Electron Microscopic Studies of Radiation-induced Leukemia in Mice: Virus Release following Total-Body X-ray Irradiation

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SUMMARY

Within a few days after total-body X-ray irradiation, leukemia virus particles could be observed in several organs of irradiated C3Hf mice. This was observed not only after fractionated irradiation (5 × 150 R), but also after a single (250 R) total-body X-ray irradiation. Electron microscopic examination of several organs from the irradiated mice, such as spleen, lymph nodes, and bone marrow, revealed the presence of virus particles budding from the cell membranes; mature and immature type C particles were also present in the intercellular spaces.

Leukemia virus particles were also consistently present in organs of eight C3Hf mice with radiation-induced leukemia. The distribution of virus particles in organs of mice with radiation-induced leukemia was similar to that observed in mice with spontaneous or virus-induced leukemia; however, the number of virus particles observed in organs of mice with radiation-induced leukemia was lower than that observed in mice with passage A (Gross) virus-induced leukemia.

These observations contrasted sharply with results of electron microscopic examination of organs of nonirradiated, normal C3Hf mice, which revealed the presence of usually only a few isolated virus particles in the thymus and occasionally also in lymph nodes or in the bone marrow.

INTRODUCTION

It was demonstrated in previous studies that a filterable and transmissible virus could be isolated from radiation-induced leukemia in mice. The isolated virus could be passed serially by filtrates inducing leukemia following inoculation into susceptible mice (5–12). It was therefore concluded that leukemia induced in mice by total-body X-ray irradiation was actually caused by an activation of a latent virus carried by the irradiated hosts.

Electron microscopic studies demonstrated the presence of virus particles in organs of mice in which leukemia had been induced with the passaged virus, such as passage X in C3Hf mice (3) or a similar passaged virus in C57 Black mice (1, 2). No virus particles could be found in organs of mice with primary radiation-induced leukemia in these studies. In another study carried out on RF mice (13), the presence of virus particles was observed in mice in which leukemia was induced either by total-body X-ray irradiation or with the passaged radiation-activated virus.

In the present study virus particles were found in several C3Hf mice with primary radiation-induced leukemia. Furthermore, experiments reported here revealed the rather unexpected presence of budding virus particles in lymph nodes, spleen, and bone marrow of the irradiated mice within only a few days after the irradiation, i.e., a long time prior to the development of leukemia.

MATERIALS AND METHODS

Mice from our colony of the C3Hf inbred line received fractionated total-body X-ray irradiation, 150 R each, at weekly intervals for 4 to 5 consecutive weeks, a total of 600 or 750 R respectively.

Since female mice were found to be more susceptible than males to the induction of leukemia by X-ray irradiation, with few exceptions only female mice were used in this experiment. The irradiation was started when the animals were 3 to 5 weeks old.

In the first group the animals were kept for observation until they developed radiation-induced leukemia. In a second group the animals were sacrificed 8 to 40 days after the last fractionated irradiation, and their organs were then used for electron microscopic studies. In a third group the animals received only a single total-body X-ray irradiation of 250 R. One to 16 days after the irradiation, the animals in this group were sacrificed and their organs were embedded and used for electron microscopic studies.

The technical factors were as follows: 200 kv, 9.5 mm Cu and 1 mm Al filter, 1.5 mm Cu IVL, 100 cm distance (target to midplane of mouse), 18 ma, 15 R/min (rate of output), and a portal size of 15 x 15 cm. Irradiation time was 10 min for 150 R and 16 min 40 sec for 250 R. The irradiation was performed at the Radiation Therapy Service of this Hospital (Dr. B. Roswit, Chief).

First Group: Fractionated Total-Body X-ray Irradiation Followed by Development of Primary, Radiation-induced Leukemia. Six female and 2 male mice from our C3Hf colony, at ages 4 to 5 weeks received fractionated total-body X-ray irradiation, 150 R each, at weekly intervals for 4 consecutive weeks, total 600 R. All mice developed leukemia between 5
and 14.5 months of age. The leukemic mice were sacrificed, and their organs, such as thymus, spleen, lymph node, and bone marrow, were removed and processed for electron microscopy.

