Composition and Structure of Cytoplasmic Globules in Leukemic Lymphocytes

By John Laszlo, Herbert J. Gerber and Joachim R. Sommer

The purpose of this paper is to describe certain similarities between globules observed in leukemic lymphocytes and globules in plasma cells referred to as Russell bodies. Demonstration of structural and chemical identity of these globules would further strengthen the concept of a transitional pathway between lymphocytes and plasma cells. Indeed, if normal lymphocytes can differentiate into plasma cells, then transitional stages might be anticipated during malignant lymphocytic proliferation. In the following report, an instance of subacute lymphocytic leukemia is described in which many of the lymphocytes contained cytoplasmic globules. The structure and composition of these globules is described.

Case Report

VA Hospital #X. X. A 49-year-old Negro handyman was first admitted to this hospital in February 1964 because of cervical and axillary lymph node enlargement, left upper quadrant pain, and 40-pound weight loss during the three months prior to admission. On physical examination the cervical, axillary, and inguinal lymph nodes, liver and spleen were found to be enlarged. The hemoglobin was 11.3 Gm. per cent, hematocrit 37.5 per cent, reticulocyte count 0.5 per cent, platelet count 86,500/mm.³ and white cell count 52,000/mm.³ The WBC differential revealed 88 per cent lymphocytes, most of which were small and contained scanty cytoplasm, and 10 per cent of the lymphocytes were large and had nucleoli (Fig. 1). Marked vacuolization of the lymphocyte cytoplasm was noted. The bone marrow was replaced with a predominance of small lymphocytes and 15 per cent large lymphocytes with nucleoli (lymphoblasts). Remaining laboratory studies were within normal limits except for the serum electrophoretic pattern, which showed a mild hypoalbuminemia, hypogammaglobulinemia (0.6 Gm. per cent), and an elevation of beta globulins. The composition of urinary protein was normal as determined by electrophoresis. A left submandibular lymph node was biopsied and the architecture was found to be obliterated with a monotonous infiltrate. This infiltrate was comprised of small, densely staining lymphocytes. A diagnosis of subacute lymphocytic leukemia was made and the patient was treated with methotrexate.

Since that time, the patient has been seen regularly in the Hematology Clinic and readmitted on six occasions for recurrent infections including pneumonia, prostatitis, cellulitis, abscesses of the extremities, and one episode of septicemia. He was treated with courses of methotrexate, prednisone, chlorambucil and streptonigrin and required numerous blood transfusions. At the time of the report he felt improved and was gaining weight. His generalized lymphadenopathy had not increased, the liver was no longer palpable, but the...
spleen was palpable 2 cm. below the left costal margin. When he was last seen in December 1965, his hemoglobin was 8.7 Gm. per cent, with a white count of 58,700 almost all of which were abnormal lymphocytes. His platelet count remained between 2000 and 4000. He is now being treated with fluoxymestrone and prednisone.

MATERIALS AND METHODS

Light Microscopy

Films of the peripheral blood, bone marrow, and lymph node aspirates were stained with Wilson's stain. Freeze-dry preparations were made according to the procedure of Weinstein et al.\(^2\) For bromphenol blue,\(^3\) ninhydrin-Schiff,\(^4\) and PAS staining (with and without diastase digestion)\(^5\) of the frozen-dried cells, the preparations were postfixed in Carnoy's solution for 15 minutes. For the ninhydrin-Schiff and Millon's staining, the frozen-dried cells were left unfixed prior to staining. For the alkaline and acid phosphatase reactions,\(^6\) the frozen-dried cells were postfixed in cold acetone for 5 minutes. For the demonstration of SH groups the ferric cyanide reactions was performed.\(^5\) Sections of the lymph node were made from paraffin-embedded material after fixation in Zenker's solution.

