Functional Cytogenetic and Cytochemical Study of the Leukemic Reticulum Cells

By L. T. Yam, G. L. Castoldi, M. B. Garvey and W. J. Mitus

Leukemic reticuloendotheliosis is a malignant condition characterized by generalized proliferation of the reticulum cells and their derivatives which appear in the peripheral blood. This condition was first recognized by Ewald in 1923, and since then many other reports have appeared. Mitus et al. have presented histochemical findings in these cells. Rabinowitz and Schrek and Schrek and Donnelly added further observations on the phase-microscopic appearances, motility, and response to irradiation of these cells. The cells were found to be much more resistant to x-ray exposure than the normal lymphocyte.

The present report deals with observations on the morphology, cytochemistry, karyotypes, responsiveness to phytohemagglutinin stimulation, and phagocytic properties of cells from the peripheral blood and spleen of a patient with leukemic reticuloendotheliosis.

Case Report

This 48 year old laborer was first admitted to the New England Medical Center Hospitals (NEMCH) on December 17, 1966, because of pancytopenia and splenomegaly. Six months prior to admission he had been seen elsewhere because of mild fatigue, back pain, and general malaise. Splenomegaly, leukopenia, and a progressive increase of cells interpreted as "lymphocytes" in his bone marrow were then discovered.

On admission to NEMCH, a soft and nontender spleen was palpable at the left iliac crest. The liver was palpable at the right costal margin. There was no lymphadenopathy. Laboratory examination revealed a hemoglobin of 9.9 Gm./100 ml., a hematocrit of 29 per cent, a red blood cell count of 2,940,000/mm³ and a platelet count of 51,000/mm³. Reticulocyte count was 2.3 per cent and a white blood count was 3,600/mm³ with 7 per cent neutrophils, 73 per cent lymphocytes, 1 per cent eosinophils, 1 per cent bands, and 18 per cent reticulum cells. On Wright-Giemsa stain, the neoplastic reticulum cells (or...
their derivatives) appeared as round and occasionally elongated cells 10 to 20 μ in diameter with irregular cytoplasmic edges. The nucleus was round, sometimes slightly indented, had a distinct nuclear membrane and a chromatin which was coarser than that of a blast but finer and less clumped than that of a lymphocyte. Nucleoli were infrequent, but were occasionally seen (Fig. 1). The serrated cytoplasmic edge was appreciated better when Wright-Giemsa stain was made slightly alkaline. A bone marrow aspirate revealed myeloblasts 0.4 per cent, promyelocytes 1.1 per cent, myelocytes 0.8 per cent, metamyelocytes 0.9 per cent, juvenile 0.5 per cent, neutrophils 0.2 per cent, plasma cells 2.3 per cent, lymphocytes 4.3 per cent, megakaryocytes 0.3 per cent, proerythroblasts 1.6 per cent, basophilic erythroblasts 6.3 per cent, polychromatophilic erythroblasts 33.2 per cent, orthochromatic erythroblasts 15.9 per cent, mast cells 0.1 per cent, endothelial cells 0.2 per cent, and reticulum cells 28.4 per cent. These were identical to the reticulum cells described in peripheral blood. The patient was discharged without therapy.

Over the next three weeks he developed episodes of fever, lethargy, and fatigue; the liver and spleen increased rapidly in size. He was readmitted on January 11, 1967. On examination his spleen was firm and extremely tender. The liver was palpable 8 cm. below the right costal margin.

Laboratory investigations at that time revealed a hemoglobin of 8.2 Gm./100 ml., a hematocrit of 26 per cent, a platelet count of 7,000/mm³, reticulocyte of 1.5 per cent. White blood cell count was 2,869/mm³ with 8 per cent neutrophils, 62 per cent lymphocytes, 5 per cent monocytes, and 25 per cent reticulum cells. There were 34 erythroblasts per 100 leukocytes. A bone marrow was aspirated from the sternum with difficulty; only a few spicules could be obtained. Differential count of marrow smears revealed promyelocytes 0.3 per cent, myelocytes 0.1 per cent, metamyelocytes 0.3 per cent, juveniles 0.1 per cent, neutrophils 0.4 per cent, plasma cells 1.1 per cent, lymphocytes 4.8 per cent, megakaryocytes 0.1 per cent, proerythroblasts 0.1 per cent, basophilic erythroblasts 2.2 per cent, polychromatophilic erythroblasts 3.5 per cent, orthochromatic erythroblasts 2.6 per cent, endothelial cells 0.1 per cent, and reticulum cells 84.4 per cent.

