Storage Cells of Spleen and Bone Marrow in Thalassemia: An Ultrastructural Study

By C. A. Beltrami, I. Bearzi, and G. Fabris

In the spleens and bone marrows of patients with thalassemia, we have consistently observed PAS-positive histiocytes that contain numerous cytoplasmic bodies surrounded by a single membrane and filled with bundles of fine fibrils (typical thalassemia storage cells). In addition to the characteristic cytoplasmic bodies, some histiocytes contained leukocytes, erythrocytes, platelets, and plasma cells in different stages of digestion (transitional cells). These findings suggest that the storage cells in thalassemia are macrophages that are unable to metabolize completely the products of phagocytized blood cells. In the spleen the histiocytes were closely associated with plasma cells. When stained by the Giemsa method, the storage cells appeared as "sea-blue histiocytes." The histologic, histochemical, and ultrastructural features distinguish the storage cells of thalassemia from Gaucher's cells and from storage cells of other disorders in which there is excessive destruction of formed elements of the blood, i.e., chronic myelogenous leukemia and idiopathic thrombocytopenic purpura.

CHARACTERISTIC HISTIOCYTES have been described in the bone marrow and spleen of subjects with thalassemia major.1 These cells have been reported to stain positively for PAS and acid phosphatase activity and to have primary autofluorescence.2-4.5 The cytoplasmic positivity for PAS has been attributed to mucopolysaccharides3 or glycoprotein material.4 Fabris et al.6 and Gordon et al.7-8 have described the ultrastructural aspects of the histiocytes found in thalassemia. A number of histologic studies have suggested that these histiocytes may be specific for thalassemia.9-10 This report presents further electron microscopic studies of the spleen and bone marrow of patients with thalassemia and compares the histiocytes of thalassemia with the storage cells observed in other disorders.

MATERIALS AND METHODS

The material for study was obtained from 20 spleens removed surgically from patients with homozygous \( \beta \)-thalassemia. The age of the patients ranged from 2 to 16 yr. In addition, five bone marrow aspirates were examined by electron microscopy. Thin sections were examined after staining with periodic acid-silver methenamine.11 This procedure accentuates membranes, and black structures usually correspond to PAS-positive material. Other histologic, histochemical, and ultrastructural techniques used have been described previously.5-6

From Institute of Pathological Anatomy, University of Ferrara, Ferrara, Italy.

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RESULTS

The characteristics of thalassemia storage cells studied by light microscopy have been described in detail. Our observations confirm their constant arrangement in nests and their close connection with plasma cells (Fig. 1). In addition, we have found that the storage cells appear as "sea-blue histiocytes" when stained by the Giemsa method.

Electron microscopic study of spleens of thalassemic patients showed that the histiocytes presented two different morphologic aspects: typical cells and transitional cells. The typical thalassemia storage cell (Fig. 2) was a large histiocyte measuring 50–80 μ in diameter. The nucleus had an eccentric location and irregular contours. The nucleolus was seldom detectable. The cytoplasm was filled with inclusion bodies of medium electron opacity that were invariably enclosed by a single membrane (Fig. 2). They varied from small and round to large and irregular. The cytoplasmic inclusion bodies contained a variable number of fine fibrils set in a pale matrix (Fig. 3). The fibrils were often arranged in parallel, simulating a tubular structure. The inclusion bodies stained positively with periodic acid methenamine silver (Fig. 4). The plasma membrane of the histiocytes was irregular and had numerous projections and infoldings. Mitochondria were restricted to the scanty cytoplasm between the bodies, and they had well-developed cristae. Short profiles of rough endoplasmic reticulum were also scattered throughout
Fig. 2. Spleen. Typical thalassemia storage cell. Cytoplasm is filled with inclusions containing fibrillar material (*) surrounded by a single membrane. Rough endoplasmic reticulum and mitochondria are dispersed throughout cytoplasm. × 4200.
Fig. 3. High-power view of typical cytoplasmic body showing fibrillar structure. × 59,200.

