GENETIC CONTROL OF RADIATION LEUKEMIA VIRUS-INDUCED TUMORIGENESIS

II. Influence of *Srlv-1*, a Locus not Linked to *H-2**

By DANIEL MERUELO,‡ MIRIAM LIEBERMAN, BEVERLY DEAK, and HUGH O. McDEVITT

(From the Division of Immunology, Department of Medicine, and the Department of Radiology, Stanford University School of Medicine, Stanford, California 94305)

It was more than a generation ago that studies from the laboratories of MacDowell (1), Furth (2), and others first indicated that virus-induced leukemogenesis in mice is under genetic control. Much subsequent study had demonstrated that a number of well-characterized Mendelian loci affect leukemogenesis by what appear to be distinct effects at different steps between virus expression or infection, and neoplasia. For example, the *Akv-1* and *Akv-2* genes, discovered in a study of hybrids of high spontaneous leukemia incidence mice, AKR, with low incidence strains, e.g. C57BL, govern viral induction and/or expression (3, 4). The *Fv-1* locus, first described by Lilly (5) in studies with Friend leukemia virus (FV),[1] appears to affect in vivo and in vitro replication of all naturally occurring C-type RNA viruses (6). *H-2* linked genes appear to affect the course of virus-induced leukemogenesis, rather than the initial virus infection and replication (7). Progress in analyzing these genetic controls has made it apparent that further understanding of multigenic regulation of virus-induced leukemogenesis will require greater knowledge of gene-gene interactions.

During the course of experiments dealing with *H-2*-linked effects on resistance to radiation leukemia virus (RadLV)-induced leukemia, one strain, B10.AQR(n4), was found to be highly susceptible to the disease. This was an unexpected result since this strain expresses the $H\text{-}2D^d$ allele, which usually confers resistance to RadLV. In this study, evidence indicates that a single locus in the B10.AQR(n4) genome, distinct from other presently defined loci affecting virus-induced leukemogenesis, such as *Fv1-*, *Fv-2*, and *H-2*, confers susceptibility to RadLV-induced tumorigenesis. The susceptible allele of this locus, which shall be defined as *Srlv-1* (susceptibility to RadLV), is dominantly expressed.

* Supported in part by NIH grant AI 07757 and NIH contract CB 43941.

‡ Supported by a postdoctoral fellowship from the Damon Runyon-Walter Winchell Memorial Cancer Fund, DRG-30-F for the period of 1974–1976, and by a NIH postdoctoral fellowship for 1976–1977.

[1] *Abbreviations used in this paper:* FV, Friend leukemia virus; *Fv-1*, gene governing relative spleen focus response to F-S strain of FV; *Fv-2*, gene governing relative spleen focus response to F-B strain of FV; r, resistance; RadLV, radiation leukemia virus; s, susceptibility; SFFV, spleen focus-forming virus; *Srlv-1*, susceptibility to radiation-leukemia virus-1.

This system is particularly interesting because the *Srlv-1* locus overrides the protection against neoplasia conferred by the *H-2D*^{d} allele.

Materials and Methods

Mice. B10.AQR(n8) mice were generously supplied by Dr. Jan Klein, University of Texas Southwestern Medical School, Dallas, Texas. All other mouse strains (Table II) were bred and maintained in our own colonies.

Viruses. RadLV preparations were cell-free extracts from virus-induced lymphoid tumors of C57BL/Ka mice, made as previously described (8–10). FV preparations were kindly supplied by Dr. Frank Lilly, Albert Einstein College of Medicine, Bronx, N. Y., and Dr. Bruce Chesebro, National Institutes of Health, Rocky Mountain Laboratory, Hamilton, Mont.

Virus Inoculation and Scoring for Leukemogenesis. All animals received RadLV intrathymically at 3–6 wk of age and were scored for leukemogenesis after autopsy as described previously (8).

H-2 Typing of F_2 Progeny. Contract antiserum D-17 [(D1.C × AKR.M)F_1 anti-DBA.1; i.e., anti-*H-2K*^{q}] and an [(A.TL × B10.S(5R))F_1 anti-B10.A; i.e., anti-*H-2K*^{k}] antiserum raised in our laboratory were used to *H-2* genotype F_2 progeny by the hemagglutination assay (8, 11).