Second Group: Fractionated Total-Body X-ray Irradiation. Mice Sacrificed Prior to Development of Radiation-induced Leukemia. Thirteen female C3H mice received total-body X-ray irradiation, 150 R each, at the age of 3 to 4 weeks for 5 consecutive weeks, total of 750 R. The irradiated mice were sacrificed at 8, 15, 19, 22, 23, 27, 30, 36, or 40 days after the last irradiation. Samples from 10 mice were examined: fragments of thymus, spleen, lymph node, and bone marrow were removed and processed for electron microscopy.

Third Group: Single Total-Body X-ray Irradiation. Mice Sacrificed Prior to Development of Radiation-induced Leukemia. Nine female C3H mice received a single dose of total-body X-ray irradiation, 250 R, 3 to 4 weeks of age. The mice were sacrificed 1, 2, 3, 4, 7, 9, 11, 14, or 16 days after irradiation. Fragments of thymus, spleen, lymph node, and bone marrow were removed from the irradiated mice and processed for electron microscopy.

Controls. Fifteen normal, nonirradiated C3H female mice 1 to 3 months of age served as controls to the irradiated mice.

Preparation of Specimens for Electron Microscopy. Immediately after the donor mice were sacrificed by ether inhalation, fragments of thymus, spleen, lymph nodes and bone marrow were removed and fixed either directly in 1% phosphate-buffered osmic acid for 1 to 1.5 hours on ice, or alternatively in 4% glutaraldehyde for several days or weeks, followed by osmic acid. The specimens were dehydrated in successive changes of 50 to 100% ethanol, immersed in propylene oxide, and embedded in Epon. The tissues were then sectioned with a diamond knife using a Porter-Blum microtome and placed on uncoated 300-mesh copper grids. The sections were lightly coated with carbon, stained with uranyl acetate and lead hydroxide, and examined in an RCA EMU-3G electron microscope at 50 kv (D. G. Feldman).

RESULTS

Radiation-induced Leukemia. Twelve tissue specimens which included spleen, bone marrow, lymph node, and thymus from 8 leukemic mice were examined for the presence of leukemia virus particles (Table 1). In 9 specimens studied, virus particles were present in relatively small numbers appearing singly or in small groups. In 3 specimens, two of spleen and one of lymph node, particles appeared more frequently and in larger groups. Budding particles and doughnut-like immature C particles were most frequently observed; mature C particles containing nucleoids were present but in lesser numbers. Electron micrographs of spleen (Figs. 1, 2) and lymph node (Figs. 3, 4) illustrate groups of virus particles consisting of particles budding from the cell membrane (arrows), doughnut-like immature C particles (d), and mature C particles with nucleoids (n). In Fig. 1 one of the doughnut-like particles (lower right) is not completely detached from the cell and is still connected by a narrow bridge of cytoplasm. The particle containing a nucleoid in Fig. 2 appears to have a faint tail-like attachment.

The average diameter of doughnut-like immature C particles was about 95 m, the diameter of the mature C particles with nucleoids was approximately 105 m.

150 R at 5 Weekly Intervals; Mice Sacrificed 8 to 40 Days after Last Irradiation. Electron microscopic study of spleen, bone marrow, lymph node, and thymus from mice at various intervals ranging from 8 to 40 days after the fifth irradiation revealed the presence of virus particles in all but 2 specimens examined (Table 2). Leukemia virus particles were observed in spleen from irradiated mice 8, 15, 22, 23, 27, 30, 36, and 40 days after the last irradiation. Particles also appeared in bone marrow 22 days after the last irradiation. Particles were observed in lymph node 15, 23, 30, 36, and 40 days after the last irradiation. In thymus, particles were observed 8, 15, and 22 days after the last irradiation.

