Eosinophilic Leukemia
A Morphologic and Histochemical Study

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IN SPITE of the rarity of eosinophilic leukemia, many investigators have reported a number of morphologic alterations of the eosinophils present in the blood and/or bone marrow of patients with eosinophilic leukemia.6,11,13,16,18,19,21,24,26,29,31,34,36,39,40,42,45,47,53 The cytologic alterations have included a sparcity of eosinophilic granulation in the mature eosinophils, asynchronous nuclear-cytoplasmic maturation and vacuolated cytoplasm. In some instances the eosinophils have a tendency to be larger than normal, to have larger, coarser eosinophilic granules and to exhibit hypersegmentation.

In view of the apparent lack of correlated morphologic and histochemical studies of the unusual cells of eosinophilic leukemia, it is the purpose of this report to illustrate and characterize many of the bizarre and unusual morphologic and histochemical features exhibited by the leukemic eosinophils from an individual with terminal acute eosinophilic leukemia.

MATERIALS AND METHODS

Blood and bone marrow specimens were obtained from a 38-year-old man, studied by the Hematology Division of the University Hospital, and diagnosed as acute eosinophilic leukemia. Because of the leukopenic blood picture, the majority of morphologic and histochemical studies were done on bone marrow samples obtained prior to the initiation of chemotherapy. Subsequent confirmatory observations were also carried out during the early phase of the patient's brief clinical course after the initiation of chemotherapy (6-mercaptopurine riboside, prednisone and massive steroids). Normal human blood and bone marrow films served as parallel histochemical controls and for the histochemical comparison of normal and leukemic eosinophils.

The following morphologic and histochemical procedures were performed on the bone marrow samples:

Both unstained vital and supravital (neutral red and Janus green) films were examined by means of brightfield and phase contrast microscopy; Wright's-stained films also were examined. Marrow fragments fixed in buffered 1 per cent osmium tetroxide were embedded in maraglas, sectioned, stained with lead hydroxide and examined with the RCA EMU 3-f electron microscope.

Air-dried films were employed in all histochemical procedures. After appropriate fixation, the following histochemical methods were employed: (a) Fast green at pH 2 following fixation with fresh acetic-ethanol or with formalin vapor for protein;46 (b) fast green at pH 8.1 after acetic-ethanol fixation for basic protein;41 (c) the modified Sakaguchi reaction for arginine after formalin vapor fixation;12 (d) the Biebrich scarlet method for
arginine after 2 hours fixation in 95 per cent ethanol;28 (e) the Morel-Sisley diazotization method for tyrosine after acetate-ethanol fixation;33 (f) the Rosindole method for tryptophane and indole derivatives following formalin vapor fixation;29 (g) the dihydroxy-dinaphthyl-disulphide (DDD) method for protein-bound sulphhydrys following formalin vapor fixation;2 (h) the p-bromaniline method for histamine after 3 days fixation with formalin vapor at 60°C;32 (i) the periodic acid-Schiff reaction after formalin vapor or methanol fixation for glycogen and glycoproteins with salivary amylase digestion employed as the control for the localization of diastase-labile glycogen;5,27 (j) the Gram iodine procedure for preformed glycogen;44 (k) the Quaglino and Hayhoe technic for phosphorylase activity using 30 seconds fixation in 70 per cent acetone with appropriate controls;4 (l) the naphthol AS-MX phosphate-Fast blue RR method with formalin-methanol fixation for alkaline phosphatase activity;3 (m) the naphthol AS-BI phosphate-paraosanilin procedure after formol-calcium fixation for acid phosphatase activity;22 (n) the Wachstein-Meisel technic after formol-calcium fixation for adenosine triphosphatase activity;41 (o) the Wachstein-Meisel method, without prior fixation for glucose-6-phosphatase activity;41 (p) the naphthol AS-D chloroacetate method with formalin-methanol fixation for non-specific esterase activity;37 (q) aminopeptidase activity using 0.5 per cent osmium tetroxide in dimethylformamide as the fixative with leucyl-hetanaphthylamide hydrochloride or the more sensitive L-leucyl-4-methoxy-beta-naphthylamide substrate;38 (r) the succinic, lactic, glucose-6-phosphate and 6-phosphogluconate dehydrogenases, DPNH and TPNH diaphorases methods following fixation with 40 per cent or 95 per cent acetone using nitro BT and appropriate substrate solutions;43 (s) the Graham method after fixation with formalin-ethanol for peroxidase activity.23

