STUDIES OF GENETIC TRANSMISSION OF MURINE LEUKEMIA VIRUS BY AKR MICE

I. Crosses with *Fv-1*^{n} Strains of Mice

By WALLACE P. ROWE

(From the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014)

(Received for publication 27 June 1972)

Over the past 20 yr, evidence has progressively mounted that murine leukemia virus (MLV)[1] or its genetic determinants are ubiquitous in both high-leukemic and low-leukemic strains of mice, and that viral genetic determinants are heritable components present in all of the cells (1–9). However, little is known of the state of the viral genetic determinants in the cells, i.e. if they are present as one or more DNA and/or RNA copies, and if they can be carried as elements of the host chromosome. In high-leukemic strains, such as AKR, infectious virus and viral antigens are present throughout life, while low-leukemic strains generally show little or no antigen or infectious virus until late in life. In some low-leukemic strains, virus-related antigens can be detected in embryos or in certain postnatal tissues in the absence of demonstrable infectious virus (10–13). The differences between inbred strains of mice in frequency and age when viral expression becomes detectable are sufficiently marked to allow genetic analysis of the transmission of viral antigen or infectivity.

The previously reported genetic studies have been based on viral antigen expression; in these, the segregation patterns differed with the antigen under study. The G_{IX} antigen, a possibly virus-coded cell surface alloantigen which in normal animals is expressed primarily on thymocytes, requires two unlinked genes for its expression (12). One of these genes is semidominant, and in crosses between 129 and C57BL/6 mice was found to be located in linkage group IX. Its expression requires the presence of a dominant gene, whose linkage is not yet established.

In recent studies of the expression of the group-specific (gs) antigen of MLV in the spleen of offspring obtained from crosses between AKR and C57L mice, Taylor et al. (14) observed two patterns. In F_1, F_2, and first-generation backcross mice, the occurrence of antigen indicated that the AKR mouse was contributing two unlinked dominant genes for its expression, either of which alone caused the appearance of gs

---

[1] *Abbreviations used in this paper:* Bc1, first backcross; gs, group specific; GSA, Gross soluble antigen; IdU, 5-iododeoxyuridine; METC, mouse embryo tissue culture; MLV, murine leukemia virus; NIH, National Institutes of Health; p.f.u., plaque-forming units.

antigen. However, the frequency distribution of spleen gs antigen in multiple inbred lines derived from the F_2 generation indicated that there was only one gene for gs antigen expression, and that another AKR gene must also be present in order for infectious virus to be formed.

Genetic studies have also been reported (15) on the occurrence of the Gross soluble antigen (GSA), a virus-specific antigen detectable in the serum of high-leukemic mice. Although GSA was transmitted by AKR to F_1, F_2, and backcross mice, the penetrance was not complete and showed a marked maternal influence. Consequently, no attempt was made to interpret the results in terms of gene segregation ratios.

The naturally occurring MLV of AKR mice is readily detected as infectious virus by tissue culture techniques (16). Since virus is uniformly present, is in high titer, and appears much earlier in life in AKR than in low-leukemic mouse strains, it was feasible to carry out genetic studies of transmission of MLV by AKR mice using appearance of virus infectivity in young animals as an end point. Particularly useful in this regard was the finding that the bone of the tail was high in virus, providing a readily accessible tissue which could be sampled repeatedly.

TABLE I

Mouse Strains Used in Studies of Hybrids between AKR and Low-Virus Mice

Strain	Abbreviation	Genotype (Linkage group)				
		Fv-1 (?)	*Fv-2* (II)	*H-2* (IX)	Albino (*c*) (I)	*Hbb* (I)
AKR/J	AK	*n*	*S*	*k*	*c*	*d*
C57BR/cdJ	BR	*n*	*r*	*k*	+	*s*
C57L/J	L	*n*	*r*	*b*	+	*s*
DBA/2J	D2	*n*	*S*	*d*	+	*d*
NIH		*n*	*S*		*c*	
BALB/cN	C	*b*	*S*	*d*	*c*	*d*
C57BL/10J	BL	*b*	*r*	*b*	+	*s*
B10.BR/Sn		*b*	*r*	*k*	+	*s*
B10.D2(old)/Lw		*b*	*r*	*d*	+	*s*

We have studied progeny of crosses of a number of mouse strains with AKR by quantitating the amount of virus in the tail at 2 wk and again at 6–12 wk of age. The low-virus mouse strains used in these crosses were chosen to allow evaluation of the influence of several genes known to be of importance in MLV infection or in experimental or spontaneous leukemogenesis, i.e. *H-2*, *Fv-1*, and *Fv-2*, and to follow a viral genetic marker by using mouse strains known to carry virus of a host range type different from that carried by AKR. Table I shows the strains which were used. This report describes the results of virus testing of F_1, F_2, and first and second backcross generations of crosses of AKR with other mouse strains having the $Fv\text{-}1^n$ allele (17, 18), in which the AKR strain of MLV (N-tropic) (19) should have maximal opportunity for replication. Crosses with $Fv\text{-}1^b$ mice are described in the accompanying report.

