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Host-Gene Control of C-Type RNA Tumor Virus: Inheritance of the Group-Specific Antigen of Murine Leukemia Virus

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

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ABSTRACT **Genetic studies were aimed at elucidation of the mechanism of inheritance of the group-specific antigen of the murine leukemia virus. Two approaches have been used. First, a classical Mendelian hybridization experiment was performed with mice of the high-leukemia AKR strain and the low-leukemia C57L strain; the first filial (F_1) generation hybrids, the second filial (F_2) generation hybrids, and the backcrosses to the two parental strains. Second, a number of partially inbred lines derived from the F_2 generation of the same cross were used. The results of these studies demonstrate a specific genetic regulation of expression of group-specific antigens. Genes permissive to the expression of the antigen are dominant or semidominant to their nonpermissive alleles. It appears that two dominant genes carried by the AKR strain, but not by the C57L strain, are necessary for the presence of a complete virus; one of these controls the expression of group-specific antigen.**

That genetic factors play a major role in the etiology of spontaneous tumors in mice is evident from the existence of inbred strains with different frequencies of characteristic tumors (1). In mice, RNA tumor viruses of the C-type have now been established as causes of various leukemias and sarcomas; they have also been found associated with carcinomas (2, 3). The hypothesis that such RNA tumor viruses are transmitted from parent to offspring and controlled by host genetic factors has gained support (2, 4–6). Environmental factors cannot be ignored, however, since aging, radiation, and chemical carcinogens may modify viral expression and block immunological surveillance (7–14). The goal of the research reported here is to investigate the nature of the genetic dependence of virus expression by means of a hybridization experiment involving the high-leukemia AKR/J strain and the low-leukemia C57L/J strain. Data are presented on the inheritance of group-specific antigen of C-type RNA viral genomes and on the expression of infectious virus.

MATERIALS AND METHODS

Breeding Methods. AKR/J and C57L/J mice were mated to produce the reciprocal F_1 hybrids that were splenectomized at 4–5 weeks of age; spleens were prepared for testing for group-specific antigen of murine leukemia virus. After recovery from surgery, the mice were mated either *inter se*, to produce the F_2 generation, or to one or the other of the parental strains to produce all possible reciprocal backcross progenies, taking into account both sex and reciprocal type of the F_1-generation mice. Only first litters were used. Subsequently, some F_2-generation mice were mated *inter se* to establish a set of recombinant inbred lines (15); these are designated AKXL lines. Brother and sister mice were mated for three generations, with random selection of pairs of siblings each generation. Sixth-generation mice were set aside for group-specific antigen, virus isolation, and other testing. The reason for developing these recombinant inbred lines is to obtain a genetically fixed, segregant population that facilitates the analysis of genetic segregation, recombination, and function. At the tested generation, these AKXL lines are expected to be fixed at about 50% of the loci that segregate in the cross. Individual mice are expected to be homozygous at 75% of these loci.

Complement-Fixation Test for Group-Specific Antigen of Murine Leukemia Virus. The presence of group-specific antigen of murine leukemia virus in mouse spleens was determined with the complement-fixation test. The spleens were suspended 1:10 (w/v) in Eagle's minimum 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 the complement-fixation test (16–18).

It is now known that the internal proteins of the C-type RNA viruses carry both intraspecific group-specific antigen and interspecific group-specific antigen, or cross-reacting determinants, as an integral part of their structure (20–22). The internal proteins are soluble and ether-resistant; thus they are distinct from the envelope proteins of C-type viruses.

Three types of antisera, two prepared in rats and one prepared in guinea pigs, were used to detect the group-specific antigen by the complement-fixation test. Antiserum pool MSV-26 was derived from inbred Fischer rats carrying transplantable tumors induced by the Moloney variant of murine sarcoma virus (MSV). This antiserum had been selected for reactions giving relatively high titers (1:80–1:160), with intraspecific group-specific antigen and for negative reactions (at 1:10) with the other antigens that are present in normal and tumor tissues. MSV-IX 322 is a tumor-exudate pool from a rat infected with Moloney sarcoma virus that was used in a limited number of sample retests. Both MSV-26 and MSV-IX 322 sera gave identical reactions with the third antiserum used, a monospecific guinea pig serum, prepared by hyperimmunization of the animal with

Abbreviation: MSV, murine sarcoma virus.

highly purified, group-specific antigen that had been separated on gels and isoelectrofocused.

