Transplantable Granulocytic Leukemia In Strain 13 Guinea Pigs

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ABSTRACT

The occurrence of a granulocytic leukemia in 1 of 40 female strain 13/N guinea pigs given N-nitroso-N-butylurea continuously in their drinking water for 21 weeks is reported here. This leukemia has been successfully transplanted in this guinea pig strain for 13 transplant generations by i.p. inoculation of leukemic blood or marrow cells. Macroscopically and microscopically, this leukemia resembles the chronic myelogenous form in humans. Histochemical studies showed, however, that unlike the human leukemic cells those in the leukemic guinea pigs are alkaline phosphatase positive. Electron microscopic studies of the guinea pig leukemic cells revealed the presence of numerous intracisternal A-type particles that are not found in corresponding normal leukocytes.

INTRODUCTION

Granulocyte leukemia in the guinea pig is a rare disease. Although lymphocytic leukemia in guinea pigs has been well documented, to our knowledge there are no reports in the literature of either the spontaneous or induced forms of granulocytic leukemia in this species. As part of an ongoing study in our laboratory of the biochemical aspects of granulocytic leukocyte development in the bone marrow, an attempt was made to induce granulocytic leukemia chemically in strain 13 guinea pigs with BNU, a chemical known to cause a high incidence of granulocytic leukemia in rats (14). In the preliminary phase of the work, a dose-response study was carried out in order to determine the toxic level of this drug in guinea pigs. During the course of this preliminary study, 1 of the 40 guinea pigs tested developed granulocytic leukemia. This neoplasm has been successfully maintained in strain 13/N guinea pigs by i.p. transplantation of either whole blood or washed bone marrow cells from leukemic animals.

This communication reports on the general biological and histopathological characteristics of this granulocytic leukemia, which is now in its 13th transplant generation. Electron micrographs showing the presence of virus-like particles in the blood leukocytes of these leukemic guinea pigs are also presented.

MATERIALS AND METHODS

Female strain 13/N guinea pigs were obtained from Frederick Cancer Research Center, Frederick, Md. They were housed in metal cages (5/cage) with wood shaving bedding in an environment-controlled room at 22°C and 50% relative humidity. The animals were fed Teklad Standard Diet (ARS-Sprague Dawley Division, Winfield, Iowa) supplemented with kale to supply the necessary vitamin C. Repeated screenings of these guinea pigs for murine viruses were negative.

BNU, synthesized by nitrosation of N-butylurea (17), was kindly supplied by Dr. E. K. Weisberger of the Carcinogen Metabolism and Toxicology Branch, National Cancer Institute, Bethesda, Md. The procedure used for continuous p.o. administration of BNU in the drinking water of guinea pigs was similar to that used by Odashima (13) for rats. Five groups of 10 guinea pigs each receiving 0, 0.025, 0.050, 0.100 or 0.200% BNU, respectively, in their drinking water were studied.

Hemacytometer counts of WBC were obtained on the peripheral blood sampled weekly from the hind foot pads of guinea pigs and on heart blood samples used to transplant the leukemia. Leukocyte differential counts were carried out on the blood smears stained on a Hematek slide stainer (Ames Co., Elkhart, Ind.). Leukemic blood smears were stained for peroxidase (8) and alkaline phosphatase (1). Tissues removed at necropsy were fixed in 10% buffered formalin for at least 48 hr, and routine methods were used to prepare slides for histology.

Whole blood for transplantation was collected by cardiac puncture. Heparin (Eli Lilly and Co., Indianapolis, Ind.) was added to the blood at a final concentration of 50 USP units/ml. Bone marrow for transplantation was removed aseptically from the femur of each leukemic guinea pig. The marrow from the femur was dispersed in 3 to 4 ml of sterile 0.15 M NaCl with a sterile pipet and centrifuged at 1500 rpm for 5 min in an International centrifuge refrigerated at 4°C. The supernatant was discarded, and the cells were resuspended in a final volume of 2 ml sterile 0.15 M NaCl.