Electron Microscopy

Heparinized whole blood (20 ml.) was removed by venapuncture and the red cells were settled out by gravity. The leukocyte-containing plasma layer was removed and the cells centrifuged and washed in 0.85 per cent NaCl solution. The cells were resuspended in 4 per cent glutaraldehyde\(^7\) in 0.05 M phosphate buffer (pH 7.4) and fixed in the cold for 16 hours. Thereafter, the cells were washed in distilled water for 2 hours with several changes and then postfixed in osmium tetroxide\(^8\) and embedded in Maraglas\(^9\) after dehydration. Thin sections were cut with a Porter-Blum ultramicrotome, deposited on 300-mesh naked grids, stained with lead citrate, and viewed with an RCA EMU3-F electron microscope at magnifications between ×5000 and ×32,000, using 50 kv. accelerating voltage and objective aperture of 35 microns.

RESULTS

Stained films of peripheral blood (Fig. 1) and marrow were consistent with subacute lymphocytic leukemia. Cytoplasmic globules were distributed throughout the cytoplasm of many of the abnormal lymphocytes, as many as 10–30 often occurring within one cell (Fig. 2). The globules were clearly visible by positive phase contrast microscopy (Fig. 3), revealing different degrees of optical density. By light microscopy, some were so small as to be barely discernible, while the rest varied in size up to approximately 5 microns in greatest diameter (Figs. 3 and 10).

The staining reactions are summarized in Table 1. The staining of the globules with bromphenol blue varied considerably from cell to cell and within a single cell (Figs. 6 and 7). More sharply defined and intensely stained globules (using bromphenol blue) were occasionally observed (Figs. 6 and 7) with, or occasionally without, the association of less intensely stained material. The globules appeared to have crystalloid shapes when examined by direct oil immersion microscopy of unfixed frozen-dried cells. These crystalloid shapes were not seen when the cells were fixed and/or stained after freeze-drying, presumably due to rehydration incident to these procedures. Globules in
lymphocytes of the peripheral blood stained strongly with PAS and resisted diastase digestion (Fig. 4). Likewise, sections of the lymph node showed innumerable cells containing PAS-positive globules (Fig. 5). Some of these were minute and within the sparse cytoplasm of small cells appearing to be

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<th>Stain Applied</th>
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<tr>
<td>PAS</td>
<td>++</td>
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<td>PAS after digestion</td>
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<td>Bromphenol blue</td>
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<td>Alcian blue</td>
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<tr>
<td>Oil red 0</td>
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<td>An occasional granule unrelated to the globules</td>
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<td>Acid phosphatase</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>-</td>
<td>An occasional granule in a few cells unrelated to the globules</td>
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<td>Wilson</td>
<td>-</td>
<td>Typical hematologic staining reaction</td>
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<td>Ferric cyanide (SH groups)</td>
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<td>(-)</td>
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— not stained.
(-) stain negligible.
++ strong reaction.

Fig. 1.—Peripheral blood. Wilson's stain. (× 900)
Fig. 2.—Peripheral blood, Wilson's stain. Note numerous globules of varying sizes in the cytoplasm. (× 450)

Fig. 3.—Peripheral blood, positive contrast phase microscopy. Note multiple globules of varying sizes in one cell. The other cells represent the majority of the leukemic cell population. (× 1050)

Figs. 4–7.—See figure legends on facing page.
lymphocytes, whereas others were very large, resembling Russell bodies that grossly distended the host cells (Fig. 5).

In electron microscopic preparations, the leukemic cells were closely packed and rather uniform in appearance. The nuclei were round or ovoid and lobulations were common (Fig. 8). A few mitotic figures were seen and extranuclear spindle fibers were distinguished in several instances (Fig. 10). The chromatin was arranged in clumps along the nuclear envelope (Fig. 8), and the envelope itself was unremarkable except for the paucity of nuclear pores (Fig. 12). The pores were covered by a membranous septum (Fig. 13).

