Cytoplasmic Inclusions in Acute Hemocytoblastic Leukemia: Case Report

By L. G. Saraiva, G. P. Bourboul, M. Silveira, J. D. Prospero and J. J. Angulo

IT IS THE purpose of this paper to report a case of acute hemocytoblastic leukemia in which we observed primitive cells containing peculiar cytoplasmic inclusions. The descriptive name “para-hemocytoblast” is hereafter applied to these cells because their morphologic type corresponds to that of hemocytoblasts, and where, furthermore, anomalous inclusions are found. Although anomalous intracellular structures in leukemic human blood cells have previously been reported, it seems that the findings of Terrasse et al. most nearly resemble those presented here.

CLINICAL SUMMARY

J. B. S., a 56 year old, Brazilian-born, white farmer was admitted to the Sixth Medical Infirmary of the Central Hospital, Santa Casa de Misericordia, on May 23, 1959. The patient complained of weakness, edema of the legs, dyspnea on marked effort and pallor of three years’ duration. Five months before admission, these manifestations had become more intense and others appeared, including fever, dizziness, headache, epigastric pain and weight loss. The past medical history and family history were non-contributory.

On physical examination there was marked pallor. There were no palpable lymph nodes. The heart was enlarged and the examination of the lungs was not remarkable. The liver edge was felt 2 cm. below the right costal margin; its edge was smooth, firm, and tender. The spleen was not felt. The neurologic examination was normal. Chest x-ray showed accentuation of bronchovascular markings and increase of the left ventricle. Roentgen examination of the bones was normal.

The patient was observed in the hospital for a period of 54 days with no relevant alteration of the clinical or laboratory findings. Treatment consisted of dietetic-hygienic measures, antibiotics, blood transfusions and adrenocorticosteroids. Despite the therapy the anemia became more severe and the number of blast cells increased in the blood and bone marrow (table 1, Blood-A, B). The patient died on July 15, 1959.

LABORATORY EXAMINATIONS

Laboratory findings and the electrophoresis data are shown in tables 1 and 2, respectively. Biochemical, bacteriological, immuno-hematologic and coagulation tests were repeatedly made during the hospitalization and they did not change significantly. Hematologic studies were made weekly and the most representative results are presented in table 1, Blood-A. Preparations of peripheral blood and bone marrow were examined unstained by phase contrast microscopy and polarized light microscopy as well as by Leishman and cytochemical stains. Material from the bone marrow was examined by electron microscopy. The morphological findings were essentially the same in the different specimen collections.
### Table 1

