The Fate of Parental Preleukemic Cells in Leukemia-susceptible and Leukemia-resistant F1 Hybrid Mice*

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SUMMARY

(AKR X C3H)F1 mice were found to develop a high (89 per cent) incidence of spontaneous lymphoid leukemia. (AKR X C57BL)F1 hybrids had a low (8 per cent) spontaneous incidence of lymphoid leukemia but a significant (>16 per cent) incidence of reticulum-cell sarcomas. Pooled 6-month-old preleukemic AKR spleen cells were injected intraperitoneally into 2- to 5-day-old (AKR X C3H)F1 and (AKR X C57BL)F1 hybrid mice. Lymphoid leukemia of AKR type occurred in the (AKR X C3H)F1 mice after a short latent period similar to that which would have occurred if the cells used had been allowed to remain in the AKR donors. The appearance of reticular tumors of AKR type in (AKR X C57BL)F1 recipients was delayed by an average period of 40 weeks, and when they finally occurred two-thirds were reticulum-cell sarcomas, not lymphoid leukemias.

The results indicate that leukemia-resistant (AKR X C57BL)F1 mice can retard the development of neoplasia in preleukemic AKR cells and can possibly influence the type of reticular tumor finally developing from the injected cells.

Lymphoid leukemia in the AKR mouse is probably a virus-induced disease, the virus concerned being that described by Gross (8). With the use of passaged Gross virus, evidence was obtained (5) of a primarily maternal passage of this virus in C3H mice. In the AKR mouse, however, the tendency to develop leukemia is transmitted almost equally by the male and female parent.

Host conditioning factors are of critical importance in determining the development of the disease (12). In particular, the presence of thymus tissue is necessary if infection by the virus is to result in neoplasia of the lymphoid cells of the host (4, 10, 16).

The crossing of high-leukemia strain mice with low-leukemia strains usually results in F1 hybrids which have an intermediate incidence of spontaneous leukemia (7, 9). The present report concerns two AKR F1 hybrid strains, one (AKR X C3H)F1 having a high incidence, the other (AKR X C57BL)F1 an unusually low incidence of spontaneous leukemia. There is some evidence to suggest that the thymus in the latter strain may not behave in a fashion typical for thymuses of high-leukemia strain mice (14), and this could in part be responsible for the failure of these mice to develop a significant incidence of leukemia.

There are probably many additional factors involved in the resistance to leukemia of the (AKR X C57BL)F1 hybrid mouse. In an attempt to analyze some of the factors involved, the effect has been studied of injecting AKR cells into hybrid mice of both the above types. The rationale behind this approach is the fact that parental strain cells will grow in F1 hybrid mice which thus serve as an "in vivo tissue culture," permitting the continued life history of the injected cells.

In the present experiments, cells from AKR mice late in the preleukemic period have been injected, and observations have been made on the capacity of these cells to progress to the stage of neoplasia in the two types of F1 hybrid host.

MATERIALS AND METHODS

Mice.—Mice used were of the strains AKR, C3H, and C57BL, (AKR X C3H)F1, and (AKR X C57BL)F1. The AKR mice were originally ob-

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tained in 1958 from Dr. Jacob Furth, then at the Children's Cancer Research Foundation, Boston; the C3H and C57BL strains were originally obtained from the Imperial Cancer Research Fund, London. All three strains have been maintained at the Hall Institute by brother-sister mating for more than fourteen generations. The current incidence of lymphoid leukemia in these three strains is: AKR, 98 per cent (182 of 196 mice; mean latent period, 8.8 months); C3H, 8 per cent (nine of 120 mice; mean latent period 17.8 months); C57BL, 0.5 per cent (one of 200 mice; mean latent period, 14 months). The hybrid mice were produced by mating virgin AKR females with males of strains C3H or C57BL. The mice used in the present experiments were from the first or second litters of such matings.

