Synergistic Action of Radiation and Virus in Induction of Leukemia in Rats

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SUMMARY

More than 50% of Wistar/Furth (W/Fu) strain rats, which received combined treatment with a fractionated total-body X-irradiation and an injection of a mouse leukemia virus (Gross virus), developed leukemia within 7 months following treatment. The majority of the induced leukemias were thymic lymphomas with a short latency period similar to those observed in susceptible strains of mice and W/Fu rats after the neonatal inoculation of this virus. Also, a few cases of nonthymic lymphoid leukemia and myeloid leukemia of the chloroma type occurred with a longer latency period.

Persistence and multiplication of the inoculated virus in leukemic rats were suggested by the development of leukemia in recipients which were inoculated at birth with cell-free filtrates prepared from these leukemic rats. In contrast to these findings, no leukemia occurred among rats which received either fractionated total-body X-irradiation alone or virus inoculation alone.

As to the mechanism of leukemogenic synergism of radiation and virus, it is suggested that radiation acts as a modifier for both the physiologic state of the target cell of the virus and the immunologic responsiveness of the host. Thus, it might promote an interaction between the target cell and virus and a proliferation of antigenically altered leukemic cells.

INTRODUCTION

Implication of leukemogenic virus in radiation leukemogenesis in mice has been suggested by several investigators (5, 10, 16, 17, 19, 25). It has also been demonstrated in our laboratory that leukemias induced in a low leukemic strain of mice either by total-body X-irradiation or by injection of radiostrontium (90Sr) could be transmitted to mice of the same strain by neonatal inoculation of leukemic cell-free extracts or filtrates (18).

However, an analysis of the role of the virus in the radiation leukemogenesis in mice seems to be not wholly satisfactory due to the fact that many mouse strains are naturally infected with the leukemogenic virus and that total-body X-irradiation alone produces leukemia at a high rate. On the other hand, it seems to be advantageous to pursue such an analysis in rats because the incidence of radiation-induced leukemia is generally low (27), and thus far a rat leukemogenic virus has never been isolated.

The present paper describes data obtained from our experiments on synergistic action of total-body X-irradiation and mouse leukemogenic virus in induction of leukemia in rats.

MATERIALS AND METHODS

Animals. The Wistar/Furth (W/Fu) strain of rats was used. One litter of young W/Fu rats was generously given by Prof. Jacob Furth, Department of Pathology, College of Physicians and Surgeons, Columbia University, New York; their offspring have been produced by brother-sister mating in our laboratory since 1962. The frequency of spontaneous leukemia in this strain has been reported to be low, and it occurs only in older rats (22).

Factors of X-irradiation. X-irradiation was provided with a 180 kvp X-ray generator, the factors being 25 ma, HVL 1.18 mm Cu, with 0.5 mm Cu and 0.5 mm Al filters; it delivered 50 R/min at the TSD 65 cm.

Virus. A leukemic female W/Fu rat which had been inoculated at birth with a rat-adapted passage A Gross leukemia virus (29) was obtained by courtesy of Dr. Hiromitsu Okano, Cancer Institute, Kyushu University, School of Medicine, Fukuoka, Japan. A leukemic filtrate containing the virus was prepared from the rat by a procedure described by Levinthal et al. (24). The virus has been maintained in our laboratory through successive cell-free passages in W/Fu rats by inoculating 0.1 ml of leukemic filtrate intraperitoneally within 48 hours after birth of the animals. The virus preparation was stored in an electric deep freezer at -100°C until used.

In the principal experiment, 76 rats of both sexes were divided into 5 groups. In the first group, the rats received 600 R of total-body X-irradiation delivered in 4 doses of 150 R each at 5-day intervals. Irradiation was started when rats were 4 to 5 weeks of age. Then they were inoculated intraperitoneally with 0.4 ml of Gross virus a few hours after the last X-irradiation. In the second group, rats received the fractionated total-body X-irradiation alone, and the third group of rats was inoculated with the virus alone with identical doses and time schedules as the first group. In addition, two litters of newborn rats were inoculated intraperitoneally, within 48 hours after birth, with 0.05 ml of the same lot of the virus preparation in order to certify the leukemogenic activity of the virus. Two litters of untreated rats served as controls for all of the experiments.

