Alterations in Morphology and Growth Pattern in a Transplantable Leukemia of Rats

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SUMMARY

A transplantable Wistar rat leukemia of a mononuclear type is described. Its growth patterns and morphological characteristics during 17 serial passages are considered. The leukemic cell was characterized by lymphoid or monocytoid morphologically recognizable features, phagocytic properties, nuclear karyorrhexis, and an abortive attempt towards myeloid transformation. Severe anemia with bone marrow erythroid hypoplasia was often associated with the disease. In several instances during serial passage, the neoplasm assumed a solid sarcomatous pattern, with or without accompanying blood changes. The morphological peculiarities of this leukemia are compared with those of other mononuclear leukemias.

INTRODUCTION

Spontaneous leukemias rarely occur in the laboratory rat (2—4, 9, 11). In recent years, however, considerable attention has been paid to both induced and spontaneous leukemias in this species. Acute, subacute, and chronic forms of myelogenous leukemia, with or without chloroma (6, 8, 13), as well as other less common types of leukemia, have been described at some length (1, 3, 15). In addition, an unusual leukemic condition in Wistar-Furth rats was seen by Moloney et al. (8) in connection with their investigation of a leukemic condition resembling but not identical with the rat leukemia resembling but not identical with the Wistar strain, from the Food and Drug colony. The original donor rat was a 2-year-old Food and Drug Wistar male that had received a 125-µg dose of DMN¹ at 1 year of age. One hundred thirty males and 23 females less than 70 days of age (young) and 17 males and 10 females over 70 days and up to 1 year old were used. The age distribution is given in Table 1.

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All rats received i.p. inoculations of leukemic material and were anesthetized with ether and killed by exsanguination when signs indicative of the terminal stages of the disease became evident.

Collection of Specimens. Blood samples for hematological studies performed during the induction period were taken directly from tail tips of anesthetized rats. For final determinations, as well as for inoculation purposes, blood was collected from the opened abdominal cavity at the iliac bifurcation of ether-anesthetized rats with the use of 10% EDTA, disodium salt. Peritoneal fluid was obtained with a separate syringe. Blood from a single donor or from samples pooled from several rats of the same generation and peritoneal fluid from a single donor were used. The dose was 0.2 ml. Small pieces of tumor or spleen were either fresh or quick-frozen, thawed, and macerated in a few drops of Ringer's solution; 0.2 to 0.5 ml of tumor cells or 1.0 ml of spleen cells suspensions were injected.

Hematological and Histological Preparations. Leukocyte (WBC) counts were made on a Coulter electronic counter. HGB was determined by the cyanmethemoglobin method according to standard procedures (16). Blood, bone marrow, and peritoneal fluid coverslip smears, as well as imprints of hematopoietic organs, liver, and tumor, were stained with Leishman stain, modified for staining highly cellular specimens in this laboratory. For each blood and bone marrow smear, 200 and 500 cells, respectively, were counted under an oil immersion lens. Quantitative and morphological characteristics of platelets were estimated from coverslip smears.

All rats were necropsied, except for a few cases in which marked autolysis precluded histological assessment. Tissues were fixed in formalin-0.9% NaCl solution, and sections were stained with hematoxylin-phloxine-saffron.

RESULTS

The original donor rat had normal HGB (15 g/100 ml) and packed cell volume (45%), with a few nucleated erythrocytes.

¹The abbreviations used are: DMN, dimethylnitrosamine; HGB, hemoglobin; AL, acute leukemia; AL-T, acute leukemia and tumor; L-T, preleukemic cells and tumor present; L, preleukemic cells present; T, tumor present, without leukemic manifestations; AMN, abnormal mononuclear cell.

MATERIALS AND METHODS

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reduced platelets, and a WBC count of 166,000, with 88% leukemic cells.

A bone marrow assay disclosed the following distribution of cells: leukemic cells, including frank blasts, 43.2%; granulocytic series, 26.8%; lymphocytes, 7.0%; others, 0.4%; erythroid series, 22.6%. The leukemic cells varied in size, nuclear shape, and amount of fairly deeply stained and granular cytoplasm. In many cells, the nuclear structure resembled the myeloid series. Nucleoli ranged from prominent to discrete. No cytoplasmic granules were observed.