OBSERVATIONS

The accompanying photomicrographs illustrate the marked degree of morphologic variation and stages of eosinophilic development of the leukemic cells of the bone marrow. Comparison of the morphologic features of these leukemic eosinophils with eosinophils at various stages of maturation obtained from the blood and bone marrow of normal individuals reveals marked alterations of the leukemic cells. Such changes are most striking in the living cells visualized by phase microscopy (figs. 1–8). Many of the leukemic eosinophils are larger than normal (figs. 1–8) and not infrequently contain very large eosinophilic granules (figs. 2, 4, 5). These large granules may occasionally have an irregular contour as well as varying in size and optical density (figs. 2, 5). Asynchronous nuclear-cytoplasmic maturation is evident throughout the period of differentiation but is most apparent in the large mature multilobed eosinophils (figs. 1, 2). These bizarre cells may possess 3-4 irregular nuclear lobes and contain relatively few eosinophilic granules in their abundant cytoplasm. The granules present in these cells tend to localize around the nucleus while the remainder of the hyaloplasm is homogeneous and essentially devoid of organelles. Occasionally ill-defined vacuole-like zones are evident between the granules of some of the developing leukemic eosinophils.

Eosinophilic granules arise in the region of the cytocentrum in the young eosinophilic promyelocytes and myelocytes with variation in size and density of their granules being clearly evident (figs. 1, 3, 4, 6–8). The eosinophilic granules appear to enlarge in size and increase in optical density during their elaboration and maturation (figs. 4–7). Spherical mitochondria are
Figs. 1–4.—See legend, facing page.
numerous in the early eosinophilic myelocytes (figs. 1, 3, 4, 6, 7) but gradually
decrease in number during maturation. The nuclei of the developing eosino-
phils have thin nuclear membranes, delicate chromatin patterns and possess
one or two prominent nucleoli. The more mature eosinophils exhibit ameboid
activity and a scattering of mitotic figures are evident in the eosinophilic
myelocytes. Charcot-Leyden crystals as well as free eosinophilic granules
are conspicuous features in the vital films.

Although less obvious, morphologic alterations are evident in the neutro-
phil lineage. These changes include a shift to the left with the majority of
neutrophilic promyelocytes and myeloblasts (figs. 2, 3, 7), like the leukemic
easinoophils (figs. 1–8), possess more delicate chromatin patterns and occasion-
ally more numerous nucleoli than comparable normal cells.

Electron microscopy did reveal several interesting features in the leukemic
easinoophils including the presence of prominent fibrillar formations in the
cytoplasm of several of the leukemic eosinophils (figs. 11, 12) and extensive
accumulations of cytoplasmic glycogen (figs. 11, 12). The internal crystalline
structure of the eosinophil granules is evident (figs. 9, 12) and smooth
endoplasmic reticulum appears relatively abundant in the leukemic cells
(figs. 9, 10, 12).

Histochemically, the most striking alterations evident in the leukemic
easinoophils include (1) the tremendous cytoplasmic accumulations of glyco-
gen (figs. 17, 18), and (2) the presence of elevated phosphorylase activity in
many of the glycogen-laden cells (figs. 19, 20). The greatest glycogen deposi-

Unstained living cells from the bone marrow of acute eosinophilic leukemia.

Fig. 1.—A young eosinophilic myelocyte showing the eosinophilic granules clus-
tered near the cytocentrum and a more peripheral localization of spherical mito-
chondria. The large cell illustrates the multilobed granule-deficient eosinophil.
This cell exhibits ameboid activity. The eosinophilic granules localize around the
lobed nucleus while the remainder of the cytoplasm is essentially devoid of
organelles.