Mice were housed in metal boxes with sawdust bedding, in animal rooms maintained at 75° F. They were fed Barastoc1 dog pellets and water ad libitum with supplemental carrots and greens.

Injection of hybrid mice.—Spleens were removed aseptically from 6-month-old nonleukemic AKR mice and pooled (four or five to a pool) in sterile, normal saline. These spleens were finely minced with curved scissors until the cell suspension could be aspirated through a No. 23 needle. These suspensions were then injected without delay, intraperitoneally, into litters of (AKR X C3H)F₁ and (AKR X C57BL)F₁ mice 2–5 days old. The dosage of cells injected into each recipient was not accurately established, but each mouse received approximately 20–25 mg. of minced splenic tissue. Each spleen pool was divided approximately equally between (AKR X C3H)F₁ and (AKR X C57BL)F₁ litters. A small portion of each mixed spleen pool was also injected intramuscularly into four 6-week-old AKR recipients to confirm the absence of transplantable lymphoma cells from each spleen pool used. These recipient AKR mice were killed 3 months later, all having failed to develop evidence of transplanted leukemia. In all, 28 pools were used for injection into the F₁ hybrid mice described in the present results.

Control F₁ hybrids from alternate litters were injected with an equivalent volume (0.2 ml.) of normal saline.

All babies given injections were returned to their nests without delay and were left undisturbed until weaning, when the mice were numbered and separated by sexes. These mice were then examined twice weekly for life for the development of reticular neoplasms.

Transplantation tests.—When an F₁ hybrid mouse of either group which had received injections became clinically ill with signs of a reticular tumor, the mouse was killed with ether, and if autopsy confirmed the presence of a reticular tumor appropriate tumor tissue (usually either thymus or spleen) was removed aseptically and minced in sterile normal saline. This tumor mince was then injected intramuscularly into four 6-week-old mice of the same sex, of the following strains: for tumors in (AKR X C3H)F₁ mice, to AKR, C3H, and (AKR X C3H)F₁ mice; and for tumors in (AKR X C57BL)F₁ mice, to AKR, C57BL, and (AKR X C57BL)F₁ mice.

These transplanted mice were observed for 6 months, and if no transplanted tumors had developed by this time, the mice were killed and autopsied.

Histology.—All mice dying or killed in experimental and control groups were examined histologically. Tissues were taken from relevant organs, fixed overnight at 4° C. in 10 per cent Zenker’s formalin, blocked in paraffin, and sectioned at 7 μ. Sections were stained routinely with Mayer’s acid hematoxylin and eosin. When difficulty arose in the histological classification of the original tumor in the F₁ hybrid mouse, sections were also taken from transplanted tumors in the mice transplanted to establish the genotype of the tumor cells.

RESULTS

General.—Accurate mortality data were not kept for the hybrid mice which had received injections before the age of weaning. A number of litters given injections of either spleen cells or saline were apparently eaten by their mothers during the night following injection, and in many of the litters one or two babies appeared to suffer a similar fate. Approximately one-third of the treated mice were lost in this manner. A small number of mice given injections of spleen cells during the 2d and 3d weeks of life, but the cause of death in these mice could not be clearly determined. The mice which had received injections were weaned when 4 weeks old. From this time, two of 72 (AKR X C3H)F₁ mice and five of 57 (AKR X C57BL)F₁ mice given injections of AKR spleen cells died with what histologically appeared to be chronic runt disease. These deaths occurred between the 3d and the 3d months of life, and these mice have been excluded from the calculations of the percentage incidence of reticular tumors in the mice given injections.

The remainder of the mice showed no evidence of runt disease as judged by their general appearance, subsequent body weight growth, peripheral white cell levels, and palpation of their spleens and lymph nodes.

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AKR cells in (AKR × C3H)F1 mice.—Of the (AKR × C3H)F1 mice given injections when 2–5 days of age with 6-month-old AKR spleen cells, 70 were weaned and closely observed until moribund. One hundred and twenty-four control (AKR × C3H)F1 mice, given injections, as babies, of saline, were also weaned and observed.