In the lymph node, large numbers of leukemic cells were present, but the lymphocyte, in all phases of maturation, appeared to predominate.

Cellular distribution in the spleen resembled that in the bone marrow. The leukemic cells, however, appeared to have more prominent nucleoli.

**Clinical and Hematological Characteristics of the Transplanted Leukemia**

Animals inoculated with blood during the 1st 10 generations developed signs of an acute leukemic condition that, with few exceptions, lasted less than 2 weeks. Splenic injections produced a longer survival time (29 to 40 days). The 1st diversification became manifest in the 11th passage and presented a very different picture from the preceding transfers (Table 2). The following types were observed: AL; AL-T; tumor accompanied by a slight involvement of blood and bone marrow and, in various degrees, of other hematopoietic organs (L-T); as above, but no tumor grossly evident (L); and tumor without morphologically detectable leukemia in the peripheral blood (T). The changes also included a much longer survival period in most animals of the L-T combination. The blood transfers that followed the 11th generation had a similar combination in leukemia-tumor pattern, whereas L-T group blood transfers yielded decidedly L-T rather than AL-T variety of the disease. Peritoneal fluid, on the other hand, produced AL-T in all cases, with reduced survival time (13 to 16 days).

**Peripheral Blood.** The hematological picture of animals involved in the 1st 10 passages of blood was consistent. The WBC fluctuated between 160,000 and 657,000/cu mm; leukemic cells fluctuated between 80 and 97%; and HGB fluctuated between 5.1 and 9.5 g/100 ml. Nucleated red cells, Howell-Jolly bodies, and increased polychromasia were present. Platelets ranged from normal in number to markedly reduced. The 11th through 17th generations have been studied in greater detail. The following 4 broad types of hematological response were observed in the entire study: (a) A very sudden onset of leukemia, accompanied by drastic and rapid changes in WBC, number of blasts, and HGB values. Sarcomatous masses were seen in about two-thirds of the animals in this group. (b) A more gradual course of the disease was characterized by ascending WBC values. Preleukemic cells appeared first, followed by a few leukemic cells. The condition terminated in fulminating acute leukemia. The HGB values remained normal or only slightly reduced. The animals in this group invariably had marked sarcomatous growth. (c) The characteristics of this group included the development of sarcomatous tumors with a slightly elevated WBC count and the presence of few to numerous preleukemic cells. HGB usually remained unchanged. No alterations in this picture were observed in the terminal stages. (d) This group had sarcomatous tumor masses with no detectable hematological complications in the peripheral blood.

**Bone Marrow.** This was moderately to markedly involved in AL and AL-T groups. Total replacement by the leukemic cells was not observed. Erythrogenesis was markedly impaired in AL and AL-T groups; it occasionally decreased in the L-T group and remained normal in the T group.

<table>
<thead>
<tr>
<th>Generation</th>
<th>Leukemia type of the donor</th>
<th>Material inoculated</th>
<th>Leukemia type of the recipients</th>
</tr>
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<tbody>
<tr>
<td>Original rat</td>
<td>AL</td>
<td>Blood, spleen</td>
<td>AL, L</td>
</tr>
<tr>
<td>1st–6th</td>
<td>AL</td>
<td>Blood</td>
<td>AL</td>
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<tr>
<td>6th</td>
<td>L</td>
<td>Blood</td>
<td>AL</td>
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<tr>
<td>7th–10th</td>
<td>AL</td>
<td>Blood</td>
<td>AL</td>
</tr>
<tr>
<td>10th</td>
<td>AL</td>
<td>Blood</td>
<td>AL, AL-T, L-T, L, T</td>
</tr>
<tr>
<td>11th</td>
<td>AL-T</td>
<td>Blood</td>
<td>L-T</td>
</tr>
<tr>
<td>12th</td>
<td>L-T</td>
<td>Tumor, blood</td>
<td>L-T</td>
</tr>
<tr>
<td>13th</td>
<td>L-T</td>
<td>Blood</td>
<td>L-T</td>
</tr>
<tr>
<td>14th</td>
<td>AL-T</td>
<td>Peritoneal fluid</td>
<td>AL-T</td>
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<td>15th</td>
<td>AL-T</td>
<td>Blood</td>
<td>AL-T</td>
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<td>16th</td>
<td>AL-T</td>
<td>Peritoneal fluid</td>
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<td>17th</td>
<td>AL-T</td>
<td>Blood</td>
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Lymph Nodes, Thymus, and Liver. Imprints indicated a range of from no neoplastic cells to almost total replacement by the leukemic cell (regardless of manner of development).

