CORRELATION OF EARLY MURINE LEUKEMIA VIRUS TITER AND H-2 TYPE WITH SPONTANEOUS LEUKEMIA IN MICE OF THE BALB/c × AKR CROSS: A GENETIC ANALYSIS*

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The AKR mouse strain was developed by selective breeding for a high incidence of spontaneously occurring leukemia, starting from a randombred stock in which the incidence of the disease was low to moderate (1). The success of the breeding program strongly suggested that genetic factors play a role in the occurrence of the disease. This implication was amply confirmed in studies of crosses between high- and low-leukemia mouse strains (2).

After the discovery by Gross (3) that the tissues of AKR mice yield a virus capable of transmitting the disease, it soon became clear that great variations exist from one strain of mice to another in the degree of susceptibility to the leukemogenicity of the virus. The analysis of the complex genetic control of this phenomenon has proceeded rapidly in recent years (4).

A major advance in this genetic analysis came from the development of a rapid assay for the infectivity in vitro of murine leukemia virus (MuLV), the XC cell plaque assay (5). With this assay it could be demonstrated that AKR mice first showed detectable levels of MuLV from the perinatal period and attained high adult levels by 6 wk of age (6). Mice of low-leukemia strains, by contrast, were generally devoid of detectable infectious MuLV, at least until relatively late in life. From studies of crosses of AKR with low-leukemia, low-MuLV mouse strains, it appears that two autosomal loci, Aku-I and Aku-2, are the determinants of MuLV expression in AKR mice (7-10). Recent studies lend support to the idea that these prototypic "V" genes of the mouse consist of chromosomally integrated DNA copies of the complete RNA MuLV genome (11).

The presence of these Aku genes is strongly associated with spontaneous tumors in segregating generations of the cross, AKR × C57L (12). Since in this cross no major regulatory genes exist which can suppress MuLV expression, the presence of an Aku gene is tantamount to the presence of the virus. The studies we now report investigated the relation between the expression of endogenous MuLV and spontaneous leukemia in a segregating population of mice all of which

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possess two or more copies of these Akv genes but which vary genotypically in respect to regulatory genes capable of interfering with the expression of infectious MuLV. The findings indicate that about half of the mice were MuLV-positive (titer range: $10^{8.2} - 10^{9}$) at 6 wk of age and that the level of virus expression at this age strongly correlates with the occurrence of spontaneous leukemia much later in life.

Materials and Methods

Experimental Design. Table I summarizes the relevant characteristics of mice of the AKR and BALB/c parental inbred strains and their F₁ progeny. AKR mice show a near 100% incidence of spontaneous leukemia, whereas BALB/c mice show only a very low and much later incidence of the disease; the low BALB/c level of the disease is dominant in F₁ hybrids. Similarly, AKR mice express high levels of N-tropic MuLV in their tissues at 6 wk of age, whereas both BALB/c and F₁ mice show at most very low levels of the virus (8, 9). We therefore bred and observed the backcross generation, (BALB/c × AKR) F₁ × AKR, for a possible correlation between MuLV expression and spontaneous leukemia.

| Trait               | AKR                  | (BALB/c × AKR) F₁ | BALB/c               |
|---------------------|----------------------|------------------|----------------------|
| Leukemia incidence  | 95% (8-11 mo)        | 4% (>1 yr)       | low (>1 yr)          |
| MuLV expression     | 100% (10⁸⁺) (6)      | 83% (10⁸⁻) (9)   | 20–60% (low titer late in life) |
| Akv-1 and Akv-2     | Aku-1/Aku-1          | Aku-1/−          | Aku-2/−              |
| Fu-1 type           | Fu-1⁺/Fu-1⁺          | Fu-1⁺/Fu-1⁺      | Fu-1⁺/Fu-1⁺          |
| H-2 type            | H-2⁺/H-2⁺            | H-2⁺/H-2⁺        | H-2⁺/H-2⁺            |