Materials and Methods

Mice.—The parental strains, their sources, and relevant genetic markers were shown in Table I. The National Institutes of Health (NIH) Swiss mouse is not an inbred strain, but was used because it was the only strain which combined albino, *Fv-1*^{n}, and apparent absence of endogenous infectious MLV. The virologic characteristics of the low-virus parental *Fv-1*^{n} strains are shown in Table II; the comparable studies of AKR were described previously (16).

Hybrid mice were bred in our laboratory; the majority were first litters, though some second litters are included. The designations of the hybrid strain combinations are given with the maternal strain first. Mice were tested for virus in the tail at 2 wk and again at 6 wk of age. Specimens were obtained by clipping about 1 cm off the tail; the specimen was briefly immersed in diethyl ether to destroy any surface virus. Scissors were also decontaminated between animals by dipping in ether.

Virus Testing.—Tail specimens were held on ice until the ether had evaporated, and 2% suspensions were prepared by grinding with a mortar and pestle and suspending in Eagle's basal medium with 20% veal infusion broth. After clarification by low speed centrifugation in the cold, the extract was stored at −70°C until tested. Infectivity was assayed by inoculating 0.2 or 0.4 ml of the extract onto a 1-day old secondary culture of NIH Swiss mouse embryo cells; the cultures were treated with 25 μg/ml diethylaminoethyl (DEAE)-dextran (Sigma Chemical Co., St. Louis, Mo.) for 1 hr and rinsed once before the specimen was added. Growth and maintenance medium was 10% inactivated (56°C, 30 min) fetal calf serum in Eagle's minimal essential medium, with 2 mM glutamine and antibiotics. Medium with unheated serum was used for the first 24 hr after inoculation of the specimen. Cultures were developed for plaques by the UV-XC procedure (20) on the 6th or 7th day after infection. In dishes showing large numbers of plaques, the count was estimated by comparison with a set of standards. Titers are expressed as the $\log_{10}$ of the number of plaque-forming units (p.f.u.) per 0.4 ml of 2% extract.

TABLE II

Virus Testing of Low-Virus Fv-1^n *Mouse Strains*

Strain	Tissue	No. with N-tropic virus/ No. tested Age (in months)				No. with B-tropic virus/ No. tested Age (in months)		
		1-3	4-6	7-12	13-16	1-3	7-12	13-16
C57BR	Tail	0/30		1/6	0/10	0/19	0/5	
	Pool*	1/24		1/10	0/10	0/18		0/10
C57L	Tail		0/18	0/16				
	Pool			0/16				
DBA/2	Tail	0/23		4/20			0/20	
	Pool			4/20			0/20	
NIH	Tail	0/20		0/19				

* Pool = pool of spleen, thymus, mesenteric lymph node, and femur.

Determination of H-2 and Hbb Type.—*H-2* type was determined by the hemagglutination method, using antisera provided by Dr. Frank Lilly and the Reference Reagents Branch of the National Institute of Allergy and Infectious Diseases. *Hbb* type (single or diffuse hemoglobin) was determined by acrylamide gel electrophoresis of carboxyhemoglobin; the tests were kindly done by Miss Phyllis Fabisch and Dr. Hilton B. Levy.

Tests for Activation of Virus in Mouse Embryo Tissue Culture (METC) by 5-Iododeoxy-

uridine (IdU).—METC were prepared from individual 15- to 17-day embryos to determine from what proportion of the embryos infectious MLV could be activated by IdU (7). Embryos were immersed briefly in diethyl ether to remove possible contaminating surface virus, and were then minced and the cells dispersed with 0.25% trypsin. Petri dishes (50 mm; Falcon Plastics, Oxnard, Calif.) were seeded with 2 × 10^6 cells; plating efficiency was 10%. On the following day, two or more cultures were treated with 5 mcg/ml IdU, which was held in the medium for 42 hr. 6 or 7 days after beginning IdU treatment, treated and control cultures were exposed to UV irradiation (1800 ergs/mm^2) and overlaid with 4 × 10^5 NIH METC. 4 or 5 days later the cultures were developed for plaques by the UV-XC procedure (20).

