Host-Gene Control of Type-C RNA Tumor Virus Expression and Tumorigenesis in Inbred Mice

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ABSTRACT We analyzed the relationship of genetic factors determining the expression of endogenous type-C RNA tumor viruses and other host-gene markers to tumorigenesis. A hybridization experiment was performed with mice of strains AKR/J and C57L, the first filial (F1) generation hybrids, the second filial (F2) generation hybrids, and the backcrosses to the two parental strains. The results demonstrated a highly significant and predictable association between the expression of complete infectious virus or the viral group-specific (gs) antigen in spleens of young mice and tumorigenesis later in life. Most of the tumors were thymic leukemia and reticulum sarcoma, but other mesenchymal, tumors were also observed. Tumors occurred preferentially in gs-antigen- or virus-positive mice of all crosses; in the C57L-backcross and F2 mice segregating for gs-antigen and virus expression, a few gs-antigen-negative mice developed reticulum cell sarcomas. At the time of their occurrence, the mice were all gs-antigen-positive, and most had virus as well.

A minor effect of the major histocompatibility locus, H-2, on leukemogenesis was found in the F1 mice. Several tumor types were also found that we have never observed in the two parental strains. Our data provide the most direct biological evidence in favor of the viral oncogene theory. Thus, from the presence or absence of expression in early life of splenic gs antigen or virus, we can predict whether or not a tumor is likely to develop later in life. These findings suggest that the genome of endogenous type-C RNA viruses is the major determinant for tumorigenesis although they provide no clues about the factors responsible for the various histological types.

In studies on the inheritance of murine type-C RNA tumor (leukemia) virus (MuLV), we demonstrated specific host genetic regulation of expression (1). Two autosomal dominant genes determine the group-specific (gs)-1 antigen and complete the virus. Strains lacking these genes must contain the viral glycoprotein in covert form because gs antigen is frequently observed in old mice of such “switched-off” strains as C57BL/10Sn, SWR/J, and C57L/J (2); gs antigen may also be induced in negative mice with carcinogenic and other chemicals, and occasionally complete leukemogenic virus is recovered from “spontaneous” or carcinogen- and radiation-induced tumors (3–5).

Although the oncogenicity of type-C RNA tumor viruses upon inoculation into appropriate hosts is unequivocal, the role of the expression of endogenous oncogenic viruses in

Abbreviations: MuLV, murine type-C RNA tumor (leukemia) virus; CF, complement fixation; MSV, murine sarcoma virus; RCS, reticulum cell sarcoma; BC, backcross gs, group specific.

“spontaneous” and chemical carcinogenesis is at most inferred from studies of gs-antigen or virus induction, and cell-free tumor transmission. To determine unequivocally a relationship, if any, of endogenous viral expression to tumorigenesis we performed a genetic hybridization experiment involving a strain with a high incidence of leukemia, AKR/J, and one with a low incidence, C57L/J, their reciprocal F1 hybrids, and the F2 and backcross generations of this cross. We found a highly significant association of gs-antigen or complete virus expression(s) and oncogenesis, i.e., from the presence or absence of complete infectious virus or gs antigen in spleens of young adult mice, we could predict whether or not individual mice will develop a tumor later in life. Furthermore, we found a weak association of the major histocompatibility locus (H-2) and of three other host genes with tumorigenesis.

MATERIALS AND METHODS

Mice and Breeding Methods. The parental strains, AKR/J and C57L/J, were obtained from the production colonies of The Jackson Laboratory. Mice of F1 and segregating generations were separated by sex at weaning, individually ear-marked, and housed five per cage in stainless steel cages, 5 × 11 × 6 inches. Water and Old Guilford Laboratory chow were freely provided. Cages were changed at weekly intervals, when fresh bedding and water were provided. AKR/J and C57L/J mice were mated reciprocally to produce the two kinds of F1 mice. Parental and F1 mice were spleenectomized at 4–6 weeks of age and then set aside for tumor development; the same procedure was followed for the F2 and backcross mice. Their spleens were then tested for gs antigen and complete virus (1).

