Acute Lymphoblastic Leukemia With Unusual Cytoplasmic Granulation: A Morphologic, Cytochemical, and Ultrastructural Study

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The classification of the acute leukemias depends mainly on the morphologic and cytochemical evaluation of the blast forms. One of the main accepted morphologic criteria in the differentiation between acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) is the absence of granules in the blast cells of ALL. We evaluated a patient with ALL in whom granules were present in the cytoplasm of 35% of the blast cells, as seen in AML. Cytochemical evaluation was performed, including periodic acid-Schiff reaction, Sudan black B, α-naphthyl acetate, α-naphthyl butyrate, naphthol AS-D chloroacetate, and acid phosphatase stains. The results of these studies confirmed the morphologic impression and diagnosis of ALL. Ultrastructural evaluation revealed that the granules consisted of many tiny vesicles closely packed together in a proteinaceous matrix, resembling to some extent the inclusions described in lymphocytes in the Chédiak-Higashi syndrome, but clearly different. The morphologic, cytochemical, and ultrastructural studies of this unique case are presented in detail. To our knowledge, this is the first time that such granules have been described in blast cells of ALL.

CASE REPORT

A 45-year-old white female with a negative past medical history presented with right upper-quadrant pain. Physical examination revealed hepatosplenomegaly with the border palpated 7 cm below the right costal margin. There was no splenomegaly or lymphadenopathy. The hemogram was as follows: hemoglobin level, 14.7 g/dL; WBC count, 158,000/μL; differential cell count, 78% blasts, 3% segmented neutrophils, 4% bands, and 15% mature lymphocytes; and platelet count, 264,000/μL. Many of the blast cells had large cytoplasmic granules, the rest were typical lymphoblasts without granulation. The blood chemistries (Sequential Multiple Analyzer–12, Techicon) were normal. A spinal tap yielded cerebrospinal fluid that was free of cells. The chest roentgenogram was unremarkable; liver and spleen scans revealed mild hepatosplenomegaly, and the serum electrophoresis showed slight hyperalbuminemia without other abnormalities.

The bone marrow was markedly infiltrated with typical lymphoblasts, and mild mature lymphocytosis was also present. The diagnosis of ALL, L1, using the French-American-British classification was made in spite of the presence of the blast forms with coarse granulation. Cytochemical studies were performed that confirmed this diagnosis.

Treatment was instituted with vincristine and prednisone after informed consent was given by the patient, and complete remission was obtained 6 weeks later. Bone marrow aspiration at this time revealed marked mature lymphocytosis of uncertain significance. Prednisone therapy was tapered, but shortly thereafter, the patient relapsed and was given vincristine and prednisone again with an immediate response. Treatment was continued for 6 weeks, after which prednisone was tapered again. Complete remission was confirmed by bone marrow aspiration, and maintenance chemotherapy with methotrexate and 6-mercaptopurine was begun with the consent of the patient and continued until the patient relapsed again 3 months later. Reinduction with vincristine and prednisone was unsuccessful this time, and treatment with doxorubicin and arabinosyl cytosine was instituted.

CNS involvement was confirmed and treated with cranial radiation. Sepsis ensued, and the patient was treated aggressively with antibiotics including cephalosporins and aminoglycosides. Antifungal agents were administered for systemic candidiasis, and platelet and granulocyte transfusions were given with poor response. Multiple complications occurred, including hepatitis, which precluded further therapy. Supportive treatment was continued until death supervened from complications approximately 1 year after the initial diagnosis of acute leukemia.

MATERIALS AND METHODS

Blood smears and cytochemical studies. The peripheral blood smears and bone marrow preparations were stained with Wright’s-Giemsa stain. The cytochemical studies performed included Sudan black B stain, periodic acid–Schiff reaction (PAS), naphthol AS-D chloroacetate esterase, α-naphthyl acetate esterase, α-naphthyl butyrate esterase, and acid phosphatase.