Spleen. The spleen presented a more uniform picture, with a predominantly leukemic cell population in AL and AL-T variants and very few to none seen in the remaining groups.

Morphology of the Leukemic Cell

The mononuclear leukemic cells in the peripheral blood were characterized by a certain degree of morphological variation. There were 2 major recognizable leukemia cell types.

Type I. This was a large (20-μ), undifferentiated cell with fairly abundant cytoplasm and an indented, convoluted, or round nucleus. The nucleus was usually rather lightly stained, with fine reticuloc to disorganized chromatin, uneven areas of condensation, abnormally clear parachromatin, and distinct nucleoli (1 to 2 nucleoli present). These cells resembled monoblasts and therefore are considered as monocytic leukemic cells (Figs. 1 and 2).

Type II. These cells were smaller and more uniform in size and shape with round, oval, or slightly indented nucleus, coarser and clumpier nuclear chromatin, discrete nucleoli, and deeply basophilic cytoplasm. On the basis of these characteristics, Type II cells were defined as “lymphoid leukemic.”

In both type cells, an occasional inclusion body or engulfed red cell was seen. Few cells contained reddish granules, and some cells developed a central nuclear vacuole reminiscent of the myeloid transformation in the normal rat (5) (Fig. 3). All samples contained few to numerous cells, with karyorrhectic or lysed nuclei, buds of cytoplasm that retained nuclear remnants, and occasional budding cytoplasmic projections. Mitotic figures were frequent.

The so-called preleukemic cell was about the size of a young lymphocyte, basophilic, with a round, oval, or slightly indented nucleus and an absent or very poorly visible nucleolus. The preleukemic cell seemed to be more atypical than immature.

The leukemic cells seen in the bone marrow, thymus, lymph nodes, spleen, and liver preparations had the same morphological pattern as was observed in the peripheral blood (Figs. 4 to 7).

The abdominal fluid smears from animals with minimal blood involvement usually contained leukemic cells (Fig. 8). The tumor imprints manifested 1 or more varieties of the leukemic cells previously described (Fig. 9). No Auer bodies were observed.

The leukemia was categorized according to whichever type of cell predominated. In many rats there was a mixed cell population consisting of both monocytoid and lymphoid leukemic cells with rare myeloid transformation.

In the 1st 10 passages, inclusive, all the morphological types were fairly evenly distributed. Beginning with the 11th generation, however, monocytoid and mixed types were more frequently seen.

Pathology

The spleen of the donor rat was greatly enlarged, firm, and pulpy, measuring 6 x 2 cm (Fig. 12). The mesenteric and anterior mediastinal lymph nodes were slightly enlarged. Multiple petechiae occurred on the mucosal surface of the bladder, the central nervous system, and the stomach. There was a 2-mm mass on the dorsal aspect of the right kidney extending into the kidney surface for about 2 mm. The liver was highly mottled but contained no gross tumors.

Rats dying from acute leukemia were emaciated in varying degrees, the tissues were pale, and their spleens were invariably enlarged. Generalized lymph node enlargement, uniform enlargement and mottling of the liver, and ulcers on the gastric mucosa, with a generalized hemorrhagic gastroenteritis, often were seen. Petechiae were present in most organs and tissues, particularly in the lung, gastrointestinal mucosa, pericardium, and brain. The gross anatomical features of the disease were altered at the 11th generation, when the solid or sarcomatous tumor type evolved. In the early stages, there was a thin film of opaque tissue growing on the serosal surface of different tissues and organs of the peritoneal cavity. As the disease progressed, these structures became heavily infiltrated with a dense, smooth, white, glistening tumor that invaded and distorted the various organs and supporting structures (Fig. 13).

