The Development of Leukemia in Lethally Irradiated Mice Protected with Cells from Mice of a High Incidence Leukemia Strain

By ESTHER FINCHER HAYS AND EDWARD M. WHITE

These studies were instituted to determine if cells from healthy mice of a high incidence leukemia strain (AKR) would manifest their leukemic potential in a foreign host. Previous experiments carried out by Miller1 and in our laboratory2 showed that mice of a low incidence leukemia strain which were made immunologically tolerant with neonatal injection of spleen and thymus cells from healthy AKR mice did not develop leukemia. It was concluded that although the tolerant mice were cellular chimeras and maintained viable populations of AKR cells, these donor cells were apparently not capable of undergoing leukemic transformation. It was postulated that cells of the thymus which are capable of leukemic change did not survive the transfer. It has been shown by Miller3 that an intact AKR thymus graft in a tolerant C3H host will produce generalized leukemia, frequently of AKR genetic type. These several studies, then, lead to the conclusion that the thymus from the AKR mouse must be transplanted as an intact organ in order for leukemia to develop, and that suspensions of viable lymphoid or hematopoietic cells from healthy AKR mice can survive, but are not leukemogenic, in a tolerant host.

The experiment reported here was designed to test the leukemic potential of normal AKR cells in a foreign host environment using another experimental method. Adult animals of a low incidence leukemia strain (C3HeB) were lethally irradiated and protected with viable cells from a high incidence leukemia strain (AKR). These animals were then observed for the development of leukemia for 43–45 weeks post-irradiation. They were tested for persisting viable allogeneic cells with normal AKR skin grafts 4 months following irradiation.

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MATERIALS AND METHODS

Mice

The host mice were males of the C3HeB/JAX strain. They were 7–9 weeks of age at the time of irradiation. The bone marrow and thymus cell donors were healthy 7–20-week-old AKR/JAX and C3HeB mice of both sexes. All of the C3HeB mice were obtained from the Jackson Memorial Laboratory, Bar Harbor, Me. The 7–8-week-old AKR/JAX donors were of the same source. The 17–20-week-old AKR mice were from the breeding colony maintained in this laboratory. This colony is in its 15th generation of inbreeding. Spontaneous leukemias only occur in animals over 24 weeks of age. The mice had access ad libitum to commercial rodent pellets and water containing 0.2 mg/cc. oxytetracycline hydrochloride, with glucosamine and vitamins. All mice were observed until natural death or sacrificed at 1 year of age (43–45 weeks post-irradiation).

Irradiation

The C3HeB mice were irradiated in groups of 10 with 750–800 r total body irradiation, a dose which was shown to be 100 per cent lethal to non-protected mice of this strain. The radiation factors were: 250 kv; 15 ma.; filtration 0.50 mm. Cu parabolic + 1.0 mm. al; target distance 45 cm.; dose rate 51–54 r/min.

Cell Suspensions and Injection

All suspensions of bone marrow were prepared from the femora and tibiae removed from mice sacrificed with ether. The bones were stripped of tissue and the marrow extracted by flooding the lumen with Tyrode's or Hank's solution to which had been added antibiotics (100–200 units/ml of penicillin and streptomycin), using a 25-gauge needle. The marrow suspension was then centrifuged at a low speed for 10 minutes. The supernatant fluid was discarded and the marrow cells resuspended in the physiologic solution. The thymus cells were obtained by removing the intact organ and placing it in Hank's or Tyrode's solution with the same concentration of antibiotics as noted above. The cells were then teased from the thymus, dispersed by aspirating with a 24-gauge needle, and washed one time with the salt solution. The thymus and bone marrow cells were counted and tested for viability by mixing with 0.2 per cent eosin. The unstained cells were counted as viable. The bone marrow cells were injected in 0.25 cc. amounts containing 8–10 million cells, 85–90 per cent of which were viable. When cells from the two sources were used, each 0.25 cc. injection consisted of 6–8 million bone marrow cells and 1.5–2 million thymus cells of which 85–90 per cent were viable. All of the injections were made into the tail vein, 4–6 hours after irradiation.

Skin Grafts

The skin grafts were done by Dr. W. H. Hildemann of the Department of Medical Microbiology and Immunology, UCLA School of Medicine, using the method of Billingham and Medawar. Grafting was performed on representative surviving C3HeB mice 15–19 weeks after irradiation. The grafts were placed on the right posterior thorax of the animals with the hair in a reverse direction.

Pathology and Histology

An autopsy was performed on each animal. Sections of the liver, spleen, lymph nodes, thymus and kidneys were made for microscopic examination from each of the animals dying 2 months or later after the irradiation and from representative animals dying before that time. The organs were fixed in Bouin's fluid and stained with hematoxylin and eosin.