Electron microscopic studies. Blood for this study was obtained from our patient with lymphoblastic leukemia and from one child with the Chédiak-Higashi syndrome. Samples were obtained by venipuncture and mixed immediately with citrate–citric acid–dextrose, pH 6.5 (9.3 mmol/L sodium citrate, 7.0 mmol/L citric acid, and 140 mmol/L dextrose) in the ratio of nine parts blood to one part anticoagulant. The blood was centrifuged at 100 g for 20 minutes at room temperature. Platelet-rich plasma was aspirated above the buffy coat. Leukocyte-rich buffy coat was then separated from the red cell layer by careful aspiration and transferred to a second tube. Theuffy coats from several tubes were combined and mixed with an equal volume of 0.1% glutaraldehyde in White’s saline, pH 7.3 (a 10% solution of a 1:1 mixture of 2.4 mol/L NaCl, 0.1 mol/L KCl, 46 mmol/L MgSO4, 64 mmol/L Ca(NO3)2, 4H2O, 0.13 mol/L NaHCO3, 8.4 mmol/L NaH2PO4 · 7H2O, 3.8 mmol/L...
anhydrous KH₂PO₄, and 0.1 g/L phenol red). After 15 minutes at 37 °C, the leukocyte-rich samples in glutaraldehyde were sedimented to pellets and the supernatant discarded. The cells were washed in fresh 3% glutaraldehyde and White's saline and then postfixed in osmium-containing potassium ferrocyanide. After an hour at 4 °C, leukocyte-rich samples were dehydrated in a series of alcohols and imbedded in Epon-812. Contrast of the thin-section cut in osmium-containing potassium ferrocyanide. After an hour at 4 °C, leukocyte-rich samples were dehydrated in a series of alcohols and imbedded in Epon-812. Contrast of the thin-section cut from plastic blocks on an ultramicrotome was enhanced with uranyl acetate and lead citrate. The sections were evaluated in a Phillips 301 electron microscope.

Membrane markers. Suspensions of blast cells obtained from bone marrow aspirates were studied for surface immunoglobulins (slg) by immunofluorescence with monospecific antisera against heavy chains, light chains, and polyvalent immunoglobulins (PV). Mouse rosettes were assayed as described elsewhere. Spontaneous sheep erythrocyte rosette formation (SRBC-R) was performed according to the method of Borella and Sen. Anti-T monoclonal antibodies (Becton Dickinson, Mountain View, CA) were used for the identification of T cells and subsets of T cells. The intracellular distribution of terminal deoxynucleotidyl transferase (TdT) was assayed by immunofluorescence with monospecific antibodies, the following values were obtained: Leu-1 (6.5%), Leu-la (8%), Leu-3a (3%), and Leu-4 (9%). The intracellular TdT assay was brightly positive in most cells examined. These results were interpreted as compatible with non–T-ALL.

Electron microscopy. Most of the thin sections of lymphocytes observed in samples of leukocyte-rich plasma prepared for electron microscopy revealed large cytoplasmic organelles. Only a single organelle was evident in some cells, but in many of the thin sections studied, 2, 3, 4, or 5 of the large, relatively oval-shaped structures were present (Figs 3 and 4). The substructure of the internal matrix present in the large organelles was relatively uniform. It appeared to consist of many tiny vesicles closely packed together within a proteinaceous matrix. A few of the large structures appeared to have a relatively electron-dense substructure, but the majority resembled the large organelles with the vesicular substructure. The origin of these inclusions was not immediately apparent; however, many of the fusion of the vesicular elements with adjacent organelles could be defined. They are well demonstrated in the second set of illustrations (Figs 5A to 5D). In these examples, vesicular organelles can be seen fusing with others that appear to have a relatively electron-dense matrix. Such a fusion process could result in the formation of what resembles autophagic granules, and several of these were seen in the lymphoblasts from the patient. However, the substructure of the matrix inside the large vesicular organelles was reasonably uniform. If fusion was involved in their formation, this would suggest that organelles of a similar type had fused together to form the large bodies rather than a variety of different types of organelles being involved. Thus, the precise origin of the large vesicular organelles remains uncertain, although it is quite clear they can fuse with electron-dense organelles to form complex bodies.

The similarity of the large inclusions in this patient to the organelles containing tubulelike structures found in lymphocytes of patients with the Chédiak-Higashi syndrome is quite striking. A typical giant tubule-containing organelle from a lymphocyte of a patient with the Chédiak-Higashi syndrome is shown in Fig 5E. The matrix of these organelles consists of microtubulelike structures that are packed closely together. In a sense, the matrix resembles that of the large vesicular organelles in our patient with lymphoblastic leukemia. However, it is quite clear that the tubules in the Chédiak-Higashi inclusions are different from the vesicular structures present in the matrix of the patient's large organelles. Thus, our patient appears to have a unique spongy organization of lymphocytic inclusions that are not observed under normal conditions, nor are they, to our knowledge, found in other types of human lymphocyte disorders.

RESULTS

Light microscopy. In the Wright-Giemsa–stained peripheral blood smear, the blast cells ranged in size from 10 to 15 μm in diameter. Most of the nuclei were round, but occasionally slight indentations were observed. Some of the blast forms exhibited one or two large nucleoli, and the chromatin pattern varied from finely granular to coarsely dispersed (Fig 1).