Complement-Fixation Test for MuLV gs Antigen. The presence of gs antigen or MuLV in mouse spleens was determined with the complement-fixation (CF) test. The spleens were suspended 1:10 (w/v) in Eagle’s minimal essential medium and sonicated with three 1- to 2-sec bursts at the lowest setting of a Heat Systems sonifier model 140W. These suspensions were clarified by centrifugation at 1500 rpm for 5 min and diluted 1:2 and 1:4 for use in CF (6, 7). Three antisera prepared in rats and one prepared in guinea pigs were used to detect gs antigen by CF. Antiserum pools MSV-26 and 30 were derived from inbred Fischer rats carrying transplantable tumors induced by the Moloney variant of murine sarcoma virus (M-MSV). These antisera had been selected for reactions giving relatively high titers (1:80–1:160), with gs-1 and for negative reactions (at 1:10) with other antigens.
Demonstration of Complete Infectious Virus and Virus Particles. To detect "infectious" MuLV, we used the CF test for MuLV or COMuL test (7). Duplicate secondary SWR/J mouse-embryo tissue cultures were inoculated with 0.1 ml of unsolicited 10% spleen suspension 18-24 hr after plating. 60-mm Plates were seeded with 350,000 cells each. After 18-20 days of incubation at 37°C, one plate was scraped and the cells were suspended in 0.6 ml of Eagle’s minimal essential medium and sonicated; this material was then tested for gs-1 by CF. The other plate was scraped and passed in fresh whole embryo cultures. Second-passage plates were again tested in CF at 18-21 days. Negative COMuL tests after three passages indicate absence of complete, "infectious" virus. For each assay, at all three passage levels, positive and negative controls were included; these consisted of plates inoculated with an AKR/J spleen suspension and uninoculated plates. SWR/J cells are the most permissive for AKR/J virus.

We also used the XC-plaque assay for virus isolations and titrations (8, 9). Unsolicited 10% (w/v) tissue extracts were inoculated onto SWR mouse-embryo tissue cultures in 50-mm petri dishes grown and maintained on minimal essential medium with 10% heat-unheated fetal-calf serum, 2 mM glutamine, 250 units/ml of penicillin, and 250 µg/ml of streptomycin. The cultures were exposed to 25 µg/ml of DEAE-dextran for 1 hr at 37°C and rinsed immediately before inoculation. 6 Days after virus inoculation the cultures were exposed to UV light for 25 sec from two germicidal bulbs at 60 ergs/mm² per sec, and 10³ XC cells were added per dish. 4 Days later the dishes were fixed and stained with Giemsa stain. Virus titers were obtained by comparison of the percent plaquing efficiency of reference virus standards run simultaneously on SWR cells. Plaques were recognized by the absence of growth of XC cells, a Rous sarcoma virus-induced rat tumor line in areas of syncytium formation.

Tumors and other tissues in gs-antigen- and virus-negative mice were also studied by electron microscopy for type-C virus particles. Tissues were fixed in 2% glutaraldehyde followed by dehydration with alcohol; they were then embedded in Araldite by standard procedures. The sections were evaluated in a Hitachi electron microscope, type HU-11C, at an accelerating voltage of 75 kV.

Genetic Analyses and Linkage Testing. Detailed genetic studies on the inheritance of the gs antigen and complete virus have been published (1). The fact that spleens from all F₁, and AKR-backcross (BC) mice were antigen-positive indicated that the gene(s) for the presence of the gs antigen is dominant to its allele for the absence of the antigen. However, we observed gs-antigen- and virus-negative segregants among F₂ and C57L/BC mice. In C57L/BC progeny, the ratio of gs-positive to gs-negative mice approximated a 3:1 ratio as if negative CF tests were due to simultaneous homozygosity of recessive alleles at two independently inherited autosomal genes. Results obtained in the F₃ generation tended to support this interpretation, as a 15:1 positive to negative ratio was obtained. Data on virus isolation in C57L-BC and F₂ mice suggested that most, if not all, gs-positive mice also had complete replicating virus. Thus, the segregation of gs antigen and virus in C57L-BC and F₂ mice afforded us the opportunity of examining whether the appearance of tumors later in life could be predicted from the results of CF- and virus-testing early in life.