Virus Detection. Conversion of mouse-embryo cell cultures from complement-fixing antigen-negative to antigen-positive was used for the detection of replicating murine leukemia virus. Duplicate secondary mouse-embryo tissue cultures from SWR/J, BALB/cJ, and C57L/J mouse strains were inoculated with 0.1 ml of unsonicated 10% spleen suspension at 18–24 hr after plating. AKXL mice were tested with the SWR/J embryo tissue culture only, since the SWR/J cells appeared to be most permissive for the replication of murine leukemia virus. 60-mm plates were seeded with 350,000 cells each. At 18–20 days of incubation at 37°C, one plate was scraped and the cells were suspended in 0.6 ml of Eagle's medium and sonicated. This material was tested for group-specific antigen. The duplicate plate was scraped and passed in fresh whole-embryo cultures; second-passage plates were again tested for complement-fixing antigen at 18–21 days. Positive-control cultures were inoculated with an AKR/J spleen suspension; negative-control cultures were uninoculated.

Linkage Testing. In an attempt to map the genes controlling virus expression, mice from the backcross (F_1 x AKR, F_1 x C57L) and F_2 generations were typed for the gene loci *Hbb**, *H-2*, *c*, *b*, and *ln*. The AKXL lines were typed in addition for the loci *Es-1*, *Es-3*, *Gpd-1*, *In* (23), *Thy-1*, and *Ly-2*. (*Thy-1* and *Ly-2* were formerly called the *Theta* and *Ly-B* loci, respectively, ref. 24.)

RESULTS

Strain hybridization

A large number of mice of the backcross and F_2 generations of the AKR-C57L cross were tested for group-specific antigen by the complement-fixation test (Table 1). We found no effect of such variables as sex and litter size on the occurrence of viral antigen; neither were there any significant differences among reciprocal mating types. The fact that all F_1 and AKR-backcross mice (see *Methods*) were antigen-positive indicates that the gene(s) for the presence of group-specific antigen is dominant to its allele for the absence of the antigen. Segregants that were antigen-negative were observed among the F_2 and C57L-backcross mice. In the C57L-backcross progeny, the ratio of antigen-positive to antigen-negative mice approximated a 3:1 ratio (70:29; $P = 0.3$), as if negative complement-fixation tests were due to simultaneous homozygosity of recessive alleles to two independently inherited autosomal genes. Results obtained in the F_2 generation tend to support this interpretation, as a 15:1 (49:3, $P > 0.9$) positive to negative ratio was obtained.

Attempts were made to detect replicating murine leukemia virus from a sample of spleen preparations obtained from backcross and F_2 generation mice. Mouse-embryo tissue cultures from SWR/J, BALB/cJ, and C57J/J strains of mice were used. In no case was a BALB/c culture converted to antigen-positive. BALB/c cells are known to be refractory to virus replication of the N-trop leukemia virus that is carried by such strains as AKR and C58, but are permissive to B-trop virus that is found in some spontaneous BALB/c tumors and to leukemia viruses isolated from irradiated C57BL/6 mice. 15 virus tests were performed in the AKR backcross series. All 15 of the C57L cultures were converted to antigen-positive; two of the 15 SWR cultures remained negative. In the F_2 generation, only five virus tests were attempted, and each of these samples converted both SWR and C57L cultures. 19 mice from the C57L-backcross were tested for virus; All yielded virus in SWR cultures; five samples failed to convert the C57L cultures. These results suggest that most, if not all, backcross and F_2 mice had complete, replicating virus.

TABLE 1. *Results of complement-fixation test for the group-specific antigen in crosses of AKR/J and C57L/J mice (hybridization experiment)*

Breeding groups	Rat antiserum pool MSV-26 (positive*/total)
AKR/J†	10/10
C57L/J†	0/8
(C57L x AKR)F_1	13/13
(AKR x C57L)F_1	13/13
C57L ♀ x (C57L x AKR)F_1 ♂	19/25
C57L ♀ x (AKR x C57L)F_1 ♂	19/25
(C57L x AKR)F_1 ♀ x C57L ♂	13/25
(AKR x C57L)F_1 ♀ x C57L ♂	19/24
(C57L x AKR)F_1 ♀ x (C57L x AKR)F_1 ♂	30/32
(AKR x C57L)F_1 ♀ x (AKR x C57L)F_1 ♂	18/19
(C57L x AKR)F_1 ♀ x AKR ♂	13/13
(AKR x C57L)F_1 ♀ x AKR ♂	13/13
AKR ♀ x (C57L x AKR)F_1 ♂	11/11
AKR ♀ x (AKR x C57L)F_1 ♂	13/13

* Positive = 3+ or greater at a dilution of 1:2 or higher (= 1:20 or higher actual dilution of tissue).