#### Blood

<table>
<thead>
<tr>
<th>A (Peripheral Blood)</th>
<th>B (Bone Marrow)</th>
<th>C (Chemical Analysis)</th>
<th>Immuno-Hematologic and Coagulation Tests</th>
<th>Bacteriology and Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Erythrocytes, millions/mm³</strong></td>
<td>May 220, June 185</td>
<td>May norm (10.0)</td>
<td>Amylase direct 80 μg</td>
<td>Bacteroides broth negat</td>
</tr>
<tr>
<td><strong>Hemoglobin, grams/100ml</strong></td>
<td>60</td>
<td>51</td>
<td>43</td>
<td>Blood Group B, P A</td>
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<tr>
<td><strong>Eosinopenia, %</strong></td>
<td>15 15</td>
<td>10 10</td>
<td>13 13</td>
<td>Titer of anti A 02mg</td>
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<tr>
<td><strong>MCH (pg) MCHC (g/dl)</strong></td>
<td>202-2781-2781-27</td>
<td>May 202</td>
<td>02mg</td>
<td></td>
</tr>
<tr>
<td><strong>MCV (fl) MCHC (g/dl)</strong></td>
<td>33-76 34-75 35-76</td>
<td>33-76 34-75 35-76</td>
<td>02mg</td>
<td></td>
</tr>
<tr>
<td><strong>Normoblasts per 100WBC</strong></td>
<td>0 0 0 1 2</td>
<td>0 0 0 1 2</td>
<td>04mg</td>
<td></td>
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<tr>
<td><strong>Reticulocytes %</strong></td>
<td>2 5 2 4 2 0</td>
<td>2 5 2 4 2 0</td>
<td>04mg</td>
<td></td>
</tr>
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<td><strong>Thrombocytes, thousands/mm³</strong></td>
<td>52 40 50</td>
<td>52 40 50</td>
<td>04mg</td>
<td></td>
</tr>
<tr>
<td><strong>Leukocytes, thousands/mm³</strong></td>
<td>5 9 0 7 1 6</td>
<td>5 9 0 7 1 6</td>
<td>04mg</td>
<td></td>
</tr>
<tr>
<td><strong>Metamyelocytes %</strong></td>
<td>0 0 0 0 0 0</td>
<td>0 0 0 0 0 0</td>
<td>04mg</td>
<td></td>
</tr>
<tr>
<td><strong>Band forms %</strong></td>
<td>5 2 2 4 44</td>
<td>5 2 2 4 44</td>
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<tr>
<td><strong>Segmented forms %</strong></td>
<td>170 11 10 0 40</td>
<td>170 11 10 0 40</td>
<td>04mg</td>
<td></td>
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<tr>
<td><strong>Basophils</strong></td>
<td>1 2 1 0 2 0 0</td>
<td>1 2 1 0 2 0 0</td>
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<tr>
<td><strong>Eosinophils</strong></td>
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<td>1 2 1 0 2 0 0</td>
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<tr>
<td><strong>Monocytes %</strong></td>
<td>3 6 4 0 4 0</td>
<td>3 6 4 0 4 0</td>
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<td><strong>Lymphocytes %</strong></td>
<td>700 790 70</td>
<td>700 790 70</td>
<td>04mg</td>
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<td><strong>Plasmocytes %</strong></td>
<td>1 4 1 0 0 6</td>
<td>1 4 1 0 0 6</td>
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<td><strong>Hemocytoblasts %</strong></td>
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<td></td>
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<td><strong>PARA HEMOCYTObLASTS %</strong></td>
<td>0 2 0 8 0 4</td>
<td>0 2 0 8 0 4</td>
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<td></td>
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<td><strong>Myeloblasts %</strong></td>
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<td><strong>Plasma</strong></td>
<td>0 6 7 5 9 7 0</td>
<td>0 6 7 5 9 7 0</td>
<td>04mg</td>
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<td><strong>Erythrocyte sedimentation rate</strong></td>
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<td>60 70 73</td>
<td>04mg</td>
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<td><strong>WBC/10³/ml</strong></td>
<td>160</td>
<td>168</td>
<td>172</td>
<td>160</td>
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<tr>
<td><strong>Hb/10⁹/l</strong></td>
<td>165</td>
<td>172</td>
<td>174</td>
<td>165</td>
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<td><strong>Bone fibrous tissue</strong></td>
<td>0 46 0 44 0 46</td>
<td>0 46 0 44 0 46</td>
<td>04mg</td>
<td></td>
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<td><strong>Bone marrow</strong></td>
<td>0 32 0 32 0 32</td>
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<td>04mg</td>
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<td><strong>Mechanical fragility</strong></td>
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<tr>
<td><strong>Sicca</strong></td>
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<td>negat</td>
<td>negat</td>
<td>negat</td>
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<tr>
<td><strong>LE cells</strong></td>
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<td>negat</td>
<td>negat</td>
<td>negat</td>
</tr>
</tbody>
</table>