The amount of cytoplasm varied, but many of the blasts had abundant light blue cytoplasm. Thirty-five percent of these cells had usual, large, dark purple compact granules in the cytoplasm that occurred singly or in aggregates (Fig 1) and are the subject of this report. Blast forms with unusual cytoplasmic granularity were more abundant in the peripheral blood than in the bone marrow aspirate. The granules were round, oval, or elongated, and multiple granules were always present in each cell. Auer rods were absent.

The bone marrow was markedly infiltrated with typical lymphoblasts and an admixture of mature lymphocytes. Only 15% of the blasts in the marrow showed the unusual cytoplasmic granulation as opposed to 35% in the peripheral blood.

Cytochemistry. The blast cells were negative for the Sudan black B reaction, the naphthol AS-D chloroacetate, and the α-naphthyl butyrate stain. The PAS stain revealed diffuse fine granulation in a high percentage of blasts. The α-naphthyl acetate was strong positive with a nongranular, mostly focal, reaction resistant to sodium fluoride (Fig 2). This type of reaction has not been described previously and is different from the pattern of blocks observed by Shaw and Ishmael in seven cases of ALL and from the positive focal granular reaction often associated with T-ALL. The acid phosphatase stain showed a granular positivity with an irregular distribution throughout the cytoplasm. The granules gave negative results with all the stains tested.

Membrane marker studies. The following results were obtained for the surface marker studies: SRBC-R at 4 °C, 12.5%; SRBC-R at 37 °C, 3%; PV, 16.5%; IgG, 0.5%; IgM, 9%; IgA, 0%; k, 5.5%; λ, 4.5%; and mouse rosettes, 0%. Using the anti-T monoclonal antibodies, the following values were obtained: Leu-1 (6.5%), Leu-la (8%), Leu-3a (3%), and Leu-4 (9%). The intracellular TdT assay was brightly positive in most cells examined. These results were interpreted as compatible with non–T-ALL.

DISCUSSION

Small cytoplasmic granulations have been recognized as a normal morphologic finding in large mature normal lymphocytes. In Wright-Giemsa–stained smears these granules are usually azurophilic and believed to represent lysosomes. Cytoplasmic granulations have also been described in pathologic mature lymphocytes characteristic of lymphoproliferative-
Fig 1. Peripheral blood from the patient illustrating lymphoblasts with the abnormal large cytoplasmic granules. Some of the lymphoblasts have slightly indented nuclei with prominent nucleoli (A, B). Although in all the lymphoblasts with granulation separate distinct granules were identified (C, D), in some of them fusion of several granules could also be observed (A, B, D). Wright's-Giemsa stain (original magnification × 1000; current magnification × 920).

Fig 2. Patient's bone marrow smear illustrating the positive reaction obtained with the α-naphthyl acetate stain in the lymphoblasts (original magnification × 1000; current magnification × 630).

tive disorders of T cell lineage. At the present time a distinct subtype of chronic lymphocytic leukemia (CLL) is recognized in which mature lymphocytes with abundant cytoplasm and large azurophilic granules are frequently observed. Characteristically, these lymphocytes have a strong, tartrate-inhibited positive reaction, with the acid phosphatase stain mainly localized paranuclearly. On electron microscopy, the granules closely resemble mitochondria morphologically; therefore, their origin from the latter cannot be excluded.
LYMPHOBLASTS WITH ABNORMAL GRANULATION

Fig 3. Thin section of lymphoblast from example of peripheral blood obtained from patient described in this study and prepared for electron microscopy by fixation in glutaraldehyde and osmic acid. Nucleus (N), mitochondria (M), and cytoplasm demonstrate typical lymphoid features. Two large inclusions (I) manifesting a vesicular substructure are present in the cytoplasmic matrix (original magnification x 23,000; current magnification x 13,800).

Similar abnormal large granular lymphocytes (LGL) have been described by Chan et al\(^1\) and Semenzato et al\(^2\) in patients with expansions of a unique population of lymphocytes consistently associated with neutropenia, a relatively favorable clinical course, and a T-suppressor/cytotoxic cell phenotype. Although recently Loughran et al\(^3\) described three patients in whom he was able to define the leukemic nature of the LGL, the controversy regarding its malignant or benign nature remains unresolved.\(^4\) Grossi et al\(^5\) analyzed the granules of these LGL by enzyme cytochemistry and electron microscopy. Acid phosphatase and a-naphthyl acetate of the monocytic type were detected in the granules. The granules were also identified as primary lysosomes but were not involved in the process of phagocytosis.