† AKR/J mice are complement-fixing, antigen-positive throughout life, contain infectious virus, and are leukemia-prone. All spleens were complement-fixing antigen-positive with all the rat antiserum pools tested. C57L/J mice were antigen-negative, virus-negative, and resistant to "spontaneous" tumorigenesis, even when 2–3 years of age.

AKXL recombinant inbred lines

In order to further analyze, characterize, and hopefully map the genes that control the antigen and complete virus expression, incipient recombinant inbred lines were assayed in the third generation of brother–sister mating (after the F_2 generation). A total of 142 mice from 16 independently derived inbred lines were tested for the presence of group-specific antigen (Table 2). In contrast to the backcross and F_2 generation results, the segregation of presence or absence of the antigen, within and between these partially inbred lines, was in good agreement with a single dominant gene model ($P > 0.9$). The results do not fit a two-gene interpretation ($P < 0.01$). Apparently, only one of the two dominant genes controlling presence or absence of the antigen is expressed in the AKXL lines. The results of testing mice of the AKXL lines simultaneously for antigen and infectious virus were most revealing. 110 mice from 16 lines were tested for both antigen and virus. Of these, 53 mice that were

* *Hbb*, β-chain of hemoglobin locus; *H-2*, major histocompatibility locus; *c*, albino locus; *b*, brown locus; *ln*, leaden locus; *Es-1*, kidney esterase-1 locus; *Es-3*, kidney esterase-3 locus; *Gpd-1*, autosomal glucose-6-phosphate dehydrogenase locus; *In*, inflammatory response to dimethylbenzanthracene locus; *Thy-1*, thymus antigen-1 locus; *Ly-2*, lymphocyte antigen-2 locus.

TABLE 2. *Results of complement-fixation testing of 16 partially inbred lines (AKXL) that are F_2 derived, compared with predicted frequencies of different mating types on the assumption that the expression of the group-specific antigen is due to the presence of a dominant gene*

Possible mating types*	Expected positive progeny† (%)	Probability of the four mating types among AKXL lines in the third generation of sibmating	Predicted number of line under one-locus hypothesis	Observed number of lines	Results for individual AKXL lines (positive/total‡)
					12/12, 6/6
					9/9, 5/5
A/A x —/—	100	0.425	6.8	7	9/9, 3/3
					6/6
A/a x *A/a*	75	0.176	2.8	2	11/14, 8/9
A/a x *a/a*	50	0.137	2.2	3	6/15, 4/8
					7/13
a/a x *a/a*	0	0.263	4.2	4	0/14, 0/4
					0/11, 0/4

* *A* is used as a generalized symbol for a dominant gene; *a*, for its recessive allele; —, for either *A* or *a*.

† Expected proportion of positive-antigen scores under the hypothesis that positive scores are due to the presence of a single dominant gene. Complement-fixation tests were performed with MSV-26 serum; the rat antiserum was selected for high-titered reactions to group-specific antigens-1. A number of specimens were retested with guinea pig antiserum with virtually identical results as those obtained with MSV-26 serum.

‡ Numbers represent number of positive over the total of the tested mice of single sibships in different AKXL lines. Each of the lines was tentatively assigned to one of the mating types; since some of the 100% positive lines were classified on the basis of a small number of progeny, the parametric ratio in one or more of these lines might be 3:1 or 1:1. The 16 RI lines tested comprise a total of 142 mice.

antigen-positive were also virus-positive, and 37 mice that were antigen-negative were also virus-negative. 16 mice were antigen-positive, but virus-negative. Six of the latter came from a single line in which none of the other types were represented, and 10 came from four additional lines. Of 110 mice, only four were antigen-negative, but virus-positive. These results indicate that there is a strong, but imperfect, association between the presence of viral antigen and complete virus. Thus, positivity for group-specific antigen is generally necessary, but not sufficient, to obtain complete virus. It appears that two dominant genes carried by AKR are necessary for the presence of complete virus; the first controls the presence of group-specific antigen and the second cooperates in some unknown way with the first to complete viral synthesis. The function of this second locus might be either to supply some additional virus components, or to control the synthesis of RNA–protein components into a complete particle. More indirect mechanisms are not ruled out.