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The experimental and control animals were kept in metal cages in an air-conditioned animal room (22 ± 2°C) and were fed with commercial pellets and tap water ad libitum. When the occurrence of leukemia was suspected by the development of dyspnea with thoracic distention, splenomegaly, enlargement of the peripheral lymph nodes, or the appearance of abnormal cells in the peripheral blood, the rat was sacrificed and carefully autopsied. Tissues for microscopic examination were stained with hematoxylin and eosin. Blood films and tissue imprints were stained routinely with May Grünwald-Giemsa stain. When necessary, peroxidase activity was estimated with McJunkin's stain, and alkaline phosphatase was stained with hematoxylin and eosin. Blood films and tissue imprints were stained routinely with May Grünwald-Giemsa stain. When necessary, peroxidase activity was estimated with McJunkin's stain, and alkaline phosphatase was estimated by the Naphthol AS-MX method described by Tomonaga et al. (34). The experiment was terminated on the 400th day after treatment. All rats living at the termination were also examined in the same manner as the leukemic rats.

**RESULTS**

**Prevalence of Leukemia.** The experimental groups and the occurrence of leukemia in various groups up to 400 days following treatment are summarized in Table 1. The neonatal inoculation of Gross virus in this strain of rats showed a high leukemogenicity as previously reported (29). All 15 rats developed typical thymic lymphomas, with or without blood invasion, within 80 days after viral inoculation. However, the susceptibility of the rat to the virus appeared to rapidly decrease as the age of the rat increased; no leukemia occurred when the virus was inoculated at 7 to 8 weeks of age. Fractionated total-body X-irradiation started at 4 to 5 weeks of age was also ineffective in eliciting leukemia up to 400 days following the termination of irradiation. Adenocarcinoma of the breast occurred in two female rats of this group about 9 and 12 months after irradiation, respectively. No tumor development was observed in control rats.

In contrast to the above groups, 11 out of 20 rats (55.0%) that received a combined treatment of X-irradiation and virus inoculation developed leukemia with a latency from 77 to 213 days. Seven cases of the induced leukemias occurred with a shorter latency period, from 80 to 120 days, and originated in the thymus; these cases were similar to those observed in susceptible strains of mice (9) and W/Fu rats after neonatal virus inoculations. Three of the 4 remaining cases were nonthymic lymphoid leukemias, and one was myeloid leukemia of the chloroma type; the latency period of the myeloid leukemia was much longer than that of the thymic lymphoma. There was no clear difference in the prevalence of leukemia between sexes because the number of the rats was small, 6 cases in females (50.0%) and 5 cases in males (62.5%) respectively.

**Leukemogenicity of the Cell-free Filtrates Prepared from the Leukemic Tissue of the Induced Leukemia.** Two leukemic cell-free filtrates were prepared from 2 thymic lymphomas induced by a combination of X-irradiation and Gross virus injection. These preparations were inoculated into each of two litters of newborn W/Fu rats within 48 hours after birth to test their leukemogenic activity. All but one (13 out of 14) of the recipients developed typical thymic lymphomas with a latency period of from 65 to 110 days, thus indicating the persistence and multiplication of the Gross virus originally inoculated in the host.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats of each sex</th>
<th>No. of rats that contracted leukemia (%)</th>
<th>Latency (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray + virus</td>
<td>12 F 8 M</td>
<td>6 (50) 5 (62.5)</td>
<td>77-213</td>
</tr>
<tr>
<td>X-ray alone</td>
<td>5 F 7 M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virus alone</td>
<td>6 F 9 M</td>
<td>9 (100)</td>
<td>80-90</td>
</tr>
<tr>
<td>Virus alone + X-ray</td>
<td>9 F 6 M</td>
<td>6 (100)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>6 F 8 M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Incidence of leukemia in W/Fu rats treated with X-rays and/or Gross virus. F, female; M, male.

600 R total-body X-irradiation delivered in 4 doses of 150 R each at 5-day intervals, started at 4-5 weeks of age.

Intraperitoneal injection of 0.4 ml of the passage A Gross virus a few virus a few hours after the 4th X-irradiation (at 7-8 weeks of age).

Intraperitoneal injection of 0.05 ml of the passage A Gross virus within 48 hours after birth.