Both parents of this backcross generation possess the Akv-1 and Akv-2 genes, the AKR parent being homozygous and the F₁ heterozygous for these genes. Since the genes are dominant, this difference in zygosities is not a significant factor in the phenotypic differences noted between the parents or among the backcross mice in relation to MuLV expression or the development of leukemia. On the other hand, differences among these mice at the Fu-1 and H-2 loci do influence their phenotypes. The AKR parents are homozygous Fu-1⁺/Fu-1⁺ and H-2⁺/H-2⁺, whereas the F₁ parents are heterozygous Fu-1⁺/Fu-1⁺ and H-2⁺/H-2⁺. The Fu-1⁺ allele confers relative resistance to cellular infection with N-tropic MuLV, and the H-2⁺ haplotype is less favorable for viral leukemogenesis than H-2⁻.

Mice of the backcross generation, which should include half Fu-1⁺/Fu-1⁺ homozygotes and half Fu-1⁺/Fu-1⁻ heterozygotes, were not typed directly for this difference, which in our standard procedure would require challenging them with exogenous MuLV. In addition, the parental strains do not differ at the Gpd-1 locus, which is closely linked to Fu-1 and could thus serve as a marker for it (13). Rather, the mice were tested individually for the presence of endogenous N-tropic MuLV in their tissues at 6 wk of age. The major genetic factor influencing this trait in this cross is the presence or absence of the Fu-1⁺ allele. H-2⁺ typing of the backcross mice was carried out directly.

Mice. Backcross progeny of (BALB/c × AKR) F₁, 50, and AKR, 53, were bred and maintained at Albert Einstein College of Medicine from parental strain mice originally obtained from The Jackson Laboratories, Bar Harbor, Maine. The majority of the backcross mice were from first litters, and they were housed three to four mice per cage during the observation period. The mice were observed for the development of leukemia until the youngest were 600 days old; the experiment was then terminated, since the disease had become a rare event among the survivors. When in extremis the mice were
sacrificed and autopsied. The leukemias were generally characterized by extreme enlargement of the thymus often associated with pronounced enlargement of the lymph nodes and occasionally with moderate enlargement of spleen and liver.

**Virus Testing.** At 6 wk of age a 1 cm terminal segment of the tail was cut off each mouse; the segments were immediately frozen, stored at -75°C and packed in dry ice and shipped to the National Institutes of Health for virus testing. Tail segments were rinsed with ether, ground with a chilled mortar and pestle, and suspended in cold Eagle’s basal medium containing 20% veal infusion broth. The extracts were centrifuged, and the supernates were tested for infectious MuLV by the XC cell method (5) using secondary cultures of NIH Swiss mouse embryo cells. Titers are expressed as the number of plaque-forming units per 0.4 ml of approximately 2% extract. It is probable that the storage and shipping of the tail segments before extraction and virus testing had some effect of lowering the observed virus titers, by comparison with what one would expect from fresh tissues examined immediately.

**H-2 Typing.** The hemagglutination method of Gorer and Mikulska (14) with minor modifications was used to determine the H-2 type of the mice. Serum from (AKR x C57BL/6) F1 mice hyperimmunized with cells of the chemically induced BALB/c sarcoma, Meth A, detects antigens of the H-2b haplotype, transmitted from the BALB/c grandparent to about 50% of the backcross population studied. To one drop of this antiserum, diluted 1:100 and 1:400 in a saline solution of 1.8% dextran (mol wt ~115,000), was added one drop of a dilute suspension of washed erythrocytes in 50% heat-inactivated fetal calf serum in saline. The mixture was agitated and incubated at 37°C for 90 min without further agitation, and the sedimented erythrocytes were examined for agglutination by low-power microscopy.