Virus Susceptibility Test. Mice were scored as being either susceptible or resistant at *Fv-2* according to the results of the spleen focus assay (12). Mice were injected in the tail vein with various FV dilutions and killed 9–10 days later. Their spleens were removed and weighed, fixed in Bouin's solution for about 3 h, and examined for macroscopic foci. Under these conditions, susceptible mice showed markedly enlarged spleens with many foci, whereas resistant mice showed spleens of near normal size with no or very few foci.

Results

A Single Locus, Srlv-1 Confers Dominant Susceptibility to RadLV-Induced Neoplasia. In the preceding publication (8), we showed that C57BL/10-derived congenic strains of mice carrying the *H-2D*^{d} allele are resistant to RadLV-induced leukemogenesis, and that resistance is dominant. One exception to this rule is strain B10.AQR(n4). As shown in Fig. 1, and Table I, this mouse strain is highly susceptible to the disease. In addition, [B10.AQR(n4) × B10.A)F_1 [susceptible(s) × resistant(r)] mice are susceptible to RadLV-induced neoplasia, demonstrating that susceptibility is dominant.

The *H-2* chromosome (*H-2*^{v1}) carried by B10.AQR(n4) was derived by recombination in a (B10.A × T138)F_1 heterozygote (13, 14). The crossover occurred between the *H-2K* and *Ir-1A* loci, such that it derived the *H-2K*^{q} allele from T138, and the *Ir-1A*^{k} allele (and *H-2D*^{d} allele) from B10.A. Progeny carrying the recombinant chromosome were backcrossed four times to strain B10, and then intercrossed to establish the B10.AQR(n4) line. Since 8–15 backcrosses are required to produce a truly congenic line (15), intercrossing after only 4 backcrosses to B10 potentially fixes T138-derived alleles at loci not linked to *H-2*.

The first clue that a locus not linked to *H-2* determined susceptibility to RadLV-induced leukemogenesis came from studies with strain B10.AQR(n8). This strain was derived from the eighth backcross generation, and would be expected to carry less of the genome foreign to the original B10 stock. Table I compares the mortality incidence of B10.AQR(n8) mice with B10.AQR(n4) animals. It is clear that the n8 generation, like other strains expressing the *H-2D*^{d} allele, is resistant to neoplasia. This finding suggests that a locus not linked to *H-2* confers susceptibility to RadLV-induced leukemogenesis, and that the dominant allele carred by B10.AQR(n4) has been lost after four additional backcrosses to B10.

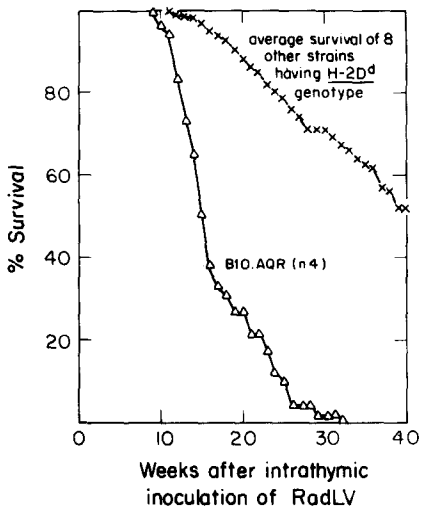

FIG. 1. Percent survival to leukemogenesis vs. weeks after intrathymic inoculation of RadLV into B10.AQR(n4) (*H-2D*^{d}, see Table I) compared to the average incidence for eight other strains having *H-2D*^{d} genotype [B10.T(6R), B10.S(7R), B10.A, B10.A(3R), B10.A(5R), B10.A(18R), B10.HTT, and B10.D2]. None of the eight strains used to calculate the average leukemia incidence had a survival curve significantly different from the average curve. B10.AQR(n4) had a survival curve typical of other highly susceptible strains, such as B10.S and B10.G(8).

TABLE I

B10.AQR(n4) Carries A Dominant Locus Conferring Susceptibility to RadLV-Induced Neoplasia

Strain	No. of animals tested	Mean survival time after RadLV inoculation	Leukemia incidence 22 wk after RadLV inoculation
		wk	%
B10.AQR(n4)	48	17	79
B10.AQR(n8)	8	32*	0
B10.A	59	31*	19
[B10.AQR(n4) × B10.A]F_1	18	18	78

* Values may be higher since animals not leukemic at termination of experiment (approximately 40 wk) would probably have survived longer.