Twenty-four hours after admission he underwent splenectomy for spontaneous rupture of the spleen. At the time of surgery, he was placed on cortisone acetate, 100 mg. every eight hours, and this medication was continued in reducing doses for the subsequent thirteen days.

The spleen weighed 2,940 Gm. The capsule was thin and the surface smooth. Cut section revealed a reddish, soft, infiltrated and meaty parenchyma. Lymphoid follicles and trabeculae were scanty. There were extensive areas of hemorrhage and of fresh and organized infarction. Near one pole there was an area of necrosis of parenchyma with rupture of the capsule. Microscopically, there was a uniform and diffuse proliferation of proliferation...
of reticulum cells affecting cords of Billroth and spilling into sinusoids (Figs. 2 and 3). These cells measured 10 to 15 μ, were oval, and had fairly abundant amphophilic cytoplasm. Their nuclei were round with frequent indentations. Nuclear chromatin was rather loose and nuclear membrane distinct. Nucleoli were not prominent. In areas where these cells were less closely packed, the irregular cytoplasmic border was seen. Lymph follicles were virtually absent, only a few small lymphocytic aggregates were seen. Cytoplogic examination of spleen imprints stained with the Wright-Giemsa stain revealed lymphocytes 6.2 per
Fig. 4.—Reticulum cells from spleen imprints. Wright-Giemsa stain. 1,300×.

cent, neutrophils 1.0 per cent, plasma cells 1.1 per cent, and reticulum cells 91.6 per cent. These reticulum cells resembled those seen in the peripheral blood and bone marrow (Fig. 4).

**MATERIALS AND METHODS**

The cells were obtained from peripheral blood and from the spleen. The following morphologic and cytochemical studies were performed: Wright-Giemsa, PAS with and without diastase digestion,8 methyl green pyronin,9 Sudan black B,10 peroxidase,11 alkaline phosphatase,12 acid phosphatase,13 β-glucuronidase,14 lactic and succinic acid dehydrogenase.15 Dihydroorotic acid dehydrogenase was performed according to our modification* of Cohen's method.16 Supravital17 and phase-contrast examinations were performed on fresh specimens.

Phagocytic activity was studied by carbon particles and latex particles methods. The particles were added to cell suspensions and the preparation was incubated at 37 C. for one hour. Monocytes from normal subjects and from patients with monocytic leukemia (Schilling type) served as controls.

Response to phytohemagglutinin (PHA) and the elimination of cells by gelatin-cotton filtration were studies according to the method of Yam et al.18 Cytochemical studies of cells in cultures with and without PHA were also performed. The PHA responsiveness of the cells was called the transformation index and was expressed as a percentage of absolute number of PHA reacting cells per milliliter of culture medium at seventy-two hours to a total number of viable lymphocytes per milliliter at zero hours. (In the splenic preparation, the percentage was to a total number of viable round cells per milliliter of medium at zero hours.) The viability of cells in cultures was tested by the trypan blue method.19 Controls consisted of blood from five normal individuals and from three patients with chronic lymphocytic leukemia.

Chromosomal studies were performed using the direct method of Kiossoglou et al.20 and the short-term culture method described above.18 Sex chromatin body (drumstick) was looked for in five hundred neutrophils according to the method of Kosenow.21

*Fixation: Acetate buffer at 0.03 M and acetone 60 per cent (pH 4.2). Incubation mixture: addition of DPN (2.26 × 10^-4 M) pH of incubating mixture 7.8.
Table 1.—Cytochemical Study of Lymphocytes, Monocytes, and Neoplastic Reticulum Cells