### Bacteriology and Serology

- **Gastric broth**: negat
- **Blood agar**: negat
- **Selenite**: negat
- **Blood culture**: negat
- **Stool culture**: negat
- **Urine culture**: negat
- **Kahn - VDL - Wassermann**: negat
- **Mocchado - Guillermo**: negat
- **Peral - Bannall**: negat
- **Siles - Feldman**: negat
- **West - Felix**: negat
- **Urine**: negat
- **Specific gravity**: 1025
- **Reaction**: acid
- **Bacillus**: negat
- **Acetone body**: negat
- **Acetone body**: negat
- **Bacillus - Jones protein**: negat
- **Bile acids - pigments**: negat
- **Doubly refractile bacillus**: negat
- **Glucose - Hemoglobin - Indican**: negat
- **Phenylalanine - phenylalanine**: normal
- **Creatinine - phenylalanine**: normal
- **Coagulation time**: 10 min
- **Urobilinogen**: 1/10
- **Sediment - not present**: negat

### Feces

- **Occult blood**: negat
- **Proteins**: 100% negat
- **Helminthes (ovum)**: negat
Table 2.—Paper Electrophoresis Data

<table>
<thead>
<tr>
<th></th>
<th>2850</th>
<th>140</th>
<th>140</th>
<th>400</th>
<th>3070</th>
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<tr>
<td>PROTEIN-BOUND</td>
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<tr>
<td>LIPID</td>
<td>132</td>
<td>414</td>
<td>36</td>
<td>582</td>
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<tr>
<td>PROTEIN-BOUND</td>
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<td>37</td>
<td>30</td>
<td>59</td>
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<tr>
<td>CARBOHYDRATE</td>
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<td></td>
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</table>

**Cytohematology:** Inclusion-bearing blast cells, called here “parahemocytoblasts,” varied from 0.2 to 0.8 per cent in the peripheral blood and from 4.0 to 13.0 per cent in the bone marrow (table 1, Blood-A, B). The nuclear and cytoplasmic characteristics correspond to those of hemocytoblasts (Ferrata’s nomenclature) and in addition they showed the anomalous inclusions already mentioned. Some “para-hemocytoblasts” exhibited accentuated basophilia of the cytoplasm and an increase of the nucleolus/nucleus ratio. In the peripheral blood the chromatin structure of “para-hemocytoblasts” was coarser than in the bone marrow.

In the cytoplasm of “para-hemocytoblasts” one or more round, or sometimes oval, inclusions were found. Their diameter commonly ranged from 1.2 to 9.0 micra, but reached 20 micra in some cells (fig. 27). The larger inclusions distorted the nucleus which then presented a concavity corresponding to the inclusion (figs. 14, 18, 19, 22, 24–27). About 58 per cent of the “para-hemocytoblasts” contained a single inclusion (figs. 2, 3, 14, 15, 17–19, 22 and 27), 25 per cent contained two inclusions (figs. 13, 16, 20, 23, and 25) and 17 per cent presented three or more (figs. 4, 24 and 26). Also their localization in the cytoplasm was variable, at times in opposition (fig. 23). When a “para-hemocytoblast” contained two or more inclusions, one of them was usually larger than the others (figs. 16, 23, 24 and 26). The inclusions exhibited a regular variation in their reaction to Leishman stain. In general, the smaller inclusions (figs. 1, 10, 15–17) stained a uniform dark rose color, while the larger ones were lighter in color and paler in the center. Stainability increased from the center to the periphery (figs. 3, 4, 18, 19, 22, 24 and 26). Staining of some inclusions, particularly of the giant ones (fig. 27), was extremely light giving the impression of a vacuole which was not confirmed under phase microscopy.

In addition to the peculiar inclusions, some cells also contained typical azurophilic granules, thus indicating a more mature form, i.e. the myeloblast (figs. 1 and 23). These granules gave a positive reaction in peroxidase and Sudan Black B tests, while the inclusions were negative in the same tests (see Cytochemical tests below). Some cells in mitotic activity containing an inclusion were also observed (fig. 21). In bone marrow preparations, 0.4 to 2.3 per cent of reticulum cells (table 1, Blood-B) (figs. 5–7, 9–11) and some
Figs. 1–11.—Photomicrographs of peripheral blood and bone marrow (Leishman stain).