**DISCUSSION**

The present study clearly demonstrates that both radiation and virus are necessary for the induction of leukemia in the young rats which, however, failed to develop leukemia following the single application of either agent. Furthermore, cell-free transmissibility of the induced leukemia suggested that the inoculated virus had been multiplying in X-irradiated rats, leading to malignant transformation of the target cells. Several hypotheses have been proposed for the mechanism of radiation carcinogenesis (7, 35). In view of the fact that radiation itself is a potential mutagen, it is reasonable to speculate that radiation-induced mutation in the hematopoietic cells may be responsible for the development of leukemia in an irradiated subject. In our present study with young rats, however, this does not seem to be the case. X-irradiation alone did not lead to the development of leukemia, at least during an observation period of 400 days. A hypothesis first proposed by Gross (11) that, radiation in mice acts merely as an activator of a latent leukemogenic virus carried by the host, seems also not to be applicable to our data with rats. The hematopoietic cells of the young adult rats never showed a neoplastic response upon inoculation of the concentrated active virus (Gross virus), which is highly leukemogenic in newborn rats of the same strain. It should be emphasized that total-body X-irradiation may act as a modifier for the physiologic state of the target cell, providing a favorable environment for viral multiplication rather than merely activating "latent virus."

Kaplan (20) has postulated three simultaneous effects of
total-body X-irradiation in induction of thymic lymphoma in C57BL mice: mobilization of the latent leukemogenic virus into the thymus from other body sites through the bloodstream; injury to the thymus followed by a vigorous regeneration; and damage to the bone marrow, which in turn interferes with a smooth regeneration of the injured thymus, leading to a state of maturation arrest in which a large number of immature lymphoid cells are made available to interact with mobilized virus for a sufficient period of time. In this process, he has defined the regenerating thymic lymphoid cells as the target cell of the virus, and an interaction between target cell and virus takes place within the thymus. This idea seems to be supported by recent studies of Kaplan and his coworkers (14) in which they demonstrated that lymphomas consistently developed at a high rate in the inoculated lobe when a leukemogenic virus (RadLV), originally recovered from radiation-induced leukemia of C57BL mice, was inoculated directly into one lobe of the thymus in situ of newborn C57BL mice. Kaplan also showed that the thymic lymphocytes of the newborn C57BL mice undergo infection by RadLV in vitro, which was followed by neoplastic transformation to lymphoma cells when the infected cells were transplanted into adequately conditioned recipients (21).

However, the origin of the thymic lymphoid cells, which emerge during the middle stage of embryogenesis, still seems to be under dispute. The question is whether they have been derived from the epithelial cells of the thymic rudiment or have migrated into the thymus from other sites of the body (1, 32). It is now well established that, in adult mice, a depleted thymus is repopulated by bone marrow cells, at least under conditions where there has been injury in the myeloid-lymphoid complex (6, 26, 31). Moreover, an experiment recently reported by Ball (2) strongly suggests that at least some progenitor cells of thymic lymphomas induced by a chemical carcinogen (7,12-dimethylbenz(a)anthracene) are derived from bone marrow. Taking these observations into consideration, it is speculated that bone marrow and spleen cells are also eligible as target cells of the virus in addition to those in the thymus, and that they can interact with the virus in situ. This interpretation may be supported by recent observations that cell-free extracts of bone marrow and thymus derived from mice shortly after X-irradiation are leukemogenic (13), and that virus particles of C type could be observed in almost equal numbers in various hematopoietic organs within a day or two after total-body X-irradiation(12).

It has been demonstrated that, in newborn rats, thymic lymphoid cells are most susceptible to the Gross virus, but this virus also possesses an affinity for the myeloid cells, though to a lesser extent (23). In our previous studies (33, 36), it was shown that, in adult RF mice, total-body X-irradiation followed by an injection of either Gross virus or Moloney virus significantly increased the induction rate of myeloid leukemia without affecting that of thymic lymphoma. We ascribed this phenomenon to the acceptance of viruses by the myeloid cells during a heightened proliferative activity following X-irradiation. Similarly, Graffi (8) has shown that the incidence of myeloid leukemia was elevated either by X-irradiation or by polycyclic hydrocarbons given to the Graffi virus-infected mice.

The present experimental results may be attributed to a sequence in which X-irradiation alters the hematopoietic system of young adult rats, reverting the system to the neonatal state during the recovery phase. This confers a susceptibility to the Gross virus on the rats which in turn leads to the eventual development of thymic lymphomas in most cases and to myeloid and nonthymic lymphoid leukemias in other cases.

The effect of irradiation (15) and inoculation of leukemogenic viruses (3, 4, 28, 30) in suppressing immunologic responses has been well documented. A dual effect by both agents might also contribute to making the host tolerant to the inoculated virus and to facilitating the proliferation of leukemic cells which might have acquired a new antigenic property.

REFERENCES


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