Linkage testing

No association between any of the genetic markers and the viral parameters was detected. Thus, close linkage between the virus controlling loci and the marker loci *c*, *b*, *ln*, *H-2*, *Hbb*, *Es-1*, *Es-3*, *Gpd-1*, *Thy-1*, *Ly-2*, and *In* can be excluded. Since C-type RNA tumor virus functions are evidently affected by several genes that may mimic each other, placing these genes in the linkage map is especially desirable. The AKXL lines are ideally suited for this purpose. We are continuing to type these lines for additional segregating loci.

DISCUSSION

Interpretation and implications of findings

Our results show that the presence of group-specific antigen of murine leukemia virus is subject to host-gene regulation. Expression of the antigen is dominant or possibly semidominant to lack of expression. Presence or absence of intraspecific group-specific antigen segregates into discrete, nonoverlapping classes in the cross of AKR by C57L. These results suggest that strain AKR mice carry two dominant genes, lacking in C57L, that confer high levels of antigen. In incipient recombinant inbred lines of mice derived from the F_2 generation, the results are more consistent with a single-gene model for the control of group-specific antigen. This discrepancy is not yet explicable, but might relate to extracellular transmission of virus from AKR and F_1 mice to susceptible progeny. Simultaneous testing for antigen and replicating virus indicates that expression of group-specific antigen is in general a necessary, but not sufficient, condition for the presence of complete virus. Another dominant gene is evidently necessary for completion of virus synthesis. We have deferred assigning gene symbols to any of these hereditary factors until we can more completely define them in terms of number, function, and location.

Evidence that the gene(s) controlling group-specific antigens is probably regulatory rather than structural comes from the observations that the antigen is more prevalent in old (>8 months) than young mice of such low-expression strains as C57BL/10Sn, BALB/cCr, and C57L/J (9, 26); group-specific antigen may also be induced with chemical carcinogens and with radiation (2–5, 10, 12, 26).

Whether or not the structural loci for group-specific antigen and other viral constituents are integrated into the host chromosomes is uncertain, although considerable indirect evidence now favors this hypothesis (2, 4, 5, 26). The extreme dependence of group-specific antigen and complete virus on the genotype of the host, demonstrated in our data, suggests a very intimate relationship between viral and host genomes. If the structural locus for group-specific antigen is in fact integrated, it may be adjacent to the regulator

gene (27). Possible examples of this type of arrangement in mice exist for the delta-levulinate dehydratase gene (28), and TL-alloantigens of the *Tla* locus (29).

If complete virus depends on the simultaneous presence of two genes segregating in the AKR–C57L cross, one of which controls group-specific antigen, and another that is necessary for synthesis of complete virus, then it may be possible to recover virus in the hybrid produced from crossing the antigen-positive, virus-negative AKXL line to the antigen-negative AKXL lines. If one of the antigen-negative AKXL lines carries the gene required for virus completion, we would expect to see genetic complementation, permitting synthesis of complete virus in the F_1-generation hybrid of two virus-negative lines. Such demonstration of the recovery of complete virus in the F_1 hybrid would amount to genetic rescue. Appropriate matings to test this possibility have been made.

Relationship to other work

Inheritance of the group-specific antigen of avian leukosis virus has been studied (30). Mendelian inheritance of a single gene was found in chick embryos of chicken flocks free from resistance-inducing factor (RIF); presumably the gene determines the presence or absence of group-specific antigen-a. It is now known that a second antigenic determinant, group-specific antigen-b, occurs that may be analogous to the interspecific murine antigen (31). In fact, as many as four major internal polypeptides of avian RNA tumor viruses have been isolated and characterized; two of these are immunologically stronger than the others, but all four have been found to have properties of group-specific antigen (32).