We also typed the backcross- and F₂-generation mice for several other genetic loci, to try mapping the genes controlling expression of antigen and complete viruses and to search for additional genetic determinants that may be relevant to tumorogenesis. The H-2 genotype was ascertained by erythrocyte agglutination (10). The antisera against H-2k was prepared by immunizing C3H-H-2k mice with C3H/Disn spleen, thymus, and lymph node (11). The antisera against H-2b was obtained from C3H/Disn mice immunized with the same organs from A.SW and C57BL/10Sn mice. A few mice for Thy-1 (Theta) and Ly-2 (Ly-B) were serotyped by in vitro absorption of cytotoxic antisera (11). Preliminary experiments with non-H-2 antigens showed that frozen thymus could be teased upon thawing to yield cells with good specific absorptive capacity. These cells were counted and used at single, or occasionally several, concentrations to absorb separately antisera specific for Thy-1.1 (Theta-AR), Thy-1.2 (Theta-C3H), Ly-2.1 (Ly-B.1), and Ly-2.2 (Ly-B.2).

Mice from the F₂ and backcross to C57L/J were typed with respect to the electrophoretic variant of the β-chain of hemoglobin after hemolysate-alkylation (12). The backcross mice were classified as either Hbb*1/Hbb* or Hbb*2/Hbb*. Hbb* and Hbb*2, respectively, are the symbols for the alleles determining single (C57L/J type) and diffuse (AKR/J type) electrophoretic hemoglobin patterns. The F₂ generation was also Hbb typed. Mice produced by backcrossing to AKR were not typed since the albino locus (c) that is closely linked to Hbb is segregating in the cross.

The zymogram technique was used for analysis of loci determining allozymes; these were serum and kidney esterases (Es-1 and Es-2), dipeptidase (Dip-1), and autosomal glucose-6-phosphate dehydrogenase (Gpd-1) (13). Additional markers used were the three coat color genes, albinism (c), brown (b), and leaden (ln).

Pathological Evaluation. When the mice set aside for tumor development were moribund they were killed. Tumors and other tissues were removed for diagnostic pathological and virological evaluation. We recorded deaths from all causes, and all diagnoses were verified histologically. The slides were read without knowledge of the phenotype with respect to gs antigen or virus.

RESULTS

Development and Types of Tumors. Strains AKR/J and C57L/J were selected for this experiment for two reasons: AKR/J mice are leukemia-prone, and their spleens are strictly CF-positive and contain infectious virus throughout life. C57L/J mice are gs-antigen- and virus-negative, and highly resistant to tumorogenesis even when 2-3 years old (14). All of 34 AKR/J mice (100%) developed leukemia within 1 year or less, whereas 4 of 21 C57L/J mice (19%) developed reticulum cell sarcomas (RCS) at a mean age of nearly 2 years.
(Table 1). We found 277 tumors among 382 mice; tumors occurred in the progeny of all crosses, including types that were never observed in the parental strains. 10 Different tumor types were found, although most were thymic leukemia and RCS. Thus, both AKR/J and C57L/J strains must contribute additional genes that determine the development of tumors other than those characteristically occurring in these strains, including hepatoma, pulmonary adenoma, hemangioido-thelioma, and epidermoid (squamous cell) carcinoma. All but one F1 mouse died with tumors, and one mouse had both an RCS and a mammary adenocarcinoma. Since RCS occurred more often than leukemia in the F1 hybrids, RCS development, which is characteristic of strain C57L/J in old age, appeared to be dominant over leukemia development. In contrast, the AKR-backcross genotype conferred greater susceptibility to leukemia than RCS development, and leukemia developed significantly earlier in life (409 ± 22 days) than in BC-C57L/J (564 ± 22 days) and F1 (471 ± 25 days mice). Also, leukemia was fatal at younger ages than RCS independent of the genotype (Table 1).