RESULTS

The results are summarized in figure 1 and table 1. Three groups of mice
were employed. Group I was given AKR bone marrow. Group II received AKR bone marrow plus thymus cells, and Group III was protected with isogeneic marrow. There was a 33–61 per cent mortality within the first 6 weeks in all groups. The most common findings in these animals at autopsy were apparently sequellae of irradiation, including pneumonia, diarrhea, and hemorrhage. No evidence of leukemia or other neoplasms was observed in the animals dying during this period. No animals showed weight loss with splenomegaly and lymphoid atrophy which led us to conclude that a graft vs. host syndrome did not develop.

Group I consisted of 55 animals irradiated with 750–800 r and given AKR bone marrow. Post-irradiation sequellae resulted in the death of 35 mice within 6 weeks. Of the 20 survivors, four developed leukemia 24–43 weeks after irradiation. These leukemias were all similar to the lymphocytic neoplasm occurring spontaneously in AKR mice and the cells grew progressively in AKR but in C3HeB mice when transferred. One mouse in this group developed a parotid gland tumor and an epithelial thymoma at 22 weeks after irradiation. The thymoma was transferred by cells and did not grow in either strain. Another animal developed bilateral parotid tumors and an adrenal tumor. The adrenal neoplasm did not grow in either strain after cell transfer.

Group II consisted of 57 C3HeB mice irradiated with 750–800 r and pro-
Table 1.—The Incidence of Leukemia and Other Tumors in C3HeB Mice Irradiated and Protected with Allogenic (AKR) and Isogenic Cells

<table>
<thead>
<tr>
<th>Cells Injected</th>
<th>Total Animals Irradiated</th>
<th>Survivors Over 6 Weeks</th>
<th>Survivors with Leukemia</th>
<th>Survivors with Other Tumors</th>
<th>AKR Skin Graft*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKR bone marrow</td>
<td>55</td>
<td>20</td>
<td>4 (20%)</td>
<td>2</td>
<td>5/5</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKR bone marrow plus thymus</td>
<td>57</td>
<td>27</td>
<td>10 (37%)</td>
<td>1</td>
<td>6/6</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3HeB bone marrow</td>
<td>33</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0/9</td>
</tr>
</tbody>
</table>

*Number of viable grafts/number of animals grafted.

ected with AKR bone marrow to which AKR thymus cells had been added. The sequellae of irradiation resulted in the death of 30 animals within the first 6 weeks. Of the survivors, 10 developed leukemia from 14–38 weeks after irradiation. These leukemias all resembled the AKR spontaneous lymphocytic lymphoma and all involved the thymus. When transferred by cells, they grew progressively in AKR but not in C3HeB mice. One mouse in this group developed an epidermoid carcinoma, at 25 weeks post-irradiation. This tumor was probably of esophageal origin and did not grow after cell transfer to C3HeB or AKR mice.

Group III was the control group, and consisted of 33 mice irradiated with 750–800 r. This group was protected with bone marrow from 7–8-week-old C3HeB donors. Eleven animals died within the first 6 weeks from post-radiation sequellae. No leukemia or tumors developed in the 22 survivors.

Fifteen of the survivors in Group I received bone marrow from AKR mice of 7–8 weeks of age and five from 17–20-week-old donors. All four leukemias developed in the animals receiving the 7–8-week-old marrow at 24 to 43 weeks after irradiation. Twenty-two of the survivors in Group II received thymus cells from 7–8-week-old donors, with six leukemias developing at 24–34 weeks after irradiation. The incidence and onset of leukemia in the animals receiving young cells in the two groups therefore was comparable. The remaining five animals in Group II were given thymus cells from 17–20-week-old donors. Four of these five animals developed leukemia at 14, 22, 33 and 38 weeks post-irradiation.

At 15–19 weeks post-irradiation, five mice from Group I, six mice from Group II and nine mice from Group III were grafted with skin from healthy 2-month-old AKR male mice. All of the grafts on the mice given AKR cells (Groups I and II) survived until the time of natural death or sacrifice, a period of 4–6 months. They grew abundant hair crops which, in a few instances became thinner with age. The grafts on the mice protected with C3H bone marrow (Group III) were erythematous at 2 weeks when the dressings were removed and were all completely rejected at 4 weeks after grafting.
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DISCUSSION

These experiments have shown that cells were present in the injected AKR hematopoietic and lymphoid tissue which could manifest their leukemic potential in a foreign host. The AKR cellular origin of the leukemias observed was confirmed by cell transfer. Uphoff and Law\(^5\) reported a 10 per cent incidence of leukemia of donor tissue origin in lethally irradiated mice of low leukemia strains protected with AKR bone marrow.

The assumption was made that after lethal irradiation and injection with viable hematopoietic cells, the animals surviving would have repopulated their marrow and lymphoid tissue with donor cells. Evidence to support this assumption is found in the studies of Welling et al.\(^6\) who reported that the proportion of donor erythrocytes in allogeneic radiation chimeras increased from the time of their injection onward so that by 40–50 days the treated animals had exclusively donor type red cells. They observed no reversion of the chimeric state.