Formal genetic proof that a single locus determining susceptibility to the neoplasia is not linked to *H-2* was achieved by segregation analysis. [B10.AQR(n4) × B10.A]F_1 mice were intercrossed, and the F_2 generation analyzed. Antisera specific for the *H-2K*^{q} product [expressed by B10.AQR(n4)] (see Table II) and the *H-2K*^{k} produce (expressed by B10.A) (see Table II) permitted identification of the *H-2* haplotype(s) carried by the progeny.

Table III shows the expected leukemia incidence of [B10.AQR(n4) × B10.A]F_2 mice as predicted by three different modes of inheritance of susceptibility to RadLV. Susceptibility may result from (*a*) a dominantly expressed *H-2*-linked locus; (*b*) a single, dominantly expressed locus (*Srlv-1*) not linked to *H-2*; or (*c*)

TABLE II

Haplotype Origin of Region Carried by Strains Used in the Present Work

Strain	*H-2* haplotype	Haplotype origin of region								
		H-2								
		K	A	B	J	E	C	S	G	D
B10.A	$H\text{-}2^a$	*k*	*k*	*k*	*k*	*k* |	*d	*d*	*d*	*d*
B10.AQR	$H\text{-}2^{v1}$	*q* |	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*d*
B10.S	$H\text{-}2^s$	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*
B10.G	$H\text{-}2^q$	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*	*q*
B10.T(6R)	$H\text{-}2^{v2}$	*q*	*q*	*q*	*q*	*q*	*q*	*q* ⋮	? ⋮	*d*
B10.S(7R)	$H\text{-}2^{f2}$	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s* |	*d*
B10.A(3R)	$H\text{-}2^{f3}$	*b*	*b*	*b*	*b* |	*k*	*d*	*d*	*d*	*d*
B10.A(5R)	$H\text{-}2^{f5}$	*b*	*b*	*b* |	*k*	*k*	*d*	*d*	*d*	*d*
B10.A(18R)	$H\text{-}2^i$	*b*	*b*	*b*	*b*	*b*	*b*	*b* ⋮	? ⋮	*d*
B10.HTT	$H\text{-}2^{f3}$	*s*	*s*	*s*	*s* |	*k*	*k*	*k*	*k*	*d*
B10.D2	$H\text{-}2^d$	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*

* Vertical bar indicates crossover position.

TABLE III

Outcome of an F_2 Segregation Analysis Designed to Determine the Number and H-2-Linkage of Gene(s) Conferring Dominant Susceptibility to RadLV

Hypothesis considered	Postulated genotypes		Mortality incidence 22 wk after RadLV inoculation		No. of mice tested	Result from χ^2 test	
	Parents	F_2	Expected	Observed			
			%	%			
One dominant *H-2*-linked locus confers susceptibility to RadLV. Thus only F_2 mice of the $H\text{-}2^{a/a}$ genotype should be resistant to neoplasia.	B10.AQR:	$H\text{-}2^{v1/v1}$	$H\text{-}2^{v1/v1}$	79	57	7	
	B10.A:	$H\text{-}2^{a/a}$	$H\text{-}2^{a/a}$	19	60	10	$P < 0.005$ (hypothesis rejected)
	F_1:	$H\text{-}2^{v1/a}$	$H\text{-}2^{v1/a}$	78	64	14	
One dominant, non-*H-2*-linked locus (*Srlv-1*) confers susceptibility to RadLV. Therefore, F_2 offspring of all *H-2* genotypes should be 3/4 susceptible and 1/4 resistant.	B10.AQR:	$Srlv\text{-}1^{s/s}$ $H\text{-}2^{v1/v1}$	$H\text{-}2^{v1/v1}$	64	57	7	$P > 0.650$ (hypothesis not rejected)
	B10.A:	$Srlv\text{-}1^{r/r}$ $H\text{-}2^{a/a}$	$H\text{-}2^{a/a}$	64	60	10	
	F_1:	$Srlv\text{-}1^{s/r}$ $H\text{-}2^{v1/a}$	$H\text{-}2^{v1/a}$	64	64	14	
Two dominant, non-*H-2*-linked loci (*Srlv-1*, *Srlv-2*) confer susceptibility to RadLV. F_2 mice of all *H-2* genotypes should therefore be 15/16 susceptible and 1/16 resistant.	B10.AQR:	$Srlv\text{-}1^{s/s}$ $Srlv\text{-}2^{s/s}$ $H\text{-}2^{v1/v1}$	$H\text{-}2^{v1/v1}$	75	57	7	
	B10.A:	$Srlv\text{-}1^{r/r}$ $Srlv\text{-}2^{r/r}$ $H\text{-}2^{a/a}$	$H\text{-}2^{a/a}$	75	60	10	$P < 0.010$ (hypothesis rejected)
	F_1:	$Srlv\text{-}1^{s/r}$ $Srlv\text{-}2^{s/r}$ $H\text{-}2^{v1/a}$	$H\text{-}2^{v1/a}$	75	64	14	