Fig. 2.—Two large multilobed granule-deficient eosinophils. A portion of an
easinoophil containing large irregular dense granules (left) and a myeloblast (right)
exhibiting numerous mitochondria; a vesicular nucleus with several nucleoli also is
illustrated.

Fig. 3.—The asynchronous development of the nucleus and cytoplasm is evident
in the eosinophilic myelocyte. The nucleus shows signs of lobulation but still has
the immature nuclear characteristics and nucleoli. A myeloblast (upper left) and a
young neutrophilic myelocyte (lower left) exhibit several morphologic variations
as compared with normal myeloblasts and young neutrophilic myelocytes; e.g.
slightly larger in size, more vesicular nucleus and more prominent nucleoli.

Fig. 4.—An eosinophilic promyelocyte with newly formed eosinophilic granules
clustered about the cytocentrum. These granules show some variation in size and
optical density. Granule variation is evident also in the eosinophil in the lower
portion of the field. Asynchronous nuclear-cytoplasmic maturation is suggested in
both of these leukemic eosinophils.
Figs. 5–8.—See legend, facing page.
tion occurs in the multilobed granule-deficient eosinophils (fig. 18). These cells yield both intense Gram iodine and PAS-positive (diastase-labile) reactions; phosphorylase activity also is greatest in these bizarre cells (fig. 19). The extent of glycogen deposition in the leukemic eosinophils is quite variable; some eosinophils contain little or no demonstrable glycogen (fig. 17). In those cells containing high concentrations of glycogen, the eosinophilic granules are essentially PAS-negative although their is a tendency for the glycogen to adhere to or coat these granules. In contrast with most normal eosinophils, many of the leukemic eosinophils contain eosinophilic granules which are faintly to moderately PAS-positive (saliva-resistant) (figs. 17, 18). Phosphorylase activity necessary in glycogen synthesis is demonstrable in many of the leukemic cells. The degree of cellular phosphorylase activity is variable but appears to be confined to the intergranular cytoplasm (fig. 19, 20). There is a tendency for the more mature eosinophils and eosinophilic myelocytes to exhibit greater phosphorylase activity than the more immature forms. Myeloblasts and promyelocytes exhibit weak phosphorylase reactions localizing diffusely in their cytoplasm (fig. 19).

In general, the histochemical reactivity of the leukemic eosinophils for various proteins and amino acids is similar to that observed in normal mature and developing eosinophils. The eosinophilic granules stain strongly for proteins, arginine, tryptophane and protein-bound sulphydryls and yield a moderate reaction for tryosine with the technic employed. The variation observed intracellularly and between cells in the histochemical reactivity of the eosinophilic granules is well illustrated by the Fast green reaction for proteins and the DDD reaction for sulfhydryls (figs. 15, 16). The acidophilic quality of the eosinophilic granules is evident in both supravital and Wright's-stained preparations (figs. 13, 14). Eosinophilic myelocytes with basophilic staining granules are evident in some of the leukemic eosinophils examined in Romanowsky stained films. Similar mixed granule eosinophilic myelocytes also may be seen in normal bone marrow preparations. Such basophilic staining gran-
Figs. 9–12.—See legend, facing page.
ules possibly represent immature or developing eosinophilic granules. Cytoplasmic basophilia decreases during the maturation of the leukemic eosinophils with only traces of basophilia evident in the mature eosinophils. In the glycogen-laden cells, the presence of cytoplasmic basophilia is confined to those regions of the cytoplasm free of glycogen. In the multilobed granule-deficient eosinophils, the granule-free cytoplasm is completely unstained (fig. 13) except for a faint basophilic tinge along the plasma membrane. The eosinophilic granules of the mature eosinophils and eosinophilic myelocytes exhibit a strong reaction for sulfhydryls whereas the hyaloplasm is only faintly stained. The hyaloplasm of the myeloblasts and eosinophilic promyelocytes also exhibits a light diffuse reaction for protein-bound sulfhydryls. In addition to the more intensely colored eosinophilic granules in the eosinophilic promyelocytes, a few smaller and less reactive granules may be distinguished in the cytoplasm. Histamine could not be demonstrated in any of the leukemic eosinophils with the technic employed.

