An Electron Microscopic Study of the Bone Marrow of the Rat in an Experimental Myelogenous Leukemia

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Alterations in the vascular sinus and hematopoietic compartment of rat bone marrow were observed with electron microscopy during the pathogenesis of an acute myelogenous leukemia. As the disease progresses, the sinus wall becomes damaged and disintegrates; normal hemic elements disappear, and the marrow compartment becomes packed with leukemic myeloblasts. Virus-like particles are present in intercellular spaces and appear to bud from leukemic cells.

THE SHAY CHLOROLEUKEMIA, an acute myelogenous leukemia in Wistar rats, first induced by gastric instillation of methylcholanthrene, can be transferred to rats by the passage of viable cells but not of ultrafiltrates. In this disease, splenic and hepatic enlargement may occur but changes in the bone marrow resulting in complete destruction of normal vascular and hematopoietic relationships are predictable and consistent. The present study demonstrates, by means of electron microscopy, progressive structural changes in vascular sinuses and hematopoietic compartments of the bone marrow and extends findings that suggest a viral basis for the leukemia. We describe a pattern of progressive reduction in numbers of the cellular layers in the walls of sinuses and the total obliteration of sinuses in this distinctive pathological process.

MATERIALS AND METHODS

Thirty male Long-Evans rats weighing 180–200 g were injected intravenously with $10 \times 10^6$ chloroleukemic cells. Cell suspensions were prepared as previously described. Twenty animals survived and are the basis of our study. Three to four animals were sacrificed daily 7–12 days following the inoculation of chloroleukemic cells. Animals were anesthetized with ether and both femora removed. Previous studies have shown no significant differences in marrow cellularity between right and left femora. Bone marrow was removed for electron microscopy from the right femur as described below. The left femur was split longitudinally; using a small paint brush dipped in serum, duplicate marrow smears were made on pre-cleaned slides. One slide was treated with May-Grünwald stain and the other with benzidine and counterstained with hematoxylin. Marrow myelograms based on 1000 nucleated cells...
were performed for each animal, and the percentage of leukemic myeloblasts in each marrow smear assessed. The percentage of nucleated red blood cells in 1000 nucleated marrow cells was also determined for each animal from the benzidine-treated slide. Earlier studies have shown that the one parameter consistent and predictable during pathogenesis is the alteration in bone marrow myeloblast content with percentages increasing from near zero to over 90%. The 20 surviving animals in this study were grouped as follows: early stage (less than 10% leukemic myeloblasts), intermediate stage (10–50%); advanced stage (50–70%); and late stage (over 70%). In this study, only one animal was found to be in the intermediate stage of the disease. It is of interest to note that most often leukemic rats are found to be in either early or late stages. The intermediate phase is apparently a short-lived interval not readily captured at the time of autopsy.

Under ether anesthesia the right femur was removed, held vertically and split longitudinally by a sharp blow applied to a single edge razor. This procedure took little more than 1 min. The split bone with marrow exposed was placed in Karnovsky’s formaldehyde-glutaraldehyde mixture for approximately 5 min, during which the surface of the marrow was hardened. The marrow was then gently eased out of the bone and floated into a fixative, using sharpened wooden sticks or dental picks. After approximately 5 min, the marrow was cut transversally into disks about 1–2 mm thick. The tissue was then dehydrated and embedded in Araldite. Liver, spleen, thymus, and lymph nodes were removed from many animals and processed for electron microscopy.

Thick sections were cut on a Porter Blum Ultramicrotome II and stained with toluidine blue. Thin sections were then cut from appropriate blocks by this microtome and studied in a Siemens Elmiskop I.

**Observations**

**Leukemic Myeloblasts**

Leukemic myeloblasts had a large nucleus, well developed nucleolus, numerous ribosomes, Golgi apparatus, small granules, moderate number of mitochondria and endoplasmic reticulum (Figs. 8 and 9A). The nuclear membrane often projected into the cytoplasm. This projection contained a small amount of nucleoplasm and enclosed a part of the cytoplasm (Figs. 8 and 9A).

**Early Stage**

Femoral bone marrows from animals in the early stages of the disease (less than 10% myeloblasts) were virtually normal in appearance. Numerous well developed vascular sinuses were present (Fig. 1A and 2); the trilaminar wall of the sinus was usually visible (Fig. 2). Erythroid and myeloid elements in all stages of maturation occupied the hematopoietic space. Small clusters

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**Fig. 1.**—(A) Early stage of leukemic bone marrow (near normal). Vascular sinuses (S) are many and well developed. They form a communicating system of vessels. The hematopoietic spaces contain various cell types. A few fat cells are present. $\times 200$. (B) Late stage of leukemic bone marrow. Very few vascular sinuses remain and virtually the entire hematopoietic space is occupied by leukemic cells. Very few granulocytes are present. $\times 200$. (C) Advanced stage of leukemic bone marrow. The number of vascular sinuses is greatly reduced, and leukemic cells lie in their lumens (arrows). The hematopoietic spaces are largely filled with leukemic cells. A few granulocytes are also recognizable. A branching arteriole is present in the upper part of the field. $\times 200$. (D) Advanced stage of leukemic bone marrow. In this higher power, leukemic cells (short arrows) appear in passage across a sinus wall. Note the cell in mitosis (long arrow) lying against the adventitial surface of a sinus. A few granulocytes may be seen in the hematopoietic space in the upper left part of the field and in the sinus. $\times 525$. 