The final incidence of leukemia in the group being 96 per cent. The first case of leukemia in the control group occurred at 22 weeks of age; thereafter, the incidence increased at a gradual rate, reaching a final level of 89 per cent at 92 weeks.

When possible, tissue from leukemic experimental (AKR × C3H)F1 mice was injected into AKR, C3H, and (AKR × C3H)F1 recipients to establish the genotype of the neoplastic cells. Of the 67 cases of leukemia occurring in this group, 46 were so tested. Of these, twenty proved to be neoplasms arising from the injected AKR cells, whereas 26 were of host origin. The ages at which these various neoplasms occurred in the recipient mice are indicated in Chart 2. It may be seen that the earliest neoplasms occurring in these mice tended to be of AKR origin, whereas those occurring in older mice tended to be of host origin. Of the twenty cases demonstrated to have arisen from the injected AKR cells sixteen (80 per cent) were thymic and generalized, and four were nonthymic in distribution. It can also be noted that the leukemias of (AKR × C3H)F1 type in the experimental group occurred at an earlier age than did leukemia in the control group—i.e., there had been acceleration of development of spontaneous leukemia in these recipients.

Twenty-four of the leukemias occurring spontaneously in the control group of (AKR × C3H)F1 mice were also subjected to test transplantation in AKR, C3H, and (AKR × C3H)F1 mice. Sixteen of these grew on transplantation only in (AKR × C3H)F1 mice, as was expected. However, eight leukemias also grew in AKR mice, and three of these leukemias also grew in C3H mice. Thus, one-third of spontaneous F1 leukemias in this cross appeared capable of growing progressively in one or both parental strains. This troublesome failure of (AKR × C3H)F1 leukemias to obey the laws
of transplantation has been noted previously by Furth et al. (2). In some of these exceptional cases, although tumor growth in the parental strain was progressive, leading to death of the animal, it was apparent that some histo-incompatibility was being encountered by the neoplastic cells. The latent period to death was considerably longer than that for the isologous transplants, and the transplanted leukemia did not disseminate, instead forming very large thigh tumors. An attempt was made to improve the efficiency of the transplantation tests by incorporating \((\text{AKR} \times \text{C57BL})F_1\) mice into the battery of test mice. However this hybrid proved as susceptible as AKR mice to the growth of transplanted \((\text{AKR} \times \text{C3H})F_1\) leukemias. These results with the transplanted patterns of the spontaneous \((\text{AKR} \times \text{C3H})F_1\) leukemias throw some doubt on the validity of accepting all apparently long-lived. At the time of writing, one-third of the control group were still alive at the age of 120 weeks. Two types of reticular tumors were encountered in these mice lymphoid leukemia (of thymic or nonthymic type) and reticulum-cell sarcoma of type A (1). With the exception of a single case of lymphoid leukemia occurring at 28 weeks, no reticular tumors occurred until 78 weeks of age. Between 78 and 120 weeks of age, an additional eight cases of lymphoid leukemia and eighteen cases of reticulum-cell sarcoma occurred (Chart 3).

Test transplantation of both types of spontaneous \((\text{AKR} \times \text{C57BL})F_1\) reticular tumors revealed a more satisfactory situation than encountered in the \((\text{AKR} \times \text{C3H})F_1\) mice. All spontaneous neoplasms tested were found to grow on transplantation only in \((\text{AKR} \times \text{C57BL})F_1\) recipients and never in the parental strains.

In the experimental group given injections of AKR spleen cells, no lymphoid leukemias occurred before 22 weeks, but by 68 weeks of age 35 per cent of these mice had developed lymphoid leukemia. Of the nineteen cases of lymphoid leukemia occurring in this group, thirteen were subjected to transplantation analysis. Nine were found to have originated from the injected AKR cells and four from host cells. Chart 4 shows the age of occurrence of these various lymphoid neoplasms.

