Leukemic Reticuloendotheliosis

By Sidney Trubowitz, Bertha Masek, and Julio M. Frasca

The anatomical and functional features of the hairy cells of leukemic reticuloendotheliosis were investigated by time lapse cinematography; phytohemagglutinin stimulation; adherence to siliconized surfaces; a variety of cytochemical methods; and phase, transmission, and scanning electron microscopy. The scanning electron microscopic image supports the morphologic evidence for a relationship between the hairy and the reticular cells.

In 1923 EWALD described a rare and unusual form of chronic leukemia whose outstanding features are a very large spleen, in the absence of significant lymphadenopathy, and the presence of a circulating mononuclear cell with many cytoplasmic projections. This unique cell and its singular surface has invited much attention, and considerable morphological, chemical, and physiological detail has been accumulated and interpreted in support of a close kinship with the reticular cells. More recently, Rubin concluded from studies of the anatomical and functional features of the cells of a patient with leukemic reticuloendotheliosis (LRE) that the cells, though possessing properties of both reticular cells and lymphocytes, should be more properly classified as lymphocytes and designated the disease chronic reticulolymphocytic leukemia.

In the present report we wish to present some studies on the cells of a patient with LRE and, in addition, to demonstrate the surface ultrastructure of these cells as seen with the scanning electron microscope.

Case Report

A 55-yr-old merchant was admitted to the St. Elizabeth Hospital, Elizabeth, N.J., on November 14, 1969, for the investigation of an abdominal mass. Except for mild diabetes discovered in 1960, controlled with orinase, and hypertension in 1967, treated with an antihypertensive agent, the patient enjoyed good health until the autumn of 1969 when he became aware of a vague abdominal discomfort. In the next 2 mo he noted some weight loss and fatigue, but more alarming, a feeling of fullness and hardness of the abdomen. A rapid increase in the size of his girth led him to visit his physician.

The patient was an alert, coherent white man in no distress. His temperature was normal and remained so during his entire period of hospitalization. Blood pressure was 160/100, pulse was regular at 84. He displayed mild pallor of the conjunctivae. The heart was normal in size and a grade II soft blowing systolic murmur was heard at all.
valve areas. A large, hard, smooth mass filled the left half of the abdomen. The mass was freely movable and extended from the costal margin to the pelvic brim and to the midline. The liver was not enlarged and there was no lymphadenopathy.

The laboratory data included the following: hemoglobin, 10.5–11.5 g/100 ml; hematocrit determinations, 30–35%; average red blood counts 3,900,000/cu mm; white blood cell counts, 11,000–13,000/cu mm; reticulocytes, 1.5–2.5%. About 90% of the cells in the peripheral blood smear were large mononuclear cells (Fig. 1). The red blood cells showed marked anisocytosis and mild poikilocytosis. Examination of urine was normal. Serum calcium was 8.8 mg/100 ml, phosphorus 4.5 mg/100 ml, glucose 150 mg/100 ml, BUN 25 mg/100 ml, uric acid 6–8 mg/100 ml, total protein 7.8 g/100 ml, albumin 4.5 g/100 ml, total bilirubin 1.0 mg/100 ml, alkaline phosphatase, 35 mU/ml, LDH 175 mU/ml, and SGOT 48 mU/ml.

The chest X ray was normal. The intravenous pyelogram revealed a mass in the left flank, compressing the left kidney, but outside the retroperitoneal region. A scan, following administration of 3 MCI of sulfur colloid 99TC, showed a normal liver but a large spleen corresponding to the mass seen on the pyelogram.

An EKG showed deep Q3, which was felt to be indicative of left ventricular hypertrophy. In the bone marrow smear more than 90% of the cells were identical with the large mononuclear cells in the peripheral blood.
In brief, this patient exhibited the typical clinical features of leukemic reticuloendotheliosis. This disease has an incidence of less than 2% of all the leukemias, usually appears between the age of 50–70, and is more common in men. The onset is insidious, with weakness, easy fatigue, repeated infections, and left upper quadrant discomfort. About 95% of the patients have massive splenomegaly, which is the site of frequent perisplenitis, splenic infarct, and rupture. Hepatomegaly is also common, estimated to be present in about 50% of the subjects. Lymphadenopathy is strikingly infrequent. About 70% have mild anemia, generally normocytic and normochromic. Leukopenia is seen in 50% of the patients and elevated white cell count (15,000–30,000) in about 20%. Platelets are decreased in about 70% of the cases. The marrow is difficult to aspirate and shows marked increase in “reticular” elements and a decrease in the hematopoietic compartment. The spleen size ranges from 2500 to 3800 g.
The hallmark of the disease is the presence of mononuclear cells in the blood, marrow, and spleen. The cells show numerous short villi and a characteristic flagellated appearance when examined under the phase microscope (Fig. 2). These cells have been variously designated as “hairy” cells, neoplastic lymphoid reticular cells or reticuloendothelial cells.

On December 8, 1969, a 3300-g spleen was removed. The organ measured 33 × 15 × 15 cm and showed numerous, multiple, pressure lobulations. The capsule showed focal zones of fibrous thickening. The cut surface revealed multiple, well-circumscribed nodules of extremely soft hemorrhagic tissue. A touch preparation of the cut surface of the spleen is shown in Fig. 3.

