Neoplastic Lymphoid Reticulum Cells in the Peripheral Blood: A Histochemical Study

By W. J. Mitus, I. B. Mednicoff, B. Wittels and W. Dameshek

LYMPHOID organs (lymph nodes, spleen, etc.) may be said to contain at least two types of leukocytes, i.e., lymphocytes and reticulum cells. Neoplastic proliferations of the lymphoid organs, which may be either lymphocytic or reticulum cell in type, are either leukemic or aleukemic. Although the lymphoproliferative tumors (lymphosarcoma) are frequently associated with the dissemination of these cells in the peripheral blood (leukemic picture), solid tumors of the reticulum cells (reticulum cell sarcoma, reticuloendotheliosis) do not usually show this characteristic. It is possible that the syncytial arrangement of the reticulum cells in the organs prevents them from being readily washed out into the peripheral blood. Nevertheless, in rare cases of generalized proliferation of the reticulum cell system some of the neoplastic cells may be found in the peripheral blood, sometimes in large numbers (reticulum cell leukemia, leukemic reticuloendotheliosis).

The reticulum cells vary considerably in their morphologic appearance under normal conditions. This is not surprising in view of the various degrees of maturation of these cells which one may encounter in normal tissues and even more so on account of the various cytologic directions into which these cells may differentiate (e.g., bone marrow elements, cells of the lymphocytic series, plasma cells, fibroblasts). The reticulum cells which appear in the peripheral blood in some of these cases of generalized reticulum cell proliferations (reticulum cell leukemia, leukemic reticuloendotheliosis) are as a rule relatively small and may resemble lymphocytes, at least superficially. It is possible that this small variety is more readily detached and thus swept into the circulation, while their larger counterparts are trapped and retained in the lymphoid structures. As these cells in some respects resemble lymphocytes (or, indeed, may be differentiating towards them), it is not strange that they have often been confused with the cells of the lymphoid series, especially of the atypical variety. Their existence or even their identity are still not universally accepted. The variety of names given to them, their precursors or related cells indicates a lack of uniformity of nomenclature and a certain degree of uncertainty regarding their character. Berman and more recently Sundberg discuss the interrelationships of the cells bearing these various names. It has been suggested by Ewald that the cells of "leukemic reticuloendotheliosis" are derived from the primitive reticulum cells which in the normal course of events would have differentiated into lymphoblasts, histiocytes and myeloblasts.
and subsequently into mature forms of these lineages. Bouroncle et al.² expand this theme further, but retain its fundamentals. Berman also believes that primitive undifferentiated reticulum cells are the cells involved in this condition, but states that they more often acquire a more differentiated appearance, (lymphocytoid, monocytoid), and in this unusual (abnormal) form appear in the peripheral blood.¹⁰ The findings in our study are in line with those of Berman. The cells under discussion have a more mature appearance than the primitive looking hematopoietic reticulum cells (stem cell, hemocytoblasts), especially in regard to the character of the nuclear chromatin and infrequent presence of nucleoli. We believe that they represent an abnormal (malignant) type of maturation of the primitive “fixed” (tissue) reticulum cell (hemohistioblast) (table 1). The term neoplastic lymphoid reticulum cells (LR cells) appears reasonable because it implies membership in the reticulum cell series, resemblance to lymphocytes and malignant nature.

**Morphologic Characteristics**

*Stained preparations* (Wright-Giemsa): The cells were on an average 1½ to 2½ times the size of lymphocytes. They were round or slightly oval. In most of the cells the cytoplasmic edge was irregular (figs. 1A and B) and small pseudopod-like projections were commonly seen. The cytoplasm stained blue or grayish-blue and in some of the cells several azurophilic granulations were present (figs. 1A and B). These were heavier than the fine granulations of monocytes. The nuclei were round or slightly oval and approximately one-half the size of the cell, in contradistinction to the monocytes with their in-