Fig. 4. After periodic acid-silver methenamine staining, the reaction product is found only in cytoplasmic inclusion bodies. × 4200.
Fig. 5. Spleen. In this transitional cell there are large phagocytic vacuoles containing blood cell debris and bodies (arrow) containing fibrillar material. P1, plasma cell. × 5400.
the cytoplasm. Plasma cells were intermingled with the histiocytes and were usually arranged in small clumps of a few cells. Sometimes their ergastoplasm was greatly distended and contained a low-density, fine, flocculent material that occasionally condensed into crystals. Plasma cells that had undergone degeneration and necrosis were also observed.

The transitional cells (Fig. 5) represented an intermediate stage between normal reticuloendothelial cells and typical thalassemia storage cells. They measured 20–30 μ in diameter. Their large nuclei were often located eccentrically and usually contained a single compact nucleolus. The cytoplasm contained a conspicuous Golgi complex usually located near the nucleus. Centrioles were observed in many of the cells near the nucleus; however, no cells in mitosis were seen. The rough endoplasmic reticulum was well developed, and there were free ribosomes. The cytoplasm was crowded with phagocytic material, including erythrocyte debris, platelets, plasma cells, and leukocytes at different stages of digestion.
Fig. 7. Bone marrow. A macrophage - erythroblast island. Macrophage contains characteristic inclusions, some of which contain fibrillar material (*). MN, macrophage nucleus; E, erythroblast; LE, late erythroblast; (arrows), fingerlike projections of the macrophage. × 4200.

Large phagocytic vacuoles sometimes showed small evaginations filled with bundles of fine fibrils similar to those in the cytoplasmic bodies (Fig. 6). Transitional histiocytes also contained typical cytoplasmic bodies, and their number was related inversely to the number of phagosomes.

Bone marrow specimens also contained both transitional and typical histiocytes. In contrast to the spleen, the transitional cells in the bone marrow were invariably surrounded by erythroblasts (Fig. 7), and they contained numerous erythroblasts at different stages of digestion in their cytoplasm. The plasma membrane frequently presented fingerlike projections surrounding some of the neighboring erythroblasts, which often showed regressive changes. The transitional histiocytes also had typical cytoplasmic inclusion bodies that often contained deposits of ferrogenous micelles.
Table 1. Characteristics of the Storage Cells Present in Thalassemia, Gaucher’s Disease, Chronic Myelogenous Leukemia, Syndrome of the Sea-Blue Histiocyte, and Idiopathic Thrombocytopenic Purpura

<table>
<thead>
<tr>
<th>Location</th>
<th>Thalassemia</th>
<th>Gaucher’s Disease</th>
<th>Chronic Myelogenous Leukemia</th>
<th>Sea-Blue Histiocytosis</th>
<th>Idiopathic Thrombocytopenic Purpura</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Bone marrow, spleen</td>
<td>Bone marrow, liver, spleen, lymph nodes</td>
<td>Bone marrow, liver, spleen, lymph nodes</td>
<td>Bone marrow, spleen</td>
<td>Bone marrow, spleen, lymph nodes</td>
</tr>
<tr>
<td>PAS</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>PAS after diastase</td>
<td>3+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>Alcian blue</td>
<td>2+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Metachromasia with toluidine blue</td>
<td>2+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan black B</td>
<td>ND</td>
<td>ND</td>
<td>2+</td>
<td>2+</td>
<td></td>
</tr>
<tr>
<td>Baker’s acid hematin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Luxol fast blue</td>
<td>3+</td>
<td>ND</td>
<td>3+</td>
<td>ND</td>
<td>2+</td>
</tr>
<tr>
<td>Acid fastness</td>
<td>2+</td>
<td>ND</td>
<td>3+</td>
<td>ND</td>
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<tr>
<td>Danielli</td>
<td>2+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
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<tr>
<td>Perl’s iron</td>
<td>2+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Autofluorescence</td>
<td>Blue to yellow</td>
<td>ND</td>
<td>ND</td>
<td>Golden yellow</td>
<td>Golden yellow</td>
</tr>
<tr>
<td>Giemsa</td>
<td>Blue</td>
<td>Pale blue</td>
<td>Pale blue</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>3+</td>
<td>3+</td>
<td>Similar to Gaucher’s cell, but material in sacs is fibrillar instead of tubular</td>
<td>2+</td>
<td></td>
</tr>
<tr>
<td>Ultrastructure</td>
<td>Cytoplasm filled with numerous roundish bodies containing bundles of fine fibrils set in a pale matrix</td>
<td>Cytoplasm filled with round irregular elongated sacs containing varying numbers of osmophilic smooth-walled tubules</td>
<td>Similar to Gaucher’s cell, but material in sacs is fibrillar instead of tubular</td>
<td></td>
<td>Cytoplasm contains rounded densely osmophilic masses, myelin figures, and lipid droplets</td>
</tr>
</tbody>
</table>