The postoperative course has been uneventful. The peripheral blood picture remains unchanged and the patient is asymptomatic.

**Other Investigations**

*Time-lapse Microcinematography*

Preparations of the patient’s blood were studied as previously described. Cell locomotion was characterized by the formation of veil-like cytoplasmic projections that undulated in random fashion about the cell perimeter. The movement of the cell was quite erratic but did tend to move in one general direction at the rate of about one cell diameter in 6-8 min. Motion was similar to previous descriptions and reminiscent of the general movement of monocytes rather than lymphocytes.

*Phytohemagglutinin Stimulation*

About 10.0 ml of blood were obtained from the patient by venesection into a syringe previously wetted with phenol-free heparin (Panheparin, Abbott). Two ml of the supernatant plasma-cell suspension were introduced into a flask containing 6 ml TC 199 (Difco); 0.2 ml of phytohemagglutinin M (Difco) were added and the mixture incubated at 37°C for 72 hr. At termination of incubation, cell suspensions were prepared, appropriately washed, smeared on slides with a sable brush, and stained with May-Grunwald-Giemsa.

Evaluation of the transformation process was accomplished by counting three approximately equal groups of 1000 cells each at the ends and center of the smear.

No transformed cells were detected in duplicate cultures.

*Histochemical Examinations*

Blood smears were prepared and stained for alkaline phosphatase, acid phosphatase, nonspecific esterase, periodic acid Schiff, toluidine blue, and iron, as previously described. Air-dried smears were stained for peroxidase by the Coomassie technique. Formal vapor-fixed smears were stained in Sudan black (saturated solution in 70% alcohol) for 30 min at 60°C. Slides were washed well in distilled water, dried thoroughly, rinsed briefly in xylol and mounted in synthetic resin HSR.

**Results**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>Negative</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>Positive</td>
</tr>
<tr>
<td>Nonspecific esterase</td>
<td>Negative</td>
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<tr>
<td>Sudan black</td>
<td>Negative</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>No metachromasia</td>
</tr>
</tbody>
</table>
The histochemical responses are in keeping with those described for the lymphocyte.

**Adherence to Siliconized Glass Bead Column**

Siliconized glass beads were prepared as columns in syringes and adhesiveness studied according to the technique of Brandt. Cells were exposed to the beads for 10 min at 37°C. Before filtration the white blood cell count was 17,600/cu mm with 90% mononuclears. After filtration these figures were 17,200/cu mm and 88% mononuclear cells.

Failure of adherence to the siliconized beads is much more characteristic of lymphocytes than of monocytes.

**Transmission Electron Microscopy**

Blood from the patient was collected in syringes treated with Panheparin (Abbott) and kept in an ice bath for 1 hr. The red blood cells were sedimented and the plasma-white blood cell suspension was transferred to a centrifuge tube and washed twice with White's saline. Between washes the sample was centrifuged at 300 rpm. After the second wash, the sample was centrifuged at 800–1000 rpm, the saline was removed, and the white blood cells were fixed in 1% buffered OsO₄ (cacodylate buffer) for ½ hr. The fixed button of white blood cells was broken into small clumps, stained in block

![Fig. 5.—Low-magnification transmission electron micrograph illustrating the numerous slender cytoplasmic extensions of the LRE cells. In contrast, at the upper left, is a neutrophil with a relatively smooth surface. × 2800.](image-url)
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and processed for embedding in Epon. Thin sections were cut on the LKB microtome and doubly stained.

Again, by transmission electron microscopy the LRE cells are small, mononuclear, with numerous cytoplasmic projections at their periphery (Fig. 5). These projections range in length from 0.5 to 3.0 $\mu m$ and in width from 0.1 to 0.5 $\mu m$. Most of these are long and slender, but clublike and pyramidal shapes also occur. In diameter the cells range from 8 to 12 $\mu m$ and the slightly indented oval nuclei are usually centrally located. Marginal chromatin in the nuclei ap-

Fig. 6.—At higher magnification the LRE cell shows numerous vesicles and granules of various sizes throughout the cytoplasm, and the cytoplasmic extensions. The many mitochondria have well-defined cristae. Numerous ribosomes are scattered throughout the cytoplasm. $\times$ 10,500.
pears condensed and nucleoli are sometimes visible. A pair of centrioles in their typical orientation at right angles to each other is frequently seen adjacent to the nuclear indentation.

The cytoplasm is characterized by the presence of numerous vesicles which range in diameter from 80 to 280 m$m$ (Fig. 6). In general, the larger vesicles appear translucent and the smaller ones appear opalescent. Occasionally, some of the vesicles are present in the cytoplasmic extensions at the cell periphery. Also seen are some small electron-dense granules about 50 m$m$ in diameter.