Apparently RCS originated in the mesenteric lymph node, but involved most other organs of the endoreticular system as well. The tumorous mesenteric lymph node may reach a size 6 × 6 cm, and large well-defined tumor masses occur in the liver. In contrast, leukemias involved primarily the thymus, but the spleen and peripheral lymph nodes were also enlarged. Leukemias occurred exclusively in gs antigen, virus-positive mice; this was true for all other tumors, including epithelial tumors, and all but several of the RCS occurring in originally negative BC-C57L mice.

Six mice had two types of tumors each. All but one were associated with RCS and consisted of a mammary adenocarcinoma, hepatoma, hepatocarcinoma, and epidermoid carcinoma. One mouse had both a pulmonary adenoma and a subcutaneous lipoma. These findings confirm previous observations that the appearance of one type of tumor does not always exclude that of another (3), and transformation events may occur simultaneously and independently in two different types of tissues or organs (15).

**Relationship of gs-Antigen and Virus Expression to Tumorigenesis.** Spleens from F1 and from AKR-backcross mice at 4–6 weeks of age were uniformly positive with respect to both gs antigen and virus, as is the AKR/J parent strain. C57L/J mice are uniformly gs-antigen negative, and negative mice occurred in the backcrosses of F1 mice to C57L/J (BC-C57L) mice and in the F2 generation. In fact, the ratio of gs-antigen-positive to gs-antigen negative mice in the backcrosses was about 3:1 (192:58), and that of the F2 was 15:1 (70:4). Complete infectious virus was isolated from the spleens of all AKR-backcross mice. In a sample of F2 and C57L-backcross mice, virus was isolated from all mice that were gs-antigen positive, suggesting that most, if not all of them, had complete replicating virus; no virus was isolated from gs-antigen-negative BC-C57L and F1 mice.

Segregation in C57L-BC mice and F2 with respect to gs

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>AKR/J</th>
<th>C57L/J</th>
<th>F1</th>
<th>BC-AKR</th>
<th>BC-C57L</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymic leukemia</td>
<td>34</td>
<td>(240 ± 11)</td>
<td>2</td>
<td>33</td>
<td>54†</td>
<td>30</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
<td>4</td>
<td>(458 ± 11)</td>
<td>6*</td>
<td>13</td>
<td>62†</td>
<td>22</td>
</tr>
<tr>
<td>Mammary adenocarcinoma</td>
<td>2</td>
<td>(744 ± 60)</td>
<td>62</td>
<td>1</td>
<td>1</td>
<td>(521)</td>
</tr>
<tr>
<td>Pulmonary adenoma</td>
<td>1</td>
<td>(854)</td>
<td>3§</td>
<td>1</td>
<td>(694 ± 39)</td>
<td>(876)</td>
</tr>
<tr>
<td>Lipoma</td>
<td>1</td>
<td>(396)</td>
<td>1</td>
<td>(510)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatoma, hepatocarcinoma</td>
<td>1</td>
<td>(768)</td>
<td>1</td>
<td>(590 ± 63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermoid carcinoma</td>
<td>1</td>
<td>(396)</td>
<td>1</td>
<td>(851)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangioido-thelioma</td>
<td>1</td>
<td>(403)</td>
<td>1</td>
<td>(403)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>1</td>
<td>(643)</td>
<td>1</td>
<td>(643)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total no. tumors/ total no. mice</td>
<td>34/34</td>
<td>4/21</td>
<td>13/14</td>
<td>46/65</td>
<td>124/178</td>
<td>56/70</td>
</tr>
<tr>
<td>Percentage of mice with tumors</td>
<td>100</td>
<td>19</td>
<td>93</td>
<td>71</td>
<td>70</td>
<td>80</td>
</tr>
</tbody>
</table>

Values in parentheses are survival times in days ± SE.

