Genetically dominant resistance in mice to 3-methylcholanthrene-induced lymphoma

(murine leukemia viruses/chemical carcinogenesis)

ALLEN MAYER*, FRANK LILLY†, AND MARIA L. DURAN-REYNALS‡

*Department of Pathology, New York University School of Medicine, New York, New York 10016; and Departments of †Genetics and ‡Pathology, Albert Einstein College of Medicine, Bronx, New York 10461

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ABSTRACT Mice of the RF/J strain are highly susceptible to induction of thymic lymphoma by skin painting with 3-methylcholanthrene (MCA), whereas mice of the 129/J and 1/Ln) strains are resistant. Resistance was the dominant trait in F1 mice of crosses of RF with each resistant strain. Analysis of the lymphoma incidence in MCA-painted backcross populations indicated segregation of a single dominant gene for resistance in both crosses. None of these strains show inducibility of the aryl hydrocarbon hydroxylase enzyme system, a phenotype attributed to the dominant Ahb gene which is also known to influence susceptibility to MCA-induced lymphomas. The occurrence of the disease in these backcrosses was independent of the hosts' phenotype at either the H-2 or Fv-1 locus, both of which have shown an influence on susceptibility to murine leukemia virus-associated lymphoma in other experimental systems.

The Ah locus in mice governs inducibility of the aryl hydrocarbon hydroxylase (AHH) enzyme system (1) in response to treatment with 3-methylcholanthrene (MCA), and it also exerts a major influence on susceptibility to tumorigenesis by skin painting with this hydrocarbon carcinogen (2). Strains carrying the dominant Ahb allele for AHH-inducibility are highly susceptible to development of skin tumors as a result of MCA skin painting (3). In contrast, AHH-noninducible mice, homozygous for the recessive Ahd allele, rarely develop skin tumors but respond to MCA skin painting with the development of thymic lymphoma (2). However, the various AHH-noninducible mouse strains display marked heterogeneity in their susceptibility to MCA-induced lymphomagenesis. Some strains (e.g., RF/J) have a characteristic high incidence of lymphoma after MCA skin painting, whereas others (e.g., 129/J) have low incidences. This observation indicates that genetic factors in addition to Ah govern susceptibility to MCA lymphomagenesis.

We now report the results of preliminary studies of the genetic basis of this polymorphism in susceptibility to MCA-induced lymphoma among AHH-noninducible mice. Our findings suggest that a single gene with a dominant allele for lymphoma resistance governs this polymorphism. Furthermore, in the two strain combinations studied, neither H-2b nor Fv-1 alleles showed a capacity to interfere with the MCA-induced disease, although both have shown significant effects on susceptibility to lymphoma in systems in which it occurs spontaneously or is induced by inoculation with various strains of murine leukemia virus (MuLV).

MATERIALS AND METHODS

Mice. Mice of inbred strains were obtained from The Jackson Laboratory (Bar Harbor, ME). F1 and backcross mice were bred in our colony. By convention, the female parent is placed first in designating F1 and backcross mice. All mice in these studies were of the homozygous Ahd type (AHH-noninducible), and female mice were used exclusively.

MCA Treatment. Female mice were treated with MCA beginning at 12–15 weeks of age (4). The flanks and backs of mice were shaved and then painted (marten fur brush) with a 1% (wt/vol) solution of MCA (Eastman) in benzene. MCA application was repeated daily for 5 consecutive days.

Lymphoma Incidence. After MCA treatment, mice were observed daily for onset of symptoms indicative of lymphoma, including labored breathing and enlarged peripheral lymph nodes. Mice displaying these symptoms were sacrificed and autopsied. Gross enlargement of the thymus, often accompanied by enlarged spleen and lymph nodes, was taken as the criterion for lymphoma. Data are presented for the first 6 months after MCA treatment; lymphoma incidences did not significantly increase after this period.

Fv-1 Typing. Individual mice of the (1 × RF)/F1 × RF backcross were typed for the alleles they carried at the Fv-1 locus by determining their phenotype with respect to the glucose-6-phosphate dehydrogenase specified by the closely linked Gpd-1 locus. The Fv-1 and Gpd-1 genes display less than 1% recombination (5). RF mice transmit the Gpd-1b allele; 1 mice transmit the Gpd-1b allele. These codominant alleles code for enzyme variants displaying different electrophoretic mobilities on starch gels (6). Gpd-1a/Gpd-1b heterozygous backcross mice were distinguished from their Gpd-1a homozygous littermates by starch gel electrophoresis of aqueous kidney tissue homogenates. Gpd-1a homozygotes were inferred to be homozygous for the Fv-1a allele transmitted by RF; Gpd-1a/Gpd-1b heterozygotes were considered to carry both Fv-1a and the Fv-1b allele of the 1 strain.

