Role of the AKR Gene Locus AKv-1 in Susceptibility to Chemical Induction of Thymic Lymphomas

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ABSTRACT

Various strains of mice demonstrate widely differing susceptibility to chemical induction of thymic lymphomas, in both timing and incidence. In AKR mice tumors appear very early and at high incidence after a single dose of N-methyl-N-nitrosourea, while in other strains they appear later and at lower incidences. In an attempt to determine the potential role of AKR ecotropic murine leukemia virus loci in this process, congenic mice of NFS/N background, into which the highly productive ecotropic murine leukemia virus loci AKv-1 or AKv-2 has been transferred, were challenged with N-methyl-N-nitrosourea. Although they had a lower incidence of thymic lymphomas than did the parental donor AKR, the NS.AKv-1 mice had a tumor incidence twice that of NFS/N or NS.AKv-2. However, no difference in timing was noted, and these three strains demonstrated tumor appearance much later than that of AKR/N. It is suggested that the presence of the AKv-1 loci, or a gene of the closely associated genomic region, increases the number of target cells that are susceptible to N-methyl-N-nitrosourea.

INTRODUCTION

A series of events involving viral expression, cellular infection, and multiple genetic recombinations occur during the evolution of spontaneous thymic lymphomas of the AKR mouse (1, 2). AKR mice are also highly susceptible to the induction of these tumors by chemical agents and young mice exposed to a single dose of the carcinogen MNU develop thymic lymphomas within 2–3 months, achieving a final incidence in excess of 90% (3–5). This is much earlier than spontaneous thymic lymphomas in strains that do not express endogenous, promotional factors.

Kozak and Rowe (9) have developed congenic strains of mice that carry high expression AKv loci from AKR/N on an NFS/N background. These congenic lines demonstrate high titers of XC-detectable virus in tail extracts and high inducibility in cultured cells. The NSF/N parental line is negative in both instances (10).

In the present study, I compared the responsibility of these congenic lines to MNU induction of thymic lymphomas to determine the potential role of specific ecotropic MuLV loci (AKv-1 and AKv-2) in the pathogenesis of these tumors and to determine whether factors other than known viral loci might also contribute to the process. It appears from the results that a specific, high-titer viral locus, AKv-1, contributes to the final incidence but not the timing of thymic lymphomas when present in a common genetic background. It is suggested that the increase in incidence results from an increase in available target cells, whereas the timing of appearance may depend on endogenous, promotional factors.

MATERIALS AND METHODS

Breeding stock of the congenic lines were a gift from Dr. Janet W. Hartley. AKv-1 and AKv-2 are chromosomal loci containing sequences of ecotropic murine leukemia viruses (9). Each has been established in the NFS/N strain, which has no ecotropic virus-inducing loci (10). The resulting congenic lines, NS.AKv-1 and NS.AKv-2, were highly positive by the XC assay (11) after receipt in our laboratory, the NFS/N, negative. The assay is translated into four quantitative values, 1+ to 4+, and in all of the tests performed on the mice used in these experiments, NS.AKv-1 and NS.AKv-2 demonstrated a similar range of values, 2+ to 4+. Assay of the stocks at the end of the experiments confirmed the persistence of the original viral status. NSF/N, NS.AKv-1, and NS.AKv-2 mice were bred continuously in this laboratory. AKR/N mice were obtained from the National Cancer Institute Cancer Treatment program. NSF/N × AKR/N-F1 and AKR/N × NFS/N-F1 mice were also bred in this laboratory.

Freshly prepared MNU was administered i.p. as a single dose of 75 mg/kg to female mice at 7 weeks of age. Each mouse was palpated weekly by a single observer and was sacrificed as soon as a tumor was detected. Every thymic lymphoma or thymus was sectioned and examined histologically and the experiment was terminated when the mice reached 8 months of age to avoid the difficulty of distinguishing between induced and spontaneous tumors in AKR/N. However, additional groups of the other strains have been followed to 1 year of age without a significant increase in incidence. These strains demonstrated relatively low incidences of spontaneous (untreated) thymic lymphomas.

For examination of the effect of MNU on thymus weight and cellularity, mice of each line were treated at 7 weeks of age with 75 mg/kg as in the induction experiments. Mice were then sacrificed at intervals, and the thymuses were dissected free of other tissues, weighed, and prepared for histology. Untreated 11-week-old mice were used as a control for mice examined at 3 and 4 weeks after MNU treatment.

RESULTS

Incidence of Thymoma after MNU. The incidences of thymic lymphomas could be roughly grouped as high, intermediate, and low (Table 1). Thus, AKR/N and NFS/N × AKR/N F1, had average incidences of 96 and 80%, respectively; AKR/N × NFS/N F1, and NS.AKv-1, 62 and 59%; and NFS/N and NS.AKv-2, 27 and 26% respectively. Multiple runs were performed on each line since moderate variation in incidence
occurred in each run. The earliest appearance of thymoma was also in AKR/N and NFS/N × AKR/N F1 mice. However, only in AKR/N, which demonstrated a mean time to appearance of 93 days and a relatively tight range of 63–119 days, was there a statistically significant difference from other strains.

