Chloroma (Granulocytic Sarcoma) Without Evidence of Leukemia: Facilitated Light Microscopic Diagnosis

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Localized tumors composed of immature cells of the myelogenous series have been recognized for many years as an uncommon manifestation of granulocytic leukemia. The histologic diagnosis of chloroma (granulocytic sarcoma) may be extremely difficult when the myeloblastic cells are poorly differentiated and the tumor lacks the characteristic green color. The diagnostic difficulty may be further compounded when the granulocytic sarcoma develops before there is peripheral blood or bone marrow evidence of leukemia. Previous criteria for the diagnosis of chloroma have been ambiguous because of the capricious nature of the hydroperoxidase activity and the lack of definitive histochemical criteria. In this case, a combination of Sudan black B and myeloperoxidase histochemical staining and ultrastructural evaluation was applied. The light microscopic histochemical studies suggested the presence of Phi bodies and rods both in the formalin-fixed tumor and in the cells derived from the subsequent pleural effusion; this was confirmed by electron microscopy, which demonstrated the periodicity of the crystalline rod substructure. These observations show that light microscopic histochemical studies can facilitate the diagnosis of granulocytic sarcoma or chloroma in the absence of peripheral blood or bone marrow manifestations of leukemia.

CHLOROMA or granulocytic sarcoma is a rare tumor that may develop in patients with aggressive forms of acute or chronic granulocytic leukemia. The histologic diagnosis may be difficult with light microscopy and standard Romanowsky staining techniques, since the myeloblastic cells are usually poorly differentiated. The diagnosis may be particularly difficult when the granulocytic sarcoma is observed before peripheral blood or bone marrow abnormalities are recognizable and when it is not green. In such cases, the distinction of granulocytic sarcoma from an inflammatory reaction to a nonhematopoietic tumor may not be possible. In a rat chloroma chemically induced by dimethylbenzanthracene, ellipsoidal rods could be consistently observed by electron microscopy in the myeloperoxidase-positive tumor cells. There have been cases where electron microscopy has helped substantiate the diagnosis of chloroma by defining the morphology of the myeloblastic cells or by defining the presence of Auer rods. However, no readily applied satisfactory criteria have been proposed for establishing the diagnosis by light microscopy. The present study shows that this can be done, even where Auer rods are not visible with a Romanovsky stain, by the readily demonstrable hydroperoxidase-positive Phi bodies and rods. Ultrastructural studies confirmed the tumor's granulocytic origin despite the absence of peripheral blood or bone marrow stigmata of leukemia.

MATERIALS AND METHODS

Case Description

The patient, a 33-yr-old white married man, had had excellent health until November 1977 when he was noted to have a tumor involving the right anterior chest wall. The tumor was resected and appeared as an undifferentiated neoplasm without the typical green color of granulocytic sarcoma. There were no demonstrable peripheral blood or bone marrow abnormalities. After establishment of the diagnosis of granulocytic sarcoma, the patient was treated with electron beam irradiation to a total dose of 3500 rad.

He continued to do well until, in the summer of 1978, dull low back pain was experienced. Extensive evaluation at that time revealed no specific etiology for his symptoms. His back pain became worse, and with the development of dull pain in the right lumbar region in November 1978, he was found to have increased lower extremity deep tendon reflexes with loss of anal sphincter control. Radiographs of the spine were interpreted as normal. A complete block from the level of T9 to T11-T12 was demonstrated by myelography. In December 1978 he underwent a laminectomy with debulking procedure (neoplastic tissue revealing morphology similar to the chest wall tumor). Postoperative irradiation was given to a total dose of 3000 rad in 3 wk. Neurologic examination improved dramatically.

A right pleural effusion (3000 cc removed by thoracentesis) was also observed during this November hospitalization. Immature granulocytic cells were observed in histochemical and electron microscopic studies of the effusate, although no peripheral blood or bone marrow evidence of leukemia was observed.

Currently, the patient is on a chemotherapeutic regimen consisting of vincristine, thioguanine, cytosine arabinoside, and doxorubicin. He continues to show no clinical or laboratory evidence of leukemia.