The leukemic eosinophils do not exhibit alkaline phosphatase, glucose-6-phosphatase nor chloroacetate esterase activities with the procedures employed. A moderate reaction for acid phosphatase activity is evident in most of the eosinophilic granules; in some instances acid phosphatase activity appears to be localized about the periphery of certain of these granules. Acid phosphatase activity appears somewhat more intense in the multilobed granule-deficient eosinophils than in the other leukemic eosinophils with acid phosphatase activity localizing in the eosinophilic granules and the hyaloplasm surrounding these granules (fig. 21). Adenosine triphosphatase activity may be localized within the eosinophilic granules of the leukemic cells (fig. 22) but appears to be less intense than that observed in normal eosinophils. Aminopeptidase activity appears slightly greater in leukemic than in normal eosinophilic leukemias.

Bone marrow from acute eosinophilic leukemia fixed in 1 per cent buffered osmium tetroxide, embedded in maraglas, with sections stained with lead hydroxide and examined with the RCA EMU 3-f electron microscope.

Fig. 9.—A leukemic eosinophil or eosinophilic myelocyte showing a number of eosinophilic granules (e) which exhibit some evidence of the crystal-like internal structure. A number of profiles of smooth endoplasmic reticulum and a portion of the nucleus (n) are also evident. X13,500.

Fig. 10.—This cell probably represents a myeloblast or very early eosinophilic promyelocyte. Although there is no evidence of distinct eosinophilic granules, several small granule-like structures (arrow) are visibly associated with the Golgi complex. Mitochondria (m) are numerous as well as profiles of both smooth and rough endoplasmic reticulum. X13,000.

Fig. 11.—A portion of a leukemic eosinophil or eosinophilic myelocyte shows a sparcity of eosinophilic granules (e), an abundance of granular aggregates of glycogen (g), a tangential section of the nucleus (n), and the presence of an extremely prominent fibrillar formation (f). X13,000.

Fig. 12.—Another leukemic eosinophil or eosinophilic myelocyte with eosinophilic granules (e), small clusters of glycogen (g), smooth-surfaced vesicles (v) and tangential and cross-sections through fibrillar formations (f) X24,000.
Figs. 13–16.—See legend, facing page.
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 eosinophils and is confined to the more central regions of the non-granular cytoplasm. The eosinophilic granules in all stages of eosinophil maturation yield strong peroxidase reactions.

Eosinophilic granules fail to react following the histochemical procedures for the various oxidative enzymes studied. The oxidative enzymes, except for succinic dehydrogenase, do not appear to have a particulate localization but produce a homogenous coloration of the hyaloplasm (fig. 24). Lactic and glucose-6-phosphate dehydrogenase, and DPNH diaphorase (fig. 24) activities are greater in the leukemic eosinophils than 6-phosphogluconate dehydrogenase and TPNH diaphorase activities. Succinic dehydrogenase activity is comparatively weak in the leukemic eosinophils and probably resides in the mitochondria distributed randomly in the cytoplasm (fig. 23). Although difficult to evaluate, lactic, glucose-6-phosphate dehydrogenase and DPNH diaphorase activities may be slightly greater in many of the leukemic eosinophils than in normal eosinophils at various stages of maturation.

DISCUSSION

By the use of many histochemical procedures correlated with phase and electron microscopy, it has proved possible to characterize in detail some of the morphologic and chemical alterations evident in the leukemic cells in acute eosinophilic leukemia. Essentially, all of the morphologic alterations observed by other investigators concerning the leukemic cells of eosinophilic leukemia have been detected and further elaborated in this report. The only previously reported results of histochemical reactions of leukemic eosinophils show that the granules in blood and bone marrow films are peroxidase positive and periodic acid-Schiff positive in tissue sections. Histochemical reports concerning the eosinophils from non-leukemic patients with eosinophilia appear to be lacking in the literature.