*ND, not done.
STORAGE CELLS OF SPLEEN AND BONE MARROW

DISCUSSION

Thalassemia storage cells are similar but not identical to the storage cells that have been described in other conditions, i.e., Gaucher's disease, chronic myelogenous leukemia, idiopathic thrombocytopenic purpura, and the "syndrome of the sea-blue histiocyte." In all of these conditions, the storage cells probably represent histiocytes that have phagocytized erythrocytes, leukocytes, or platelets. Products of the ingested cells probably accumulate in the cytoplasm of the histiocytes because of an absolute or relative insufficiency of catabolic enzymes. The typical storage cells described in these disorders present morphologic, histochemical, and ultrastructural differences. Table 1 and Fig. 8 outline the distinguishing features of these different storage cells. By contrast, the transitional cells, which are intermediate between normal reticuloendothelial cells and the typical cells, contain only partially digested remnants of different types of cells and do not have specific features.

The typical storage cells in thalassemia have a distinctive morphology. They are filled with cytoplasmic inclusion bodies that contain fine fibrils arranged in parallel and, thus, simulating tubular structures. These storage cells probably derive from phagocytosis of erythrocytes and their precursors by histiocytes that are unable to metabolize them completely. The accumulation of ingested material in the histiocytes may result from an excess of material to be catabolized and/or a genetically determined predisposition involving enzyme systems responsible for breakdown of erythrocytic debris.

When stained by the Giemsa method, the thalassemia storage cells resemble those of the syndrome of the sea-blue histiocyte, which is characterized by splenomegaly and abundant numbers of sea-blue histiocytes in the bone marrow and spleen. Kattlove et al. have described the ultrastructural features of a sea-blue histiocyte observed in a patient with sickle cell disease. These cells contained numerous membrane-bound inclusions in which there was material ranging from intact erythrocytes to fine fibrils. Rywlin et al. have presented electron micrographs of sea-blue histiocytes observed in a case of hyperlipoproteinemia. The cytoplasm of these cells contained many ceroid granules as whorls of electron-opaque unit membranes that are identical to myelin figures. Although splenomegaly and storage cells are constantly observed in both thalassemia major and minor, it seems unreasonable to include this anemia in the syndrome of the sea-blue histiocyte. The hallmarks of this syndrome are not specific, and it probably includes several conditions.

The exact nature of the material that accumulates in thalassemia storage cells is at present under investigation. Zaino et al. have considered these histiocytes to be Gaucher's cells, because the stored material has a tubular ultrastructure and because the spleen of one patient with thalassemia contained a slight increase in glucocerebroside. Our electron microscopic studies do not support this view, and we have not found typical Gaucher's cells in the cases described in this report or in the spleens and bone marrows of more than 150 thalassemic patients' studies by light microscopy. We believe...
Fig. 8. Schematic representation of storage cells present in thalassemia, Gaucher's disease, CML, and ITP. (A) Thalassemia storage cell: the cytoplasmic bodies contain bundles of fine fibrils. (B) Gaucher cell: cytoplasmic inclusions are characterized by smooth-walled tubules. (C) "Pseudo-Gaucher cell" of chronic myelogenous leukemia: cytoplasmic sacs containing dense, round deposits and tubular-like structures that have a fibrillar pattern. (D) Storage cell in idiopathic thrombocytopenic purpura: cytoplasm contains osmophilic masses and myelin figures.
that the ultrastructure of storage cells observed in thalassemia serves to distinguish them from the storage cells of other conditions.

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