**Results**

**Leukemia vs. MuLV.** Of the 335 (BALB/c x AKR) F1 x AKR backcross mice for which complete MuLV and H-2 typing results were obtained, 111 (33.1%) developed leukemia during the 19-21 mo observation period (Table II). All but 12 of these leukemic mice showed pronounced enlargement of the thymus, and their spleens and/or lymph nodes were usually involved as well. 31 mice (9.3%) died of causes other than leukemia, and 16 mice (4.8%) died of unknown causes since they were not autopsied. Of the 111 leukemic deaths, 96 occurred among the 173 mice which had shown detectable levels of MuLV in their tissues at 6 wk of age; 15 leukemic deaths occurred among the 162 MuLV-negative animals. This difference in leukemia incidence—55.5% vs. 8.7%, respectively—is highly significant ($\chi^2 = 80.7; P \ll 0.001$).

Among the MuLV-positive mice the virus titers observed ranged from $10^{3.8}$ to $10^{4.8}$. Table III and Fig. 1 show that there was a marked correlation between MuLV titer and leukemia incidence, the animals with the highest virus titers showing the highest and earliest incidence of the disease. Fig. 2 depicts this relationship graphically, illustrating the remarkable similarity of these results to a dose-response titration pattern.

**Leukemia vs. H-2 Type.** The data in Tables II and III also demonstrate that the H-2 type of the backcross mice was a further factor of importance in the occurrence of leukemia among these mice. 71 of 166 H-2b/H-2b homozygotes (42.8%) developed the disease, whereas only 40 of 169 H-2b/H-2b heterozygotes (23.7%) did so. This influence of H-2 type was highly significant ($\chi^2 = 13.8; P < 0.001$), although it was a less important factor than the level of expression of MuLV.

**Leukemia vs. Sex.** Since leukemia is known to occur somewhat more frequently among females than among males of the AKR strain, the backcross population was also analyzed for a possible sex difference in the occurrence of the
### Table II

**Leukemia Incidence in (BALB/c x AKR) x AKR Mice**

| Sex | Mice | H-2 Type | N | Leukemic | Nonleukemic | Not autopsied | Alive |
|-----|------|----------|---|----------|-------------|---------------|-------|
| ♀   | +    | kk       | 54 | 35 (64.8%) | 1           | 5             | 13    |
|     |      | kd       | 41 | 24 (58.5%) | 1           | 2             | 14    |
|     |      | Subtotal | 95 | 59 (62.1%) | 2           | 7             | 27    |
| -   |      | kk       | 29 | 5 (17.2%)  | 1           | 1             | 22    |
|     |      | kd       | 54 | 1 (1.9%)   | 2           | 2             | 49    |
|     |      | Subtotal | 83 | 6 (7.2%)   | 3           | 3             | 71    |
| Total |     |          | 178 | 65 (36.5%) | 5           | 10            | 98    |
|♂   | +    | kk       | 40 | 26 (65.0%) | 2           | 0             | 12    |
|     |      | kd       | 38 | 11 (28.9%) | 6           | 4             | 17    |
|     |      | Subtotal | 78 | 37 (47.4%) | 8           | 4             | 29    |
| -   |      | kk       | 43 | 5 (11.6%)  | 14          | 1             | 23    |
|     |      | kd       | 36 | 4 (11.1%)  | 4           | 1             | 27    |
|     |      | Subtotal | 79 | 9 (11.3%)  | 18          | 2             | 50    |
| Total |     |          | 157 | 46 (29.3%) | 26          | 6             | 79    |
|       |      | Grand total | 335 | 111 (33.1%) | 31          | 16            | 177   |

Statistical analysis (2 x 2 $\chi^2$ method):

- Leuk Nonleuk |
  - MuLV+        | kk  |
  - MuLV-        | kd  |

| Leuk | Nonleuk |
|------|---------|
| 96   | 77      |
| 15   | 147     |

| Leuk | Nonleuk |
|------|---------|
| kk   | 71      |
| kd   | 40      |
| ♀    | 65      |
| ♂    | 95      |