Unilateral nephrectomy of the backcross mice at age 6–8 weeks provided tissue for Gpd-1 typing.

H-2 Typing. Individual backcross mice were tested for their haplotype at H-2, the major histocompatibility complex of the mouse, by hemagglutination (7). Anti-H-2b antisera was obtained by immunizing (BALB/c × C3H)F1 hybrids (H-2d/H-2k) with EL4 lymphoma cells (H-2b). This same antisera was used to detect the H-2d haplotype, which is strongly crossreactive with H-2b.

Abbreviations: MCA, 3-methylcholanthrene; MuLV, murine leukemia virus; AHH, aryl hydrocarbon hydroxylase.

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RESULTS

Crosses Between 129 and RF Mice. RF/J mice (H-2^b, Fv-1^a) developed a high incidence of thymic lymphocytic lymphoma after skin painting with MCA (Fig. 1; Table 1) (4). By 6 months after painting, 92% of treated mice had died from the disease, compared with <10% in untreated RF mice at a corresponding age.

129/J mice (H-2^b, Fv-1^a) developed a much lower incidence of lymphoma in response to MCA treatment (2). Only 10% of treated mice developed lymphoma within 6 months after painting. Untreated 129 mice had a negligible incidence of lymphoma at this age. (129 X RF)F1 hybrid mice displayed the low lymphoma incidence of the 129 parental strain upon MCA painting. The resistance of 129 mice was therefore transmitted as a dominant trait to these F1 progeny.

Studies of the (129 X RF)F1 X RF backcross were conducted in order to determine the number of suppressing genes carried by 129 mice and in order to discern the effect of the H-2^b haplotype on MCA-induced lymphomagenesis. The H-2^b allele is known to exert a dominant restriction with respect to many MuLV-induced lymphomas (5-10). Backcross mice had a MCA-induced lymphoma incidence intermediate between that of the susceptible RF parent and that of the resistant (129 X RF)F1 parent (Fig. 1). By 6 months after treatment, 50% of these backcross mice had died from lymphoma. This result suggests that 129 mice transmit a single dominant gene for resistance to MCA-induced lymphomagenesis.

In order to determine if the H-2 type of the mice plays a role in susceptibility to the disease, backcross mice were tested for the presence of erythrocyte antigens governed by the H-2^b haplotype. No association between H-2 type and the occurrence of lymphoma was observed (Fig. 2; Table 1); the lymphoma incidence of heterozygous H-2^b/H-2^k backcross mice was similar to that of H-2^k homozygotes. This experiment demonstrates that the H-2^b haplotype of 129 mice does not exert a suppressive effect on the development of MCA-induced lymphoma.

Crosses Between I/LnJ and RF Mice. I mice (Fo-1^b, H-2^a) proved to be particularly susceptible to the toxic and debilitating effects of MCA painting because all painted I mice died shortly after treatment. (1 X RF)F1 mice, however, were resistant both to these short-term effects and to the induction of lymphoma. At 6 months after MCA skin painting, only 21% of F1 mice developed lymphoma (Fig. 3; Table 2). The susceptibility of RF mice to MCA induction of lymphoma therefore appears to be recessive in this cross. The intermediate lymphoma incidence of (1 X RF)F1 X RF backcross mice (Fig. 3) indicates the presence of a single dominant gene for resistance transmitted by I/LnJ mice.

The lymphoma incidence in (1 X RF)F1 X RF backcross mice was analyzed to determine if heterozygosity at either Fo-1 or H-2 was associated with suppression of MCA-induced lymphoma in the I X RF cross. In the (1 X RF)F1 X RF backcross, about half of the population were Fo-1^a homozygotes and the remaining half were Fo-1^a/Fo-1^b heterozygotes. These two subpopulations were distinguished by testing individual mice for their phenotype at the closely linked Gpd-1 locus. RF mice carry the Gpd-1^a allele, and I mice carry the Gpd-1^b allele. Gpd-1^a homozygous backcross mice were inferred to be

Table 1. MCA-induced lymphoma incidence in crosses between 129 and RF mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Deaths from lymphoma*</th>
<th>Other deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-2^b/k</td>
<td>H-2^a/k</td>
</tr>
<tr>
<td>RF/J</td>
<td>37/40</td>
<td>92%</td>
</tr>
<tr>
<td>129/J</td>
<td>2/21</td>
<td>10%</td>
</tr>
<tr>
<td>(129 X RF)F1</td>
<td>4/27</td>
<td>15%</td>
</tr>
<tr>
<td>(129 X RF)F1</td>
<td>10/83</td>
<td>12%</td>
</tr>
<tr>
<td>X RF</td>
<td>19/36</td>
<td>53%</td>
</tr>
<tr>
<td>(53%)</td>
<td></td>
<td></td>
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</tbody>
</table>