Crystalline cytoplasmic inclusions in mature lymphocytes have also been observed in lymphoproliferative disorders.\(^6\)-\(^9\) In one of the cases reported by Laszlo et al,\(^10\) the inclusions proved to be accumulations of glycoprotein and represented a stage in the evolution of Russell bodies similar to those seen in plasma cells. In two other cases with CLL reported by Clark et al,\(^11\) the inclusions were crystalline structures that stained for specific immunoglobulins by immunofluorescence. The published data indicate that crystallization of intracellular material appearing as globular, tubular, fibrillary, or rod-shaped inclusions within peripheral blood lymphocytes in lymphoproliferative disorders is not an uncommon event. When present, the crystals represent aggregates of \(\gamma, \lambda, \mu, \text{or} \kappa\) chains that in some cases have been antigenically identical to the membrane-bound immunoglobulin present in the cell surface.\(^12\)-\(^13\)

The classic distinctive giant granulations seen in the granulocytes of the Chédiak-Higashi syndrome can also be seen in the lymphocytes of patients with this disorder. The physical similarity of the giant granules present in this syndrome to the normal granulation present in normal granulocytes has been documented by Bessis et al\(^14\) in light and electron microscopic studies. The cytochemical profile of these giant granules is also compatible with normal granulocytic particles whose histochemical and biochemical evaluation have proven them to be lysosomes.\(^15\)-\(^17\) In addition, White,\(^18\) using techniques of ultrastructural histochemistry, corroborated that the Chédiak-Higashi giant granules were abnormal lysosomes.

Other types of giant inclusions were also observed by...
White\textsuperscript{20} in mature lymphocytes of patients with the Ch\'ediak-Higashi syndrome. The giant size of the inclusions apparently was the result of fusion of smaller organelles. Since inclusions were negative for acid phosphatase, White postulated the possibility that giant lysosomes could have fused with nonlysosomal organelles to produce compound inclusions. Similar inclusions had previously been reported by Hoving et al\textsuperscript{24} in 1968 in a patient with chronic rheumatoid arthritis and leukopenia. Subsequently, Huhn\textsuperscript{25} reported similar organelles in the peripheral blood lymphocytes of healthy individuals. The electron microscopic studies of these giant granules revealed a characteristic tubular substructure completely different from the vesicular structure characteristic of the giant granules in our case.

The atypical lymphocytes observed in infectious mononucleosis may also contain large cytoplasmic azurophilic granules that on electron microscopy consist of parallel arrays of microtubulelike structures and have been referred to as parallel tubular arrays (PTA) in the literature.\textsuperscript{26} These structures had been identified by Huhn,\textsuperscript{25} Hoving et al,\textsuperscript{24} and White\textsuperscript{20} in their studies of the large granules observed in cases of rheumatoid arthritis, in normal individuals, and in the lymphocytes of cases of the Ch\'ediak-Higashi syndrome, respectively. The PTA appear to represent normal organelles occurring in lymphocytes at a particular stage of their life cycle.

To our knowledge, coarse cytoplasmic granulation or the presence of inclusions in lymphoblasts has never been previously reported, with the possible exception of Rosen et al\textsuperscript{27} who reported the only instance we have been able to find of unusual cytoplasmic inclusions in the blasts of a patient with acute leukemia. Unfortunately, these authors could not determine the type of leukemia in their case since the blasts had morphologic features of both lymphocytic and granulocytic tissue; neither TdT or immunologic studies were performed. In the Wright’s-Giemsa-stained films, the inclusions appeared as oval, pale blue, yellow-tinged organelles about 2 to 4 \textmu m in diameter. As in our case, the inclusions gave negative results with all the tested stains, including PAS, Sudan black B, $\alpha$-naphthyl acetate, oil red 0, and toluidine blue. These authors concluded that the granules described were not specific for any particular type of leukemia. The observed inclusions appear to be similar to the ones reported by Coppola and O’Connor\textsuperscript{21} in a patient with a
myeloproliferative disorder. In both instances the inclusions were morphologically different from the ones observed in our case when studied with the light microscope in Wright's-Giemsa stained preparations.

In summary, we have described a well-documented patient with ALL in whom blast cells containing large cytoplasmic granules were present. The Wright's-Giemsa staining characteristics as well as the electron microscopic features of the granules were distinctly different from other lymphocyte granules and inclusions thus far reported in the literature. Although in the electron microscopic studies the granules resembled the granules described by White in the mature lymphocytes of the Chédiak-Higashi syndrome, the vesicular ultrastructure of the granules present in our case is different, and to our knowledge, hitherto undescribed. The rare occurrence of these granulations in lymphoblasts should be taken in consideration when performing the morphologic study of cases of acute leukemia. Morphologists should recognize this rare entity to avoid confusion in the proper classification of blast forms. On the other hand, the important role of cytochemistry and membrane marker studies in the characterization of blast cells is dramatized by the possible presence, albeit rare, of nonspecific abnormal granulation in lymphoblasts.

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