The marked morphologic and histochemical alterations in the leukemic
Figs. 17–20.—See legend, facing page.
cells of eosinophilic leukemia suggest an impairment of cellular maturation, impairment and/or aberration in granule formation, as well as an alteration and/or impairment of normal carbohydrate metabolism. Morphologically, the leukemic eosinophils exhibit an increase in cell size, asynchronous nuclear-cytoplasmic development with incomplete premature nuclear lobulation evident in the young eosinophilic myelocytes and hypersegmentation of the nucleus in some of the mature eosinophils. Eosinophilic granules vary in size, number, contour, and optical density. Submicroscopically, prominent fibrillar formations are evident in some of the eosinophils further suggesting the leukemic nature of these cell forms. Although poorly developed fibrillar structures have been occasionally seen in normal developing neutrophilic myelocytes, extensive development of such fibrillar formations has been observed only in leukemic cells. The exact nature of the fibrillar formation is unknown but may be related to the endoplasmic reticulum and/or possibly the Golgi membranes.

The marked deposition of glycogen in the leukemic eosinophils is quite remarkable, and in our experience represents the largest cellular accumulation of glycogen observed in the cells of the blood or bone marrow in any hematologic condition thus far studied. It would be expected from the descriptions and illustrations of other investigators, when compared with our findings, that the multilobed eosinophils which they reported as granule-deficient with abundant clear hyaloplasma also would yield strong reactions for glycogen. A tendency for an inverse relationship between the extent of cytoplasmic glycogen deposition and the relative number of granules in the more mature leukemic eosinophils was observed. Phosphorylase activity necessary in the active synthesis of glycogen is markedly elevated in the leukemic cells. The distribution and degree of phosphorylase activity closely parallels the distribution and the relative amount of glycogen present in the leukemic cells. Since glucose-6-phosphatase activity could not be detected in these abnormal cells, it seems likely that the glycogen is being stored rather than being re-utilized in other pathways of


Figs. 17, 18.—The marked variation and tremendous accumulation of intracellular glycogen (black) are evident in the numerous leukemic eosinophils shown at various stages of maturation. The non-glycogen PAS-positive (saliva-resistant) reactivity of some of the eosinophilic granules may be seen in figure 17 (upper left) and to a lesser extent in figure 18 (upper left). The cytoplasm of two multilobed granule-deficient eosinophils (figure 18, top and right center) are almost filled with dense amorphous masses of glycogen. Periodic acid-Schiff reaction following fixation with formalin vapor.

Figs. 19, 20.—Variation and cytoplasmic localization of phosphorylase activity in the leukemic eosinophils. Eosinophilic granules are non-reactive. A multilobed granule-deficient eosinophil showing intense phosphorylase activity is illustrated in figure 19 (upper right). The scanty diffuse reactivity of a myeloblast is shown also in figure 19 (top center). Quaglino and Hayhoe method for phosphorylase activity.
Figs. 21–24.—See legend, facing page.
carbohydrate metabolism. The presence of phosphorylase, slightly elevated oxidase enzyme activities, absence of distinct cellular degenerative changes and amoeboid activity of the more mature eosinophils suggest that the leukemic cells are metabolically active. Such evidence suggests that glycogen formation results from faulty carbohydrate metabolism rather than reflecting cellular degeneration.

Other comparatively minor alterations in the histochemical composition of the leukemic eosinophils and eosinophilic granules have been mentioned in this report. Most of these differences between the leukemic and normal eosinophils represent only slight variations in the relative intensity of histochemical reactivity of the eosinophilic granules. Not infrequently such variations in reactivity may be correlated with the variation in the size and optical density of these granules.

