Observations on Leukemia in Wistar Furth Rats

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SUMMARY

Spontaneous leukemia was found to occur in 15.8 to 22% of inbred Wistar Furth rats, and the leukemia was characterized by an unusual type of mononuclear cell containing distinct reddish granules. Enlargement of the spleen, leukemic infiltration with lobular hyperplasia of the liver, and severe hemolytic anemia were other features of the disease. Average age at death was 22 months; administration of 3-methylcholanthrene to young rats increased, but total-body X-ray markedly decreased, the rate of leukemia. The high incidence of leukemia, long "latent" period, and distinctive cell type are features which provide a unique opportunity for investigation of leukemogenesis in this species.

INTRODUCTION

Spontaneous leukemia is considered to be a rare disease in the rat, and comparatively few cases have been noted following exposure to radiation, 3-methylcholanthrene (MCA) and other leukemogenic agents. However, Huggins and others (4, 12) have recently reported a high incidence of leukemia in Long Evans rats following administration of 7,12-dimethylbenz(a)-anthracene (DMBA). Moreover, in our laboratory during studies carried out over the past 4 years, spontaneously occurring leukemia was found in 15.8 to 22% of old, untreated, inbred Wistar Furth (W/Fu) rats. With few exceptions this leukemia was of an unusual mononuclear cell type, and the late stage of the disease was accompanied by a severe hemolytic anemia. Unique features of this leukemia and results of experiments with agents influencing leukemogenesis in W/Fu rats are presented in this paper.

MATERIALS AND METHODS

Inbred Wistar Furth (W/Fu) rats used in these studies were maintained 2 in a cage and fed a Purina Laboratory Chow diet. Results of initial studies on 332 four-month-old W/Fu rats treated with total-body X-ray, 3-methylcholanthrene (3-MCA), or a combination of these agents were previously reported (8).

As shown in Table 1, additional observations have been carried out on 164 untreated female and 50 male W/Fu rats, 101 rats treated with a total dose of 180 mg of 3-MCA at 12 months of age, 71 rats treated with 90 mg of 3-MCA at 4 months of age followed by 150 R total-body X-ray, and 100 rats treated with 80 mg of 3-MCA at 2 months of age. The 3-MCA was dissolved in olive oil and administered in doses of 5 to 10 mg daily (except on weekends) by stomach tube. The radiation factors were: 250 Kvp, 15 ma Thorasi I filter (0.25 Cu; 1 AL) 50 cm, TSO, HVL 2.00 mm Cu, TD 430 rads. Rate 30—32 rads per minute. All animals were examined and weighed at least once a week. Prior to treatment, white blood counts and differentials were obtained from tail blood. These studies were repeated 7 days following X-ray treatment and after the last dose of 3-MCA. Subsequently, leukocyte counts (using a Coulter counter) and differentials were carried out at monthly intervals. If a rat became sick, developed leukocytosis, anemia, or abnormal cells in the peripheral blood, white blood counts and careful examination of Wright-stained blood smears were done as frequently as indicated. In some cases, as an additional aid to cell identification, histochemical methods for detection of leukocyte alkaline phosphatase, verdo-peroxidase, and esterase activity were employed.

In order to obtain adequate whole blood specimens and fresh tissues for histologic, cytologic, and cytogenetic studies, rats were sacrificed by ether anesthesia in the terminal stage. Autopsies were carried out on animals sacrificed or found dead; grossly, the size and weight of organs and presence of enlarged glands and tumors were noted. Tissues were generally fixed in 10% unbuffered formalin solution and stained with hemotoxylin and eosin. Imprint and paint brush smears were made on cover slips from the bone marrow, spleen, liver, enlarged lymph nodes, and tumors if present. Smears were air dried and stained with Wright and Geimsa stains; histochemical stains were also done on cover slip preparations (8). Chromosome studies were carried out on material obtained from peripheral blood, bone marrow, spleen, liver, employing methods described by Nowell (9) and Hungerford (5), and the system of classifying rat chromosomes advocated by these authors was used in this study.

