The Immunocapacity of the AKR Mouse

Betty J. Hargis and Saul Malkiel

Children's Cancer Research Foundation, Inc. [B. J. H., S. M.], and Department of Pathology, Harvard Medical School [S. M.], Boston, Massachusetts 02115

SUMMARY

Mice of the high leukemia strain, AKR, possess immunocompetent lymphocytes and are capable of participating in both immediate and delayed types of hypersensitivity reactions. These responses can be demonstrated undiminished throughout the life-span of the animal, even extending into the leukemic stage. A 35% mortality from anaphylaxis occurred in 2-month-old AKR mice, and an 87% mortality occurred in those AKR mice older than 7 months. Spleen cells from AKR mice aged 1 to 11 months produced a graft-versus-host reaction in newborn CFW mice.

INTRODUCTION

A correlation has often been observed between immunodepression and the development of cancer. In mice infected with passaged leukemia-inducing viruses, immunological disturbances have been noted before leukemia actually develops. Thus, mice infected with Gross leukemia virus (23), Rauscher leukemia virus (28), or Friend disease virus (3) all have shown abnormalities in antibody-producing capacity when stimulated by diverse antigens. However, Murphy and Syverton (21) have presented evidence that resistance or susceptibility to a variety of infectious agents does not correlate generally with genetic predisposition to leukemia. The AKR strain of mouse which had spontaneously developed a generalized lymphoid leukemia was found to have a severely depressed immune response to sheep erythrocytes (19). On the other hand, preleukemic AKR mice were capable of (a) a nearly normal immune response to sheep red blood cells (19), (b) a normal resistance to infection (19), and (c) rejection of tumor transplants (18). In the present experiments, the AKR mouse was used, since the long latency and high incidence of spontaneous lymphocytic leukemia and the longevity of the diseased animal permits the controlled assay of immunocompetence to be carried out during various stages of this disease. The objective of this research was to examine the immunocompetence of the leukemic AKR mouse with respect to its capacity to manifest an anaphylactic response and with respect to the capacity of its spleen cells to elicit the GVH2 reaction. The leukemic cell has been described as a malignant form of lymphocyte (10).

MATERIALS AND METHODS

Animals. AKR mice purchased from The Jackson Laboratory, Bar Harbor, Maine, and CFW mice purchased from Carworth Farms, Inc., New City, N. Y., were maintained on Purina laboratory chow and allowed water ad libitum. Progeny from the respective strains were used for these experiments.

GVH Reaction. The GVH assay which determines the immunocompetence of lymphocytes (29) was utilized in this study. Spleens from AKR mice of various ages were used to produce a GVH reaction in CFW newborn mice. For some experiments, AKR mice, up to 7 days of age, were used as control recipients. A suspension of viable cells was prepared in chilled Hanks' balanced salt solution by passage of the spleen through the mesh screen of a Swinny syringe; viability of the cells was determined by the eosin dye-exclusion test (26). The newborn recipients were injected i.v. within 24 hr of birth with 0.1 ml of a suspension adjusted to contain approximately 4 X 10^6 viable cells. The experiment was terminated when death from an apparent disease process occurred; all living members of the litter were sacrificed at that time. This usually was not before Day 9. If death had not already occurred, the litters were sacrificed no later than Day 14 (29). Leukocyte counts and total body, splenic, and thymic weights were obtained. The thymus, spleen, and liver were fixed in Tellyesniczky's solution, sectioned, and stained with hematoxylin and eosin.

Anaphylaxis. Adult mice from both the AKR and CFW strains were sensitized by a single i.p. injection of a mixture of 0.03 ml of HS and 6 x 10^9 BPV Phase I cells (16). On the 10th day following this sensitization, a challenge dose of 0.1 ml of HS was injected i.v.

RESULTS

GVH Reaction. The body weight index (Chart 1) is the average total body weight of a recipient CFW litter as compared with normal uninjected controls of the same age. Approximately 11 normal mice were sacrificed every other

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Chart 1. The body weight index is the average total body weight of a recipient CFW litter compared with normal uninjected controls of the same age. *Horizontal line*, theoretical body weight index of 1.0.

The apparent discrepancy in the body weight index was calculated in the same manner as was the thymic index (Chart 3). A value of greater than 1.3 is considered to be indicative of a GVH reaction (29). The apparently discrepant high values in Chart 3 at the 7-month donor age were indices from recipient animals that developed leukemia. The spleens and livers of these animals were greatly enlarged, and the white counts were extremely high. The highest index at the 10-month donor level also represents a leukemic litter. The livers of these mice were extensively replaced by tumor growth.