Figs. 1–4.—Peripheral blood. “Para-hemocytoblasts” with one or more inclusions whose size, shape and stainability varies. Fig. 1 shows, in addition to an inclusion (arrow), azurophilic granules (peroxidase and Sudan Black B positive) thus indicating a more mature cell. In the blood preparations the chromatin is coarser and nucleolus are not so evident as in cells from the bone marrow.

Figs. 5–11.—Bone marrow. Several reticulum cells exhibit inclusions of varying morphology in their cytoplasm. Fig. 8 shows a macrophage containing an intensely stained inclusion.
macrophages (fig. 8) exhibited the inclusions. Sometimes the inclusions were found, without morphologic alteration, outside of seemingly disrupted cells. Auer bodies or vacuoles associated with the inclusions were not found.

*Phase Contrast Microscopy:* The inclusions appeared as more or less clear structures which were homogeneous, well circumscribed and surrounded by mitochondria. They could be easily differentiated from vacuoles. The larger inclusions caused marked distortion of the nucleus.

*Polarized Light Microscopy:* No birefringence of the inclusions was noted.
Electron Microscopy: Ultrathin sections of bone marrow aspirated from the iliac crests were examined under the electron microscope. The presence of inclusions with a single or sometimes double limiting membrane was easily seen (figs. 28–32) and their internal structure evidenced. A detailed presentation of these findings will be made in a forthcoming publication.

Cytochemical Tests: The presence of polysaccharides, mucoproteins, lipids, phospholipids, neutral fat, deoxyribonucleic acid, peroxidase and phosphatases was investigated by the use of periodic acid-Schiff, toluidine blue, Sudan Black B, Baker, Nile blue sulfate, Feulgen, Sato-Sekiya, Washburn and Wachstein technics, respectively. The inclusions subjected to P.A.S. test showed a slightly positive result, that proportion was 20–25 per cent, which was not abolished by previous treatment with saliva. The other tests were negative.

Necropsy: Performed two hours after death, the necropsy disclosed the following macroscopic alterations: enlargement of the heart (380 grams), chiefly of the left ventricle, hepatomegaly (1,800 grams), enlargement of the kidneys (200 grams each), chronic gastric ulcer and tumefaction of the mesenteric lymph nodes.

Histologically, the main changes were characterized by intense and diffuse infiltration of the bone marrow (figs. 33 and 34), mesenteric lymph nodes (fig. 38) and spleen (fig. 37) by blast cells and small foci of these cells in the liver (portal spaces) (fig. 36) and kidneys. The structure of the bone tissue was preserved. The capsule of the lymph nodes revealed a discrete infiltration (fig. 38). In the spleen the white pulp was only represented by the central arterioles (fig. 37). In Leishman stained touch preparations of those organs, the blast cells were identified as hemocytoblasts. The peculiar inclusions described in a previous chapter were observed only in bone marrow imprints (fig. 35).

Discussion

There was no difficulty in arriving at the diagnosis of hemocytoblastic leukemia in this case, since the hematologic and clinical findings were quite typical. The histologic picture corresponded to acute leukemia and showed diffuse infiltration of immature cells in the hemopoietic organs while infiltration was focal in the liver and kidneys.

The inclusions were noted in only a fraction of the hemocytoblasts and in...
an even smaller fraction of reticulum cells and macrophages (table 1, Blood-A, B). Inclusions were not found in promyelocytes, myelocytes, metamyelocytes, band and segmented forms, plasma cells, lymphoid cells or cells of the erythrocytic and thrombocytic series. Inclusions were not observed either in epithelial cells found in smears of material from the mouth, tracheobronchial tree and genito-urinary tract. Touch preparations of material from organs collected at autopsy were extremely helpful since they showed the inclusions
while the standard fixed tissue preparations of the same organs failed to do so.

