Ultrastructural Study of Acute Lymphocytic Leukemia: Comparison With Immunologic Studies

By Alan D. Glick, Bonita K. Vestal, John M. Flexner, and Robert D. Collins

Leukemic cells from 29 cases of acute lymphocytic leukemia (ALL), studied for T and B cell markers by the use of sheep erythrocyte rosetting and surface immunoglobulin determinations, were examined by electron microscopy. The majority of patients (76%) were found to have non-T, non-B neoplasms composed predominantly of relatively small, inactive-appearing cells with frequent nuclear folds. T cell cases (21%) were associated with mediastinal masses and were predominantly composed of large, active-appearing cells with nuclear irregularity and little rough endoplasmic reticulum. One case of B cell origin was not morphologically distinct from the non-T, non-B cell cases.

Analysis of neoplastic lymphoid cells by immunologic techniques is now accepted as an essential component of the investigation of lymphoid malignancies. Using these techniques it has been found that B cell lymphoid neoplasms are far more common than T cell neoplasms and include chronic lymphocytic leukemia, multiple myeloma, and follicular center cell lymphomas. T cell neoplasms include thymic lymphoma (malignant lymphoma of convoluted lymphocytes), mycosis fungoides, and node-based T cell lymphomas. The cell of origin in Hodgkin disease remains undefined.

Although most cases of acute lymphocytic leukemia (ALL) are not identifiable as T or B cell based, some cases contain cells that form rosettes with sheep erythrocytes, react with antithymocyte serum, or bear surface immunoglobulin (SIg). This heterogeneity in immunologic characterization in ALL has not been related to morphologic variations. Light-microscopic studies have not shown consistent differences between immunologic subgroups with respect to cell size, nuclear indentation, or cytoplasmic reaction with periodic acid–Schiff (PAS) stain. However, acid phosphatase positivity appears to be definitely associated with T cell ALL.

The present study attempts to correlate the ultrastructural appearance of neoplastic cells in ALL with immunologic studies.

Materials and Methods

Neoplastic cells from 29 patients with untreated acute leukemia, diagnosed as ALL by a combination of morphologic and cytochemical studies, including PAS, Sudan black, and esterase stains, were examined. The majority of cases in all immunologic subtypes (80%) were positive for blocklike or granular PAS staining.

Immunologic studies were carried out on cell suspensions prepared from bone marrow filtrate,
blood, and lymph node biopsy material. Cells were isolated by Hypaque-Ficoll differential centrifugation. Methods for detection of erythrocyte rosette (ER) formation and SIg have been previously described. Trypsinization studies were performed by methods described previously.

Electron microscopy. Small fragments of fresh bone marrow were fixed in 2% glutaraldehyde in Tyrode's buffer at pH 7.3, postfixed in 1% osmium tetroxide, dehydrated in graded alcohol solutions, and transferred through propylene oxide to Araldite. Sections were stained with uranyl acetate and lead citrate and examined with a Philips EM 200 electron microscope.

Measurements. Cell diameters were measured by an ocular micrometer using thick sections prepared from Araldite-embedded blocks.

RESULTS

Non-T, Non-B ALL—General Features

In 22 patients (15 male) (76% of the total), the tumor cells lacked SIg and did not form ER (Table 1). This group includes 5 adults; 17 patients were young children or teenagers with ages ranging from 6 mo to 17 yr. Mediastinal masses at time of diagnosis were noted in 4 patients (18%) in the non-T, non-B cell group.

Electron microscopy. Of 22 patients in the non-T, non-B group (Table 1), 18 had neoplasms composed predominantly of small cells or had mixtures of small and large cells. In 7 patients the vast majority (80%) of the neoplastic cells were small cells measuring 6-8 μm in diameter. These cells contained very little cytoplasm and had nuclei with margined chromatin and nucleoli without prominent nucleolonema (Fig. 1). Nuclei were irregular with prominent folds, in-

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<th>Mediastinal Mass</th>
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*Rosettes in cytocentrifuge preparation contained nonneoplastic-appearing lymphocytes.

†Deceased patient.
dentations, bridges, and pockets. The cytoplasm contained variable numbers of mitochondria, occasional profiles of rough endoplasmic reticulum, and numerous ribosomes, primarily singly distributed. Large masses of glycogen particles or other cytoplasmic inclusions were often encountered. Perinuclear microfilament bundles were frequently seen. Rarely, dense granules were found in the Golgi region. Larger cells (10–12 μm in diameter) were also present in these 7 patients but were few in number. These cells had abundant cytoplasm, often containing aggregated ribosomes, and had more regular nuclei showing partially dispersed chromatin and prominent nucleoli (Fig. 2).

Eleven patients had neoplasms composed of a mixture of the above two cell types without small or large cell predominance (Table 1). These cells ranged from 6 to 14 μm in diameter (average ~9 μm). The other fine-structural features were as described above.

