Pseudo-Chediak-Higashi Anomaly in a Case of Acute Myeloid Leukemia: Electron Microscopic Studies

By M. Tulliez, J. P. Vernant, J. Breton-Gorius, M. Imbert, and C. Sultan

The formation and fine structure of giant granules in neutrophil promyelocytes of a patient with a variant of acute myelogenous leukemia were investigated by electron microscopy. The patient presented with large lymph nodes and disseminated intravascular coagulation (DIC). By light microscopy, numerous giant granules, resembling those of Chediak-Higashi syndrome (CHS), were present, but Auer bodies could not be found. By electron microscopy, these giant granules were seen to be formed by fusion of azurophilic granules, as in CHS; however, they were different from the large granules of CHS, since they contained numerous microcrystalline structures like those of Auer bodies. However, the crystalline cores of these granules exhibited a periodicity different from that of Auer bodies of acute promyelocytic leukemia. This clinical and hematologic syndrome (giant granules, enlarged lymph nodes, and DIC may represent a variant of acute promyelocytic leukemia.

The CHEDIAK-HIGASHI SYNDROME (CHS) is a rare autosomal recessive disorder characterized by partial oculocutaneous albinism and an increased susceptibility to pyogenic infections. The striking feature of the disease is the presence of giant lysosomes in most lysosome-containing cells, especially in blood and bone marrow granulocytes.1,2

In these cells, the giant granules contain the myeloperoxidase and lysosomal enzymes as azurophilic granules.3 Electron microscopy (EM) studies have suggested that they occurred by fusion of azurophilic in promyelocytes.4 Later, during maturation, the process of fusion continues, giving autophagic vacuoles in polymorphonuclear leukocytes.3

In granulocytic cells of acute or chronic leukemias, some authors have described large granules resembling those of CHS. This pseudo-CHS anomaly has been found in rare chronic myelogenous leukemia and in some acute myeloid leukemias.5-8 EM studies have been limited to a single study9 and revealed the concomitant presence of giant granules and Auer bodies.

We have had the opportunity to observe recently a case of acute granulocytic leukemia with several unusual features. Large lymph nodes and disseminated intravascular coagulation (DIC) were found, and the blast cells contained numerous giant granules, but typical Auer bodies were not seen by light microscopic examination.

Our EM studies revealed that, as in CHS, the giant granules of the promyelocytes are formed by the fusion of azurophilic granules. However, unlike the giant
granules in CHS, they contain numerous very thin structures that resemble Auer bodies.

MATERIALS AND METHODS

Case Report

A 22-yr-old patient was admitted to Henri Mondor Hospital on July 12, 1976, with sternal pain, bleeding tendency, and weakness. He had splenomegaly and diffuse lymph node enlargement; moderately sized nodes (2-3 cm) were present in the axillae, the neck, and the groins.

Initial hemogram showed hemoglobin (Hb) 16.3 g/dl, hematocrit 46%, platelets 40.10⁹/liter, WBC 3.9.10⁹/liter. The differential count was 15% myeloblasts with giant purple cytoplasmic granules, 5% myelocytes, 6% band forms, 32% polymorphonuclear leukocytes, 2% monocytes, and 40% lymphocytes. Bone marrow aspiration revealed 93% myeloblasts with the same granules. Lymph node aspiration revealed the presence of the same myeloblasts.

Coagulation data suggested DIC (Table 1). Plasma lysozyme was 21 μg/ml (normal 11.5 ± 4 μg/ml), and urine lysozyme was 2.5 mg/liter; LAP (leukocyte alkaline phosphatase): score 4.

Platelet transfusions and heparin therapy (150 U/kg/24 hr) were started on July 13 followed by chemotherapy (daunomycin 100 mg/sq m x 3 days and cytosine arabinoside 100 mg/sq m x 5 days).

The patient received daily platelet transfusions and heparin. The DIC progressed but without clinical bleeding or defibrination and disappeared on July 21. Heparin was stopped on July 22. The patient received a daily leukocyte transfusion, and aplasia was achieved without bleeding or infectious problems. A complete remission was obtained and the patient was discharged with maintenance therapy (monthly, daunomycin and cytosine arabinoside).