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**Table 1.—Scheme Indicating Probable Relation of Neoplastic Lymphoid Reticulum Cells to the Development of Hematopoietic Cells**

<table>
<thead>
<tr>
<th>Primitive, Fixed Reticulum Cell</th>
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</thead>
<tbody>
<tr>
<td>Neoplastic Lymphoid Reticulum Cells</td>
</tr>
<tr>
<td>Hematopoietic Reticulum Cell (Hemocytoblast)</td>
</tr>
<tr>
<td>Megakaryocytic</td>
</tr>
</tbody>
</table>

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Fig. 1A, B and C.—Lymphoid reticulum cells from the peripheral blood of patients with malignant generalized proliferation of the reticulum cells. Wright-Giemsa stain. × 1200.

dented or reniform nucleus filling usually 7/10 of the cell. Frequently they were located eccentrically. The nuclear membrane was thick and unusually distinct. The nuclear chromatin was spongy, heavy in appearance, less clumped than the chromatin of lymphocytes, but not so fine as the chromatin of the blast cell (figs. 1A, B and C). It was distinctly different from the fine but loosely woven network of monocytic nuclear chromatin. Many of the cells showed indistinct nucleoli; only rarely a distinct nucleolus was present. Occasionally elongated forms with tapering ends were seen.

*Phase microscopy and supravital preparations:* The neoplastic lymphoid
The purpose of this study was to examine these cells cytochemically and reticulum cells were 1½ to 2½ times the size of lymphocytes. The cells had irregular borders and slow movements with pseudopodic extrusions were seen (figs. 2A, B and C). However, the cells did not exhibit actual mobility. Numerous mitochondria were present throughout the cytoplasm. Occasional neutral red staining granules and vacuoles were seen near the nucleus. The nuclei were round or slightly oval, approximately one-half the size of the cells and frequently eccentric in position. Nuclear membrane was heavy and very distinct. Nuclear chromatin had a spongy appearance.

Fig. 2A, B and C.—Lymphoid reticulum cells from the same patients. Peripheral blood. Phase microscopy. × 1200.
compare their reactions with those of the cells of the lymphocytic series, with monocytes and with "fixed" (tissue) reticulum cells in an effort to establish their respective relationships.

**Material and Methods**

Neoplastic lymphoid reticulum cells were found in the peripheral blood of five patients, all of whom were adults with splenomegaly as a prominent feature. In all cases, there were varying indications of bone marrow failure usually with low platelet counts, and hypopcellular marrows infiltrated with abnormal RE cells. White blood counts varied from 3,200/cu. mm. to 18,800/cu. mm., and the percentage of reticulum cells in the blood from 6 per cent to 90 per cent. In one patient, reticulum cell sarcoma developed as a terminal manifestation.

In addition, monocytes were examined in numerous smears of normal people, in many examples of monocytosis and in 10 cases of monocytic leukemia. Fifteen cases of well substantiated lymphosarcoma with the presence of abnormal lymphocytic cells in the peripheral blood were also studied. In addition, fixed reticulum cells were investigated in tissue sections and imprints in four surgically removed normal spleens, in 10 spleens of patients with hemolytic anemia which showed reticuloendothelial hyperplasia, and in 14 pathologic lymph nodes. The following stains and examinations were performed:

**Blood smears and imprints**: Phase microscopy, supravital staining, Wright-Giemsa stain, P.A.S. stain with and without diastase digestion, Methyl-Green-Pyronin, Sudan Black, Dithizone, Reactive Sulphydryl Groups (in smears fixed in 10 per cent neutral formalin), Peroxidase, Lactic dehydrogenase-D.P.N. diaphorase (in smears fixed in formol-calcium), Alkaline Phosphatase, Acid Phosphatase, nonspecific Esterase, Phosphorylase and Acridine Orange for fluorescence.