<table>
<thead>
<tr>
<th></th>
<th>Normal Lymphocytes</th>
<th>Monocytes</th>
<th>Pathologic Reticuloendothelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase*</td>
<td>+ + / 0</td>
<td>+ + / + + + +</td>
<td>+ / + / +</td>
</tr>
<tr>
<td>β-glucuronidase*</td>
<td>+ + / 0</td>
<td>+ / + + + + +</td>
<td>+ + / + +</td>
</tr>
<tr>
<td>Periodic acid-Schiff reaction*</td>
<td>+ / 0</td>
<td>0 / +</td>
<td>0 / +</td>
</tr>
<tr>
<td>PAS-diastase*</td>
<td>0 / 0</td>
<td>0 / 0</td>
<td>0 / 0</td>
</tr>
<tr>
<td>Methyl green-pyronin</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sudan black</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>0</td>
<td>0 - +</td>
<td>0</td>
</tr>
<tr>
<td>Lactic acid dehydrogenase</td>
<td>++</td>
<td>++</td>
<td>++ + +</td>
</tr>
<tr>
<td>Succinic acid dehydrogenase</td>
<td>++</td>
<td>++</td>
<td>++ + +</td>
</tr>
<tr>
<td>Dihydro-orotic acid dehydrogenase</td>
<td>++</td>
<td>++</td>
<td>++ + +</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

*Granular activity/nongranular activity.

Table 2.—Elimination of Neutrophils, Monocytes, and Reticulum Cells by Gelatin-cotton Method

<table>
<thead>
<tr>
<th></th>
<th>12/19/67</th>
<th>1/17/67</th>
<th>1/30/67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>Before*</td>
<td>After</td>
<td>Before*</td>
</tr>
<tr>
<td></td>
<td>Elimination</td>
<td></td>
<td>Elimination</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.2</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Neutrophiles</td>
<td>7.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Others</td>
<td>0.2</td>
<td>0.2</td>
<td>12.8</td>
</tr>
<tr>
<td>Reticulum cells</td>
<td>21.4</td>
<td>3.8</td>
<td>25.4</td>
</tr>
<tr>
<td>Reticulum cells†</td>
<td>17.8</td>
<td>4.6</td>
<td>20.0</td>
</tr>
</tbody>
</table>

*Separation made by setting syringe in upright position for one hour. Gelatin not used.
†By phase-contrast microscopy.

RESULTS

Cytochemical Studies
Results are presented in Table 1. The reticulum cells were similar to, but not identical with, the monocytes. Both had high acid phosphatase and beta-glucuronidase activity and a characteristic pattern of distribution. These enzymes were low in the lymphocytes from untreated patients with chronic lymphocytic leukemia. PAS stain was diffusely positive in the reticulum cells and monocytes from normal subjects and patients with monocytic leukemia. It was granular in positive lymphocytes from normal subjects and patients with chronic lymphocytic leukemia.

Phase-Contrast Microscopy
The lymphocytes from normal subjects and patients with chronic lymphocytic leukemia were round and had a smooth cytoplasmic margin. The reticulum cells showed distinct cytoplasmic projections which changed constantly. Pressure exerted on the cells caused them to spread more, and the cells assumed the appearance previously described by Bouroncle et al. and Mitus et al. 
Increased thickness of the preparations enhanced the “hairy projections” described by Schrek et al.7

Supravital Stain

The reticulum cells had mitochondria and small neutral red granules in their cytoplasm. Lymphocytes from normal subjects and patients with chronic lymphocytic leukemia had less neutral red granules. The monocytes from normal subjects and patients with monocytic leukemia frequently contained large masses of neutral red in their cytoplasm.

Phagocytic Activity

Leukemic reticulum cells showed no phagocytic activity of carbon and latex particles. The monocytes from normal subjects and patients with monocytic leukemia phagocytized these particles.