Eight months later, a relapse occurred with several unusual features. He has numerous large nodes (5-6 cm) containing only myeloblasts. The bone marrow was very cellular, with only 15% myeloblasts containing the same giant granules. The peripheral blood was normal. A chromosomal study of the bone marrow did not show any abnormality, but banding techniques were not performed. On retreatment with the initial therapy, a complete remission was again obtained. Six months later, a second relapse occurred that was not responsive to chemotherapy, and the patient died in November 1977.

Light Microscopic Studies

Routine hematologic tests were performed according to Dacie and Lewis.⁹

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<th>Biologic Data and Treatment</th>
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<td><strong>TREATMENT</strong></td>
<td>Daunomycin</td>
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<tr>
<td><strong>TRANSFUSION</strong></td>
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<tr>
<td><strong>COAGULATION STUDIES</strong></td>
<td>Fibrogenes (mg/%)</td>
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<tr>
<td><strong>PLATELETS</strong> x 10⁹/ℓ</td>
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<td><strong>LEUKOCYTES</strong> x 10⁹/ℓ</td>
<td>4</td>
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<tr>
<td><strong>TEMPERATURE</strong></td>
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**Ultrastructural Studies**

Bone marrow samples were immediately fixed in 1.25% glutaraldehyde in 0.1 M phosphate buffer for 30 min, post-fixed in 1% osmium tetroxide in phosphate buffer for 30 min at 4°C, dehydrated in ethanol and propylene oxide, and embedded in epon. Thin sections were prepared on a Reichert OM U2 microtome, stained with uranyl acetate and lead citrate, and examined with a Philips EM 300 electron microscope.

**RESULTS**

**Light Microscopic Studies**

The smears were obtained before any chemotherapy. Blast cells ranged in size between 25 and 35 μ. Nucleus was large, round or oval, with a delicate chromatin pattern and a conspicuous nucleolus. The cytoplasm was basophilic. Some cells contained vacuoles and phagocytosed red cells. Granules were abundant and made of different types, including small azurophilic granules scattered through the cytoplasm; large dense, purple and well limited granules, often located at one pole of the cell (Fig. 1 A and C); and very large granules that looked like a coarse block of azurophilic material (Fig. 1B). Auer rods were not observed.

In the peripheral blood, the blast cells were rare with very few granules; large granules were not observed in the rare mature granulocytes. On aspiration of the lymph nodes, the same blast cells were found but with less numerous inclusions.

Peroxidase stain was positive in all blast cells. The large granules were strongly positive.

During the remission, the granulocytic precursors did not contain any giant granules. At the time of the relapse, the cell morphology was exactly similar to that of the initial phase.
Fig. 2. (A) Giant granules in a blast cell. Two different types of granules: one circular granule with homogenous matrix and denser round masses near the periphery; another granule with heterogenous matrix (× 6930). (B) Giant granule in a blast cell; heterogenous matrix with numerous rods (× 17,760).
**Electron Microscopic Studies**

The blast cells had a round or clefted nucleus. The cytoplasmic granulations were often clustered at one pole of the cell, not intermixed with the numerous mitochondria. The striking feature was the presence of giant granules in the cytoplasm of most of the blast cells. These giant granules were very numerous, as many as six in the same section. They were sometimes very large (5-μm diameter) and were very polymorphic; many were circular or more often with an irregular outline. Some were thin rods, short or very long, with one or several round protuberances. Their structure was highly heterogeneous. Round dense masses were often observed (Figs. 2A and B, 3A). Numerous rods were present in the matrix of most of the giant granules (Fig. 2B). On some sections, these rods appeared transversally sectioned (Fig. 2A), in which case they were seen to be very numerous, often more than 30 in one giant granule.

With high magnification, the longitudinal sections of these rods revealed a longitudinal regular striation with a periodical alternation of dark lines separated by clear spaces (Fig. 3B). The periodicity of these dark lines was about 75 Å, suggesting that the giant granule is constituted of many subunits. This ultrastructure was similar to that of the Auer bodies, but true Auer bodies (long, 6 μ, and very narrow, 0.2 μ, with the same periodical structure) were observed very rarely.