With only minor variation, every thymic lymphoma conformed to the diagnostic criteria for undifferentiated, malignant lymphoma (12). This includes the stary sky pattern and monocellular infiltrates.

**Table 1** Incidence of thymic lymphoma induced by MNU a

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of runs</th>
<th>Total mice</th>
<th>Average incidence (%)</th>
<th>Mean timed appearance (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>9</td>
<td>64</td>
<td>96</td>
<td>93 (63–119)</td>
</tr>
<tr>
<td>NFS × AKR</td>
<td>5</td>
<td>24</td>
<td>80</td>
<td>109 (76–160)</td>
</tr>
<tr>
<td>AKR × NFS</td>
<td>3</td>
<td>13</td>
<td>62</td>
<td>140 (88–154)</td>
</tr>
<tr>
<td>NS.AKv-1</td>
<td>4</td>
<td>17</td>
<td>59</td>
<td>150 (86–171)</td>
</tr>
<tr>
<td>NFS</td>
<td>5</td>
<td>33</td>
<td>27</td>
<td>135 (90–170)</td>
</tr>
<tr>
<td>NS.AKv-2</td>
<td>3</td>
<td>19</td>
<td>26</td>
<td>155 (145–195)</td>
</tr>
</tbody>
</table>

* MNU was administered at 75 mg/kg i.p. at 7 weeks of age to female mice.

**Table 2** Thymus response to MNU

<table>
<thead>
<tr>
<th>Strain</th>
<th>0 h b</th>
<th>48 h b</th>
<th>72 h b</th>
<th>1 week c</th>
<th>4 weeks d</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR/N</td>
<td>(8) 0.134</td>
<td>(6) 0.038 (28%)</td>
<td>(6) 0.036 (27%)</td>
<td>(6) 0.050 (37%)</td>
<td>(6) 0.089 (66%)</td>
</tr>
<tr>
<td>NFS/N</td>
<td>(7) 0.068</td>
<td>(2) 0.056 (82%)</td>
<td>(6) 0.048 (71%)</td>
<td>(4) 0.048 (71%)</td>
<td>(3) 0.053 (78%)</td>
</tr>
<tr>
<td>NS.AKv-1</td>
<td>(5) 0.070</td>
<td>(3) 0.038 (54%)</td>
<td>(3) 0.038 (54%)</td>
<td>(3) 0.038 (54%)</td>
<td>(3) 0.066 (94%)</td>
</tr>
<tr>
<td>NS.AKv-2</td>
<td>(5) 0.060</td>
<td>(3) 0.034 (57%)</td>
<td>(3) 0.030 (50%)</td>
<td>(4) 0.032 (53%)</td>
<td>(3) 0.048 (80%)</td>
</tr>
<tr>
<td>AKR × NFS</td>
<td>(5) 0.110</td>
<td>(3) 0.026 (24%)</td>
<td>(2) 0.027 (25%)</td>
<td>(6) 0.028 (26%)</td>
<td>(3) 0.076 (69%)</td>
</tr>
<tr>
<td>NSF × AKR</td>
<td>(11) 0.084</td>
<td>(3) 0.036 (43%)</td>
<td>(3) 0.047 (56%)</td>
<td>(5) 0.045 (54%)</td>
<td>(3) 0.076 (90%)</td>
</tr>
</tbody>
</table>

* Average wet weight in grams of whole thymus at 7 weeks of age. First numbers in parentheses, number of mice.

**DISCUSSION**

It was originally suggested by Frei (3) that the early appearance and high incidence of chemically induced thymic lymphomas in AKR mice were the result of their endogenous viruses, acting as a hit in a multihit sequence. Subsequently, Mayer and Dorsch-Hasler (8) and others (4, 6, 7) have presented evidence that indicates that the expression of infectious, endogenous, ecotropic viruses is not required for a carcinogen to evoke these tumors. However, this evidence did not rule out a potential role of the viral genome itself in the process. Further, there is substantial evidence that the chemically induced thymic lymphomas of AKR mice differ in cell lineage from those of spontaneous origin and fail to demonstrate the mink cell focus viruses of the latter, which result from recombinant events (4).

None of the studies that have analyzed the components of carcinogen induction of thymic lymphomas have revealed the mechanisms that account for the early appearance and high incidence in the AKR strain. Recently, it was reported MNU-induced thymic lymphomas appeared at an earlier time in AKR/J × C57L/J F1 than in C57L/J mice (13). A similar finding was noted in the current study where thymic lymphomas appeared earlier in NFS × AKR F1 than in NFS/N mice (Table 1). That the earlier appearance of these tumors was not related to the presence of either of the highly productive, ecotropic MuLV loci was evident from the failure of tumors in either NS.AKv-1 or NS.AKv-2 to be detected earlier than those of NFS/N.