In all specimens of bone marrow and thymus where particles were found, in all but one sample of spleen, and in 3 specimens

2 The irradiated mice were examined, and gently palpated, twice weekly; gradual development of enlarged lymph nodes and of a palpable, enlarged spleen represented usually the first signs of the onset of leukemia. Most of these mice also developed thymic lymphomas. The presence of a thymic tumor could be suspected only in the more advanced cases by difficulty in breathing. Peripheral (tail) blood examination was useful but had only a limited diagnostic value, since it did not consistently reveal presence of abnormal blood cells. However, in terminal phases of the disease, examination of peripheral blood revealed in most of the leukemic animals an elevated white blood cell count, presence of lymphoblasts, and a moderate or moderately severe anemia.

3 Instead of using exclusively arbitrary designations by alphabet letters, we have preferred to employ also descriptive terms indicating the morphology of the observed virus particles. According to classification terminology suggested by W. Bernhard (The Detection and Study of Tumor Viruses with the Electron Microscope. Cancer Res., 20: 712-727, 1960), and more recently modified (Classification of Oncogenic RNA Viruses. J. Natl. Cancer Inst., 37: 395-397, 1966), the term "doughnut-like" particle employed in our study corresponds to "immature C" particle and the term "particle containing a nucleoid" employed in our study corresponds to "mature C" particle.
Table 2

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Age mice sacrificed (mo.)</th>
<th>No. of days after last irradiation</th>
<th>Spleen</th>
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Distribution of virus particles in organs of C3Hf mice observed 8 to 40 days following fractionated (5 x 150 R at weekly intervals) total-body X-ray irradiation. Approximate average number of virus particles per grid square using 300-mesh grids: blank space, not examined; +, less than 5 (few); ++, 5-10 (moderate).

Table 3

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Distribution of virus particles in organs of C3Hf mice observed 1 to 16 days following a single (250 R) total-body X-ray irradiation. Approximate average number of virus particles per grid square using 300-mesh grids: blank space, not examined; +, no particles observed; +, less than 5 (few).
thus far observed budding from vacuolar membranes of mega-
particle was found in one specimen only; no particles were
9 days after irradiation appears in Fig. 15.

containing a nucleoid proximal to an erythroblast from spleen
row 7 days after irradiation. A mature C virus particle (n)
located only after a prolonged and diligent search. Fig. 16 illus-
trates a budding particle (arrow) in a normal lymph node.

Eleven samples of lymph node were examined. In 3 spec-
imens budding and doughnut-like immature C particles could
be found; however, they were extremely sparse and could be
located only after a prolonged and diligent search. Fig. 16 illus-
trates a budding particle (arrow) in a normal lymph node.

Examination of 3 specimens of thymus revealed the presence
of a few scattered budding or immature C virus particles in
2 specimens.

DISCUSSION

The mechanism of the development of radiation-induced leu-
kemia was studied. It has been known that, following total-body
X-ray irradiation, mice of certain low-leukemic strains such
as C3Hf or C57 Black develop a high incidence of leukemia.
More recently a virus could be recovered from mice with radia-
tion-induced leukemia (5-12).

In experiments reported in this study, characteristic C virus
particles could be found consistently in organs of 8 C3Hf mice
with radiation-induced leukemia. The distribution of virus parti-
cles in organs of C3Hf mice with radiation-induced leukemia
was similar to that observed in mice with spontaneous leu-
kemia or in those with passage A (Gross) virus-induced leukemia. However, the number of virus particles found in
organs of mice with radiation-induced leukemia was relatively
low, comparable to that observed in Ak mice with spontaneous
leukemia, but lower than that observed in organs of mice in
which leukemia had been induced with the passage A (Gross)
mouse leukemia virus.

Normal mice often carry a latent potentially leukemogenic
virus. However, in normal healthy C3Hf mice, the presence of
virus particles could be detected almost exclusively only in the
thymus (4) and only very occasionally also in bone marrow and
lymph nodes.