Reticulum-cell sarcomas occurred in the experimental group during approximately the same age period as that when the lymphoid leukemias were developing. Thus, between the 26th and 72d weeks of age 37 per cent of the experimental group developed reticulum-cell sarcomas, and the final incidence of this tumor, at the death of the last animal in this group at 116 weeks, was 54 per cent (Chart 3). In morphology, these reticulum-cell sar-
comas in no way differed from the spontaneous cases. Both liver and spleen were grossly involved by nodular masses of neoplastic reticulum cells, and commonly the mesenteric node was grossly enlarged by firm, gray neoplastic tissue (Figs. 2, 4). Involvement of other lymph nodes was variable, and the thymus was only involved microscopically by occasional deposits in the medullary regions. Twenty-eight reticulum-cell sarcomas occurred in the experimental group, and of these 22 were subjected to transplantation analysis. Eighteen were found to be AKR in origin and four to have originated from host (AKR X C57BL)F\textsubscript{1} cells. The distribution by age of these tumors is shown in Chart 4.

Lymphoid leukemia in both F\textsubscript{1} hybrids, at the stage when it was clinically detectable, was a rapidly progressive disease, killing the animals within 1–2 weeks. It was noted during the transplantation tests that AKR lymphoid leukaemias arising in (AKR X C57BL)F\textsubscript{1} mice grew equally rapidly on transplantation to AKR and (AKR X C57BL)F\textsubscript{1} mice, indicating that the latter F\textsubscript{1} mice exerted no retarding influence on the growth of fully developed AKR neoplastic lymphoid cells.

In contrast, reticulum-cell sarcomas in (AKR X C57BL)F\textsubscript{1} mice appeared to develop very slowly, the disease being clinically apparent for many weeks before the animals became moribund. This growth rate difference persisted on transplantation, the mean time to death for animals with reticulum-cell sarcoma being 5–6 months (versus 4–8 weeks for animals with transplanted lymphoid leukaemias). Again AKR-type reticulum-cell sarcoma arising in (AKR X C57BL)F\textsubscript{1} mice grew equally slowly in AKR and (AKR X C57BL)F\textsubscript{1} mice.

**Adult AKR mice given injections of spleen pool cells.**—The 6-week-old test AKR mice given injections intramuscularly of the various spleen pools failed to develop evidence of transplanted leukaemia over the 3-month observation period. The criteria for transplanted leukaemia were the development of nonthymic lymphoid leukaemia with excessive enlargement of the lymph nodes draining the injection site, with or without a thigh tumor at the site of injection. This suggests the absence of transplantable neoplastic cells from the spleen pools injected into the baby mice. The adult test mice did, however, develop an increased incidence of thymic lymphomas with or without generalization. When these mice were killed at the age of 18 weeks, a 19 per cent incidence of lymphoma of this type was noted. The incidence of lymphoma in a control group of stock AKR mice which were killed when they were the same age was only 1 per cent.

These findings agree with earlier observations (15) on the accelerated development of leukaemia in adult AKR mice given injections of preleukemic AKR thymic cells.

A similar phenomenon was encountered in the AKR mice given injections of lymphoid leukaemic cells during the transplantation tests to determine the genotype of the tumor cells. Those AKR mice which received injections of (AKR X C3H)F\textsubscript{1} and (AKR X C57BL)F\textsubscript{1} leukemic cells and failed to develop transplanted leukaemia did, however, exhibit an accelerated incidence of thymic lymphoma.