two dominantly expressed loci (*Srlv-1*, *Srlv-2*) not linked to *H-2*. The leukemia incidence predicted for each mode of inheritance can be computed by multiplying the expected genotype frequency by the leukemia incidence previously observed for this genotype (see Table I). For example, if it is assumed that a single, non-*H-2*-linked locus confers susceptibility to RadLV, all possible *H-2*

haplotypes, namely y_1/y_1, a/a, and y_1/a, will be associated with genotypes s/s, s/r, and r/r at the non-*H-2* locus (where s stands for the susceptible allele, and r for the resistant one) with a frequency of 0.25, 0.50, and 0.25, respectively. Only the r/r genotype will confer resistance, while the *H-2* genotype of F_2 progeny will be irrelevant in determining resistance or susceptibility to RadLV. The leukemia incidence predicted for this mode of inheritance may be computed by mutiplying the expected genotype frequency by the leukemia incidence previously observed for this genotype (in this case it is $[(0.25 \times 79\%) + (0.50 \times 78\%) + (0.25 \times 19\%)] = 64\%$). The difference between the expected and observed results for each mode of inheritance was analyzed by the χ^2 test (16).

Data presented in Table III suggest that a single, dominant locus, which is not linked to *H-2* confers susceptibility to RadLV. We tentatively designate this locus, *Srlv-1*, and assign two alleles, s denoting susceptibility and r denoting resistance. (The possibility, not shown in Table III, that two genes are operative, either one of which is sufficient to confer susceptibility, may also be ruled out by similar analysis.)

The Locus Conferring Dominant Susceptibility to RadLV-Induced Leukemogenesis is not Fv-2. FV inoculation induces erythryoleukemia, the outstanding feature of which is a logarithmic increase in spleen weight, beginning almost immediately after virus inoculation (7, 17). The splenomegaly results from the proliferation of erythrocyte precursor cells and is accompanied by the appearance of macroscopic foci on the surface of the spleen as early as 6–9 days after infection. The component of FV capable of inducing spleen foci is designated spleen focus-forming virus (SFFV). The host phenotype for SFFV is governed by the gene *Fv-2*, which has been mapped to chromosome 9. *Fv-2* seems to play a negligible genetic role in governing the response of mice to leukemia viruses other than SFFV, probably because most other murine luekemia viruses infect nonerythroid cells. Therefore, *Fv-2* probably does not affect RadLV-induced leukemogenesis. However, although strain AQR does not carry the translocation maker, it was derived from a recombination event between strains B10.A and T138, a translocation stock (13) involving chromosomes 9 and 17. Since *Fv-2* is associated with chromosome 9 and confers dominant susceptibility, and since CBA which is $Fv\text{-}2^s$ (susceptible) was one of the parent strains used in deriving T(2;9) 138Ca (14), the possibility that susceptibility to RadLV in B10.AQR(n4) is due to *Fv-2* was investigated.

FV was inoculated at various dilutions into BALB/c ($Fv\text{-}2^s$, susceptible), B10.A(1R) ($Fv\text{-}2^r$, resistant), and B10.AQR(n4). Since B10.AQR(n4) appears to be as resistant as B10.A(1R) to splenomegaly and focus formation B10.AQR(n4) mice carry the $Fv\text{-}2^r$ allele (Fig. 2). The *Srlv-1* locus is thus distinct from the *Fv-2* locus.