TABLE III

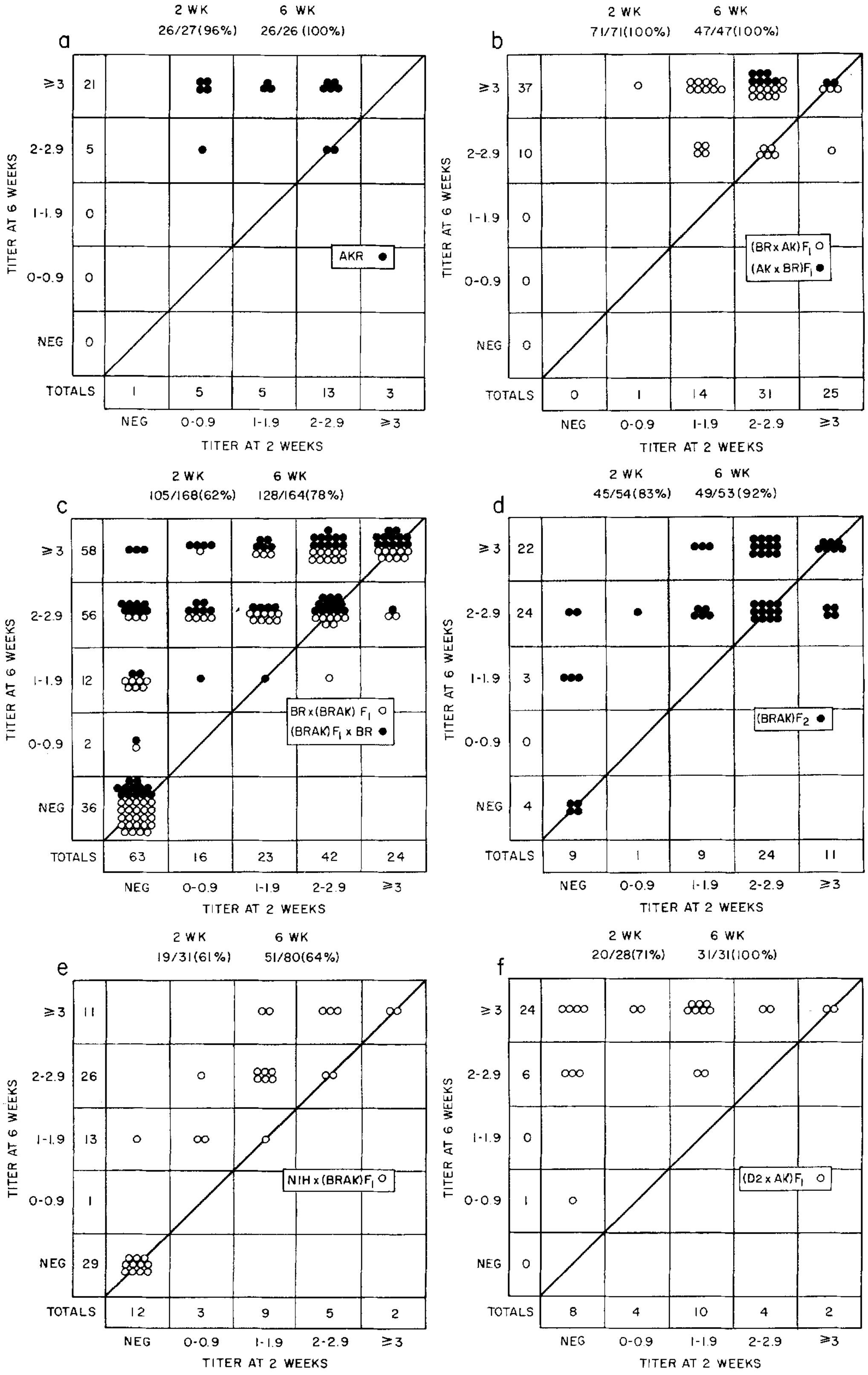
Virus in Tail Extracts of F_1, F_2, and Bc1 Hybrids of Low-Virus $Fv\text{-}1^n$ Mice with AKR