* By 6 months after MCA treatment.
Table 2. MCA-induced lymphoma incidence in crosses between I/LnJ and RF mice

<table>
<thead>
<tr>
<th>Strain</th>
<th>Deaths from lymphoma*</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-2^k/h</td>
<td>H-2^k/h</td>
</tr>
<tr>
<td>(I × RF)RF)F_1</td>
<td>6/28</td>
<td>(21%)</td>
</tr>
<tr>
<td>(I × RF)RF)F_1</td>
<td>23/34</td>
<td>18/31</td>
</tr>
<tr>
<td>RF × (I × RF)F_1</td>
<td>13/17</td>
<td>10/15</td>
</tr>
</tbody>
</table>

* By 6 months after MCA treatment.

Homozygous Fv-1\textsuperscript{a}; Gpd-1\textsuperscript{a}/Gpd-1\textsuperscript{b} heterozygotes were taken to be Fv-1\textsuperscript{a}/Fv-1\textsuperscript{b} heterozygotes. Both of these backcross subpopulations had similar lymphoma incidences, excluding a suppression of MCA leukemogenesis by heterozygosity at the Fv-1 locus (Fig. 4; Table 2). Such heterozygosity at Fv-1 has repeatedly been shown to exert a potent inhibition on both MuLV infection and associated viral lymphomagenesis (11, 12). This experiment indicates that Fv-1-regulated viral infection is not involved in MCA induction of lymphoma.

These backcross mice were also typed for their H-2 genotype, to discern any possible H-2 effect. The presence or absence of the segregating H-2\textsuperscript{f} haplotype had no effect on MCA lymphomagenesis in this backcross (Fig. 5; Table 2).

Similar results were observed in the reciprocal RF × (I × RF)F\textsubscript{1} backcross population (Table 2), although fewer mice were used in this cross.

**DISCUSSION**

Our results indicate that both the 129/J and I/LnJ mouse strains carry single dominant genes which, in crosses with the susceptible RF/J strain, are capable of protecting mice against thymic lymphomagenesis by skin painting. No evidence was obtained to indicate whether or not these resistance genes map at the same locus in the two resistant strains. In neither case was linkage detected between lymphoma resistance and either the Fv-1 or H-2 locus, an observation that may be pertinent to the etiology of MCA-induced lymphoma because alleles at these two loci are known to be capable of conferring resistance to the development of MuLV-induced lymphoma.

In mice of the AKR strain, which shows a nearly 100% incidence of spontaneous thymic lymphoma early in life (∼9 months of age), the occurrence of this neoplasm has been shown to be associated with the presence of endogenous, chromosomally located, MuLV genomes, such as Akv-1 (13, 14), which are transmitted as Mendelian dominant characters. However, possession of such MuLV genes is not in itself sufficient to generate the disease because several dominant genes have been shown to be capable of suppressing its occurrence in crosses of AKR mice of low-incidence strains. In some crosses this dominant lymphoma resistance has proved to be due to interference with the infectious spread of endogenous MuLV in somatic tissues, an effect mediated by the Fv-1\textsuperscript{b} allele (12). In other cases, this resistance has been due to genes mapping at the H-2 locus and most frequently associated with the H-2\textsuperscript{b} haplotype (12, 15, 16). These H-2-associated resistance genes appear to have little influence on infectious expression of endogenous MuLV genomes but rather govern in a poorly defined manner the capacity of the host to mount an effective immune response to MuLV-associated antigens of lymphoma cells.

Both normal and lymphomatous tissues of AKR mice show a characteristic pattern of infectious MuLV expression, including both ecotropic MuLV (infectious mainly in mice or mouse cells) detectable in the XC plaque assay (17) and xenotropic or polytropic MuLV (infectious in cells of nonmurine species) detectable by immunofluorescence after infection of mink lung cells (18). However, unpublished results from our laboratory indicate that normal and lymphomatous tissues of RF mice contain only low levels of XC-positive ecotropic MuLV and no MuLV infectious for mink cells. This observation, together with the absence of an effect of Fv-1 or H-2 on MCA lymphomagenesis in the present studies, suggests that, if endogenous MuLV is etiologically involved in MCA induction of RF thymic lymphoma, either the virus or the mechanism of its generation appears to be different from its analogue in AKR spontaneous lymphoma.

The lymphoma resistance conferred by the dominant gene(s) revealed in these studies might act in any of a number of hypothetical ways. It is not ruled out that it might represent a hitherto undetected allelic difference at the Ah locus among AHH-noninducible mice and that this new allele, like Ah\textsuperscript{b}, alters the metabolic fate of MCA in a manner that protects mice from the lymphomagenic action of the carcinogen.
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