Two patients showed a definite predominance of the large cell type (cases 13 and 15, Table 1). One of these patients (case 15) showed cells very similar to those described in the large cell T cell ALL group (see below). Two additional patients (cases 1 and 19, Table 1) showed unusual cells that resembled transformed lymphocytes (Fig. 3). These cells had regular nuclei with dispersed chromatin, large prominent nucleoli, and cytoplasm with numerous polyribo-
Fig. 2. Non-T, non-B cell ALL. Large cell (left) with more dispersed chromatin than highly folded small cell (right). × 17,400.

some aggregates, and resembled cells seen in Burkitt lymphoma. In one of these patients (case 1, Table 1) most cells contained large accumulations of glycogen (Fig. 4).

**T Cell ALL—General Features**

Six patients (five males) (21% of the total) were in this group (Table 2). The number of tumor cells forming ER varied from 37% to 86%. All of these patients were young children or teenagers (ages 7 mo to 15 yr). Mediastinal masses were present at the time of diagnosis in all but one patient (case 6, Table 2).

**Electron microscopy.** Only one patient (case 1, Table 2) had a neoplasm composed principally (90%) of small cells. Although these cells resembled normal peripheral blood lymphocytes with rounded nuclei, marginated chromatin,
Fig. 3 (top). Cell from case 19, Table 1. Polyribosomes, arrow. x 17,400.

Fig. 4 (bottom). Cell from case 1, Table 1; polyribosomes, arrow. Glycogen accumulation (*) extracted during specimen preparation. x 16,100.
Table 2. T and B Cell Cases

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<th>Case Number</th>
<th>Age (yr)/Sex</th>
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*Immunologic studies revealed 54% of the cells stained for polyvalent Ig; 0% for IgG, 0% for IgA, 56% for IgM, 34% for IgD, 52% for κ light chains, and 18% for λ light chains. After SIg was removed by trypsinization, the cells resynthesized SIg after overnight incubation (47% IgM, 25% IgD, 43% κ, and 5% λ).
†Deceased patient.

and few prominent nucleoli (Fig. 5), the cytoplasm often contained a considerable number of polyribosome aggregates. Rough endoplasmic reticulum profiles were generally absent. These cells were most similar in appearance to cells in the normal thymic cortex.20

The remaining patients with T cell ALL (cases 2-6, Table 2) had neoplasms in which large cells predominated. The majority of the cells measured greater than 10 μm in diameter and contained large folded nuclei with dispersed chromatin and prominent nucleoli. Cytoplasm was abundant and contained many aggregated polyribosomes (Fig. 6). Very little rough endoplasmic reticulum was noted in the cells. Dense granules were often noted in the Golgi region. In addition to these large cells, occasional small cells resembling those in case 1 (Table 2) were found.

The non-T, non-B cells in case 15 (Table 1) were indistinguishable from those large T cells described above, especially in nuclear folding and paucity of rough endoplasmic reticulum profiles. In case 15 the percentage of rosette-forming cells was only 2%, although a mediastinal mass was present. In three other patients with non-T, non-B cell ALL and mediastinal masses (Table 1) leukemic cells did not resemble the large T cells described above.

B Cell ALL

The one patient in this group (case 7, Table 2) had cells with a monoclonal SIg (IgM-κ) at the time of diagnosis. Morphologically, the cells resembled those in the non-T, non-B cell mixed category and showed no distinctive feature allowing their recognition as B cells. Specifically, they did not have the morphologic features of Burkitt lymphoma cells.19

Clinical Course

All patients were treated with vincristine and prednisone. In patients under age 14 yr, there were few differences in therapeutic response rates between immunologic subtypes (see Tables 1 and 2). All children went into remission. Within the non-T, non-B cell group the average total survival for children was 15.6 mo, average duration of remission was 12.3 mo, and the average number of relapses was 1.3. In the T cell group the average total survival was 15.6 mo,
average duration of remission was 6.8 mo, and the average number of relapses was 1.2. These results indicate a slightly poorer response rate for T cell patients. The one B cell patient survived for 24 mo, had a remission duration of 18 mo, and had 4 relapses.

Adult patients had considerably poorer responses. In the non-T, non-B cell group two patients never entered remission. The average duration of remission was 7.1 mo, average number of relapses was 0.5, and average total survival was 8.4 mo. The one adult T cell patient did not enter remission and died after 4 mo.

The average white blood count at presentation for the total non-T, non-B cell
Fig. 6. T cell ALL, large cells; Golgi region, G; granules, arrows. × 13,800. Inset: Toluidine blue. × 600.
The present study corresponds to previous studies of ALL in predominance of childhood cases. Although electron-microscopic findings indicated a heterogeneous picture within all three immunologic categories, certain features remained distinguishable. In this study, non-T, non-B cell leukemia usually was a neoplasm composed of small cells or a mixture of small and large cells, in contrast to T cell neoplasms, which were more often composed of large cells. The one B cell neoplasm in the series was not distinguishable from those in the non-T, non-B cell group. Significant morphologic differences between childhood and adult leukemic cells were not recognized in this group of cases.