RESULTS

Incidence of Leukemia

As shown in Table 1, observations were made on 50 males and 768 inbred female W/Fu rats including 332 previously
Table 1

<table>
<thead>
<tr>
<th>No. % rats</th>
<th>Agent used</th>
<th>Sex</th>
<th>Age treated (months)</th>
<th>No. leukemic</th>
<th>% leukemic</th>
<th>Duration (days)</th>
<th>Age at death (months)</th>
<th>Final peripheral WBC</th>
<th>% with abnormal cells</th>
<th>Av. spleen wt. (gm)</th>
<th>% with nodular liver</th>
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<tr>
<td>120</td>
<td>180 mg 3-MCA</td>
<td>F</td>
<td>4</td>
<td>13</td>
<td>10.8</td>
<td>11</td>
<td>20</td>
<td>20,000 – 67,800</td>
<td>43,650</td>
<td>49.6</td>
<td>5.0</td>
</tr>
<tr>
<td>145</td>
<td>450 R</td>
<td>F</td>
<td>4</td>
<td>2</td>
<td>1.4</td>
<td>6.5</td>
<td>16</td>
<td>42,000 – 380,000</td>
<td>75.0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>67</td>
<td>450 R + 180 mg 3-MCA</td>
<td>F</td>
<td>4</td>
<td>2</td>
<td>2.8</td>
<td>10</td>
<td>18.5</td>
<td>40,000 – 41,000</td>
<td>55.0</td>
<td>2.0</td>
<td>50</td>
</tr>
<tr>
<td>101</td>
<td>180 mg 3-MCA</td>
<td>F</td>
<td>12</td>
<td>18</td>
<td>17.8</td>
<td>25</td>
<td>23</td>
<td>14,600 – 92,000</td>
<td>37,570</td>
<td>43.0</td>
<td>3.6</td>
</tr>
<tr>
<td>71</td>
<td>90 mg 3-MCA + 150 R</td>
<td>F</td>
<td>4</td>
<td>10</td>
<td>16.9</td>
<td>36.7</td>
<td>18.8</td>
<td>21,000 – 116,000</td>
<td>60,000</td>
<td>72.0</td>
<td>3.4</td>
</tr>
<tr>
<td>100</td>
<td>80 mg 3-MCA</td>
<td>F</td>
<td>2</td>
<td>38</td>
<td>38.0</td>
<td>30.6</td>
<td>24.4</td>
<td>3,000 – 118,000</td>
<td>43,000</td>
<td>48.0</td>
<td>5.1</td>
</tr>
<tr>
<td>164</td>
<td>None</td>
<td>F</td>
<td>None</td>
<td>26</td>
<td>15.8</td>
<td>24.3</td>
<td>21</td>
<td>14,000 – 167,000</td>
<td>62,800</td>
<td>56.6</td>
<td>4.2</td>
</tr>
<tr>
<td>50</td>
<td>None</td>
<td>M</td>
<td>None</td>
<td>11</td>
<td>22.0</td>
<td>18</td>
<td>23</td>
<td>14,000 – 180,000</td>
<td>59,000</td>
<td>48.0</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Clinical and laboratory data on 120 leukemias occurring in 818 W/Fu rats. Of the 120 leukemias, 117 were acute mononuclear cell, 2 lymphocytic, and 1 chronic granulocytic leukemia. 3-MCA, 3-methylcholanthrene.

reported. A total of 120 leukemias, all but 3 of them of the mononuclear cell type (AMN), were found in these animals. Among 164 untreated control female rats followed until sacrifice or natural death, there were 26 (15.8%) leukemias. Female W/Fu rats were more readily available than male animals; however, 50 male W/Fu rats were followed throughout their life span, and 11 developed leukemia with characteristics similar to those noted in the female rats.

In a group of 120 rats treated with 180 mg of 3-MCA at the age of 4 months and 101 treated in a similar fashion at the age of 12 months, leukemia developed in 13 (10.8%) and 18 (17.8%) respectively. In marked contrast, when 2-month-old rats were treated with 80 mg of 3-MCA, 38 of 100 animals developed AMN leukemia.

When a series of 145 rats was exposed to 450 R total-body X-ray at the age of 4 months, only 2 (1.4%) leukemias were noted. Among 67 four-month-old W/Fu rats exposed to 450 R total-body X-ray and then treated with 180 mg of 3-MCA, only two leukemias resulted. However, in this case the combined therapy was quite toxic, and many animals died before leukemia could develop. On the other hand, when 71 W/Fu rats at the age of 4 months were first fed 90 mg of 3-MCA and then exposed to 150 R total-body radiation, 16.9% of the animals developed leukemia. Few early deaths resulted from this schedule, and of the 10 leukemias, 7 were of the AMN type; one rat had chronic lymphatic, one acute lymphatic, and another chronic granulocytic leukemia. The rat with granulocytic leukemia (No. 2307) had chloromatous tumors intra-abdominally at the time of sacrifice. This leukemia was successfully passaged intraperitoneally to W/Fu pups and was carried through 19 passages.