There was considerable variation in the leukocyte counts (Chart 4), contrary to the expected leukopenia in GVH reactions (29). It may be that a concomitant leukemia occurred in some litters, although 2 findings argue against this premise. No histological evidence of leukemia was seen in tissues examined, other than those indices already mentioned, and in only 1 control AKR litter (from an 11-month-old donor) were splenomegaly and an elevated leukocyte count found.

Five recipient CFW litters from the 10-month donor level (excluding the leukemic litter referred to above) were used as spleen donors to young AKR mice. Two of the CFW litters were apparently still healthy at Day 17; the others were sacrificed at Days 10, 14, and 15, respectively, because of the sudden occurrence of deaths in the litters. To exclude leukemia as the cause of death, susceptible AKR recipients were given a weight-adjusted dose of viable spleen cells, $2 \times 10^6$ cells/g of body weight, i.v. Thirty days later, the mice were sacrificed. The white counts, spleen, and thymus weights of these animals were in the normal range, indicating that leukemia was not induced by the transfer (34).

Chart 2. The thymic index was obtained by calculating the thymic weight of each mouse on a basis of mg/10 g body weight and dividing the average for each litter by an average normal value for the same age. *Horizontal line*, theoretical thymic index of 1.0.

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As shown in Table 1, only a 35% mortality occurred in the 2-month-old AKR mice upon challenge with HS, while 94% of the CFW mice died. However, when AKR mice of at least 7 months of age were tested, 87% died. The anaphylactic mortality for comparably aged CFW mice was 100%.

**DISCUSSION**

The present studies indicate that mice of the high leukemia strain, AKR, possess immunocompetent lymphocytes and are capable of participating in both immediate and delayed types of hypersensitivity reactions. These responses can be demonstrated undiminished throughout the life-span of the animal, even extending into the leukemic stage.

The preponderance of evidence in the literature supports the concept that an immunological defect could be responsible for the establishment of cancer (11, 14, 20, 27). With particular reference to virus-passaged leukemias, such as Friend and Rauscher, Ceglowski and Friedman (2) indicated marked interference with antibody production, as determined by the hemolytic plaque technique. Lengthening the interval between viral infection and antigen administration resulted in greater immunosuppression, with the most marked suppression being observed in animals with marked splenomegaly relatively late in the disease process. Humoral antibody suppression was not as severe as that observed on the cellular level. Similar findings were reported by Salaman and Wedderburn (24), who used Friend and Moloney viruses (25). Ransom et al. (24) have suggested that significant impairment of the immune response precedes the "clinical" onset of leukemia.

On the other hand, it has been found that a murine lymphoma cell line developing after infection with Rauscher virus has the ability to produce immunoglobulins and to carry or replicate a leukemogenic virus at the same time (33). Also, immunologically abnormal lymphoid cell clones, grown in tissue culture from an ascitic intraabdominal leukemic tumor, upon inoculation into newborn mice produced a wasting syndrome resembling classic runt disease (30). In contrast, Doell et al. (7) found that spleens from C57BL mice treated by irradiation or methylcholanthrene in a dose sufficient to induce tumors had a decreased ability to cause GVH reactions in F1 hybrids.

Specifically, with regard to the AKR mouse, Metcalf and Moulds (19) state that severe immune deficiencies are not a prerequisite of the preleukemic state. Although Friedman (8) indicated that adult AKR mice were poor responders, Hechtel et al. (13), by contrast, found that newborn AKR mice gave good early responses to sheep erythrocytes and that, once competence had been attained, the magnitude of the response was similar to that observed for several other strains of (nonleukemic) mice. Levine and Vaz (15) found AKR mice, aged 6 to 26 weeks, to be better producers of IgG, and reagin than were several other strains of mice that received injections of a very low dose of hapten-protein conjugate in Al(OH)3 gel.

In our experiments, donor animals from 1 to 11 months of age included those with greatly enlarged thymuses and/or spleens and with white blood cell counts ranging from normal to very high, characteristic of the leukemic state. Essentially

Histological changes were most evident in the CFW recipients of spleens from AKR donors of age 3 through 7 months. In the thymus, some loss or depression of subcapsular "germinal" lymphoid cells was seen. There was an apparent increase in cortical histiocytes and lymphoid karyorrhexis in some animals. Thymuses of experimental and control mice are represented in Fig. 1. In the spleen, lymphoid changes were characterized by: (a) a change in shape and a decrease in volume; (b) a change in cell populations, such as a decrease of lymphocytes and lymphoblasts with an increase in large reticular cells somewhat resembling plasma cells or immunoblasts; and (c) red pulp changes, reflected by a decrease in myeloid cells and megakaryocytes, with some animals exhibiting increased histiocytes or phagocytes (Fig. 2). In the liver, signs of hematopoietic stress (increased extramedullary hematopoiesis) were seen. There was subtle remodeling of hepatic architecture. In some cases, increased mitoses in hepatocytes were found. In general, these changes fit those reported in GVH reactions (29) and, since they were not observed in the AKR recipients, it seems unlikely that the changes were due to virus.