* One mouse with both a reticulum cell sarcoma and a mammary adenocarcinoma.
† One mouse each also had a heptoma, hepatocarcinoma, and an epidermoid carcinoma.
‡ One mouse also with a pulmonary adenoma.
§ One mouse also with a subcutaneous lipoma.
¶ Also had leukemia.
antigen and virus provided an excellent model for studies of the possible association of viral expressions and tumorigenesis. Of 178 C57L-BC mice, 124 developed tumors and 54 died from other causes. In the F₁, 56 of 70 mice developed tumors, and 14 died from nontumorous conditions. Tumors occurred preferentially (BC-C57L) or exclusively (F₁) in mice that were spleen-positive for either or both gs antigen and virus early in life (Table 2); few tumors developed in BC-C57L mice that were gs-antigen negative. Of 124 tumors developing in BC-C57L mice, 110 occurred in gs-positive mice, whereas only 14 developed in originally gs-negative mice (Fig. 1). In F₁ mice, tumors developed exclusively in gs-positive mice (56/56) and none developed in the few gs-negative mice. In both crosses, tumors were more frequent than nontumorous conditions. This association of partial (gs-antigen) or complete virus expression in early life (4–7 weeks of age) with tumor development later in life is highly significant (P < 0.001) in BC-C57L mice; too few gs-negative mice occurred in the F₁ generation to allow for a statistical analysis, although they confirmed our findings in BC-C57L mice. All gs-antigen- and virus-positive AKR/J mice and all but one mouse of the F₁ hybrids developed tumors, whereas the gs- antigen- and virus-negative C57L/J mice had a low incidence of tumors. Since thymic leukemias (153/277) and other mesenchymal tumors (265/277), particularly RCS (107/277), were the most prevalent tumors among the parental strains and their crosses, the occurrence of the few epithelial tumors (12/277) probably did not affect the statistical analyses. Thus, there was a highly significant association, at least for mesenchymal tumors and endogenous viral expression, in early life; however, all but one of the epithelial tumors developed in originally gs-antigen- or virus-positive mice. From these findings we conclude that: gs-antigen or virus expression in spleens of normal mice coincides with or parallels that in tumors of aging mice, and the endogenous type-C RNA viral genome is a major determinant of at least leukemias and RCS and perhaps of all types. Thus, the findings represent the most direct evidence in favor of the oncogene hypothesis (16, 17).

Most of the gs-antigen-negative and virus-free BC-C57L mice died from conditions other than tumors, and their survival times (743 ± 32 days) were similar to those of mice dying with tumors (714 ± 35 days). All of the tumors occurring in the mice that were originally gs-antigen and virus negative were RCS except for a single mammary adenocinoma, and all 14 of them revealed either gs antigen or virus by direct CF test, virus isolation by COMuL or XC-plaque assay, and EM evaluation. Thus, since the development of these tumors and gs antigen or virus in them was closely associated, an etiological relationship may either be indicated or suggested.

### Table 2. Occurrence of tumors in crosses segregating for the murine type-C RNA tumor virus or its group-specific antigen

<table>
<thead>
<tr>
<th>Conditions</th>
<th>BC-C57L</th>
<th>F₁</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>gs-AG+</td>
</tr>
<tr>
<td>Tumors*</td>
<td>124</td>
<td>110</td>
</tr>
<tr>
<td>Other†</td>
<td>68</td>
<td>24</td>
</tr>
</tbody>
</table>

* Tumors of all types occurring in mice that were either positive (gs-AG+) or negative (gs-AG-) for splenic gs-AG at 4–7 weeks of age.
† Mice dying from conditions other than tumors.

gs-AG+, positive for the group-specific (gs-1) antigen (gs-AG) or complete infectious virus; positive = 3+ or greater fixation of 1.8 units of complement; gs-AG-, negative for both gs-1 and virus within the limits of our assays.