Clinical and Hematologic Observations

Leukemias rarely developed before 18 months of age; the average age at the time of death for 117 cases of AMN leukemia was 22 months. Based on the appearance of leukemic mononuclear cells in the peripheral blood, the duration of the disease was variable, but in the majority of cases it was 2 to 6 weeks. No significant differences were noted in the clinical or hematologic aspects of leukemias among control rats compared to those treated with MCA, X-ray, or both agents. As shown in Table 1, the age at onset, duration of the disease, and age at death were similar in treated and untreated rats.

Few physical changes were noted until the late stage of the disease. Terminally, pallor, weakness, and fever, usually associated with pulmonary infection or pyosalphynx, were present in the majority of leukemic animals.

Splenomegaly was detected in some rats prior to other evidence of leukemia, but in the majority of cases leukocytosis with the presence of abnormal mononuclear cells in the peripheral blood smear were the earliest manifestations of the disease. The leukocyte count usually rose progressively in leukemic animals, accompanied by an increasing number of mononuclear cells. In the late stage of the disease, severe hemolytic anemia developed in the majority of animals, and this was marked by intense polychromasia with large numbers of normoblasts in the peripheral blood. While a small percentage of rats had normal or low white counts, most animals developed a leukocytosis ranging from 30,000 to 180,000 per cu mm with 20 to 90% abnormal mononuclear cells. Because of infection, hemolytic anemia, or both, a few rats did not show a typical blood picture of leukemia, and in these cases, the diagnosis was established by the postmortem findings. A very acute form of AMN leukemia, characterized by appearance of large numbers of blast cells in the peripheral blood and a course ending in death within a few days to a week, developed in a few rats.

The cells in the peripheral blood have been difficult to classify, and we have used the term abnormal mononuclear (AMN) cell leukemia. The cells measured 14 to 30 micra in diameter, the nucleus was oval, round, indented or, at times, folded or lobulated. The nucleus frequently had the appearance of a large lymphocyte with a smooth homogeneous texture, but
the more immature forms had a spongy chromatin often containing one or two large nucleoli. Cytoplasm was abundant, clear blue, and usually contained striking reddish granules which were fine to moderate or large and coarse (see Figs. 1–4).

In some cells the granules appeared to be surrounded by a cytoplasmic vacuole; the granules and cytoplasm were acid phosphatase negative. Some cells had faintly positive cytoplasmic peroxidase or esterase activity, but tests for alkaline phosphatase were uniformly negative (3).

Single cases of acute lymphatic, chronic lymphatic, and chronic granulocytic leukemias occurred among 120 leukemias and were distinguished not only by cytologic features but also by a much earlier age at onset and younger age at death. These leukemias also showed striking differences at autopsy in the extent and type of cellular infiltration of the bone marrow, spleen, liver, and lymph nodes.

Pathology

All animals were followed to sacrifice or natural death, and approximately 90% of the rats were autopsied. The great majority of deaths were due to infection, especially pulmonary abscesses and pneumonia. A detailed description of tumors occurring in the initial series of 332 four-month-old rats treated with 3-MCA, X-ray, or both was recently published (1).

The pathologic features of rats with AMN leukemia included consistent splenomegaly ranging from 1 to 15 grams, along with slight to moderate enlargement of mediastinal and mesenteric glands. In a few cases, mediastinal glands were huge, giving rise to a tumor mass with the histologic characteristics of a reticulum cell sarcoma. The bone marrow was fibrotic in two cases, but curiously, marked infiltration of the marrow with AMN cells occurred in less than 25% of the leukemic rats. The marrow on imprint smears and sections was usually pleomorphic, and marked erythroid hyperplasia was evident in most specimens.

Grossly, the liver presented a striking nodular appearance in 63% of the AMN leukemias, including treated and control animals. Microscopically, either typical nodular hyperplasia or evidence of hepatitis were present in the majority of leukemic animals. The earliest pathologic change consisted of liver cell necrosis of the coagulation type; the histologic distribution was predominantly central and midzonal. An associated feature was atrophy and disappearance of liver cells which resulted in marked dilation of sinusoids. Following this initial cell injury, proliferation and regenerative changes as well as inflammatory responses were noted combined with proliferation of bile ducts, regeneration of liver cells, and a mononuclear infiltrate. Of note, liver cell replacement was focally incomplete, especially in peripheral areas where thin-walled blood vessels replaced sinusoids. The latter became filled with leukemic mononuclear cells, a feature present to varying degrees in all cases of AMN leukemias. This infiltration, together with foci of lobular hyperplasia of the liver, constituted two of the most outstanding and common pathologic features of early and full-blown AMN leukemia in both 3-MCA-treated and control animals (see Figs. 5–8).