| Leuk | Nonleuk |
|------|---------|
| 94   | 79      |
| 72   | 90      |

$\chi^2 = 80.71$, $P < 0.001$

$\chi^2 = 13.79$, $P = 0.01$

$\chi^2 = 1.76$, $P = 0.18$

$\chi^2 = 3.27$, $P = 0.08$

### Table III

**Leukemia Incidence According to 6-Wk MuLV Titer in (BALB/c x AKR) x AKR Mice**

| MuLV titer (log$_{10}$) | Mice developing leukemia |
|-------------------------|-------------------------|
|                         | $H-2^a/H-2^a$ | $H-2^b/H-2^b$ | Total |
| 3.9-3.0                 | 13/15 (87%) | 9/10 (90%) | 22/25 (88%) |
| 2.9-2.0                 | 28/41 (68%) | 19/39 (49%) | 47/80 (59%) |
| 1.9-1.0                 | 14/22 (64%) | 2/16 (13%) | 16/38 (42%) |
| 0.9-0.3                 | 6/16 (38%) | 5/14 (36%) | 11/30 (37%) |
| Total MuLV+             | 61/94 (65%) | 35/79 (44%) | 96/173 (55%) |
| Total MuLV-             | 10/72 (14%) | 5/90 (6%) | 15/162 (9%) |
| Grand total             | 71/166 (43%) | 40/169 (24%) | 111/335 (33%) |
disease. However, the difference observed—34.8% in females vs. 29.3% in males—was not statistically significant.

**MuLV vs. H-2 Type.** Since both the presence of MuLV at 6 wk of age and H-2 type were important factors in the occurrence of leukemia, these two factors were analyzed for possible interaction between themselves. 94 of 166 $H^{-2^a}/H^{-2^a}$ homozygotes (56.6%) and 79 of 169 $H^{-2^a}/H^{-2^d}$ heterozygotes (46.7%) were MuLV-positive. This difference is of only marginal statistical significance ($x^2 = 3.3; P = 0.08$), and it appears that $H$-2 type had little influence on the early expression of MuLV in this population.

**Discussion**

The experiments of Gross (3) demonstrated that a virus isolated from the tissues of AKR mice is capable of inducing the leukemia typical of this strain. This
endogenous viral agent (MuLV) proved to be an RNA virus of C-type morphology which matures by budding from cell membranes. A virus present in AKR tissues and possessing these same properties has been detected by the XC cell assay in vitro (6). Our experiments demonstrate a strong association between the presence of endogenous, XC-detectable virus and the occurrence of leukemia. This association is particularly striking in terms of the correlation observed (Table III and Fig. 2) between the titer of the virus and the incidence of leukemia. Such a dose-response titration of naturally occurring MuLV is unique in tumor virus studies to date.

The capacity of AKR mice to produce infectious MuLV appears to be a function of the dominant Aku-1 and Aku-2 genes (8, 9) which seem to consist of chromosomally integrated DNA copies of the RNA viral genome (11). Meier et al. (12) demonstrated that, in a segregating cross of AKR with the Fv-1 compatible strain C57L, the inheritance of the Aku genes, as detected by the presence of gs antigen and infectious MuLV, was associated with the occurrence of spontaneous lymphoma and possibly other tumors. The results of our experiment go further, demonstrating that the presence of Aku genes does not lead to neoplasia unless these genes are expressed in terms of mature, infectious virus.

The suppression of MuLV replication in the cross described here is largely a function of the Fv-1° allele. This dominant allele, which is absent in AKR mice, is present in (BALB/c × AKR) F1 hybrid mice. Thus, although these two types of mice carry the Aku genes, they differ in that the hybrids, unlike the AKR parent, are restrictive for MuLV expression and rarely develop leukemia. Studies in vitro (9) indicate that embryo cells from AKR and (BALB/c × AKR) F1 mice exhibit a similar frequency of induction of N-tropic virus production following treatment with 5-iododeoxyuridine. In cultures of AKR cells, which are Fv-1 °/Fv-1 ° homozygous, the induced virus spreads rapidly to other cells, in which it replicates freely. However, in cell cultures from the hybrid mice, the induced virus spreads little if at all, largely because of the presence of the dominant Fv-1 ° allele which blocks the spread of infection (15). This mechanism is thought to be responsible also for the low levels or absence of MuLV in hybrids between AKR and Fv-1 ° mice (9), which in turn appears to be responsible for the absence of leukemia observed here.

