Spontaneous Leukemia in Fischer Rats

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SUMMARY

A series of 86 inbred female Fischer rats were followed throughout their life-span and 21 cases of a unique mononuclear cell leukemia were discovered. The disease appeared only in older rats; median age at death was 25 months. As leukemia developed, characteristic mononuclear cells, usually with distinctive reddish cytoplasmic granules, appeared in the peripheral blood. Imprint smears also disclosed a uniform involvement of the liver and spleen; lymphatic glands and other organs were invariably infiltrated. This spontaneously occurring leukemia in an inbred strain of rats affords an unusual opportunity for the study of leukemogenesis in this species.

INTRODUCTION

Leukemia rarely occurs spontaneously in most strains of laboratory rats. However, in recent studies 37 leukemias of a unique mononuclear cell type were found among 214 old, inbred Wistar = Furth rats (17.2%) (6). Subsequently, an incidence of 24.4% of a similar form of leukemia was noted in a series of inbred female Fischer rats. Hematological, cytogenetic, and pathological studies on these leukemic animals are presented in this report.

MATERIALS AND METHODS

Rats used in this study were inbred females derived from Dunning's Fischer 344 line (2) (Charles River Breeding Laboratories, Boston, Mass.). These rats were barrier bred, maintained 2 in a cage, and fed Purina laboratory chow diet with water ad libitum. Rats were weighed and inspected weekly; once a month leukocyte counts and peripheral blood smears were obtained from tail blood. White cell counts were carried out on a Coulter counter, and Wright-stained smears were carefully studied for presence of red cell or white cell abnormalities. If a rat became ill or developed splenomegaly or if hematological abnormalities were noted, blood studies were carried out at weekly intervals or as often as indicated. In the terminal stage, rats were sacrificed by ether anesthesia, and imprint smears or paintbrush smears were made from the bone marrow, spleen, liver, enlarged glands, and other tissues. Smears were stained with Wright-Giemsa and, when indicated, histochemical studies for peroxidase, esterase, and alkaline phosphatase activity were carried out (5). At the time of sacrifice, specimens were obtained from blood for serum muramidase determination (8). Cytogenetic studies were attempted on leukemic cells from peripheral blood, spleen, and liver (5). All animals were followed until sacrifice or natural death and, at autopsy, tissues were fixed in 10% unbuffered formalin solution and stained with hemotoxylin and eosin.

RESULTS

Clinical and Hematological. Among 86 rats under observation, 21 were found to have leukemia, all of which were of the unique mononuclear cell type previously described in W/Fu2 rats. The disease occurred only in older animals, the age at death ranging from 14 to 30 months with a median of 25 months. Duration of the disease, as measured by appearance of leukemic cells in the peripheral blood, averaged 5 weeks. Until late in the course, leukemic rats did not appear ill; they then developed clinical signs associated with infection and severe hemolytic anemia, the latter complication being a consistent feature of the disease. As leukemia developed, along with characteristic mononuclear cells, nucleated red cells and marked polychromasia appeared in the peripheral blood smears. Leukocyte counts were variable ranging from 18,000 to 158,000 per cubic millimeter. The leukemic mononuclear cells were large, measuring 15 to 20 μ in diameter, and contained a nucleus which was round, oval, or somewhat lobulated. In many cells the nucleus looked like that of a large lymphocyte, but more immature cells had spongy nuclei with blastlike chromatin, and often 1 or 2 distinct nucleoli were present. Cytoplasm was relatively abundant, clear blue, and frequently contained distinctive, moderate to large, reddish granules (see Fig. 1).

Unlike cells in rat granulocytic leukemia, the mononuclear cells did not contain cytoplasmic alkaline phosphatase, peroxidase, or esterase activity. The granules demonstrated no acid phosphatase activity; stains for oil red O and periodic acid-Schiff were negative. Serum lysozyme (muramidase) levels are greatly elevated in chloroleukemic rats and in human patients with monomyeloid leukemia (8). No elevation of serum lysozyme was noted in 7 leukemic

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1 This investigation was supported by USPHS Grants CA 05691-05 and C 6516 from the National Cancer Institute.

Received February 25, 1969; accepted May 7, 1969.

2 The abbreviation used is: W/Fu, Wistar-Furth.
rats studied in this investigation. In a few animals only small numbers of mononuclear cells were present in the peripheral blood, but there was evidence of hemolytic anemia and marked splenomegaly. In these cases imprint smears of the liver and other organs were infiltrated with pathognomonic mononuclear cells, and the diagnosis of leukemia was confirmed at postmortem examination.