The current report does indicate, however, that the presence of one AKR allele, AKv-1, is associated with a heightened degree of susceptibility to thymic carcinogenesis. NS.AKv-1 mice demonstrated an incidence of 59% as compared with 27% for the parental NFS and 26% for NS.AKv-2. This, despite the fact that NS.AKv-2 also demonstrated high titers of virus in the XC test. We have reported evidence that suggests that the differences in incidence or timing of tumors do not result from a differing capacity to “metabolize” MNU (14), a direct carcinogen activated by hydrolysis, nor from differing ability to repair or regenerate their cells. Thus, the cellularity of untreated thymuses of these 3 strains were not significantly different, and they did not differ in the degree of cell loss induced by MNU or in regenerative capacity. Admittedly, however, these measurements of relatively gross alterations would not detect any differences that were limited to a relatively minor population of target cells.

A number of mechanisms, all unproven, have been suggested to play a role in chemical induction of thymic carcinogenesis. These include the activation of ras oncogenes (15, 16), expression of viral antigens (17) and the induction of “new,” somatically acquired MuLV ecotropic-like loci (6). Alterations in one or several of these could contribute to differences in susceptibility. Although the AKv-1 allele might participate in any of these mechanisms, it must also be recognized that an associated portion of the proximate tip of chromosome 7 (18) is carried with this gene during the establishment of the congenic strain. The NS.AKv-1 congenic strain resulted from at least 10 backcrosses of the AKv-1 into the NFS/N strain. It has been reported that a passenger gene 10 cM from the differential locus would have an approximately 39% chance of being retained after this number of backcrosses (19). The total length of associated DNA segments could be as long as 12–20 cM. According to the most recently available mouse chromosomes map (20), histocompatibility genes 22 and 24, the feline sarcoma oncogene, and the xenotropic mink cell focus leukemia virus 35 are all present within this length of DNA (20). These and others must therefore be included in the analysis of potential
tial participants. Interestingly, the report of MNU induction of thymic lymphomas in AKR/J x C57L/J F1 suggested that their induction was linked to the albino locus on chromosome 7 (13). Although this locus is more distant from the AKV-1 than the DNA lengths suggested by the number of backcrosses used in the current experiments, the localization of the albino locus to the same arm as AKV-1 may be significant.

After exposure to a maximal, single dose of carcinogen above which toxicity to the host becomes a significant factor, the final number of tumors is determined by the number of “target cells” that can be “initiated.” If “promotion” is defined as the capacity to cause initiated cells to replicate to a critical population at risk, and the number of target cells is constant, then the effect of promotion should be predominantly on the time of tumor appearance (21, 22). Since none of the F1, strains demonstrated a time of appearance of thymic tumors that was significantly different from NFS or another F1, the greatly accelerated appearance of these tumors in AKR/N was not dependent upon the presence of the AKV loci alone. This phenomenon, like that reported by Mayer and Dorsch-Hasler (8) in other high and low susceptibility crosses, suggests that certain strains of mice such as AKR and RF possess an endogenous promotional capacity that hastens the evolution of cells from the initiated to the cancerous state. These findings are also similar to those in a genetically modulated mouse liver system (23).

The failure to detect a premalignant tumor transition in this process (Ref. 24; data from this study not included), the similarity of histiotype of the resultant thymomas, growth rates, and clinical course (24, 25), suggests that the process of “progression” was not participatory. It appears likely that the first, histologically detectable foci of altered cells are identical to those of the clinically detectable thymic tumors. This would suggest that neither progression nor promotion plays a significant role in the sequential development of chemically induced thymic lymphomas. It has also been suggested that this can occur in other systems such as chemically induced colon tumors (26–28).

Several observations from the current and previous reports can be utilized in the analysis of the phase of carcinogenesis affected by the AKV-1 allele. The presence of this AKV-1 locus [or another gene(s) in the associated length of chromosome] increased the incidence of thymic lymphomas, but did not affect their timing or phenotype. No detectable, premalignant lesion has been detected in this process (24, 25), nor was the phenotype of the final tumors different from those of the NFS/N. Therefore, it appears most likely that the presence of this portion of chromosome 7 enhances the susceptibility of a subset of cells to MNU initiation, in effect, increasing the number of target cells.

One additional, previously unreported finding supports the proposal that chemical induction of thymomas is under complex control. The sex of the parental line appears to exert a modifying component. Thus, the incidence of thymoma in NFS × AKR F1 was 80% while that of AKR × NFS F1 was 62%, although the differences were not significant.

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