Histology

Tissue was fixed in 4% formaldehyde 0.1 M NaPO₄ buffer, pH 7.0, for 24 hr, dehydrated through serial alcohols, and embedded in...
paraplast. Sections were cut at 4 μ, deparaffinized, and stained with hematoxylin and eosin or Masson’s trichrome. Additional sections were evaluated with Leder’s chloracetate-esterase technique. Thin film preparations of the pleural effusate cells were stained with Wright’s stain.

Light Microscopic Demonstration of Sudanophilia and Myeloperoxidase Activity in Tumor Tissue and in Pleural Effusate Cells

Incubation for sudanophilic particles. Formalin-fixed touch preparations or cryostat sections of tumor or films of pleural effusate cells on coverslips were immersed in the Sudan black B staining solution for 30 min according to the method of Sheehan and Storey. They were counterstained for 10 min in Papanicolaou-hematoxylin-Harris. Incubation for myeloperoxidase activity. Formalin-fixed touch preparations or cryostat sections of tumor or glutaraldehyde/formaldehyde-fixed films of pleural effusate cells on coverslips were incubated for hydroperoxidase activity as previously described.

Ultrastructural Studies

Tissue from the primary tumor and the cellular pellet from the pleural effusion were fixed in 4% glutaraldehyde, 0.1 M cacodylate, pH 7.4 (640 osmole), for 24 hr, postfixed in 1% OsO₄, 0.1 M collidine, pH 7.0, at 4°C for 1 hr, rinsed in 0.1 M cacodylate buffer, pH 7.4, and dehydrated through serial alcohols to propylene oxide and embedded in epon 812. Sections were cut at 1 μ, stained with toluidine blue, and areas for ultrathin sections selected. Sections of 200 nm were placed on 400-mesh colloidal-coated copper grids, stained with uranyl acetate and lead citrate, carbon coated, and examined in a Hitachi Hu-11E microscope at 75 kV.

RESULTS

Histologic Findings

The morphology of both the initial chest wall lesion and the tissue removed at the time of laminectomy were similar. In both specimens, an alveolar pattern of pleomorphic, malignant-appearing cells with hyperchromatic vesicular nuclei and prominent nucleoli characterized the tissue. No cytoplasmic granules or Auer rods were observed in sections prepared with Wright’s stain or Leder’s chloroacetate-esterase technique.

Sudan Black B and Myeloperoxidase Histochemical Staining

Fusiform and spindle-shaped particles that appeared to be Phi bodies were seen in many of the tumor cells (Fig. 1) and pleural effusate cells (Fig. 2). Few other granules were seen in the pleomorphic tumor cells with vesicular nuclei and multiple nucleoli. The effusate cells, however, were reminiscent of myeloblasts and promyelocytes observed in enriched leukocyte preparation from the peripheral blood of acute myelogenous leukemia patients. The sudanophilic and myeloperoxidase-positive inclusions were readily seen by light microscopy despite the absence of Auer rods in routine Romanowsky-stained specimens.

Electron Microscopic Observations

Electron microscopic evaluation of preparations of the tumor showed immature granulocytic elements (Fig. 3) in a loose stroma, suggesting the diagnosis of chloroma. In electron micrographs, the cytoplasm of many cells of both the tumor and the pleural effusion were seen to contain ellipsoidal granules with axial crystalloid and crystalline rods (Fig. 4). The substructure of the axial crystals demonstrated a periodicity identical with that seen in similar inclusions in leukemic leukocytes.

