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

(reciprocal backcross progenies/complement-fixing antigens/dominant genes/genetic markers)

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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 (F_1) generation hybrids, the second filial (F_2) 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, as well as epithelial, tumors were also observed. Tumors occurred preferentially in gs-antigen- or virus-positive mice of all crosses; in the C57L-backcross and F_2 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 F_2 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 glucose 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 "spontaneous" and chemical carcinogenesis is at most inferred from studies of gs-antigen or virus induction, and cell-free tumor transmission. To determine unequivocably 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 F_1 hybrids, and the F_2 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.

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.

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 F_1 and segregating generations were separated by sex at weaning, individually earmarked, 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 F_1 mice. Parental and F_1 mice were splenectomized at 4–6 weeks of age and then set aside for tumor development; the same procedure was followed for the F_2 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

present in normal and tumor tissues. MSV-IX 322 is a tumor-exudate pool from a rat infected with M-MSV that was used in a few sample retests. The sera gave identical reactions with the fourth antiserum used, a monospecific guinea-pig serum, prepared by hyperimmunization of the animal with highly purified gs antigen that had been separated on gels and isolectrofocused by Drs. R. V. Gilden and Stephen Oroszlan, Flow Laboratories, Inc., Rockville, Md. 20852.

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 unsonicated 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°, 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 unioculated 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). Unsonicated 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% 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° 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/mm^2 per sec, and 10^6 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 F_1 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 F_2 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 F_2 generation tended to support this interpretation, as a 15:1 positive to negative ratio was obtained. Data on virus isolation in C57L-BC and F_2 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 F_2 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 F_2-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 tumorigenesis. The *H-2* genotype was ascertained by erythrocyte agglutination (10). The antiserum against *H-2*^{k} was prepared by immunizing C3H-*H-2*^{o} mice with C3H/DiSn spleen, thymus, and lymph node (11). The antiserum against *H-2*^{b} 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-AKR), Thy-1.2 (Theta-C3H), Ly-2.1 (Ly-B.1), and Ly-2.2 (Ly-B.2).

Mice from the F_2 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*^{s}/*Hbb*^{s} or *Hbb*^{d}/*Hbb*^{s}. *Hbb*^{s} and *Hbb*^{d}, respectively, are the symbols for the alleles determining single (C57L/J type) and diffuse (AKR/J type) electrophoretic hemoglobin patterns. The F_2 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-3*), 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 tumorigenesis 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, hemangioendothelioma, and epidermoid (squamous cell) carcinoma. All but one F_1 mouse died with tumors, and one mouse had both an RCS and a mammary adenocarcinoma. Since RCS occurred more often than leukemia in the F_1 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 F_2 (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 F_1 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 F_1 mice to C57L/J (BC-C57L) mice and in the F_2 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 F_2 was 15:1 (70:4). Complete infectious virus was isolated from the spleens of all AKR-backcross mice. In a sample of F_2 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 F_2 mice.

Segregation in C57L-BC mice and F_2 with respect to gs

TABLE 1. *Incidence and types of tumors observed in the AKR-C57L crosses*

Tumor types	AKR/J	C57L/J	F_1	BC-AKR	BC-C57L	F_2
Thymic leukemia	34 (240 ± 11)		2 (458 ± 11)	33 (409 ± 22)	54† (564 ± 22)	30 (471 ± 25)
Reticulum cell sarcoma		4 (486 ± 121)	6* (744 ± 60)	13 (596 ± 25)	62‡ (670 ± 28)	22 (657 ± 24)
Mammary adenocarcinoma			2 (672 ± 183)			1 (521)
Pulmonary adenoma			1 (854)		3§ (694 ± 39)	1 (876)
Lipoma			1 (396)		1 (510)	
Hepatoma, hepatocarcinoma					1 (768)	2 (590 ± 63)
Epidermoid carcinoma					1¶ (396)	
Hemangioendothelioma					1 (851)	
Rhabdomyosarcoma					1 (403)	
Osteogenic sarcoma			1 (643)			
Total no. tumors/ total no. mice (277/382)	34/34	4/21	13/14	46/65	124/178	56/70
Percentage of mice with tumors	100	19	93	71	70	80

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 F_2s, 56 of 70 mice developed tumors, and 14 died from nontumorous conditions. Tumors occurred preferentially (BC-C57L) or exclusively (F_2) 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 F_2 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 F_2 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 F_1 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 adenocarcinoma, 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*

Conditions	BC-C57L			F_2		
	Total	gs-AG+	gs-AG−	Total	gs-AG+	gs-AG−
Tumors*	124	110	14	56	56	0
Other†	68	24	44	14	10	4

* 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.