**DISCUSSION**

In the present experiments, F\textsubscript{1} hybrid mice were used as an in vivo tissue culture system for observing the subsequent behavior of injected preleukemic parental AKR cells. Theoretically, the use of such a system incurs the risk of induction of homologous disease in the F\textsubscript{1} recipients or the possible death of the injected parental cells. In actual fact neither of these theoretical possibilities proved troublesome in the present experiments, possibly
because of the dosage of cells, the intraperitoneal route used, the age of the recipients (2–5 days), or even the inability of preleukemic AKR cells to exert full immunological competence. The persistence and probable multiplication of the injected AKR cells can be concluded from the occurrence of neoplasms of AKR type in recipient F1 hybrids—the longest period of persistence being 88 weeks in the case of an AKR reticulum-cell sarcoma occurring in an (AKR X C57BL)F1 mouse.

The present results in (AKR X C3H)F1 mice are in agreement with those reported earlier by Lorenz, Law, and Congdon (11). These workers injected young adult AKR bone marrow into young (C3H X AKR)F1 mice and found that an accelerated and increased incidence of leukemia resulted. Transplantation tests showed that some of these leukemias had arisen from the injected AKR cells.

The two F1 hybrids used as recipient animals in the present experiments differed sharply in their susceptibility to the development of spontaneous lymphoid leukemia, although both types of hybrid had AKR mothers. The subsequent behavior of the injected preleukemic AKR cells also differed sharply in the two F1 hybrids.

The AKR cells used in these experiments were from 6-month-old donors—i.e., from mice which would normally start to die from lymphoid leukemia within a few weeks (Chart 5). Evidence has been presented elsewhere (18) suggesting that lymphoid cells in AKR mice progress to neoplasia through a series of steps; the older the cells, the more likely they are to be nearer the final development of autonomous neoplasia. Thus, 6-month-old AKR cells can be assumed to be nearing final transformation to autonomous neoplasia. In the high-leukemia (AKR X C3H)F1 hybrid, AKR cells became fully neoplastic and caused advanced leukemia after a latent period as short as 8 weeks. In fact, when the AKR-type leukemias occurring in these (AKR X C3H)F1 mice are replotted in terms of the age of the cells concerned, it is seen (Chart 5) that the age at which transformation to the neoplastic state occurred coincides closely with the age at which such cells transform naturally in AKR mice. This method of plotting the data is artificial, since the curves were constructed only from those mice shown to have AKR-type neoplasms. Even so, the chart emphasizes the difference in the behavior of similar AKR cells in (AKR X C57BL)F1 hybrids. In this F1 hybrid, AKR-type neoplasms appeared an average of 40 weeks after their development in (AKR X C3H)F1 mice. This suggests that some inhibitory mechanisms may exist in (AKR X C57BL)F1 mice which can block or delay not only the "spontaneous" virus-induced leukemic transformation of (AKR X C57BL)F1 cells, but also the progression to full neoplasia of AKR cells which are already well advanced in the direction of neoplasia.

The use of the term "preleukemic" in the present experiments is perhaps open to question. It may be argued that dormant neoplastic rather than preneoplastic lymphoid and reticulum cells were present in the spleen cell inocula. The data obtained in the present study do not allow a decision to be made between these two possibilities. If dormant neoplastic cells were in fact present in the spleen suspensions, then the present results would indicate the capacity of (AKR X C57BL)F1 mice to prolong the duration of this dormant state.