DISCUSSION

Localized tumors composed of immature myeloid cells, chloromas, or granulocytic sarcomas have been
The diagnosis of chloroma may be extremely difficult by routine histologic techniques.\textsuperscript{14-16} The cells can comprise a series of cellular variants ranging from immature nongranular mononuclear cells to myelocytes. Identification depends on the degree and stage of maturation. The morphological separation from histiocytic or poorly differentiated lymphomas are frequently not sufficient to allow definitive diagnosis and may require additional histochemical techniques to identify the presence of granulocytic enzymatic activity. These techniques, however, often do not help distinguish the infiltrate from an inflammatory reaction in a “solid” tumor.\textsuperscript{2,17} In this case, the interpretation of the tumor histology included alveolar rhabdomyosarcoma, histiocytic lymphoma, amelanotic melanoma, undifferentiated neoplasm, or chloroma. Techniques that could elaborate the structure of these immature cells, and thereby render the diagnosis of chloroma unambiguous, include histochemical methods for azurophilic granules, such as Sudan black.

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**Fig. 2.** Light micrographs of pleural effusate films with Phi bodies and rods in granulocytic cells. (A) A holotypic Phi body (arrow) is seen in the myeloblast by virtue of its sudanophilia. Counterstaining with Papanicolaou-hematoxylin-Harris (bar = 10 μ). (B) Phi bodies and rods (double arrows) are seen in several myeloblasts by virtue of their myeloperoxidase activity (bar = 10 μ).

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Chloromas (granulocytic sarcomas) were originally described and so named because of their green color, thought to be secondary to the presence of myeloperoxidase (verdoperoxidase) in the neoplastic tissue. However, many granulocytic sarcomas are colorless, as was the tumor in the present case.

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**Fig. 3.** Electron micrograph of granulocytic sarcoma. The tumor consists of immature myelocytic elements with distinct primary and secondary granules (G). The nuclei (N) are prominent with distinct nucleoli (Nu) (bar = 5 μ).
Fig. 4. Electron micrograph of granulocytic sarcoma cell containing an ellipsoidal crystalloid granule. (A) This myelocytic cell contains a large rod (R) with crystalloid substructure. Numerous granules (G) and mitochondria (m) are seen. The nuclei (N) are prominent (bar = 2 \mu m). (B) Detail of rod (R) showing crystalloid substructure (bar = 1 \mu m).

B and myeloperoxidase staining techniques, and electron microscopy.

Light microscopic histochemical studies with peroxidase and Sudan black B methods suggested the presence of myeloperoxidase-positive and sudanophilic Phi bodies and rods\(^{18}\) in many of the neoplastic cells (Figs. 1 and 2). Recent studies have suggested that Auer rods, first described in 1906 by John Auer, may be formed by the extrusion of axial crystallloid material from the ellipsoidal fusiform hydroperoxidase-positive parent organelles known as Phi bodies.\(^{4,9,11,18}\) Segments of this extruding axial crystallloid may subsequently detach to form free crystalline rods. In immature granulocytes, Phi bodies may be formed by the enlargement and deformation or distention of spherical or ellipsoidal primary or azurophilic granules, which are characteristically found in granulocytic cells.\(^{19}\) Large numbers of myeloperoxidase-stained Phi bodies and rods have been readily observed with light microscopy in leukocytes taken from patients with acute myelogenous leukemia (92\%).\(^{10}\) Auer rods visualized with a Romanowsky-type stain constitute only a rather small subpopulation of these peroxidase-positive particles and are usually detected only with difficulty, being reported to occur in leukocytes of only 5%-20% of patients with acute myelogenous leukemia.\(^{20,21}\) The presence of myeloperoxidase-positive Phi bodies and rods have been reported to be diagnostic of granulocytic leukemia.\(^{10}\)

Electron microscopical evaluation of the tumor material showed the presence of immature granulocytic elements in a loose stroma. The cells contained ellipsoidal granules with axial crystallloid and crystalline rods with crystal periodicity identical with that observed in Auer rods and Phi bodies in granulocytic leukemic cells. This crystalline period identity served to verify the nature of the structures seen by light microscopy with the myeloperoxidase and Sudan black B techniques.

ACKNOWLEDGMENT

We thank Dr. Joseph Moore and Dr. John Laszlo for their contributions in this study. Ann D. Brinkhous, Peggy E. Yates, Wallace W. Ambrose, and Jessie Caulder are thanked for their excellent technical assistance. Linda Brogan is thanked for her typing of the manuscript.

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