The spleen was often not enlarged, and retrospectively this was seen to occur in those instances in which peripheral blood was not markedly involved. The organs of the thoracic cavity were generally not involved in gross alterations, except for the thymus, which was sometimes markedly enlarged, and this had caused signs of respiratory distress in the living animal. Enlargement of lymph nodes was an inconsistent feature.

Histopathology

The morphological features of the leukemic cell and the solid tumor cell were distinctly different. The solid tumor, whether accompanied by blood changes or not, grew in the pattern of a poorly differentiated sarcoma (Fig. 10). However, there were occasional areas where a fairly abundant reticulum was formed (Fig. 11). In such instances, the tumor resembled a reticulum cell sarcoma. The cells grew rapidly, infiltrating and invading serosal surfaces or organ capsules. They grew freely in the sinusoids of the liver and spleen and extended through the muscular coats of the intestine to the mucosa.

DISCUSSION

The donor rat from which the initial injection material was derived had been used in an experiment designed to evaluate the carcinogenicity of DMN. Approximately 1000 rats of different age groups from our colony had been used in experiments with this compound. Varying dose levels have been used, most of them higher than that given the rat in the present study. In addition, numerous investigations have been carried out with DMN and, to the best of our knowledge, induction of leukemia in the rat is not a feature of this carcinogen. Odashima (14) reports on leukemogenesis induced by N-nitrosobutylurea, and he quotes Dr. H. Druckey, who tested 65 N-nitroso derivatives and found that only N-nitrosothylurea produces leukemia. The possibility that DMN induced the tumor in this study is considered highly remote.
During the investigation of this leukemia, several clinical and morphological points of interest were noted.

In the 17 passages carried out, induction time seemed to depend directly on the leukemogenic and tumorigenic activity of the malignant cell. Acute leukemia and acute leukemia with sarcomatous tumor mass were produced within a shorter period of time than required for production of tumor mass alone.

The consistent pattern of the reversible leukemia-tumor growth differs from other transplantable rat leukemias reported elsewhere (3, 6, 11, 15).

Chloroleukemia, if transferred by blood, appeared to invade the recipient in an orderly manner (6, 11). Although we have observed a chloroleukemia in Wistar rats of 2 different strains, (Z. Z. Zawidzka and H. C. Grice, unpublished observations), features typical of this tumor were not detected in the present study.

In the rat, chemically induced malignant lymphoma, which in subtransfers was characterized by lymphosarcoma, lymphocytic leukemia, or both, has been reported (12). This tumor-leukemia pattern, however, depended directly on the technique of transplantation, whereas ours did not.

The transplantable acute leukemia in Fischer rats reported by Dunning and Curtis (3) and Rubini and Yakaitis (15) resembled to a certain degree the leukemia described in this paper. The leukemia studied by Dunning, if transferred i.p., also showed an involvement of mesentery and omentum, a high WBC count with predominantly malignant cells in the peripheral blood, and a comparable survival period. However, in the present study, animals with acute leukemia maintained rapidly increasing WBC to the very end and manifested no terminal regression following the peak, as Dunning observed in her study. In addition, no tumor growth without leukemic manifestations occurred in the transplantable leukemia reported by Dunning.

A definitive morphological classification presents many difficulties and, although it is discouraged by some, this method of classification is considered superior by others [Linman (7)]. No single cellular attribute was considered a valid criterion of a tentative blast classification in this study. It was based on the prevailing morphological qualities of the predominant cell that permitted separation of suggested lymphoid and monocytoid-to-reticulum cell-like types.

According to morphological characteristics, the cells of the monocytoid but not of the lymphoid variant resembled the blast of the frank monocytic leukemia, described by Dunning and Rubini. An AMN leukemia occurring in Wistar-Furth rats, investigated by Moloney et al. (8), differed from that in our study by the consistent presence of reddish granules in the AMN’s. We have observed a case of AMN leukemia that fitted perfectly that described by Moloney, in an experiment involving aflatoxin studies.