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of leukemic myeloblasts were seen sparsely distributed throughout the compartment; they were not observed in the lumen of the sinuses.

Advanced Stage

A marked reduction in the number of both normal hematopoietic cells and vascular sinuses occurred. Leukemic myeloblasts occupied most of the hematopoietic space (Figs. 1C and 4). Some normal myeloid elements in varying stages of development (Figs. 1C, 1D, and 3) were present. The walls of sinuses were disrupted and leukemic myeloblasts were often present in the lumen. The sinus wall was frequently present as a single attenuated layer and leukemic myeloblasts appeared to be in passage across the wall (Figs. 1D and 6). In certain instances the walls were swollen and disintegrated (Fig. 6). Cells in mitosis and degenerating cells in the hematopoietic compartment were not uncommon (Figs. 1D and 4). Occasionally, leukemic cells lay inside megakaryocytes (Fig. 5).

Late Stage

With the exception of a very few normal polymorphonuclear leukocytes, the hematopoietic spaces were filled with leukemic myeloblasts. Myelograms of the bone marrow from the contralateral femurs corroborated these findings. During this stage few vascular sinuses were present. The sinus walls were swollen and disintegrated (Figs. 1B, 7, and 8). These degenerative changes were much more marked than in earlier stages.

Virus-Like Particles

Virus-like particles (50–80 m\(\mu\)) lay in between leukemic cells in advanced and late stages (Fig. 9B). In addition, in two instances, virus-like particles appeared to bud from leukemic myeloblasts (Fig. 9C).

Discussion

Myelogenous leukemia is an infrequent occurrence in the rat. In 1951 Shay et al. succeeded in inducing a myelogenous leukemia in Wistar rats following long term gastric instillation of 20-methylcholanthrene. The disease has been transferred to Wistar rats and other rat strains via inoculation of leukemic cells. The sequence of pathologic change has been described in sucklings and young adults, and the hematological manifestations of the disease reviewed. A lethal myeloblastic leukemia, with a 2–3-wk time course, develops following

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**Fig. 2.**—Early stage of leukemic bone marrow. In this near normal marrow a vascular sinus (S) is shown in the upper left of the field. Note that the wall of the sinus consists of 2–3 layers in places. Elsewhere (arrows) it is but a single layer. Two neutrophils (N) appear moving into the sinus lumen through a gap. Erythroblasts (E), reticulocytes (R), basophils (B) and megakaryocytes (MEG) occur in the perivascular hematopoietic space. One of the erythroblasts (E) appears to be undergoing nuclear extrusion. The extracellular spaces between hematopoietic cells are somewhat broader than in normal marrow. They are likely filled with edema and cellular debris. × 5000.
Fig. 3.—Advanced stage of leukemic bone marrow. A vascular sinus (S) containing erythrocytes is in the center of the field. Its wall, particularly on the left, is a bicellular layer. On the right the wall is reduced to one layer which is actually perforate. Three granulocytes with a moderate number of cytoplasmic granules are to the right. No erythroblasts are present, but erythrocytes occur in the perivascular space. Leukemic cells, mostly myeloblasts, surround the sinus. The nuclear envelope of many myeloblasts projects loops into the cytoplasm exemplified by the cell in the left lower corner (white arrow). On close inspection, it can be seen that this cell lies within the rarefied cytoplasm of another cell, likely a macrophage. See Figs. 8 and 9A. × 5000.
Fig. 4.—This field represents an hematopoietic space contiguous to a sinus. A macrophage (its nucleus labeled M) is surrounded by leukemic cells and extends cytoplasmic projections between them. The macrophage contains two dead cells (D). It also contains a granulocyte (G) which may be phagocytized or emperipoietic (see Fig. 5). A cell in mitosis lies against the sinus. × 5000.
Fig. 5.—Advanced stage of leukemic bone marrow. A megakaryocyte (N1) contains two leukemic cells (N2 and N3). The leukemic cells are vital in appearance (as is the granulocyte in Fig. 4). These cells may move in and out of the cytoplasm of the larger cell, the phenomenon of emperilopoiesis. Endocytosis of this type is common in this leukemic process. × 5000.
Fig. 6.—Advanced stage of leukemic bone marrow. A leukemic cell (X) appears moving across the sinus wall (arrows). In the lumen of the sinus (S) there are leukemic cells, erythrocytes, and a macrophage. Note that the wall is, for the most part, reduced to a single cell in thickness, and is defective in several places. The adventitial layer is not evident, but the granular perisinus extracellular material may be the residue of degenerated adventitial cells. Indeed, the persistent endothelium appears somewhat damaged, and may be in the process of obliteration. × 5000.
Fig. 7 (facing page)—(A) Late stage of leukemic bone marrow. Badly damaged sinus (S): upper part contains luminal leukemic cell (X). Upper portion (above “S”) has some semblance to normal structure, but that in the lower portion is swollen, granular and disintegrated. A gap in the sinus wall is indicated by arrows. × 5000. (B) Late stage of leukemic bone marrow. The wall of a sinus is indicated by arrows. It has been reduced, for the most part, to a single layer which has suffered marked deterioration. Myeloblasts and some erythrocytes are present within and without the vessel. × 5000.