The inheritance of antigens of mammalian C-type RNA tumor viruses has not previously been determined. However, the influence of the host genotype on Gross-virus transmission and subsequent course of infection has been studied in crosses between AKR and C57BL/6 strain mice (33). Segregating populations were tested for the presence in their serum of Gross soluble antigen and Gross natural antibody. It was found that the major murine histocompatibility locus, *H-2*, exerted a major influence: the $H\text{-}2^k$ allele favored the occurrence of Gross soluble antigen, whereas the $H\text{-}2^b$ allele inclined toward occurrence of Gross antibody. Thus, the presence of Gross soluble antigen indicated immunological tolerance, whereas the presence of Gross antibody revealed lack of tolerance (33). G^+ is a viral-coded cell-surface antigen present in lymphoid tissues of high-leukemia strains, but absent in most low-tumor or virus strains; the low-tumor strains frequently develop Gross antibody with age (33–36). In contrast, no antibodies ever develop to group-specific antigens (26). Neither have antibodies been found to the homotypic group-specific antigen in feral mice, hamsters, and cats (26). Since maintenance of tolerance to group-specific antigen depends upon the continuous presence of antigen in tissues beginning with early embryonic life, and because of the genetic control of its expression, the viral genome is likely inherited in all vertebrates. The finding and identification of host genes that determine or control viral functions and are expressed during or after transformation raises obvious questions regarding the possible chromosomal integration of the viral genome, i.e., are the genes coding for the various expressions of virus and characteristics of a transformed state coded for by viral or host genes? Although no decision is now possible, there is no doubt that an intimate relationship between the viral and host genomes exists: (*a*) productive infection with murine leukemia virus is invariably associated with the presence in the serum of Gross soluble antigen and, on the cell surface, of Gross (G^+) antigen (33–36); (*b*) usually, but not always, a second cell-surface alloantigen termed G_{IX}, appears in association with infectious virus (37); (*c*) whereas neither of these is necessarily or immediately associated with cell transformation, the appearance of TL antigens in TL-negative strains is absolutely characteristic of transformation (36, 37). Thus, these genes and those relating to group-specific antigen and complete virus that are described in this paper may represent chromosomally integrated virus.

The question of whether or not the structural genes for any of these expressions are viral or host or both, as postulated in the viral oncogene theory (4), cannot be answered by these data alone, but a decision is relevant to approaches aimed at disrupting the viral-host genetic relationship. It appears that several genes belonging to various linkage groups control or code for viral genome functions.

FUTURE GOALS

The hereditary factors reported in this paper must now be identified, related to spontaneous and induced tumorigenesis, and compared to genes described by others. For example, Lilly (38) has shown the *Rgv-1* locus that causes resistance to leukemogenesis induced by Gross passage-A virus in neonatal mice is located in or just outside the K region of *H-2*. Pincus *et al.* (25) have reported that the k allele of *H-2* is permissive to the natural presence of murine leukemia virus in embryos of the B10.BR ($H\text{-}2^k$) congenic strains relative to the embryos of their B10($H\text{-}2^b$) congenic-partner strain, although virus disappears in adults.

It is now known that the N-B locus that determines the susceptibility of mouse embryo tissue cultures of different mouse strains to variants of murine leukemia virus is identical to the Friend virus-1 locus that influences susceptibility to spleen focus-formation in mice injected with Friend virus (39). Since this locus did not segregate in the cross used in our experiments, it could not have influenced group-specific antigen expression.

Group-specific antigens are subinfectious units of C-type RNA viruses (40–42); they can occur in strains of mice that never yield infectious virus. In chickens, production of an infectious form of the pseudotype of Rous sarcoma virus [RSVβ (0)] occurs only after passage through cells possessing the group-specific antigen (43). The regular expression of group-specific antigen during embryogenesis and in proliferating tissue suggests that group-specific antigens may have additional biological functions, e.g., a determining role in cell replication (26). An assessment of this possibility is being pursued. Also, the genotype-dependent degree of natural expression of virion, group-specific antigens, and tumor incidence implies the functioning of different regulatory genes. These must be identified and compared with those presumably operating in chemical carcinogenesis and aging.

We feel that the AKXL recombinant inbred lines of mice are excellent tools for studies of both pleiotropic effects of a single gene and its linkage relationship with other genes. The eventual understanding of the complexities uncovered by our study will enhance our knowledge of the intricate relationship of tumor viruses to their natural host. Such an understanding may be necessary for a rational approach to cancer prevention and control.

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