The morphology, chemical composition and ultrastructure of the inclusions are not similar to any normal component of blood cells. The latter can be seen side by side with the inclusions either under light microscopy or under the electron microscope. For these reasons and because of their definite corpuscular nature, they are classified as inclusions. Furthermore, they exhibited variation in their size, number, structure and stainability which are important features of accepted inclusion bodies. The nomenclature of “inclusion bodies” thus seems justified but since there is a controversy over the use of this term, “inclusion” is here employed. Regarding the significance of inclusion bodies, it should be remembered that they are not necessarily related to virus infection since inclusion bodies have been produced experimentally by physicochemical means. The nature of the inclusions reported here is not evident. The P.A.S. test suggests the presence of neutral polysaccharides, at least in some inclusions. The increase of the serum gamma globulin fraction and of the protein-bound carbohydrate content suggests a protein synthesizing stimulus related to the inclusions. The bacteriologic study (table 1) did not reveal a cultivatable germ while suggestions of a viral origin of the inclusions were found in the electron micrographs. Further discussion of this matter will be made in the report on the electron microscopy study.

Anomalous cytoplasmic structures in cells from the peripheral blood or bone marrow have been recognized through the use of panoptic stains in cases of human leukemia. Several of these structures differ from those reported here either because of the cell type in which they were found or because of their morphology or cytochemical reactions. However, the possibility that in some cases these differences are due to different developmental stages of the same inclusions should not be overlooked. Certain structures observed with the electron microscope in leukemic cells evidently are dissimilar to the present ones.

It may be remarked that the crystalline shape and structure, staining reactions, cytochemistry and especially their ultrastructure serve to differentiate Auer bodies from the inclusions reported here. The findings nearest to the present ones are those of Terrasse et al. who described “spherical inclusions” in the cytoplasm of hemocytoblasts from a case of hemocytoblastic leukemia although the shape, size, stainability and cytochemical tests were similar, they
Figs. 33–38.—Histologic picture (Hematoxylin-eosin) and touch preparations (Leishman stain) of necropsy material.

Fig. 33.—Hypercellular bone marrow.

Fig. 34.—Same field of fig. 33 showing the cell type in more detail.

Fig. 35.—Touch preparation of rib marrow showing one “para-hemocytoblast” with two inclusions.

Fig. 36.—Liver: the portal space shows cellular infiltrate but the hepatic cells appear preserved.

Fig. 37.—Spleen: the structure is replaced by diffuse cellular infiltration.

Fig. 38.—Mesenteric lymph node with the same diffuse cellular infiltration.
were not identical to those of the present case. Nevertheless, the ultrastructure of an inclusion presented by those authors is similar to one of those obtained in the present work (fig. 28).

SUMMARY

A case of hemocytoblastic leukemia in a 56 year old white man is reported which was characterized by cytoplasmic inclusions in some of the hemocytoblasts, reticulum cells and macrophages of the bone marrow as well as in some of the hemocytoblasts of the circulating blood. Phase and electron microscopic studies confirmed the corpuscular nature of the inclusions. Cytochemical stains gave negative results when tested for the presence of mucoproteins, lipids, phospholipids, neutral fat, deoxyribonucleic acid, peroxidase and phosphatases. The test for polysaccharides was slightly positive. Preliminary studies underway with electron microscopy suggest the possibility of a viral nature of the inclusion bodies.

REFERENCES

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In a case of pernicious anemia with 30,000 platelets, the first platelet crisis took place after 6–7 days of treatment and a second crisis after 11–12 days. The thrombelastographic patterns were typical for thrombocytopenia before the treatment and remained so when the platelets reached normal values. Only during the second phase of the platelet crisis, with a platelet count of 400,000, a complete normalization of the thrombelastographic patterns was obtained. A functional defect of the platelets first released at the beginning of the treatment was assumed, also on the basis of other data (decreased adhesiveness, prothrombin consumption, clot retraction).—P. d. N.
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