Pathology. At autopsy the tissues were pale, and there was evidence in most animals of severe pulmonary congestion and infection. Pyosalpinx was also commonly observed. The spleen was consistently enlarged, varying from 1.5 to 20 g; average weight in 21 leukemic animals was 8.4 g. Microscopically, the spleen showed infiltration with leukemic cells. Marked hemosiderin deposits were also present, and extra-medullary hematopoiesis was evident in some animals. The liver was not usually enlarged but often appeared mottled, and in 4 cases the surface was definitely nodular. Microscopically, the nodules were made up of large, pale hepatic cells surrounded by a zone of compressed normal parenchymatous cells. Nodular hyperplasia was absent in nonleukemic animals. A consistent and striking feature was typically sinusoidal hepatic infiltration by leukemic mononuclear cells. However, in a few very acute cases the liver was diffusely infiltrated. Imprint smears of the liver were especially useful because the presence of leukemic mononuclear cells, with or without granules, could readily be demonstrated in Wright-Giemsa-stained preparations. Adenopathy was variable but when present was moderate in degree in all but a few cases.

In several animals there were huge masses of glands usually involving the mediastinal and mesenteric lymph nodes. The thymus and other organs, including the pancreas, kidneys, heart, and lungs, were infiltrated at times, but this was not a consistent finding. As noted in the W/Fu rats, the bone marrow was significantly involved in less than 20% of the animals (6).

Cytogenetic Studies. Attempts to carry out chromosome studies on leukemic cells were made in 14 of 21 animals. Because of marked erythroid hyperplasia, bone marrow preparations were not used. Among leukemic cells derived from peripheral blood, spleen, and liver, few mitoses were noted, either in direct preparations or in 1- to 4-day cell cultures. Sufficient numbers of metaphase plates, adequate for chromosomal analysis, were obtained from only 4 leukemic animals. Of these, 2 had no significant numerical or morphological abnormalities. In one rat there were either 1 or 2 extra chromosomes in over 50% of the metaphases. In another, over 50% of the metaphases had 1 extra chromosome in the terminal (Autosomes 4 to 10) group.

DISCUSSION

Until recently, little use has been made of the rat for the study of leukemogenesis; indeed, the first cases of leukemia were not described in this species until 1936 (9). In 1939 Oberling et al. (7) published results of their studies on 9 leukemias occurring among 6000 autopsies on old, nonin-
bred, white laboratory rats. These authors emphasized the infrequency of spontaneous leukemia in the rat and noted that of the 9 cases, 3 were granulocytic and 6 were difficult to classify; these were described as "lymphoid." However, in 5 of 6 cases the leukemic cells contained distinct, moderately large, reddish cytoplasmic granules. Other features noted in these leukemic animals were uniform splenomegaly, lack of marked lymphadenopathy, and absence of bone marrow infiltration. In 1959 Furth (4) commented on the occurrence of 6 cases of mononuclear cell leukemia among 64 old, inbred W/Fu rats. The cytological and other features closely resembled the "lymphoid" leukemia reported by Oberling et al. (7). Detailed descriptions of this type of leukemia in W/Fu rats have been published in prior papers from this laboratory (5, 6).

The leukemia in Fischer rats is similar to the "lymphoid" form described by Oberling et al. and to the leukemias in W/Fu rats. Dunning and Curtis (2) were the first to describe leukemia in Fischer rats. They reported a case in an old, inbred female rat of the Fischer 344 line and classified it as monocytic leukemia. Previously, these authors, in a population study of malignant diseases among Fischer 9566 rats, had observed no leukemias (1). However, Dunning and Curtis pointed out that, in population studies, cases of leukemia would undoubtedly be missed in routine postmortem examinations. Recently, Jacobs and Huseby (3) investigated the occurrence of tumors in aged Fischer rats. They briefly mention neoplasms of the reticular tissue and stated that 25% of the autopsied animals had enlarged livers and spleens; other tissues were also involved with leukemic infiltrates (3).

Lack of recognition of the high incidence of leukemia in Fischer rats may be due to several reasons. Unless Wright- or Giemsa-stained peripheral blood or imprint smears from various organs are used, the leukemic cells may not be recognized because the characteristic cytoplasmic granules are not detectable in fixed tissue sections. Grossly, while the spleen is enlarged, lymphadenopathy is not striking in most leukemic animals; unless histological sections are examined, leukemic infiltrates will be overlooked. In the presence of greatly enlarged glands and obviously infiltrated organs, a diagnosis of reticulum cell sarcoma may be made histologically. However, if the peripheral blood is invaded, as it was with few exceptions in our cases, the disease should be classified as leukemia.

Because there is no known etiology for this disease in the rat, classification must now rest on morphological criteria. Leukemias of this cell type do not occur in man. On morphological and histochemical grounds, the cell cannot be classified as granulocytic, lymphocytic, or monocytic. However, the leukemic cells do have some characteristics of reticulum cells. Until further investigation casts additional light on their nature and origin, we prefer to use the nonspecific term "mononuclear cell." Studies on this leukemia are proving useful for the investigation of various aspects of leukemia, and experiments are in progress to evaluate the effect of radiation, 3-methylcholanthrene, and splenectomy on leukemogenesis in this species.

ACKNOWLEDGMENTS

We are grateful to Arlene Keon and Norma Litchfield for their excellent assistance in the hematological and cytogenetic aspects of this study. Also to Ibsen Chen for the histological preparations and John Carabitses for the microphotography.

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