The statement is made by Koller et al.\(^7\) that, in radiation chimeras, skins from the donor strain are always accepted if the hematopoietic graft of that same strain is functional. In our studies, survival of AKR skin on the allogeneic chimeras and its rejection by mice receiving isogeneic bone marrow was interpreted as indirect evidence of the persistence of AKR hematopoietic cells.

The higher incidence of leukemia in the group of mice receiving AKR bone marrow and thymus cells indicates that these preparations probably contained greater numbers of leukemic precursors, presumably contributed by the cells of the older thymus tissue, since the incidence of leukemia in animals receiving young bone marrow cells (4 of 15) and young bone marrow plus thymus cells (6 of 22) was the same (27 per cent). These observations show that the leukemic precursor cells are present outside the thymus in young animals and suggest that they accumulate in the thymus in increased numbers in older mice. The numbers of mice receiving the old bone marrow are not large enough to permit any conclusion regarding the presence of precursor cells in this tissue.

Support for the assumption that cells from 7–20-week-old AKR mice are precursors rather than fully autonomous leukemic cells is given by two observations. Saxton et al.\(^8\) found that when blood and lymphoid cells of healthy 6–7 month-old AK mice were inoculated into susceptible animals, no leukemias developed; but cells from grossly and microscopically normal 8–11-month-old mice resulted in the production of lymphoid tumors in young AK mice, indicating that autonomous leukemic cells were not present until after 7 months. In this laboratory it was shown that C3HeB mice, which were lethally irradiated and given normal AKR bone marrow with AKR leukemic thymus cells, developed generalized leukemia in 4 weeks. This time of development of leukemia is much earlier than that occurring in the animals discussed in this report where the mean age of onset of leukemia was 29 weeks after cellular inoculation and the earliest leukemia occurred at 14 weeks. The long latent period observed in these experiments suggests that the inoculated AKR cells and their progeny became leukemic at approxi-
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mately the same time they would have if left intact in the AKR mouse. The
mice donating the marrow and thymus cells would have been, on the average,
37 weeks of age at the time leukemia developed in the chimeras. In the
AKR mouse, the peak incidence of spontaneous leukemia is between 32 and
40 weeks.

Several statements can be made to explain the increased incidence of leu-
kemia in the C3H mice inoculated with AKR cells described in this report,
and the absence of leukemia in the C3H mice made tolerant with neonatal in-
jection of AKR spleen and thymus cells reported previously. The two ex-
periments are not exactly comparable, but in each, survival of AKR cells
in a C3H host was assumed to have occurred because of the demonstration
of tolerance to skin allografts as discussed above. It was felt that in the radia-
tion chimeras, based on the work of Gengozian et al., a complete repopula-
tion of marrow and thymus by AKR cells was achieved. The donor (AKR)
cells are closely associated with the regeneration and restoration to normal
function of the damaged thymus. On the other hand, although donor cells
have been shown to be present in the thymus of mice made tolerant by neo-
natal injection of allogeneic cells, they are entering an intact and actively
functioning organ and remain in the minority. Therefore, it would seem that
leukemia develops in the radiation chimera because the cells which repopu-
late and regenerate the damaged thymus form a functioning organ com-
posed principally of donor cells with a potential for leukemic transforma-
tion. In the neonatally tolerant mouse, no leukemia is seen because the num-
ber of persisting donor (AKR) cells in the thymus remains small, and it
still functions as a C3H organ. The work of Doria lends further support to
this idea. He has shown clearly that the immunologic specificity of lym-
phoid tissues is of donor type in radiation chimeras and of host type in
neonatally tolerant mice.

Since the leukemias that developed were all of AKR (donor cell) origin,
this discussion has been limited to the transmission and survival of cells with
a leukemic potential in radiation chimeras. The role of the leukemia virus
in the development of these precursor cells and/or in leukemic transforma-
tion of these cells is of great interest but not within the scope of this study.

**Summary**

These experiments show that certain AKR cells have a leukemic potential
which can develop even when they reside in a foreign host. The results indi-
cate that these cells are most prevalent in the 20-week-old AKR thymus,
but are also found to be present in AKR bone marrow as early as 7 weeks of
age.

**Summario in Interlingua**

Le hic-reportate experimentos indica que certe cellulas del linea murin
AKR possede un potential leucemic que pote disveloppar se mesmo quando
illos reside in un hospite de un altere lineage. Le resultatos indica que iste
cellulas es le plus frequente in le thymo de muses AKR al etate de 20
LEUKEMIA IN LETHALLY IRRADIATED MICE

septimanas, sed illos es etiam incontrate in le medulla ossee de muses AKR al etate de 7 septimanas.

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REFERENCES


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