Fig. 8.—Myeloblasts in the late stage of leukemic bone marrow. The myeloblasts are so tightly packed and preponderant in the cell type as to constitute a tissue. The projection of nuclear membranes into the cytoplasm is frequently present in the myeloblasts (arrows). A deteriorating reticular cell (R) is in the upper right of the field. × 5000.
intravenous administration of leukemic cells. Leukemic cells become predominant in hematopoietic sites and circulating fluids. Marked infiltration into other body tissues occur in late stages. Influx of leukemic cells into the spinal cord leads to paralysis and death. Rosin and Zajicek³ correlate the appearance of leukemic cells in the peripheral blood with paralysis of the hind legs. At the same time the bone marrow in the vertebra and in the ribs is mostly replaced by leukemic cells. The present observations indicating passage of leukemic cells into the vascular sinuses of marrow, suggest that leukemic cells in the peripheral circulation may come from the bone marrow.

Vascular sinuses, in their fullest development, display a trilaminar wall, namely endothelium, basement membrane, and adventitial cell.⁵ As the leukemia progressed, in the present study, the walls became attenuated, were disrupted, and the sinuses destroyed. Leukemic cells proliferated first in hematopoietic compartments. They then encroached upon the sinuses. The first elements in the sinus wall eliminated were the adventitital layer and the ground substance in the basement membrane. Then large gaps or damaged zones appeared in the endothelium. Finally, the sinuses vanished and the marrow was seen as a sheet of leukemic cells. Whether the swelling and the disintegration of the sinuses were due to factors released by leukemic cells, massive pressure, or other causes is not known. The destruction of sinuses may well interfere with the release of leukemic cells to the circulation and favor the exaggeration of the already massive marrow infiltration.

Many erythroblasts, reticulocytes, and leukocytes were present in the early stages of the disease. However, as the disease progressed, immature erythroid elements disappeared, leaving a few erythrocytes and mature polymorphonuclear leukocytes. Differential counts of marrows reflect the altered proportions of cell types.

Light microscopy reveals few, if any, granules in the leukemic cells, whether derived from the bone marrow or transplantable tumor. The cells conform to the structural criteria established for myeloblasts. Electron microscopy, on the other hand, revealed the presence of small cytoplasmic granules in most leukemic cells, indicating a promyelocytic cytoplasmic stage. Leukemic myeloid cells did show asynchronous differentiation however, the nucleus less mature than the cytoplasm. It is of interest to note that, over the course of many transplant generations, the number of granules in this leukemic cell type observed under light microscopy have decreased, and the cell has assumed a more myeloblastic configuration.

The projection of nuclear loops into cytoplasmic areas are frequently seen in Shay chloroleukemic myeloblasts. Similar structures have been reported in normal granulocytes,¹⁶ in human leukemic myeloblasts⁸ and cultured Burkitt

**Fig. 9.—** (A) A promyelocyte in the late stage of leukemic bone marrow. Stacks of Golgi membrane (G) and primary granules (g) are present. A nuclear projection, containing a small amount of nucleoplasm (between arrows), encloses a part of the cytoplasm. Mitochondria and ribosomes are also present. × 37,500. (B) Late stage of leukemic bone marrow. Viruslike particles lie in the extracellular space between leukemic cells. × 37,500. (C) Late stage of leukemic bone marrow. A viruslike particle appears to bud from a leukemic cell (arrow). × 37,500.
tumor lymphoblasts.\textsuperscript{10-12} We have, on occasion, observed such projections in myelocytes of normal rat marrow. While their significance is not known, we believe that they may represent formative movements preliminary to nuclear polymorphism.

The transfer of this myelogenous leukemia requires the passage of intact leukemic cells. Cell-free extracts do not induce the leukemia, and vertical transfer to the fetus is prevented by a placental barrier.\textsuperscript{13} Leukemogenic viruses have, as yet, not been isolated from the Shay chloroleukemia. Intracellular viruslike particles associated with the chloroleukemic cell have been reported;\textsuperscript{14} intracellular units of the “C” type have been suggested to be present in intraperitoneal chloroleukemic elements.\textsuperscript{15} In the present study, we have observed similar extracellular viruslike particles. We have in addition observed the apparent budding of such particles from leukemic cells. These findings clearly suggest a viral basis for this leukemia. Perhaps the titer of free virus is not sufficiently high to permit passage by ultrafiltrate rather than whole leukemic cells.

REFERENCES

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