Discussion

B10.AQR(n4) mice are highly susceptible to RadLV-induced leukemogenesis despite carrying the $H\text{-}2D^d$ allele which has been shown to confer resistance to the disease (8). F_2 segregation analysis and studies with B10.AQR(n8) strongly suggest that susceptibility is due to a locus not linked to the major histocompatibility complex, *H-2*. In addition, susceptibility is inherited in dominant fashion,

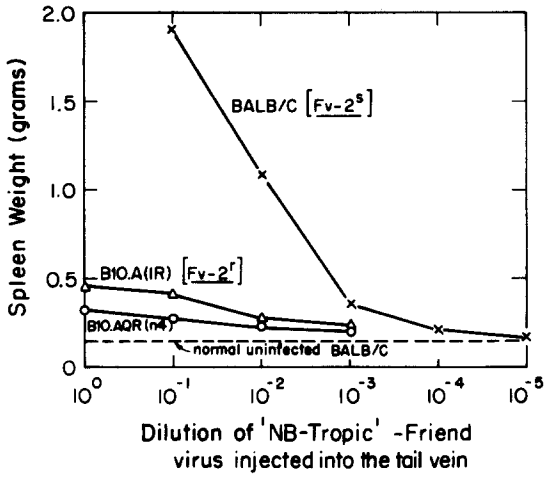

FIG. 2. The *Fv-2* phenotype of B10.AQR(n4) is resistant. NB-tropic FV was injected intravenously at various dilutions, and 9 days later mice were sacrificed, their spleens weighed and then fixed in Bouin's solution as described under Materials and Methods. BALB/c mice are $Fv\text{-}2^s$ and are highly susceptible to SFFV, whereas B10.A(1R) mice are $Fv\text{-}2^r$ and highly resistant to SFFV. The B10.AQR(n4) mice are judged to be homozygous $Fv\text{-}2^r$ since susceptibility is dominant. Each experimental point shown is the average of four mice individually tested. No animal in a group significantly differed in spleen weight from the average value given.

whereas $H\text{-}2D^d$-linked resistance to RadLV-induced tumorigenesis is dominantly expressed. The B10.AQR(n4) locus in question is not *Fv-2*, nor *Fv-1*, since RadLV is a B-tropic virus (18) and would be expected to replicate well in C57BL/10-derived strains ($Fv\text{-}1^b$). The rapid RadLV-induced leukemogenesis in B10.AQR(n4) mice, therefore, suggests that this strain is $Fv\text{-}1^b$. In addition, *Fv-1*-associated resistance is dominantly expressed. The locus carried by B10.AQR(n4) is designated *Srlv-1*, i.e., susceptibility to RadLV-1, in accordance with similar nomenclature used in defining loci conferring resistance to Gross virus, *Rgv-1* and *Rgv-2* [7].

The mechanism of action of *Srlv-1* is presently unknown, although preliminary data indicate that it might affect virus production. Supernates obtained from B10.AQR(n4)-derived, RadLV-induced tissue culture lines have 15–20 times as much reverse transcriptase activity per cell as fluids derived from B10.G, B10.S(7R), B10.S(9R), and B10.T(6R) tissue culture-adapted, RadLV-induced, tumor cells (D. Meruelo, unpublished observations). The latter four lines are derived from strains of mice with similar non-*H-2* genetic backgrounds, and show comparable levels of reverse transcriptase activity.

An important consideration is that the phenotypic expression of *Srlv-1* can override the protection provided by $H\text{-}2D^d$, since B10.AQR(n4) and $H\text{-}2D^d$ are fully susceptible. The latter is also true for (B10.AQR × B10.A)F_2 progeny carrying the $Srlv\text{-}1^s$ and $H\text{-}2D^d$ from the B10.AQR(n4) parent and the $Srlv\text{-}1^r$ and $H\text{-}2D^d$ from the B10.A parent.

These findings are suggestive of complex genetic interactions between distinct loci affecting virus-induced leukemogenesis. This is an area of great importance,

which has only recently begun to be explored (7, 19). Further studies are currently in progress to understand the mechanism of action of *Srlv-1*, as well as its precise genetic localization.

Summary

A single locus, tentatively denoted *Srlv-1* (susceptibility to radiation leukemia virus[RadLV]-1), confers dominant susceptibility to RadLV-induced leukemogenesis. *Srlv-1* is not linked to *H-2*, and appears to be distinct from *Fv-1* and *Fv-2*. Preliminary data suggest that *Srlv-1* affects virus proliferation. A striking feature of this system is that *Srlv-1* overrides the protection afforded by the *H-2D*-associated dominant resistance to RadLV-induced neoplasia.

We are most grateful to Nancy Ginzton and Diane Teece for their excellent technical assistance during this work, and to Dr. Theodore Pincus for helpful suggestions in assaying for *Fv-2* phenotype.

Received for publication 2 May 1977.

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