Cytogenetic Studies

Attempts to obtain material for direct chromosome preparations from the spleen, liver, and peripheral blood were unsuccessful in the great majority of cases. In vitro cell cultures for 1 to 4 days, with or without phytohemagglutinin, uniformly were unsuccessful. In general, mitoses were few and, when present, often failed to spread. During the initial studies bone marrow furnished numerous excellent metaphase plates. However, it became apparent that these were derived from red cell precursors participating in the marked erythroid hyperplasia associated with the hemolytic anemia. Attempts were made to study chromosomes in 63 of the 120 leukemias; suitable metaphases were obtained in only 17 animals. Significant aneuploidy was noted in 6 cases, and in 3 of these, morphologic abnormalities in the form of marker chromosomes were present. The distinctive Y chromosome, as well as the presence of marker chromosomes, in a male W/Fu rat with AMN leukemia are noted in Fig. 9.

DISCUSSION

In these experiments a high incidence of leukemia was found in W/Fu rats. We have also noted that, among inbred Fischer rats, the incidence of spontaneous leukemia was 26% while leukemia occurred infrequently in Wistar rats. The low incidence of leukemia in Long-Evans and Sprague-Dawley rats had been emphasized in the literature (12). Obviously, there is a wide difference in the occurrence of leukemia among various strains of rats. The relatively high incidence found among inbred W/Fu and Fischer rats strongly suggest the presence of either a vertically transmitted viral agent or an inherited predisposition to the disease. However, Furth has stated that continuous inbreeding by brother and sister mating did not increase the occurrence of leukemia in W/Fu rats (3, 7). Although this leukemia can be readily passaged by spleen cell suspension, repeated efforts over the past three years to transmit leukemia via cell-free filtrates have been unsuccessful in our laboratory.

Mononuclear cell leukemia in the albino rat was described by Oberling et al. in 1939 (10). Furth made additional observations in W/Fu rats; both groups of investigators emphasized the difficulties in classifying the cell type (3, 7). The cytologic and pathologic features make a myelogenous or lymphogenous origin of this leukemia unlikely. The cells, especially the more mature ones, are suggestive of a monocytic derivation. However, in humans with monocytic or mono-myelocytic leukemia, the serum and urine muramidase activity (lysozyme) is greatly elevated (2, 11). In our studies on rats with promyelocytic leukemia (chloroleukemia), we found that serum and urine muramidase was greatly increased (13). None of the rats with AMN leukemia showed elevated muramidase levels. The evidence to date favors a reticulum cell origin for AMN leukemia.

Several features of this disorder provide unique opportunities for the investigation of leukemogenesis. The long "latent" periods afford a favorable time interval in which to study the early phases of leukemia, and the distinctive cytoplasmic granules permits identification of leukemic cells, not only in
the peripheral blood but also in the liver, spleen, and lymph nodes. In an effort to localize leukemic cells in the preleukemia stage of the disease, serial liver biopsies were carried out on 40 rats at 4-month intervals, but thus far this experiment demonstrated only that liver involvement occurs relatively late in the disease. Further observations on a series of splenectomized rats are in progress.

Additional experiments are required to definitely establish that MCA significantly increases the incidence of AMN leukemia when administered to young, but not older, rats. If MCA does have a leukemogenic effect, there are several possible explanations of its \textit{modus operandi}. If a viral etiology is postulated, MCA may act as an immunosuppressive agent and enhance proliferation, release, or cellular penetration by leukemogenic viruses which are latent or have been vertically passedag in W/Fu rats. On the other hand, MCA may exert a leukemogenic effect by direct action on DNA, RNA, or other cellular constituents in a susceptible "target" cell.

It has been found that exposure of rats to ionizing radiation produces comparatively few leukemias (6). In our studies the marked reduction in the incidence of leukemia following total body radiation of 4-month-old W/Fu rats was an unexpected and interesting observation. In continuing experiments, total body X-ray of 12-month-old rats has not reduced the incidence of leukemia. These findings suggest that younger rats are more sensitive than older animals to the leukemia-inhibiting effect of X-ray. While the mechanism is not clear, it is possible that radiation destroys potentially leukemic cells or cells containing viruses capable of inducing leukemia. Studies are in progress of W/Fu and Fischer rats of various ages to further investigate the role of MCA and total body radiation on leukemogenesis.