Alternative explanations could be advanced for the delay in the appearance of AKR neoplasms in (AKR X C57BL)F1 mice. Although AKR and C3H mice belong to the same histocompatibility sub-group (H-2k), C57BL mice are H-2b. This makes the (AKR X C57BL)F1 host more foreign to injected AKR cells than the (AKR X C3H)F1 host, a fact which could in some way affect the behavior of the injected parental AKR cells. Further, it has now been shown that neoplastic lymphoid cells induced in C3H mice by passaged Gross virus contain a new antigen not present in normal mice (19). This antigenic difference was weak, but if a similar virus-induced antigen arose in AKR lymphoma cells in the environment of the (AKR X C57BL)F1 animal it might be sufficient to provoke a continuous low-grade, antigen-based elimination of newly formed neoplastic AKR cells. Gross (6) has recently demonstrated that his
passage A virus can induce lymphoid leukemia in some mouse strains and myeloid leukemia in others. In BALB/c mice he found that a significant incidence of reticulum-cell sarcoma was induced by the same virus preparations. It is conceivable that the reticulum-cell sarcomas occurring spontaneously in (AKR × C57BL)F1 mice are also induced by Gross virus transmitted by their AKR mothers. C57BL mice are known to have a tendency to develop reticulum-cell sarcoma in old age (1), and this genetically determined susceptibility may express itself in the internal environment of the (AKR × C57BL)F1 mouse. It is noteworthy, therefore, that almost two-thirds of the AKR neoplasms occurring in (AKR × C57BL)F1 mice were reticulum-cell sarcomas, not the usual lymphoid leukemias. Can the internal environment of the (AKR × C57BL)F1 mouse also alter the morphological direction taken by neoplasia in AKR lymphoid cells, even when such cells are part way along the road to lymphoid leukemia? Alternatively is there, in addition to lymphoid cells, a second population of reticulum cells in the AKR spleen potentially capable of attaining neoplasia if placed in a suitable environment? In this regard it should be noted that AKR mice occasionally develop spontaneous reticulum-cell sarcoma, usually originating in the spleen (Furth2). The artificially prolonged life span of the AKR cells in the (AKR × C57BL)F1 hosts might be of importance in allowing such a transformation to occur.

In both types of F1 hybrid host, the injection of AKR cells resulted in the accelerated development of lymphoid leukemia of host cell origin. This was much more marked in the (AKR × C3H)F1 mice than in the (AKR × C57BL)F1 mice. Indeed, in the (AKR × C3H)F1 mice this accelerated development of (AKR × C3H)F1 leukemias may have so shortened the life span of these animals as to have prevented the development of a higher incidence of AKR-type lymphoid leukemias. Latotarjet (8), Miller (17), and Salaman et al. (18) have all reported the acceleration of leukemia development in AKR mice given injections of extracts or filtrates of AKR lymphoma tissue and have ascribed the phenomenon to super-infection of the mice by naturally occurring Gross virus present in the lymphoma tissue. It seems probable that the accelerated development of leukemia in the present hosts was based on a similar mechanism—i.e., it was probably due to the virus content of the injected spleen cell suspensions. If this interpretation can be accepted, the minor degree of this occurring in (AKR × C57BL)F1 mice suggests that these mice, apart from any other distinctive features, may also be relatively resistant to infection or super-infection by the Gross virus in its naturally occurring form in AKR mice.

The accelerated development of thymic lymphoma noted in the adult AKR mice given test transplants of spleen pool cells may represent a process similar to that observed in the F1 baby mice given injections. The transformation of the injected cells may have been slower because of the adult age of the recipients and the route of inoculation used. Superinfection with naturally occurring Gross virus is also a possibility, particularly in those mice developing thymic lymphoma after the injection of incompatible F1 leukemic cells.

The above discussion has been concerned primarily with the probable leukemia-inducing effects of the Gross virus, as it exists in its natural state in AKR mice. However, it should be pointed out that the present results also indicate the important role played by the C57BL spermatozoa in determining the resistance to leukemia development of (AKR × C57BL)F1 mice—an effect which is probably chromosomally mediated.

In experiments currently in progress a further analysis is being attempted of the mechanisms underlying the resistance to the development of leukemia in (AKR × C57BL)F1 mice. Data so far obtained suggest that the thymus in (AKR × C57BL)F1 mice may function more like that of low-leukemia strain mice (14). However, this by itself cannot account for the difference in behavior of the injected AKR cells in the present experiments, since it has been previously shown that 6-month-old AKR spleen cells are thymus-responsive, not thymus-dependent, and are capable of attaining neoplasia even in thymectomized hosts (13).
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