**Anaphylaxis.** The mice of 1 CFW litter, recipients of spleen cells from a 7-month-old AKR donor, received injections at 36 days of age of BPV and HS. None of the 7 mice died, on i.v. challenge with HS 10 days later. This lack of sensitization of the "runted" mice is to be expected of animals with lymphoid atrophy.

A control litter of 8 normal CFW mice of the same age were sensitized and challenged in the same manner. Seven of the 8 mice died of anaphylaxis.

<table>
<thead>
<tr>
<th>Age (mo.)</th>
<th>CFW mice</th>
<th>AKR mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>17/18a</td>
<td>7/20</td>
</tr>
<tr>
<td>&gt; 7</td>
<td>20/20</td>
<td>13/15</td>
</tr>
</tbody>
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a No. of mice dead/no. of mice challenged.

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![Chart 4. Average leukocyte counts of recipient CFW litters at time of sacrifice.](chart4.png)

**Chart 4.** Average leukocyte counts of recipient CFW litters at time of sacrifice.

**Table 1**

Anaphylaxis in CFW and AKR mice

Sensitization was with 0.03 ml of HS and $6 \times 10^8$ BPV cells i.p.; challenge took place 10 days later with 0.1 ml of HS i.v.

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similar results in GVT reactions were obtained with all donors (with the exception of the litters noted which developed leukemia). The picture of lymphatic leukemia developing in newborn C3H mice given injections of AK leukemia is that of greatly enlarged livers and spleens, numerous greatly enlarged lymph nodes, and masses of tumors in the abdominal cavity (11). Since evidence of leukemia was found in only 1 of the control AKR recipient litters in our series, it may be that development of leukemia was facilitated by the GVT reaction (6). The production of a GVT reaction by spleen cells from animals not only had high titers of virus but also made antibody response in such mice. It seems likely that antibody formation can be carried out very efficiently by AKR cells when removed from hypothetical suppressive factors present in the leukemia-prone animal.

The higher death rate from anaphylaxis in the older AKR group may, perhaps, be explained by the observation that only 2 of the 13 older mice examined after anaphylactic death had any gross evidence of leukemia. The younger age group would have been composed of a larger percentage of leukemia-prone mice, while the older age group would, perforce, contain a larger percentage of leukemia-resistant mice. Anaphylaxis in the mouse has not been definitively associated with production of a particular type of antibody and may be dependent, in part, upon other factors: phagocytosis by the reticuloendothelial system, the quantity of enzymes and vasoactive compounds released, and the sensitivity of the strain to these substances (32).

Antigenic competition between virus and antigen has been proposed as the mechanism of leukemia virus-mediated immune suppression (9). However, Cremer et al. (5) found a relatively high percentage of antibody-producing cells to be forming viral antigen as well, indicating that both activities could occur simultaneously. Indeed, Ceglowski and Friedman (4) have suggested that immunosuppression by leukemogenic virus is not exclusively due to inactivation of antibody precursor cells. With respect to the AKR mice under present study, since transmission of the Gross virus, which is indigenous to the AKR strain, occurs early in embryonic life (12), a natural tolerance may exist, in contradistinction to viral infection later in fetal development in which limited immunological recognition would be expected (31). It is plausible that the AKR mice are tolerant to the virus and, indeed, that this tolerance is the reason the disease develops (1). Although the same theory has been the speculative explanation for other viral diseases as well, recent studies by Oldstone and Dixon (22) with mice transplacentally infected with lymphocytic choriomeningitis virus showed that these animals not only had high titers of virus but also made antiviral antibody (22). However, no data are available at present to indicate a lack of tolerance to Gross virus in AKR mice.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Thymuses of control mice and mice undergoing a GVH reaction. a, normal CFW mice; b, AKR recipients of AKR spleen cells; c, CFW recipients of AKR spleen cells. × 250.
Fig. 2. Spleens of control mice and mice undergoing a GVH reaction. a, normal CFW mice; b, AKR recipients of AKR spleen cells; c, CFW recipients of AKR spleen cells. × 250.
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