Some blast cells contained a red blood cell in a cytoplasmic vacuole (Fig. 4A) or more rarely an erythroblast. Macrophages were numerous; the cytoplasm contained many giant granules with the same ultrastructural features as those in the blast cells and many Auer rods (Fig. 4B).

**DISCUSSION**

This case of acute granulocytic leukemia is unusual because the immature cells contained striking giant granules. These cells were present in enlarged lymph nodes as well as in the marrow. The absence of giant granules during the remission obviously excluded the occurrence of leukemia as terminal evolution of a congenital CHS.

The characteristic giant granules that were seen must be compared to the granulation of CHS and to the granulations described in some leukemias reported in previous observations. The giant granules typical of CHS are not very different from these by light microscopy. Both are peroxidase positive, but in CHS, the granules are not so numerous as in our case and are usually light pink, not purple. The ultrastructural study shows that the granules are very different. The granules in CHS do not have visible crystalline structure, unlike the granules in the cells of the present patient.

Pseudo-Chediak-Higashi granulations have been reported in two cases of myelomonocytic leukemia by Van Slyck and Rebuck, in one case of acute granulocytic leukemia by Gorman and O’Connel, and in one case of chronic myelogenous leukemia with myelofibrosis by Tsai et al. These four cases are clinically different from our case, since they did not have enlarged lymph nodes or DIC. No ultrastructural studies were done on the cells.

Mintz et al. have reported one case of promyelocytic leukemia with giant lysosome like structures. The clinical description was similar to ours with typical...
Fig. 3. (A) Part of a giant granule with dense masses and numerous rods in which a periodical striation appears (× 50,700). (Inset) Fusion of two granules in the cytoplasm of a blast cell (× 55,300). (B) Detail of the longitudinal periodical striation of a rod in a giant granule (× 194,250).
Fig. 4. (A) Red blood cell in a cytoplasmic vacuole of a blast cell (x 9450). (Inset) Light microscopy. (B) Macrophage with the different types of giant granules. Some of them have an homogenous matrix, others contain longitudinal or transverse sections of rods. Many isolated Auer rods are revealed (x 5940).
DIC but without lymph nodes, and they did not obtain remission on therapy. The giant azurophilic cytoplasmic granules were similar to those seen in the cells of our patient and some Auer bodies were observed by light microscopy. On cytochemical grounds, the authors postulated a common origin for the giant granules and the Auer bodies and suggested a possible relationship of these to the coagulation disturbance.

In our patient, the giant granules seem to be the result of the fusion of azurophilic granulations (Fig. 3A inset), as observed in granules and Auer bodies of other acute myelogenous leukemias. Some of these giant granules contain crystalline structures like those seen in Auer bodies, but these do not possess the ultrastructural features observed in the usual cells of acute promyelocytic leukemia. The periodicity of the crystalline structure in the longitudinal sections is different, and the transverse sections do not show the characteristic hexagonal arrangement of tubes.

DIC may be observed in the course of some rare acute lymphoblastic, myeloblastic, and monocytic leukemias, but is nearly always present in acute promyelocytic leukemias; in our case, the presence of DIC associated with atypical granules was highly suggestive of the diagnosis of acute promyelocytic leukemia.

Enlargement of the lymph nodes, which was present at onset, was the first clinical symptom of relapse. Such lymphadenopathy is unusual in the course of an acute leukemia, either myeloblastic or promyelocytic, but may be observed in blast crisis of chronic granulocytic leukemia. In our patient, lack of anemia and the low LAP score might suggest this hypothesis, but chromosome study did not reveal the Ph chromosome.

This type of acute leukemia is different from typical acute myelogenous leukemia because of the unusual severe DIC and the great number of purple giant granules in the immature cells. It is also different from acute promyelocytic leukemia because of the absence of the Auer rods clustered like bundles of wood. The crystalline structure in the Auer bodies contained in the giant granules is highly different from that observed in acute promyelocytic leukemia. The presence of DIC in our patient and in Mintz's patient suggests that one should consider this type of acute leukemia with giant granules as a variant of promyelocytic leukemia and treat the patient with platelet transfusion, heparin, and chemotherapy.

ACKNOWLEDGMENT

We are indebted to W. Ross for his help in improving the English manuscript. We also acknowledge the photographic assistance of M. Ph. Reboul and the secretarial assistance of M. Segear.

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