**Tissue sections**: Hematoxylin and eosin, Phloxine-methylene blue, P.A.S. with and without diastase digestion, Methyl-Green-Pyronin, Alkaline Phosphatase, Acid Phosphatase, Silver stain for Reticulum (Gordon and Sweet).

**Results**

**Histochmical studies**: Results are summarized in table 2. Neoplastic lymphoid reticulum cells could be differentiated from the cells of the lymphocytic series by:

1. P.A.S. weakly positive glycogen reaction of the reticulum cells in four of the cases while lymphocytic cells were usually strongly positive. However, the lymphoid reticulum cells of one case were strongly positive.

2. Methyl-Green-Pyronin Stain: Cytoplasmic pyroninophilia was weak in the lymphoid reticulum cells, and pronounced in the lymphocytic cells. In acridine orange stains red cytoplasmic fluorescence was very marked in lymphosarcoma cells, but it was weak or absent in LR cells and in monocytes. However, normal lymphocytes were also negative.

3. Sudan Black: Granules were occasionally present in the LR cells and were absent from the lymphocytic cells.

4. Acid Phosphatase reaction was positive in the lymphoid reticulum cells (figs. 3A, B, C and D) and negative in the lymphocytic cells.

5. Nonspecific esterase reaction was positive in the lymphoid reticulum cells and negative in the lymphocytic cells.

6. Phosphorylase reaction was usually negative in the lymphoid reticulum cells and positive in lymphocytic cells, in some strongly positive.

Striking similarities were noted between the lymphoid reticulum cells from
Fig. 3.—Acid phosphatase stain (Gomori). A. Lymphoid reticulum cells from the bone marrow of one of the patients. × 1200. B. Normal lymph node imprint. Strongly positive reaction of the reticulum cells. C. Tissue sections of the spleen. Reticulum cell is strongly positive. × 1000. D. Monocytes in monocytic leukemia. Positive reaction. × 1200.

The peripheral blood and in the fixed “tissue” reticulum cells. Reactions of monocytes were very similar to those of the lymphoid reticulum cells with the exception of occasionally weakly positive peroxidase stain and weakly positive phosphorylase stain of the monocytes.
Table 2.—Cytochemical Staining Characteristics of Various Cell Types

Comparative Cytochemistry of Lymphoid Reticulum Cells

<table>
<thead>
<tr>
<th>CELLS</th>
<th>P.A.S.</th>
<th>P.A.S. Stain</th>
<th>Acidic Orange Fluorescence</th>
<th>Siderin Black</th>
<th>Eosinophil Staining</th>
<th>Lactic Dehydrogenase</th>
<th>Alkaline Phosphatase</th>
<th>Acid Phosphatase</th>
<th>Non-Specific Esterase</th>
<th>Phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoplastic Reticulum Cells (Cells of Leukemic Reticuloendotheliosis, Histocytes, Transitional Cells)</td>
<td>Diffuse</td>
<td>Neg</td>
<td>Pyroninophilic</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Granules</td>
<td>Neg</td>
<td>Pyroninophilic</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg (rare Granules)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lymphosarcoma Cells</td>
<td>Granules</td>
<td>Neg</td>
<td>Pyroninophilic</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fixed, Fixed A–C Cells</td>
<td>Diffuse</td>
<td>Neg</td>
<td>Pyroninophilic</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neoplastic Leukemic Endothelial Cells +++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Diffuse</td>
<td>Neg</td>
<td>Pyroninophilic</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neoplastic Leukemic Endothelial Cells +++</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Cells of one case showed strongly Pos Granules.
NEOPLASTIC LYMPHOID RETICULUM CELLS IN PERIPHERAL BLOOD

Dithizone stain, reactive sulfhydryl group stain, peroxidase stain, lactic dehydrogenase D.P.N. diaphorase stain and alkaline phosphatase stain did not contribute to the differentiation of the cells under discussion.