The hemolytic anemia associated with AMN leukemia is of considerable interest. Preliminary studies indicate that hemolysis was not related to the presence of warm or cold agglutinins and that the direct Coombs test with anti-rat globulin was negative. The presence of lobular hyperplasia of the liver is another unusual feature, and further studies are indicated to determine if this lesion is a form of benign hepatoma. The possible relationships of leukemia, hemolytic anemia, and lobular hyperplasia of the liver to a virus (or viral agent) remains a matter of conjecture.

Cytogenetic studies carried out on the AMN leukemia have been largely unproductive due to the low mitotic rate and poor yield of suitable metaphase preparations. As in human acute leukemia, no consistent or constant chromosome abnormalities have been noted. It is curious that once the abnormal cell line has been transplanted, as noted by several investigators (9, 14), the marker chromosome is retained in passage, and good metaphase preparations are readily available. A direct relationship between demonstrable cytogenetic damage and the occurrence of leukemia following radiation or chemical carcinogens has not been established. However, the recent observations of Sugiyama et al. (15) on the presence of consistent chromosome abnormalities in a large percent of rats developing leukemia following 7,12-dimethylbenz(a)anthracene administration is of considerable interest.

As previously noted, the distinctive Y chromosome in the male W/Fu rat constitutes a cell marker which may be useful in experiments designed to establish the presence of a leukemogenic virus in AMN leukemia (16).

\section*{ACKNOWLEDGMENTS}

The authors are grateful to Helen Higgins, Arlene Keon, and Norma Litchfield for their excellent technical assistance and to John Carabits for the photomicrography. Dr. S. C. Chen performed studies on the nature of the hemolytic anemia in leukemic W/Fu rats. We are also grateful to Dr. Francis Herzon, Radiology Department, Cambridge City Hospital, Cambridge, Massachusetts, for the radiation aspects of this investigation.

\section*{REFERENCES}

Fig. 1, 2. Leukemic mononuclear cells in the peripheral blood smears showing typical cytoplasmic granules and presence of nucleated red cells. Wright stain, × 2550.

Fig. 3. Mononuclear cell with lobulated nucleus in the peripheral blood smear. Wright stain, × 2550.

Fig. 4. Arrow points to a cytoplasmic "bud" with nuclear fragments in the peripheral blood smear. Wright stain, × 2550.

Fig. 5. Imprint smear of the liver showing typical leukemic mononuclear cells containing cytoplasmic granules. Extracellular granules are due to disruption of cells during the process of imprinting. Wright stain, × 2725.

Fig. 6. Histologic section of liver from an untreated leukemic rat (R-2076), sacrificed at 16 months of age. The 0.5-cm subcapsular nodule of atypical lobular hyperplasia (benign hepatoma) is nearly completely represented in the more central frame of the photomicrograph. It is pale and poorly lobulated, but it is well delimited from the adjacent liver tissues on the right. The latter, at this magnification, present conspicuously dilated portal-periportal thin-walled vascular channels filled with mononuclear leukemic cells. Hematoxylin and eosin, × 30.

Fig. 7. Histologic section of liver from an untreated W/Fu leukemic rat (R-2398) sacrificed at 20 months of age. The portion of atypical lobular hyperplasia (benign hepatoma) represented in the upper half of this field exhibits the characteristic cytomegalic features with large, prominent liver cell nuclei and abundant, faintly acidophilic, and faintly vacuolated cytoplasm. Compression of the normal liver cells by the regenerating nodule may be noted. Here, in contrast to the adjacent liver tissues below, sinusoids contain comparatively few mononuclear leukemic cells. Hemosiderin-laden Kupffer cells are not well evidenced. Hematoxylin and eosin, × 250.

Fig. 8. Histologic section of liver away from nodules of atypical lobular hyperplasia; same case as Fig. 6. Portal-periportal thin-walled vascular channels are markedly distended by densely packed leukemic mononuclear cells. The latter are to be seen permeating neighboring sinusoids. Hematoxylin and eosin, × 300.

Fig. 9. Karyotype of leukemic mononuclear cell. Direct preparations from the liver showing aneuploidy (43 chromosomes) with loss of one of the 19th pair of autosomes and the presence of two marker chromosomes. Note also the distinctive Y chromosome in this male W/Fu rat.
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