**Occurrence of Nonneoplastic Diseases.** Mice died from several nonneoplastic disease(s) including bronchopneumonia, vascular disease, peribronchial granulomatosis, and glomerulonephritis. The last three were the most frequent. Vascular disease consisted of generalized perivascular lymphoid cuffing and arterial hyalinizations or polyarteritis; glomerulonephritis was characterized by segmental or granular thickenings and deposits of periodic-acid Schiff-positive materials in the glomerular mesangium and basement membrane. Glomerulonephrosis, polyarteritis, and peribronchial granulomatosis may possibly represent immune complex diseases. A possible influence of sex on deaths from tumor or nontumor conditions has not been evaluated.

**Relationship of Viral Expressions and Tumor Development with Various Host Marker Genes.** Whereas the singularly most significant association relates to viral expression and tumorigenesis, we found three others that we are exploring further: (a) a "weak" relationship (0.1 > P > 0.05) between the H-2 genotype and leukemia development in F₁ mice, (b) a possible linkage effect of Dip-1 (linkage group XIII) on all tumors in both F₁ (0.1 > P > 0.05) and BC-C57L (0.2 > P > 0.1) mice, and (c) an association of a gene linked to Es-1 (linkage group XVIII) with tumor development (0.1 > P > 0.05). Whereas no interaction exists between H-2 (linkage...
Similarly, of spontaneous leukemia and the the no times leaden (In) mouse.

Both ln and Dip-1 are linked. Whether the association of Dip-1 and ln to tumor resistance is related to gs antigen or virus expression is not known.

**DISCUSSION**

**Effect of Splenectomy on Tumor Development.** Studies by other investigators (18, 19) indicated that splenectomy had no significant effect on the incidence, type, and latent period of spontaneous leukemia in mice of either AKR or C58 strain. Similarly, we found no tumor inhibition by splenectomy in two experiments involving the high-leukemia strain AKR/J and the RCS-prone strain, SJL/J. The rate of tumor development, the tumor incidence and type, as well as the survival times were no different between splenectomized and intact mice.

**Association of Endogenous Viral Expression and Oncogenesis.** Evidence for a role of endogenous oncogenic virus expression on subsequent tumor development (either "spontaneous" or chemically induced) has been mainly indirect. A relationship between virus expression and tumorigenesis must be shown to exist before development of tumors. Our results demonstrate that the probability for a young gs-antigen- or virus-positive mouse to develop a tumor is significantly greater ($P < 0.001$) than that for a gs-antigen-negative virus-free mouse. We found that the association of a positive antigen or virus status in early life with the development of a variety of tumors is highly predictable. Thus, the presence or absence of complete infectious virus or its gs antigen represents the best marker to predict whether or not an animal will develop a tumor.

The association of viral parameters controlled by the host genome with tumorigenesis is important for at least two reasons: risk groups (lines, strains, individuals) can be diagnosed before the appearance of tumors, and if both (regulatory and structural) genes for gs antigen and complete virus can be located, we may be closer to an answer regarding the possible chromosomal integration of the viral genome.

Previous findings indicated that some or all of the viral RNA also exists in the DNA of the host cell; full viral genomes can be activated by various forms of treatment and "... the development of (neutralizing) antibody to murine leukemia virus prior to the detection of overt lymphoma... suggests that unmasking of the latent leukemia virus is an indigenous actuating cause of the lymphoma" (4). Even in mice negative for gs antigen and virus expression that developed tumors later in life, tumorigenesis was accompanied by derepression of gs antigen, and complete virus in most instances. Thus, an etiological relationship can be inferred.

