and functional studies of cases of Lennert's lymphoma are necessary for evaluation of the possibility that they represent a neoplastic analog of the T suppressor/cytotoxic cell subset and a distinctive entity.

The neoplasms in Patients 18 and 23 resembled the multilobated T-cell lymphoma described by Pinkus and Said[9] and expressed intrathymic phenotypes. Once again, we are reporting, to the best of our knowledge, the first examples of the lesion to be phenotyped in this manner. Further studies are necessary before we can determine whether this neoplasm consistently expresses an intrathymic phenotype or whether it merely represents cytomorphologic features common to various stages of T-cell differentiation.

Case 17 is distinctive in that the patient presented with extensive abdominal lymph node involvement without cutaneous, mediastinal, or peripheral lymph node disease. The neoplastic cells in this case expressed the peculiar $OKT3^+T4^+T9^+T10^+$ phenotype. This phenotype would be consistent with a medullary thymic phenotype differentiating along the OKT4 pathway were it not for the reactivity with OKT9. However, since it has been suggested that OKT9 detects a cell-cycle-dependent, activation-specific antigen,[22] as well as a subpopulation of immature thymocytes, one could postulate that the OKT9 reactivity in this case is cell-cycle-dependent and is not differentiation-specific.

A number of investigators have studied the distribution of acid hydrolytic enzyme activities in lymphocyte populations, leading to the proposal of a differentiation scheme wherein maturing T cells progress from $AP^+BG^-ANAE^-$ to $AP^+BG^+ANAE^-$ and finally to $AP^+BG^+ANAE^+$.[24] The cytochemical studies described here appear to support this differentiation scheme. Most of the neoplastic cells in each of the T-cell tumors studied were AP^+, consistent with the fact that T cells at nearly all stages of maturation and differentiation express AP activity.[23,24] The expression of BG and ANAE by the neoplastic cells in these cases largely paralleled the equivalent stages of normal T-cell differentiation, as defined by the OKT antibodies: most neoplasms were BG^+, regardless of the stage of differentiation, 6 neoplasms with thymic phenotypes were $ANAE^-$, and an $OKT3^+T4^-T8^+T10^+$ neoplasm was $ANAE^+$, analogous to the medullary thymocyte. The 6 $OKT3^+T4^+$CTCLs involving lymph nodes (Table 1, Cases 8–12 and 14) represented exceptions in that they were $ANAE^-$ and occasionally BG^-. Whether this reflects a state of differentiation or activation, expansion of a normal $ANAE^-$ subset, a neoplastic anomaly, or certain other implications remains to be determined.

Finally, it should be pointed out that investigators have recently demonstrated that certain of the OKT anti-T cell monoclonal antibodies react with a number of other cell populations and are not wholly specific for T cells. OKT6 has been shown to react with histiocytes and cutaneous and nodal Langerhans cells,[56] and OKT9 appears to detect an activation-specific antigen.[22] Finally, Aisenberg and Wilkes[57] and unpublished observations from our own laboratory have shown that OKT9 and OKT10 react with some large-cell lymphomas negative for Ia antigen and positive for surface and cytoplasmic immunoglobulin that are differentiating along the plasma cell pathway. These reactivities, although of interest, should not detract from the use of OKT monoclonal antibodies in the analysis of the T-cell malignancies.

Phenotypic characterization of the T-cell malignancies, in a manner analogous to the studies performed here, should be useful in defining distinct clinicopathologic entities not appreciated by conventional clinical and histologic criteria and should

provide a basis for the understanding of their biologic heterogeneity and their clinical diversity. More importantly, analysis of the T-cell tumors should assist in the identification of the transitional stages of T-cell differentiation and minor T-cell subsets not discernible by analysis of heterogeneous populations of normal T cells. Such studies, especially those emphasizing parallel functional characterization of the phenotypically defined cell populations, will help to delineate further the heterogeneity of normal human T cells.

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