In our experience, neither extensive glycogen accumulation nor such marked morphologic alterations as observed in the leukemic eosinophils reported in this study has been observed in the eosinophils of normal individuals or individuals with various hematologic diseases including myelocytic and basophilic leukemias. Because of the variety of etiologies of persistant or transient eosinophilia, detailed morphologic-histochemical analyses of the eosinophils in these conditions seem warranted. From our limited experience and studies, the eosinophils observed in such conditions have shown little alteration from the normal pattern either morphologically or histochemically following use of limited histochemical technics including the periodic acid-Schiff reaction.

It seems obvious that further experimental histochemistry of eosinophilic leukemia, myelocytic leukemia and eosinophilias are necessary in order to determine whether the histochemical alterations reported herein represent significant alterations in the metabolism of the leukemic eosinophils in eosino-


Fig. 21.—Acid phosphatase activity is demonstrable in most of the eosinophilic granules illustrated. Some non-granular acid phosphatase activity may be localized also in the region of the cluster granules of the multilobed granule-deficient eosinophil (upper left). An eosinophilic promyelocyte containing several reactive eosinophilic granules also is shown (lower right). Naphthol AS-BI phosphate-pararosaniline method for acid phosphatase activity.

Fig. 22.—Adenosine triphosphatase activity localized in the eosinophilic granules. Wachstein-Meisel method following formol-calcium fixation.

Fig. 23.—Several eosinophilic myelocytes showing the random finely granular distribution of succinic dehydrogenase activity in the cytoplasm of these cells. Several of the eosinophilic granules in the eosinophilic myelocyte (top) appear to exhibit some reactivity around their periphery. Nitro BT method for succinic dehydrogenase activity following 40 per cent acetone fixation.

Fig. 24.—The intergranular cytoplasmic localization of DPNH diaphorase activity is shown in the eosinophilic myelocytes illustrated. Nitro BT method for DPNH diaphorase activity following 95 per cent acetone fixation.
philic leukemia or reflect only variations resulting from rapid growth and
development.

**Summary**

Correlated histochemical, phase and electron microscopic studies were em-
ployed in examining the eosinophils from a patient with acute eosinophilic
leukemia. Numerous morphologic alterations were observed in the leukemic
eosinophils and eosinophilic myelocytes. These alterations included asynchro-
nous nuclear-cytoplasmic maturation; an increase in cell size; the formation
of eosinophilic granules which vary markedly in number, size, contour, and
density; and the presence of fibrillar formations in some of the leukemic cells.
Histochemically, the major alterations observed in the leukemic cells were
the extensive deposition of glycogen in the cytoplasm and the demonstra-
tion of increased phosphorylase activity in these cells. Other minor variations
in the histochemical reactivity of the leukemic eosinophils also have been
described. Histochemical procedures included techniques for proteins, amino
acids, carbohydrates, hydrolytic and oxidative enzyme activities.

**Summario in Interlingua**

Correlationate studios histochimic e microscopic a contrasto de phase e
electronic esseva empleate in examinar le eosinophilos ab un patiente con
acute leucemia eosinophilic. Numerose alterationes morphologic esseva observ-
vate in le eosinophilos leucemic e le myelocytos eosinophilic. Iste alterationes
includeva asynchronia del maturation nuclear e cytoplasmic; un augmento
del magnitude cellular; le formation de granulos eosinophilic que variava
marcatemente in numero, magnitude, contorno, e densitate; e le presentia de
formationes fibrillar in certes del cellulas leucemic. Histochimicamente, le
major alterationes observate in le cellulas leucemic esseva le extense deposi-
tion de glycogeno in le cytoplasma e le demonstration de un augmento del
activitate de phosphorylase in iste cellulas. Altere, minus importante variatio-
tes in le reactivitate histochimic del eosinophilos leucemic es etiam
describite. Le methodos histochimic empleate includeva methodos pro le
determination de proteinas, de amino-acidos, de carbohydrates, e del activitate
de enzymas hydrolytic e oxydative.

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