Gelatin-cotton Infiltration of Cells

The results are listed in Table 2. The number of reticulum cells diminished considerably after “gelatin-cotton” separation. This was similar to the findings obtained with monocytes but unlike those obtained with lymphocytes.
Table 3.—Chromosome Studies

<table>
<thead>
<tr>
<th>Date</th>
<th>Method</th>
<th>Tissue</th>
<th>Total No. of Metaphases</th>
<th>Distribution of Chromosomes</th>
<th>Karyotyped Metaphases</th>
<th>Normal Metaphases</th>
<th>Abnormalities in the Pathologic Metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dec. 19, 1966</td>
<td>Direct</td>
<td>Peripheral blood</td>
<td>—  — — — — — — — — — — — — —</td>
<td>—  — — — — — — — — — — —</td>
<td>—  — — — — — — — — — — —</td>
<td>No metaphase found</td>
<td>Two extra chromosomes (Group C) Abnormal chromosomes in Group A G Monosomy</td>
</tr>
<tr>
<td>Jan. 12, 1967</td>
<td>Direct</td>
<td>Spleen</td>
<td>10 1 — — 1 4 4 10 — —</td>
<td>—  — — — — — — — — — — —</td>
<td>—  — — — — — — — — — — —</td>
<td>No metaphase found</td>
<td>Two extra chromosomes (Group C) Abnormal chromosomes in Group A G Monosomy</td>
</tr>
<tr>
<td>Jan. 17, 1967*</td>
<td>Culture</td>
<td>Peripheral blood</td>
<td>3 1 — — 2 — 2 2 — — — — —</td>
<td>—  — — — — — — — — — — —</td>
<td>—  — — — — — — — — — — —</td>
<td>No metaphase found</td>
<td>Two extra chromosomes (Group C) Abnormal chromosomes in Group A G Monosomy</td>
</tr>
<tr>
<td>Feb. 2, 1967†</td>
<td>Culture</td>
<td>Peripheral blood</td>
<td>21 2 1 5 13 — 11 10 — —</td>
<td>—  — — — — — — — — — — —</td>
<td>—  — — — — — — — — — — —</td>
<td>No metaphase found</td>
<td>Two extra chromosomes (Group C) Abnormal chromosomes in Group A G Monosomy</td>
</tr>
</tbody>
</table>

*On cortisone therapy.
†One week after cortisone therapy was discontinued.
Fig. 6.—Above: Metaphase plate obtained from the spleen. Below: Karyotype of the same cell. It contains forty-seven chromosomes. Two extra chromosomes are present in group C. An abnormal chromosome is present in group A (arrows).

Response to PHA

Results are given in Figure 5. The response of peripheral blood lymphocytes to PHA stimulation was normal prior to corticosteroid therapy, markedly suppressed during therapy, and reverted to normal one week after corticosteroid therapy was discontinued. Response of the cells from the spleen was less than 1 per cent. Histochemical stains of the transformed cells showed them to be identical with those transformed lymphoid cells from normal subjects.
Chromosome Studies

The chromosomes were arranged arbitrarily according to their size. The results are summarized in Table 3. No mitoses were found in the direct preparations from the peripheral blood. A relatively good number of mitoses were obtained by the direct method from the spleen preparations. All of these cells showed multiple chromosomal abnormalities characterized by the presence of consistent changes in group A (abnormal submetacentric or presence of an extra chromosome similar to those of this group), group C (two extra chromosomes), and group G (absence of one small acrocentric) (Fig. 6).

Chromosomal studies of the peripheral blood by the culture method were performed on two occasions. In the first study on January 17, 1967, few mitoses were found, probably due to an impaired transformation of lymphocytes due to steroid therapy. In the second study performed on February 2, ten metaphases showed normal karyotypes. Eleven metaphases showed inconsistent abnormalities such as elongation of chromosomes of group A and E or monosomies of other groups. In the study of sex chromatin bodies in neutrophils in peripheral blood, only one drumstick-like structure was present in five hundred cells examined.

Discussion

Cells obtained from the peripheral blood and the spleen of this patient were identical in their morphologic and cytochemical characteristics with the cells described by Bouroncle et al., Mitus et al., Rabinowitz and Schrek, and Schrek and Donnelly in cases of leukemic reticuloendotheliosis. "Filtration" studies support the view that the cells in question are of the reticulum cell variety. It is known that lymphocytes pass through the cotton filter with relative ease, but most of the monocytes are retained by it. As the number of neoplastic reticulum cells is markedly decreased following filtration, they behave in a manner similar to monocytes and unlike lymphocytes.