The cytoplasm was bounded by a single unit membrane with pseudopodial folds (Figs. 8, 9 and 12), some of which suggested the process of pinocytosis. Ribosomes were diffusely dispersed except where they were attached to tubular profiles of endoplasmic reticulum and to the nuclear envelope (Fig. 11). The endoplasmic reticulum was poorly developed and consisted only of a few elongated cisternae and small vesicles (Figs. 8, 9, 10 and 12) that were defined as belonging to the endoplasmic reticulum only if they carried ribosomes. Numerous other vesicular elements of rather uniform dimensions were observed without ribosomes (Fig. 9). Many of these were derived from the Golgi apparatus, while large ones were closer to the plasma membrane and, therefore, more likely belonged to the pinocytotic process (Fig. 12). The Golgi apparatus (Fig. 10) was fairly well developed and was generally located near the centriole (Fig. 14) in the centrosome region close to the nuclear notch. Mitochondria were few in number and lipid droplets were occasionally observed.

Of particular interest were large cisternae of varying sizes containing a homogeneous dense matrix (Figs. 8–11) similar to those described by Thiery in plasma cells. In some instances, round and very dense material was visible within this matrix (Figs. 10 and 11). The cisternal membrane was of unit membrane configuration and ribosomes were attached (Figs. 9–11). On occasional portions of this envelope, however, no ribosomal attachment was noted.

**DISCUSSION**

Reports of intracytoplasmic globule formation have appeared in the literature since 1890, when William Russell first described intracellular eosinophilic hyaline bodies in cancer cells. Kurloff described similar changes in guinea pig lymphocytes. In 1948 Pearse recorded the staining properties of both Russell bodies and Kurloff bodies, concluding that the Russell bodies in human plasma cells and the Kurloff bodies in guinea pig lymphocytes con-

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**Fig. 4.—Peripheral blood freeze-dry preparation, PAS after digestion.** The globules are red. (× 1100)

**Fig. 5.—Paraffin section of lymph node biopsy, PAS after digestion.** Note numerous Russell bodies (RB) of varying sizes. (× 1100)

**Fig. 6.—Peripheral blood, freeze-dry preparation, bromphenol blue.** Note dense globule (A) with more intensely blue staining condensed material (× 1200)

**Fig. 7.—Peripheral blood, freeze-dry preparation, bromphenol blue.** Note condensed material, particularly in one of the globules (A). The intensity of the stain in this condensed material is much less than that in Fig. 6. (× 1100)
Figs. 8 and 9.— See figure legends on facing page.
sisted of mucoprotein, probably secreted by the parent cell. In 1953 and 1954, Rappaport and Johnson described material resembling Russell bodies in lymphocytes from malignant lymphoma nodes and concluded that the globule consisted of a polysaccharide protein complex which was histochemically similar, if not identical, to the Russell bodies of plasma cells. In 1956, Zlotnick described the morular cell and the grape cell in bone marrow and peripheral blood. These were reticulum cells and plasma cells, the cytoplasm of which contained transparent vesicles or opaque bluish staining globular bodies. Subsequently, Zlotnick et al. experimentally produced grape cells and concluded that they contained a neutral mucoprotein. Quaglino and Hayhoe studied the PAS reaction in lymphoproliferative diseases. A recent report has appeared that "flaming cells" of multiple myeloma contain a glycoprotein within dilated endoplasmic reticulum. Of particular interest is the cytochemical similarity shared by the globules within plasma cells (Russell bodies, grape cells, and "flaming cells"), the globules in guinea pig lymphocytes (Kurloff bodies), and the intracytoplasmic globules that Rappaport described in malignant lymphomas. All stained with PAS and retained their stain after diastase treatment, and all exhibit a protein component. However, Izard has found myelin figures inside Kurloff bodies, whereas similar structures were not observed in the present study. Moreover, another significant difference exists in that, contrary to the Kurloff bodies, the globules described in the present communication are clearly surrounded by rough endoplasmic reticulum.