The aberrant myeloid changes in the monocytoid variant that were reminiscent of the human condition present in our leukemia were not reported in other transplantable leukemias. Other points of difference were the presence of clear to light blue homogeneous cytoplasmic masses, as well as the erythrophagocytic properties of the cells. Although the lymphoid leukemic cell appeared to engulf erythrocytes more readily, reticulum cells also contained what appeared to be remnants of ingested erythrocytes or some acidophilic droplets. Since erythrophagocytosis seems to take place in vivo in the presence of autoimmune or infection-induced hemolytic anemia (16), this suggests the possibility of a hemolytic process, as observed by Moloney in the AMN leukemia. However, the majority of our rats had markedly reduced bone marrow erythroid activity.

The monocytoid nuclear structure, the resemblance to reticulum cells, and the frank reticulum cells present in increased numbers in thymus and spleens, as well as the phagocytic properties, would suggest a reticuloendothelial character of this leukemia and would include a lymphoid variety as well.

The preleukemic cell usually preceding the phase of AL and AL-T groups and consistently appearing in L-T animals was considered to be associated with the cancer, since these cells were also present in greater numbers in the fulminating and terminal phases. In addition, transitional forms bridging the gap between the preleukemic cell and the leukemic blast were also present. This was more apparent in the lymphoid variant. However, the convincing evidence supporting the morphological recognition rests with the development of AL in the recipients inoculated with the blood of the donor, which blood showed only a few preleukemic cells and showed normal hematological parameters otherwise.

ACKNOWLEDGMENTS

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Figs. 1 and 2. Peripheral blood smear. Leukemic cells of the monocytoid type. *Arrows*, 2 cells with central nuclear vacuoles, representing the 11th and 1st generations, respectively. Leishman’s stain, X 2000.

Fig. 3. Peripheral blood smear. Leukemic cells of the lymphoid type. Two cells with basophilic and light blue cytoplasmic inclusion bodies (inset); 4th and 2nd generations, respectively. Leishman’s stain, X 2000.
Fig. 4. Spleen impression, illustrating cells that resemble reticulum cells, with cytoplasmic acidophilic material in one (arrow); 4th generation. Leishman's stain, x 2000.

Fig. 5. Lymph node impression. Arrows, erythrophagocytosis by 3 leukemic cells; 3rd generation. Leishman's stain, x 2000.

Fig. 6. Liver impression. Leukemic cells of lymphoid and monocytoid type. Mitotic figures are present; 11th generation. Leishman's stain, x 2000.

Fig. 7. Spleen impression. Leukemic cells of monocytoid type; 4th generation. Leishman's stain, x 2000.
Fig. 8. Peritoneal fluid smear containing leukemic cells; 12th generation. Leishman’s stain, × 2000.

Fig. 9. Tumor imprint. Cells of monocytoid type are identical to those seen in the peripheral blood and hematopoietic organs; 12th generation. Leishman’s stain, × 2000.

Fig. 10. Histological section; solid tumor infiltrating the smooth muscles of the small intestine. There is considerable variation in nuclear morphology. Several abnormal mitotic figures are seen; 14th generation. Hematoxylin-phloxine-saffron stain, × 128.

Fig. 11. Histological section; abundant reticulum formed by cells of the solid tumor. The reticulum is mainly around individual cells but occasionally envelops a cluster of several cells; 14th generation. Gomori’s stain, × 128.
Fig. 12. Rat with acute leukemia. The rat had received injections of blood from an AL-type rat 13 days previously. The spleen is greatly enlarged (1.66 g). Other organs and tissues appear normal; 2nd generation.

Fig. 13. Tissues from a rat that had received injections of blood from a L-T rat 37 days previously. The omentum is obliterated by dense, white, glistening tumor tissue. The tumor is adherent to the left lobe of the liver. The spleen in this animal was not enlarged; 12th generation.
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