Although the association of gs antigen and virus expression with tumor development is highly significant, it is not absolute. This finding indicates that other host genes are required for tumorigenesis in addition to those that control or regulate virus expression, and that the presence or absence of gs antigen and virus is not an all-or-none phenomenon; indeed CF-positivity and negativity denote quantitative rather than qualitative differences within the limits of our assays.

The simultaneous development of different tumor types is exceedingly rare; only 6 of 277 tumor-bearing mice developed two types of tumor. Thus, the occurrence of one tumor usually excludes development of another.

**Linkage Findings and Other Genes Affecting Tumorigenesis.** We did not detect significant associations between any of the genetic markers and the genes regulating virus expression; thus, there is no close linkage between them and the virus-controlling loci. However, we found a weak association of tumorigenesis with H-2 in F1 mice. This association is not likely fortuitous; it has also been observed in C3H × C57BL crosses (20). In addition, Lilly has shown that the Rgp-1 locus causing resistance to leukemogenesis induced by Gross passage A virus in neonatal mice is located in or just outside the K region of H-2 (21). Similarly, the Ir-1 gene, which determines immune responsiveness to certain antigens, is also located within the K region (22). The Ir-1 and Rgp-1 genes may be identical and govern the immune capacity to viral or virus-induced cellular antigens. Thus, the H-2-associated influence on viral leukemogenesis may not be due to H-2 itself but to a gene within its recombinational region. Nevertheless, the H-2-associated effect is important because the HL-A system, which is the human homologue of H-2, appears to bear a relationship to the development of lymphoid and possibly other neoplasms.

The pattern of tumor development and tumor types other than lymphoreticular neoplasms in AKR-C57L crosses suggests effects of additional genetic factors or a multigenic control of tumor susceptibility. Indeed, the appearance of tumor types other than those characteristic of these two strains suggests that each strain may contribute genes relevant to various tumors. The possible linkage effects of Dip-1, Es-1, and ln on tumorigenesis are being analyzed further with the aid of recombinant inbred strains (23). Also, whether or not Fe-2, a gene located in linkage group II that determines Friend virus infection and replication, affects tumor development is currently being analyzed (24). An effect of Fe-I, which influences the spleen focus formation in mice infected with Friend virus on either gs antigen, virus, or tumor development, could not be analyzed because it does not segregate in the AKR-C57L crosses (1, 25, 26).

**Factors Leading to Deaths Without Tumors.** We have not investigated in depth deaths from causes other than tumors. However, our frequent findings of perivascular cuffing, peri- and polyarteritis, peribronchial granulomatosis, and glomerulosclerosis parallel observations by others, which suggest that they may be due to an immune response to endogenous tumor virus. For example, Hanna et al. (27) observed an increasing incidence with age of glomerulosclerosis in RPM mice that paralleled the appearance and amount of detectable renal MuLV antigens, IgG, and beta2-globulin. Thus, they postulated that glomerulosclerosis may represent an "immune complex nephritis" similar to that in NZB mice. In addition, they found an inverse relationship between the incidence and severity of glomerulosclerosis and lymphoid neoplasia, suggesting that an immune response to endogenous
leukemia virus may, at least in part, be involved in regulation of viral oncogenesis.

The development and renal glomerular deposition of circulating immune complexes are best known in NZB mice. Several factors may be implicated; one of these is a leukemia-virus specified antigen (28). Oldstone et al. (29) detected host Ig, C3, and Gross antigen in glomeruli of AKR mice. Their findings suggest that (a) circulating Gross-antigen-anti-Gross antibody complexes occur, (b) they are subsequently deposited in the glomerular capillary walls and mesangia, and (c) classical immunological tolerance is lacking even in AKR mice naturally “infected” throughout life. Similarly, Markham et al. (30) detected the presence of IgG, beta, and MuLV-gs-1 and gs-3 antigens concentrated in a granular pattern in the renal glomeruli of 8- to 10-month-old AKR mice.

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