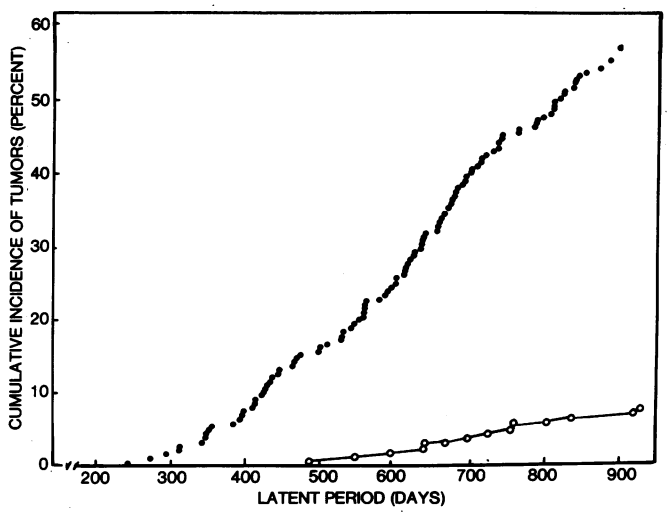

FIG. 1. Development and occurrence of tumors in BC-C57L/J mice segregating for type-C RNA tumor virus or its gs antigen. Tumors of all types preferentially occurred in mice that were originally gs-antigen or virus positive; their appearance and incidence were significantly earlier and greater than those developing in mice that were originally gs-antigen and virus negative. Twice as many tumors occurred in mice that were gs-antigen or virus positive *before* the first tumor developing in mice that were gs-antigen negative than the total cumulative incidence of tumor occurring in them. ●, mice that were originally gs-antigen or virus positive (110 tumors); ○, mice that were originally gs-antigen and virus negative (14 tumors).

Occurrence of Nonneoplastic Diseases. Mice died from several nonneoplastic disease(s) including bronchopneumonia, vascular disease, peribronchial granulomatosis, and glomerulosclerosis. The last three were the most frequent. Vascular disease consisted of generalized perivascular lymphoid cuffing and arterial hyalinizations or polyarteritis; glomerulosclerosis was characterized by segmental or granular thickenings and deposits of periodic-acid Schiff-positive materials in the glomerular mesangium and basement membrane. Glomerulosclerosis, 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 F_2 mice, (*b*) a possible linkage effect of *Dip-1* (linkage group XIII) on all tumors in both F_2 ($0.1 > P > 0.05$) 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

group IX) and gs antigen or virus expression, $H\text{-}2^k$ appears to confer greater susceptibility to leukemogenesis than $H\text{-}2^b$. For both *Dip-1* and *Es-1*, C57L/J alleles are seemingly associated with increased tumor resistance; in contrast, greater susceptibility to tumorigenesis exists for AKR/J alleles.

We are currently exploring another observation that in leaden (*ln*) mice of the F_2 generation, no tumors occurred between about 8 and 14 months of age, whereas 10/46 non-leaden mice developed tumors during the same age period. 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 F_2 mice. This association is not likely fortuitous; it has also been observed in C3H × C57BL crosses (20). In addition, Lilly has shown that the *Rgv-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 *Rgv-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 recombination regions. 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 *Fv-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 *Fv-1*, which influences the spleen focus formation in mice injected 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 endogeneous tumor virus. For example, Hanna *et al.* (27) observed an increasing incidence with age of glomerulosclerosis in RFM mice that paralleled the appearance and amount of detectable renal MuLV antigens, IgG, and $beta_{1C}$ 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, $beta_{1C}$, 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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