Cell size has been correlated with prognosis in a number of studies, with most of these studies indicating that larger cells are associated with some poor-risk parameters. Cell diameter alone may have inadequate predictive value, since volumetric studies indicate that diameter measurements may not accurately reflect cell size. In 100 cases studied by Brouet et al., meningeal relapses were more common in T cell ALL; these cases also had high leukocyte counts, usually considered an unfavorable sign. Therefore T cell cases, by cell size criteria and other indications, may have a poorer prognosis. In our study T cell patients seemed to have a shorter survival and shorter duration of remission. For each immunologic type, considering relatively few cases, adults uniformly appeared to have less favorable courses than children.

In addition to size variations between immunologic subtypes, some qualitative differences found in the present study appear significant. Within the non-T, non-B cell group cases were composed predominantly of inactive-appearing cells with marginated chromatin and scant cytoplasm. These cells have traditionally been called blasts, but they lacked the dispersed nuclear chromatin and prominent nucleoli of cells undergoing mitotic division. The larger cells in the non-T, non-B cell group had more dispersed chromatin and probably were cells in the mitotic cycle. These observations correspond to studies showing that most cells in ALL are nondividing, although all phases of the cell cycle are present.

Although the cells in non-T, non-B ("null") cell ALL are thought to be lymphoid, it is possible that some populations may represent stem cells capable of nonlymphoid differentiation. Such cells, with no membrane markers presently detectable, have also been called "undefined" or U cells, and such a designation may be more accurate.

Nuclear irregularity was easily found in most cases in all immunologic subtypes but appeared most extreme in the small cells within the non-T, non-B cell group. This appearance of nuclear irregularity was not as cerebriform as the cells in Sézary syndrome but was more irregular than the cleaved cells in follicular center-cell neoplasms. Within the T cell group, the nuclear irregularity appeared most marked in the larger cells, while small cells showed little.
indentation. The type of nuclear folding in the large T cells resembled that described in the convoluted cells in thymic lymphoma\textsuperscript{6,7} as well as those convoluted cells described in “lymphoblastic lymphoma.”\textsuperscript{3,30} Indeed, the large cells in T cell ALL in this study were not readily distinguishable from cells described in thymic lymphoma.\textsuperscript{7} The high incidence of mediastinal masses (Table 2) was also similar. These findings indicate a striking similarity between thymic lymphoma and many cases of T cell ALL.

The dense granules found in the Golgi region in T cell ALL probably correlate with the high acid phosphatase staining reported in these cases.\textsuperscript{12} The absence of significant rough endoplasmic reticulum activity in T cell cases may be useful in differentiating these from non-T, non-B cell or B cell cases. Studies of transformation in T and B cells have shown similar differences in the amount of rough endoplasmic reticulum profiles.\textsuperscript{27}

Several cases showed an apparent discrepancy between ultrastructural appearance and immunologic marking. Cells in two of the older non-T, non-B cell patients (cases 1 and 19, Table 1) resembled transformed lymphocytes with many aggregated polyribosomes, large nuclei with dispersed chromatin and large nucleoli, an appearance suggesting Burkitt lymphoma.\textsuperscript{19} However, the immunologic studies showed no Slg, and tumor was not found outside the marrow. Another non-T, non-B cell case (case 15, Table 1) was morphologically not distinguishable from T cell cases 2–6 (Table 2) but showed no conclusive surface markers. A mediastinal mass was present, and it seems likely that this case represented a nonmarking T cell neoplasm.

Although various techniques are employed for recognition of T and B cells, the methodology in this study is used in most investigative centers. Designation of a lymphoid neoplasm as to cell of origin depends upon recognition of the presumed neoplastic cell in relationship to the marker as well as the specificity of the marking technique.\textsuperscript{16} The formation of ER is generally accepted as a reliable T cell criterion, and examination of ER in cytocentrifuge preparations facilitates identification of the cell type forming rosettes.\textsuperscript{10,16} Using cytocentrifuge preparations rosette-forming cells in two non-T, non-B cell cases were interpreted as nonneoplastic small lymphocytes (Table 1). It is also possible that some cases in the non-T, non-B cell group (e.g., case 15, Table 1) might be of T cell origin and might have marked with antithymocyte serum.\textsuperscript{13} Mediastinal masses were noted in four cases in the non-T, non-B cell group, all without conclusive surface marking (Table 1). Some or all such cases may represent undetected T cell neoplasms or may indicate that the neoplastic cells have the capacity to home to the thymus but have not acquired membrane characteristics necessary to bind sheep erythrocytes.

Although the present study demonstrates several morphologic features that appear to correlate with immunologic categories, it also illustrates the heterogeneous nature of what is now called ALL. A subclassification with definite prognostic and therapeutic value awaits additional studies of much larger series of cases.

REFERENCES


27. Douglas SD: Human lymphocyte growth in vitro: Morphologic, biochemical and im-


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