A striking feature in the cytoplasm of some of the cells is the presence of large osmophilic bodies 4-6 m$m$ in diameter. These bodies are delimited by a membrane and faint membranous patterns are discerned within some of them. Large vacuoles are occasionally found and in some areas average several microns in diameter. These areas extend from the cell periphery to the nucleus and sometimes encircle the nucleus. The material within these vacuoles is dispersed as either a fine floc or as clumps. The Golgi complex appears scant, generally consisting of three to four cisternae with associated vesicles and granules. Mitochondria are abundant and of the filamentous type, with well-defined cristae traversing the short axis. By way of contrast, a lymphocyte (Fig. 7) of chronic lymphocytic leukemia is shown: the striking difference needs no description.

The buffy coat pellets of normal lymphocytes and the lymphocytes from patients with chronic lymphocytic leukemia were prepared in similar fashion but fixed as described under scanning electron microscopy.

A thin section of the patient's cells prepared for light microscopy is shown in Fig. 4. The cell villi and the cellular features are clearly seen.

Fig. 7.—This lymphocyte from chronic lymphatic leukemia shows a prominent nucleolus within its large round nucleus. The cell outline is relatively smooth. Numerous ribosomes fill the cytoplasm. $\times$ 10,500.
Scanning Electron Microscopy

Blood from the patient and from a patient with lymphatic leukemia was collected in syringes treated with panheparin (Abbott) and kept in an ice bath for 1 hr. Normal blood was similarly collected and filtered through a column of siliconized beads to remove the monocytes and polymorphonuclear cells. The red blood cells of the lymphocyte-rich filtrate and the leukemic blood samples were sedimented and the plasma and white blood cell layers
Fig. 10. (upper).—LRE cell, SEM. × 10,000.

Fig. 11. (lower).—Same cell as Fig. 10. × 20,000.

were transferred to centrifuge tubes and washed twice with White’s saline. Between washes the samples were centrifuged at 300 rpm. The saline was removed and the cell layers were fixed as follows: (1) 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3 for 1 hr at 4°C; (2) Double aldehyde (1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3) 1 hr at 4°C followed by cacodylate buffer, twice rinsed and postfixed in 1% buffered OsO4 for ½ hr at 4°C; (3) 1% buffered O3O4 (cacodylate buffer) for ½ hr at 4°C.
After fixation the cells were washed four time with 0.1 M cacodylate pH 7.3 and were stored in the buffer. The cell suspensions were placed on aluminum stubs, coated with a thin film of gold to prevent charging and placed in a scanning electron microscope.  

The surface structure of the normal lymphocyte (Fig. 8) is indeed striking and shows a rather characteristic convoluted appearance. By contrast the lymphocyte obtained from a patient with chronic lymphatic leukemia (Fig. 9) shows a smooth surface. The LRE cell surface is highly irregular, covered with spikes, and broad membrane-like structures (Figs. 10 and 11). Again, as in the transmission electron micrographs, the LRE cell appears distinct and decidedly different from the normal and leukemic lymphocyte.

**Discussion**

The circulating mononuclear cell of the disease designated leukemic reticuloendotheliosis has aroused considerable interest. The morphological characteristics are unique and suggest a relationship to the monocytic or reticular cell systems. Histochemical and functional behavior of this cell is reminiscent of the lymphocyte. The hybrid nature of the LRE cell has been noted by many investigators and expressed in their various attempts at classification e.g. lymphoid reticular cells or reticulolymphocytic cells or reticuloendothelial cells, depending on the investigator's evaluation of the score card of the cell's characteristics.

In the present report another morphological feature, the surface ultrastructure, has been investigated. The hairlike processes that distinguish this cell in phase microscopy are seen as a variety of microprojections, spikes, and flat membranes on the scanning electron micrograph, features ascribed to the macrophage surface. The information obtained from the scanning electron micrograph strengthens the entire array of anatomic evidence for the relationship between the LRE cell and the reticular cell.

It is difficult to make definitive statements, in our current state of knowledge, about the origin of the reticular or monocytic phagocytic cells. The present general consensus holds that the bone marrow contains cells that have the potential to produce the hematopoietic cells as well as the lymphocytes and the reticular elements. There is some limited evidence to suggest that thoracic duct lymphocytes can transform into Kupffer cells and that bone marrow cells may be the source of peritoneal macrophages. There is belief that the small lymphocyte-like cells in the bone marrow may, in part, represent stem cells, in that they are able to give rise to lymphocytes, macrophages, red cells, white cells, and platelets. There is also mounting evidence that negates the previously assigned stem cell role of the reticular cells.

It is conceivable that LRE may be the result of some aberration in the maturation and proliferation of the reticular cells in their differentiation from the stem cells and that at some point in this process of differentiation or de-differentiation, cells of a hybrid nature can appear.

Morphological information of any cell type is a reflection of the functional activity of the cell under study. Extrapolation from the catalog of a cell's structural and functional detail at one point in time to the cell's origin is ad-
mittedly difficult. The origin of the reticular and monocytic cells is still an important unsolved problem. Some clue may perhaps be obtained by further careful investigation by the many techniques now available of pathologic states such as LRE.\textsuperscript{17} In view of the complex problem of differentiation and our present state of ignorance one wonders whether much can be gained by changes in nomenclature.\textsuperscript{7}

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