For electron microscopy normal and leukemic peripheral blood cell pellets were fixed in 0.1 M sodium cacodylate-buffered 1.25% glutaraldehyde, pH 7.4, for 2 hr. The cells were subsequently rinsed in cold buffer and exposed to 1% chrome osmium (6) for 1.5 hr followed by 0.5% uranyl acetate, pH 4.9, for 1 hr. After fixation the pellets were dehydrated in ethanol and propylene oxide and embedded in Epon-Araldite. Ultrathin sections were cut on an LKB Ultratome equipped with a diamond knife. The sections were picked up on carbon-Formvar-coated copper grids, stained with uranyl acetate and lead citrate, and viewed in a Siemens Elmiskop 102 electron microscope at magnifications ranging from 3,000 to 30,000 diameters.

RESULTS

Brief History of Original Donor Leukemic Guinea Pig

The guinea pig that developed the original granulocytic leukemia used for transplantation was one of forty 10-week-
old guinea pigs given BNU continuously in the drinking water at concentrations of 0.025 to 0.20%. After 21 weeks the treatment with BNU was stopped since many of the animals given the higher doses of BNU began to succumb to what appeared to be toxic effects of the chemical (e.g., loss of appetite, rapid loss of weight, profuse salivations, and respiratory infections). The surviving animals were observed for 10 months thereafter, and weight change and peripheral leukocyte counts were recorded weekly. Only 1 guinea pig developed leukemia. This guinea pig, 1 of 10 that had received the highest dose of BNU, showed an increase in the leukocyte count from the normal value of 6,000 to 7,000/cu mm to one of 23,000/cu mm 24 weeks after the BNU treatment was stopped. The animal became moribund and was sacrificed 2 days later when the peripheral leukocyte count reached 37,500 cells/cu mm. The differential count of the blood smear (Table 1) was typical of that seen in chronic granulocytic leukemia in humans (18) and consisted mainly of neutrophil granulocytes at the myelocytic and more mature stages of maturation. Macroscopic examination of the original leukemic guinea pig at necropsy revealed the presence of a large gray-whitish, diffuse mass with numerous nodules in the peritoneal cavity covering both the small and large intestines. The spleen and liver were remarkably enlarged. Many small gray spots were observed in the liver and lung. Microscopically, the abdominal mass was the adipose tissue extensively and intensively infiltrated by leukemic cells (Fig. 1). The leukemic cell invasion was observed in the spleen, liver, lung, and lymph nodes. These leukemic cells represent mature granulocytes and immature neoplastic granulocytes with bizarre nuclei and nucleoli. The differential count of the bone marrow smear showed a predominance of cells in the granulocytic line over erythroid and lymphoid cells (Table 1). The distribution of granulocytic cells according to cell maturity essentially reflected that seen in the peripheral blood smear. Many of the mature cells in the marrow appeared to be abnormally large and contained hypersegmented nuclei (8 to 12 lobes).

Transplantation from the Original Leukemic Guinea Pig

Transplantation of the original leukemic cell line has been carried on successfully for 13 transplant generations. The transplantations were achieved by serial i.p. injections, into normal 3- to 7-week-old guinea pigs, of either 2 ml of leukemic blood containing 3 to 6 x 10^7 leukocytes/ml or the washed marrow cells from 1 femur suspended in 2 ml of 0.9% NaCl solution. Most of the animals receiving transplants developed peripheral leukocyte counts in excess of 30,000 cells/cu mm and leukemoid differential counts within 30 to 40 days after transplantation. All animals either died or became moribund and were sacrificed within 1 to 2 weeks after the peripheral leukocyte count exceeded 30,000 cells/cu mm. The blood leukocyte count of the animals that died of the disease exceeded 100,000 cells/cu mm in most cases. The terminal stage of the leukemia was also characterized by a pronounced anemia and a hypoplastic marrow, devoid of normal and leukemic leukocytes which had been replaced by fibroblast-like cells. In these aspects the guinea pig leukemia is very similar to the transplantable granulocytic leukemia of the rat (9).

Autopsy Findings on Leukemic Transplant Animals

Macroscopic Findings. The animals that developed leukemia with peripheral leukocyte counts greater than 40,000 cells/cu mm commonly experienced weight loss, dyspnea, weakness, and/or paralysis of the hind quarter. At autopsy, there was a small amount of viscous, slightly pink-milky, or occasionally hemorrhagic ascites in the peritoneal cavity. Numerous small tumor nodules were seen on the omentum and periurogenital and pararenal adipose tissues with petechial hemorrhages. A remarkable enlargement of the spleen and liver was uniformly observed in the leukemic guinea pigs. The liver hilus was white and infiltrated by leukemic cells. The mesentery and ilial or pararenal lymph nodes were greatly enlarged. Firm, elevated nodules of varying sizes were observed on the diaphragm.