**Discussion**

The data presented indicate that the cells which appear in the peripheral blood of the patients with some of the malignant proliferations of the hematopoietic system are of the reticulum cell variety. Their histochemical reactions are fundamentally identical with those of the tissue reticulum cells, but differ in many respects from the findings in the cells of the lymphocytic system, be they normal lymphocytes, or lymphocytes of malignant lymphocytic proliferations. As these cells show certain morphologic resemblances to the cells of the lymphocytic series, the term lymphoid reticulum cells seems to be justified. It indicates on the one hand that they are fundamentally members of the reticulum cell system, although in certain respects they resemble cells of the lymphoid series.

The neoplastic lymphoid reticulum cells in the peripheral blood are frequently not recognized as such and are usually considered to be of the lymphocytic type, especially of "abnormal" variety. Careful study of stained blood smears, preferably reinforced by phase microscopy should reveal their identity. Active movements of the cytoplasm (pseudopod formation) will distinguish them readily from the cells of the lymphocytic series in which such a high degree of cytoplasmic movement is not seen.

A close relationship seems to exist between these cells and monocytes. The few histochemical differences that are present are in degree and not in kind and might indicate that the monocyte, although fundamentally a cell of the reticulum cell variety, has been specifically modified and adapted to its function in the circulating blood. Dameshek found similar differences and relationships between histiocytes and monocytes. The cells under present investigation are in effect a neoplastic version of the histiocytes, and in many respects (including negative peroxidase stain) resemble them closely.

The neoplastic lymphoid reticulum cells in the peripheral blood give a negative stain for alkaline phosphatase. Elongated forms observed in our cases (also seen in the cases of Bouroncle et al. and termed by them "endothelial") are also negative. The endothelial cells of arteries, veins, and capillaries, i.e., the endothelial cells of vascular origin which are occasionally seen in the bone marrow smears stain strongly for this enzyme (figs. 4A and B). On the other hand, the sinusoidal lining cells of the spleen and the liver give negative reaction. It would appear that the elongated cells ("endothelial"), although neoplastic in nature, are similar in this staining characteristic to the sinusoidal lining cells of the reticuloendothelial system, and not to the endothelial cells of blood vessels which give an altogether different reaction and, as Bouroncle et al. suggest, should not be confused with them.

**Summary**

Abnormal appearing cells having the superficial appearance of large lymphocytes were found in some cases of generalized malignant proliferations of the reticulum cell system and were studied by cytochemical methods and
Fig. 4.—Alkaline phosphatase stain. A. Positively staining vascular endothelium cell in the bone marrow smear (Azo-dye). × 1200. B. Section of the spleen. Vascular endothelium is strongly positive. Cells lining the sinusoids are negative (Gomori-Takamatsu, Neutral red counterstain), × 120.

by phase microscopy. They were found to be very similar in their reactions to the “fixed” or tissue reticulum cells and in many respects to monocytes, but differed markedly from the cells of the lymphocytic system with which they are often confused. The term neoplastic lymphoid reticulum cell is applied to these cells since it indicates that they are malignant in nature and fundamentally of the reticulum cell type, although in fixed and stain material they may show some of the morphologic characteristics of the lymphocytic cells.

SUMMARIO IN INTERLINGUA

Cellulas de aere anormal—con le apparentia superficial de grande lymphocytes—esseva trovate in alicun casos de generalisate proliferationes maligne del sistema de cellulas reticular e esseva studiate per methodos cytochimic e per microscopia de phases. Esseva trovate que illos esva multo simile in lor reaactiones al “fixe” cellulas de tissu de reticulo e in multe respectos a monocytes, sed illos differeva marcatemente ab le cellulas del sistema lymphocytic con le quales illos es frequentemente confundite. Le termino “neoplastic reticulocytes lymphoidic” es applicate a iste cellulas proque illo indica que illos es de natura maligne e fundamentalmente del typo de cellulas reticular, ben que in fixate e tincturate material illos manifesta a vices caracteristicas morphologic del lymphocytos.

REFERENCES

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