We have noted similar cytoplasmic globules in three other patients with chronic lymphocytic leukemia. To our knowledge, however, this paper contains the first detailed description of such a case. On routine blood films stained with Wilson’s stain, the globules observed in the lymphocytes of this patient appeared to be clear intracytoplasmic vacuoles. Special stains, however, revealed a substance that was most likely a glycoprotein (Table 1). The cytochemical characteristics of the globules were identical in both peripheral blood lymphocytes and in cells of the lymph node. The largest globules, indistinguishable from Russell bodies and characteristic of certain functional states of plasma cells, were particularly numerous in the lymph node. However, peripheral blood lymphocytes and lymph node lymphocytes contained innumerable minute PAS-positive globules within the cytoplasm. It seems reasonable to propose, therefore, that the different sizes of globules represent stages in the evolution of the larger bodies. The occurrence of such an evolution in lymphocytic leukemia cells would be expected considering the possible functional relationship between lymphocytes and plasma cells. The present status of

Fig. 8.—Peripheral blood, several leukemic lymphocytes with and without globules. Note occasional indentation of nuclei and relatively clear cytoplasm. PV = pseudopodial veil, N = nucleus, MV = multivesicular body, ER = endoplasmic reticulum, A = globule, L = compatible with lipid granule. (× 7100) (Inset: Multivesicular body × 38,700)

Fig. 9.—Same sample as in Fig. 8. Note numerous globules surrounded by a unit membrane with many attached ribosomes. Note partially dilated cisternae of endoplasmic reticulum (ER) and smooth vesicles (V). (× 19,600)
Figs. 10–14.—See figure legends on facing page.
cytoplasmic globules in plasma cells and lymphocytes bears little similarity to the original descriptions by Russell and others. In our opinion it beclouds the issues to continue the use of these eponyms. Generic descriptions would appear preferable to future students of this problem.

Since there is little doubt that the glycoprotein of the globules occurred within the cisternae of the rough endoplasmic reticulum, we believe that the globules may well be formed de novo in the cell. The cellular protein itself may be either normal or abnormal, but its accumulation within the cell is definitely abnormal. It should be noted that no distinct polysomes were found and that there were remarkably few nuclear pores in the sections.

**SUMMARY**

Lymphocytes from a patient with lymphocytic leukemia were found to contain cytoplasmic globules. The globules varied in size, the largest appearing identical to Russell bodies. Histochemical staining was used to demonstrate the glycoprotein composition of these bodies. Electron microscopy revealed this material to be within dilated cisternae of rough endoplasmic reticulum. It is proposed that these globules are abnormal accumulations of glycoprotein which represent stages in the evolution of "Russell bodies." The generic description of these cytoplasmic globules would seem to be preferable to the continued use of eponyms.

**SUMMARIO IN INTERLINGUA**

Essu trovate que le lymphocytos ab un patiente con leucemia lymphocytic contineva globulos cytoplasmic. Le globulos variava in lor dimensiones, e le plus grandes pareva esser identic con le corpores de Russell. Tincturation histo-chimic esseva usate pro demonstrar le composition glycoproteinc de iste corpores. Microscopic electronic revelava que le material esseva situate intra dilatate cisternas de reticulo endoplasmatic. Es suggestionate que iste globulos es es possibilemente accumulationes anormal de glycoproteina representante stadios in le evolution del "corpores de Russell." Le description generic de iste globulos cytoplasmatic pare esser preferibile al uso continue de eponymos.

**ACKNOWLEDGMENT**

We wish to acknowledge the excellent technical assistance of Mr. Isaiah Taylor.

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**Fig. 10.**—Same sample as in Fig. 8. Note globule (A) containing condensed material (CM) and well-developed Golgi apparatus (G) with spindle fiber (S) in between. (× 20,300)

**Fig. 11.**—Same sample as in Fig. 8. Note attachment of ribosomes (R) to membranous envelopes of both the nucleus (N) and the globule (A) containing the condensed material (CM). (× 49,000; inset × 73,800)

**Fig. 12.**—Same sample as in Fig. 8. Note paucity of unexpanded cisternae of endoplasmic reticulum (ER) and nuclear pores (NP). PV = pinocytotic vesicle. (× 15,200)

**Fig. 13.**—Same sample as in Fig. 8. Membranous cover of nuclear pore. (× 65,600)

**Fig. 14.**—Same sample as in Fig. 8. Note characteristic structure of centriole as seen also in many other cells (× 72,500)
REFERENCES


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