In the chest a large amount of hemorrhagic pleural effusion was usually observed in the cavity. The pleura was markedly thickened by leukemic cell infiltration. The enlargement of thymus and tracheobronchial lymph nodes was commonly seen. A row of multiple tumor nodules occasionally with petechial hemorrhages was consistently observed in the parasternal tissue. The lung was often consolidated with many small grayish spots or areas. The cervical lymph nodes were also enlarged and hemorrhagic. The bone marrow was always grayish white.

Microscopic Findings. The leukemic cells that infiltrate the visceral organs in the transplant guinea pigs appear identical with those of the original leukemic guinea pig.

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<tr>
<th>Neutrophils</th>
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<tr>
<td>Smear</td>
<td>Mature</td>
<td>Myelocyte</td>
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<td>Marrow</td>
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Table 1

Differential counts of blood and bone marrow cells from original donor guinea pig with granulocytic leukemia

Smears of blood and marrow cells were prepared as described in "Materials and Methods." A total of 200 cells were counted in each smear and the percentage of distribution was calculated for each cell type. For convenience in counting the cells were grouped as follows: mature neutrophils, (segmented, band, and metamyelocyte forms); neutrophilic myelocytes; early cells, (promyelocytes and blasts); eosinophils and basophils (all stages of maturity); and RBC precursors (all stages of maturity).
The leukemic cells often show the different stages of maturation with a high mitotic index in immature forms. The bizarre nuclei are oval, kidney-shaped, edented, or lobular. In the leukemic state, infiltration of leukemic cells was observed in the bone marrow, muscle, blood vessels, liver, spleen, abdominal adipose tissues, kidneys, diaphragm, pancreas, uterus, lymph nodes, intestinal wall, heart (pericardium and myocardium), thymus, lung, eyes, and brain.

The differential counts of the peripheral blood smears of the transplant animals were similar to those observed in the original donor leukemic guinea pig. Fig. 2 shows typical immature and mature granulocytes in the leukemic blood smears. As can be seen in Fig. 2, mature granulocytes characteristically contained large cytoplasmic vacuoles, a feature rarely seen in mature cells of normal guinea pigs. Hypersegmented nuclei were also present in many of the mature granulocytes in leukemic blood smears (see Fig. 3). A positive peroxidase staining reaction was observed in most of the immature granulocytes in the blood smear, whereas only a small fraction of the mature granulocytes stained for peroxidase (Fig. 3). The histochemical reaction for alkaline phosphatase in leukemic granulocytes was similar to that obtained with normal granulocytes in that all cells at the myelocytic, metamyelocytic, band, and polymorphonuclear stages of maturation gave a strong positive reaction for this enzyme.

**Electron Microscopy.** Examination of the peripheral blood cells of the leukemic guinea pigs by electron microscopy revealed the presence of intracisternal, virus-like particles in virtually every leukemic granulocyte, whereas no particles were observed in developing or mature granulocytes found in bone marrow preparations from normal strain 13 guinea pigs (Figs. 4 and 5). In thin sections the particles appear as doughnuts formed from concentric, electron-dense shells and their diameters ranged from 75 to 80 nm. Nearly all the particles were intracellular; however, budding forms were infrequently encountered at the cell surface (Fig. 6). It is presumed that this phenomenon is followed by the release of mature particles into the intercellular spaces; however, no particles recognizable as virions were seen in the washed cell pellets from either bone marrow or peripheral blood.