An additional factor resulting in suppression of infectious MuLV was observed in the experiment described here: a maternal factor, presumably representing antiviral antibody. This effect was noted previously (9) with (BALB/c × AKR) × AKR mice, as well as with other backcrosses involving AKR and Fv-1 ° mice, and was seen when the mother was the virus-positive Fv-1 heterozygote. In the present study, of 44 litters of (BALB/c × AKR) F1, × AKR, 8, totalling 61 progeny, contained either no virus-positive mice or had at most one positive mouse with only a trace (titer < 10°) of MuLV; this is significantly different from a random distribution (P < 0.0001 by chi-square testing for heterogeneity). This maternal effect may have resulted in excess numbers of mice with low titers in other litters as well. Analysis of the data with these eight litters excluded did not alter the patterns of results.

Thus we envision two mechanisms contributing to the suppression of MuLV in this study: resistance due to the Fv-1 ° allele, and the maternal resistance factor. Both of these operate by suppressing cell-to-cell spread of virus, and there is no reason to postulate that they inhibit viral genome functions (induction of virus or synthesis of viral gene products) in cells expressing their endogenous viral
information. These considerations strongly indicate that spontaneous lymphoma in the AKR hybrids studied here results primarily from the consequences of extensive viral replication and spread.

It seems worth emphasizing that the virus testing in our experiment was carried out on tissues taken at 6 wk of age, whereas the leukemias observed occurred much later in life. It is doubtful that virus testing of tissues taken at 6 mo of age would have produced such a clear correlation. The difference in magnitude of MuLV expression between AKR and hybrids of AKR with mice carrying the Fv-1° allele is much more marked in tests at 2 wk than at 6-8 wk of age (9); also, in mice whose early viral expression is inhibited by the maternal resistance factor, virus reaches high titers during adulthood (W. P. Rowe, unpublished). It thus appears that early virus expression is a critical factor in leukemogenesis in AKR mice.

Our experiment also confirms previous studies showing that H-2 type exerts a significant influence on the occurrence of virus-induced leukemia (16). However, it is clear that H-2, unlike Fv-1, has little effect on early MuLV expression. Thus H-2 must exert its influence by a different means from Fv-1, presumably by an effect on a defense mechanism which controls the outgrowth of transformed cells, perhaps by an immunological mechanism. It is of interest that this effect of H-2 manifests itself in different titration curves for the viral induction of leukemia (see Table III). In backcross mice with high MuLV titers (>10^3), H-2 type was not a significant factor in the occurrence of the disease, since virtually all such mice developed it. Conversely, mice showing no detectable MuLV showed minimal incidences of leukemia regardless of H-2 type. Among mice with moderate MuLV levels, however, the presence of the H-29' haplotype was associated with a markedly lower incidence of the disease by comparison with mice homozygous for the H-2° haplotype.

Summary

Tissue extracts from 6-wk old mice of the AKR strain (H-2°) show high levels of infectious murine leukemia virus, and these mice show a near 100% incidence of spontaneous leukemia. In F, mice of the cross, BALB/c x AKR (H-2d/H-2k), both the occurrence of virus and the incidence of spontaneous leukemia are suppressed to very low values, due largely to the presence of the Fv-1° allele inherited from the BALB/c parent. Mice of the (BALB/c x AKR) F, x AKR backcross generation were observed for possible correlations between virus expressions at 6 wk of age, H-2 type and leukemia incidence. H-2 type showed at most a weak influence on the occurrence of infectious virus, but there was a very strong correlation between the level of virus expression and the occurrence of leukemia and a strong correlation between H-2 type and leukemia. In addition, there was a highly significant nonrandom distribution of virus-negative mice among the backcross litters, suggesting a maternal effect on virus expression.

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