Results of experiments here described also revealed that
virus particles may appear in a variety of organs, occasionally
in relatively large numbers, within a few days after total-body
X-ray irradiation of C3Hf mice. The rather sudden appearance
of virus particles in organs of mice only a few days after ir-
radiation may be interpreted by an assumption that irradiation
activates a leukemogenic virus previously carried in a latent
form by such mice. The potentially pathogenic virus triggered
by irradiation begins to form by budding and is later released
by the carrier-cells as a preliminary step in the chain of events
which may lead ultimately to the development of leukemia.
This interpretation would be consistent with the concept pre-
viously expressed (5, 6, 12) that radiation-induced leukemia
is the result of an activation of a latent virus carried by the
irradiated hosts.

The observations described in this study are also consistent
with the results of recent experiments of Haran-Ghera (7, 8),
which demonstrated that extracts prepared from organs of ir-
radiated mice of a low-leukemic strain only a few days after
irradiation of the donor animals were leukemogenic on bio-
assay tests, suggesting thereby that they contained infectious
virus particles. Inoculation of normal organs extract from non-
irradiated mice of low-leukemic strains did not induce leu-
kemia.

REFERENCES

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102-105, 1959.
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Presence of virus particles in organs of nonirradiated control
C3Hf mice. Approximate average number of virus particles per
grid square using 300-mesh grids: blank space, not examined; 0,
no particles observed; +, less than 5 (few).
Radiation-induced Leukemia

from Irradiated Mouse Thymus and Bone Marrow. Intern. J. Cancer, 1: 81-87, 1966.

Figs. 1, 2. Areas of spleen from mice with radiation-induced leukemia illustrating the presence of leukemia virus particles. A budding particle (arrow), doughnut-like immature C particles (d), and a mature C particle with a nucleoid (n) are shown. Fig. 1, × 62,000; Fig. 2, × 62,000.

Figs. 3, 4. Sections of lymph node from a mouse with radiation-induced leukemia demonstrating budding particles (arrows), a doughnut-like immature C particle (d), and mature C particles with nucleoids (n). Fig. 3, × 62,000; Fig. 4, × 62,000.

Fig. 5. Area of spleen from a mouse which received 150 R at 5 weekly intervals; 8 days after the last irradiation. × 23,600.

Fig. 5a. A higher magnification of the outlined area in Fig. 5 showing a particle (arrow) budding from the cell membrane. × 62,000.

Fig. 6. Part of an erythroblast in spleen from a mouse which received 150 R at 5 weekly intervals; 15 days after the last irradiation. A particle (arrow) appears to be budding from the cell membrane. × 62,000.

Fig. 7. Part of a plasma cell of spleen from a mouse which received 150 R at 5 weekly intervals; 27 days after the last irradiation. A leukemia virus particle (arrow) appears to be budding from the cell membrane. × 84,000.

Figs. 8, 9. Parts of 2 megakaryocytes from bone marrow from mice which received 150 R at 5 weekly intervals. Fig. 8, 15 days; and Fig. 9, 23 days after the last irradiation. Leukemia virus particles (arrows) are shown budding from vacuolar membranes. Fig. 8, × 62,000; Fig. 9, × 62,000.

Figs. 10-12. Areas of lymph node from a mouse which received 150 R at 5 weekly intervals; 15 days after the last irradiation. Figs. 10 and 11 demonstrate leukemia virus particles (arrows) budding from several areas along the cell membrane of plasma cells. In Fig. 12 doughnut-like immature particles (d) and a mature particle with a nucleoid (n) appear in an intercellular space. Fig. 10, × 62,000; Fig. 11, × 37,450; Fig. 12, × 52,000.

Fig. 13. Section of spleen from a mouse which received one dose of 250 R; one day after irradiation. A leukemia virus particle (arrow) appears to be budding from the cell membrane. × 52,000.

Fig. 14. An area from bone marrow from a mouse which received one dose of 250 R; 7 days after irradiation. A virus particle (arrow) is shown budding from the cell membrane of an erythroblast. × 62,000.

Fig. 15. Part of an erythroblast from spleen from a mouse which received one dose of 250 R; 9 days after irradiation. A mature particle (n) containing a nucleoid appears proximal to the cell × 62,000.

Fig. 16. Part of a cell from lymph node from a normal nonirradiated mouse showing a leukemia virus particle (arrow) budding from the cell membrane. × 42,800.

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