**DISCUSSION**

The development of a transplantable granulocytic leukemia in the guinea pig makes available another important model system, in addition to the 2 other major models in mice (16) and rats (9), for well-controlled biological and biochemical studies on the mechanism of myeloid leukemogenesis. Our findings indicate that some of the properties of the transplantable granulocytic leukemia in strain 13/N guinea pigs resemble those seen in chronic myelogenous leukemia in humans. The leukemic granulocytes in the blood and marrow of the guinea pig characteristically show the complete range of cell maturity, and the leukemic cells infiltrating the spleen, liver, and other body organs are exclusively granulocytes and their precursors. Also, a pronounced anemia develops and, in the terminal stage of the disease, the marrow becomes hypoplastic as the normal and leukemic cells in the marrow are replaced by fibroblast-like cells. A predominance of immature granulocytes (*i.e.*, blasts and promyelocytes), indicative of blast crisis or acute granulocytic leukemia, has not yet been observed in these animals. A notable difference between the guinea pig granulocytic leukemia and chronic myelogenous leukemia in humans is the consistent finding of a positive reaction for alkaline phosphatase in the leukemic granulocytes of the guinea pig. The human disease is characterized by a lack of staining of the majority of the granulocytes for this enzyme (10), but it has been reported that alkaline phosphatase is present in these cells in an inactive form that can be activated during cell incubation in diffusion chambers implanted in mice (4). Also, a high alkaline phosphatase activity has been reported for leukemic granulocytes in the rat (11). This enzyme has not been studied in mouse leukemia since normal mouse granulocytes apparently lack this enzyme (15).

Because of the small number of animals used in our preliminary study of the leukemogenic effects of BNU in guinea pigs, it is not possible to determine whether the disease was chemically induced. Spontaneous myelogenous leukemia in guinea pigs has not previously been reported, however, and 1 case in 40 guinea pigs treated with BNU, a known leukemogenic chemical, represents a relatively high incidence for such a rare disease. A complicating factor in this study was that most of the animals in the group of 10 from which the leukemic animal came died from the toxic effects of the BNU before this group of animals was taken off the drug. It is possible that if the BNU were removed prior to the appearance of toxic symptoms then more of the guinea pigs might have survived and developed leukemia. Further studies with larger numbers of animals would be required to test this possibility.

Our electron microscopic studies reveal the presence of intracisternal virus-like particles in the granulocytes from leukemic guinea pigs. Similar intracisternal particles have been observed in lymphoblasts of the transplantable L2C leukemia in strain 2 guinea pigs (7), in primordial germ cells from random-bred, fetal guinea pigs (12), and in tissue culture cells from chemically induced guinea pig hepatomas (3). These particles are thought to be related to type C virus particles, which are found in the plasma of L2C leukemic guinea pigs (2). A direct connection between these 2 particles has not yet been shown. Our preliminary investigation of the plasma of strain 13 guinea pigs with the transplanted granulocytic leukemia also suggests the presence of C-type virus. Whether these particles are related to the disease or simply endogenous oncornaviruses concomitantly induced by the carcinogen is not known. Experiments are in progress to evaluate the importance of these particles in the etiology of the granulocytic leukemia reported here.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the excellent technical assistance of Joan W. Nixon.

**REFERENCES**

1. Ackerman, G. A. Substituted Naphthol AS Phosphate Derivatives for the Localization of Leukocyte Alkaline Phosphatase Activity. Lab. Invest.,
Granulocytic Leukemia in Guinea Pigs


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Fig. 1. Section of the abdominal adipose tissue that is extensively and intensively infiltrated by mature and immature granulocytic leukemia cells. H & E, x 1,600.

Fig. 2. Peripheral blood smear from a guinea pig with transplanted granulocytic leukemia, showing a typical field containing immature and mature granulocytes and an erythrocyte precursor. The WBC was 111,000/cu mm, and the leukemia was in the third transplant generation. Hematek, x 880.

Fig. 3. Leukemic cells stained for peroxidase activity in same blood smear as in Fig. 2. Immature granulocytes show a strong positive reaction; mature granulocytes are negative. Hematek counterstain. x 880.

Fig. 4. An electron micrograph depicting a portion of an immature granulocyte from a normal strain 13 guinea pig. Abundant profiles of rough endoplasmic reticulum and a few developing granules are present. No intracisternal virus particles were observed in normal myeloid leukocytes. x 35,000.

Fig. 5. A portion of an immature granulocyte isolated from the blood of a strain 13 guinea pig that had been transplanted with leukemic blood. Numerous virus particles are present in the cisternae of the rough endoplasmic reticulum. These particles were present at all stages of granulocyte differentiation but appeared to be more numerous in the least-differentiated cells. x 35,000.

Fig. 6. The structure of the virus particles associated with the granulocytic leukemia at higher magnification. The particles were quite uniform, with diameters of 76 to 80 nm. Their morphology is very similar to those of the intracisternal virus particles described in L2C leukemia of strain 2 guinea pigs. Extracellular virus particles were not observed in the cell pellets; however, occasional budding forms were observed at the cell surface as shown in inset b. x 87,500.
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