The difference in response to PHA in culture shown by cells from the peripheral blood and by the cells from the spleen is probably due to the difference in the types of cells in the two specimens. Since the peripheral blood contained mostly lymphocytes and the spleen suspension contained 92 per cent reticulum cells, it is most likely the transformed cells in the cultures are primarily, if not exclusively, derived from the lymphocytes. For this reason, we considered that neoplastic reticulum cells did not transform into blasts on PHA stimulation at seventy-two hours.

The neoplastic reticulum cells from this case rarely showed phagocytic activity, while monocytes, be they from normal subjects or from patients with monocytic leukemia, had definite phagocytic properties. This impairment of phagocytic ability could be due to immaturity of the cells. At an early stage of development, the phagocytic abilities may not yet be developed, however, the morphologic picture of the reticulum cells under discussion was not that of a primitive or blastlike cell. Most of these cells were not larger than 16 μ, nuclear chromatin was rather clumpy and nucleoli were rarely seen. It is possible that these cells, although fairly advanced in maturation, are abnormal...
from the point of view of phagocytic properties, and this abnormality is an expression of a disordered function associated with neoplasia.

The presence of normal karyotypes obtained from peripheral blood cultures and the abnormal karyotypes from direct splenic preparations raises interesting questions. If we assume that the abnormal metaphase plates were derived from pathologic reticulum cells, which comprised 92 per cent of all cells, the normal chromosomal findings from the peripheral blood in leukemic reticuloendotheliosis observed by James et al.24 and by ourselves can be explained by predominant derivation of the metaphases from normal lymphocytes. This parallels the findings of culture characteristics previously described: normal transformation obtained from peripheral blood suggests the presence of normal lymphocytes, and nontransformation of the splenic material suggests abnormal cells.

In the chromosomal analysis of the present case, two general points come out quite clearly: (1) aneuploidy and the presence of abnormal karyotypes from the splenic preparations and (2) the differences between findings from the spleen and from the peripheral blood. Aneuploidy and abnormalities of karyotypes are strongly suggestive of a neoplastic nature of the cells. Consistency of the karyotypic abnormalities is in favor of monoclonal proliferation which probably evolved from a pathologic ancestral cell; however, the possibility of chromosomal rearrangement on a stabilized line cannot be excluded.

It is interesting to note that the chromosomal abnormalities do not interfere with the growth of the reticulum cells. According to the theory of Burnet,25 these cells achieve some selective advantage over other cells as far as proliferation is concerned. A similar monoclonal type of proliferation has been observed by one of us (GLC) in a case of leukemic reticuloendotheliosis with skin involvement, in which the abnormalities were limited to A group.26 There was a reciprocal translocation in group A which resulted in one fragmented chromosome and one giant chromosome. These findings are in agreement with findings in other forms of leukemia.27-29

SUMMARY

Morphologic, cytochemical, functional, and chromosomal studies were performed on cells from blood and the spleen of a patient with leukemic reticuloendotheliosis. Characteristics of these cells were further defined by histochemical studies, by their removal by the cotton-filtration process, by their negative response to PHA stimulation, and by their poor phagocytic properties. Chromosomal studies in this case showed abnormal patterns in cells from the spleen. This abnormal pattern was characterized by the presence of an abnormal chromosome in A group, extra chromosome in C group, and monosomy in G group.

SUMMARIO IN INTERLINGUA

Studios morphologic, cytochimic, functional, e chromosomal eseva effectuate in cellulas ab le sanguine e ab le splen de un paciente con reticuloendotheliosis leucemic. Le características de iste cellulas eseva definite additionalmente per medio de studios histochimic, per lor remotion per le processo de filtration a coton, per lor responsa negative a stimu-
lation con PHA, e per lor proprietates phagocytic pauc disveloppate. Studios chromosomal in iste case monstrava configurationes anormal in cellulas splenic. Le anormalitate consisteva in le presentia de un chromosoma anormal in gruppo A, un chromosoma supernumerari in gruppo C, e monosomia in gruppo G.

REFERENCES

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