Generation	Strain	2-wk tests			6-wk tests		
		No. positive/total	%	Median titer of positive mice*	No. positive/total	%	Median titer of positive mice
Parental	AKR	26/27	96	2.2	26/26	100	3.7
F$_1$	(BR × AK)F$_1$	32/32	100	2.3	37/37	100	3.5
	(AK × BR)F$_1$	39/39	100	3.0	10/10	100	3.5
	(D2 × AK)F$_1$	20/28	71	1.6	31/31	100	3.3
	(L × AK)F$_1$	35/36	97	2.4	29/29	100	3.6
	(NIH × AK)F$_1$	33/56	59	2.1	54/54	100	3.0
Total F$_1$		159/191	83		161/161	100	
Bc1 to low-virus parental strain	BR × (BR × AK)	48/84	57	2.1	57/81	70	2.7
	(BR × AK) × BR	57/84	68	1.9	71/83	86	3.0
	D2 × (D2 × AK)	22/37	59	1.3	31/35	89	2.7
	(D2 × AK) × D2	31/69	45	1.0	44/60	73	2.5
	L × (L × AK)	38/64	59	2.1	36/55	65	2.4
	(L × AK) × L	7/10	70	1.9	7/10	70	2.3
	NIH × (NIH × AK)				48/71	68	2.9
Bc1 to nonparental low-virus strain	NIH × (BR × AK)	19/31	61	1.8	51/80	64	2.5
Total Bc1		220/375	59		345/475	73	
F$_2$	(BR × AK)F$_2$	45/54	83	2.3	49/53	92	2.9

* Positive mice are those with virus at either time point. Thus, a mouse who was negative at 2 wk and positive at 6 wk is included in the calculation of the median titer at 2 wk. Titers are expressed as $\log_{10}$ p.f.u./0.4 ml of 2% extract.

RESULTS

Virus, in F_1, F_2, and First Backcross Generations.—F$_1$ hybrid mice of crosses between AKR and C57BR or C57L closely resembled AKR both in the regular early appearance of virus and the titers attained (Table III and Fig. 1). (NIH × AK)F$_1$ mice showed a tendency for appearance of virus to be somewhat delayed and for the titer at 6 wk to be slightly lower than in the other crosses. There also was a slight delay in appearance of virus in some (D2 × AK)F$_1$

a
2 WK 26/27(96%)
6 WK 26/26 (100%)
AKR ●
≥3 21
2-2.9 5
1-1.9 0
0-0.9 0
NEG 0
TOTALS 1 5 5 13 3
NEG 0-0.9 1-1.9 2-2.9 ≥3
TITER AT 2 WEEKS
TITER AT 6 WEEKS
b
2 WK 71/71(100%)
6 WK 47/47(100%)
(BR x AK)F1 ○
(AK x BR)F1 ●
≥3 37
2-2.9 10
1-1.9 0
0-0.9 0
NEG 0
TOTALS 0 1 14 31 25
NEG 0-0.9 1-1.9 2-2.9 ≥3
TITER AT 2 WEEKS
TITER AT 6 WEEKS
c
2 WK 105/168(62%)
6 WK 128/164(78%)
BR x (BRAK) F1 ○
(BRAK) F1 x BR ●
≥3 58
2-2.9 56
1-1.9 12
0-0.9 2
NEG 36
TOTALS 63 16 23 42 24
NEG 0-0.9 1-1.9 2-2.9 ≥3
TITER AT 2 WEEKS
TITER AT 6 WEEKS
d
2 WK 45/54(83%)
6 WK 49/53(92%)
(BRAK)F2 ●
≥3 22
2-2.9 24
1-1.9 3
0-0.9 0
NEG 4
TOTALS 9 1 9 24 11
NEG 0-0.9 1-1.9 2-2.9 ≥3
TITER AT 2 WEEKS
TITER AT 6 WEEKS
e
2 WK 19/31(61%)
6 WK 51/80(64%)
NIH x (BRAK)F1 ○
≥3 11
2-2.9 26
1-1.9 13
0-0.9 1
NEG 29
TOTALS 12 3 9 5 2
NEG 0-0.9 1-1.9 2-2.9 ≥3
TITER AT 2 WEEKS
TITER AT 6 WEEKS
f
2 WK 20/28(71%)
6 WK 31/31(100%)
(D2 x AK)F1 ○
≥3 24
2-2.9 6
1-1.9 0
0-0.9 1
NEG 0
TOTALS 8 4 10 4 2
NEG 0-0.9 1-1.9 2-2.9 ≥3
TITER AT 2 WEEKS
TITER AT 6 WEEKS

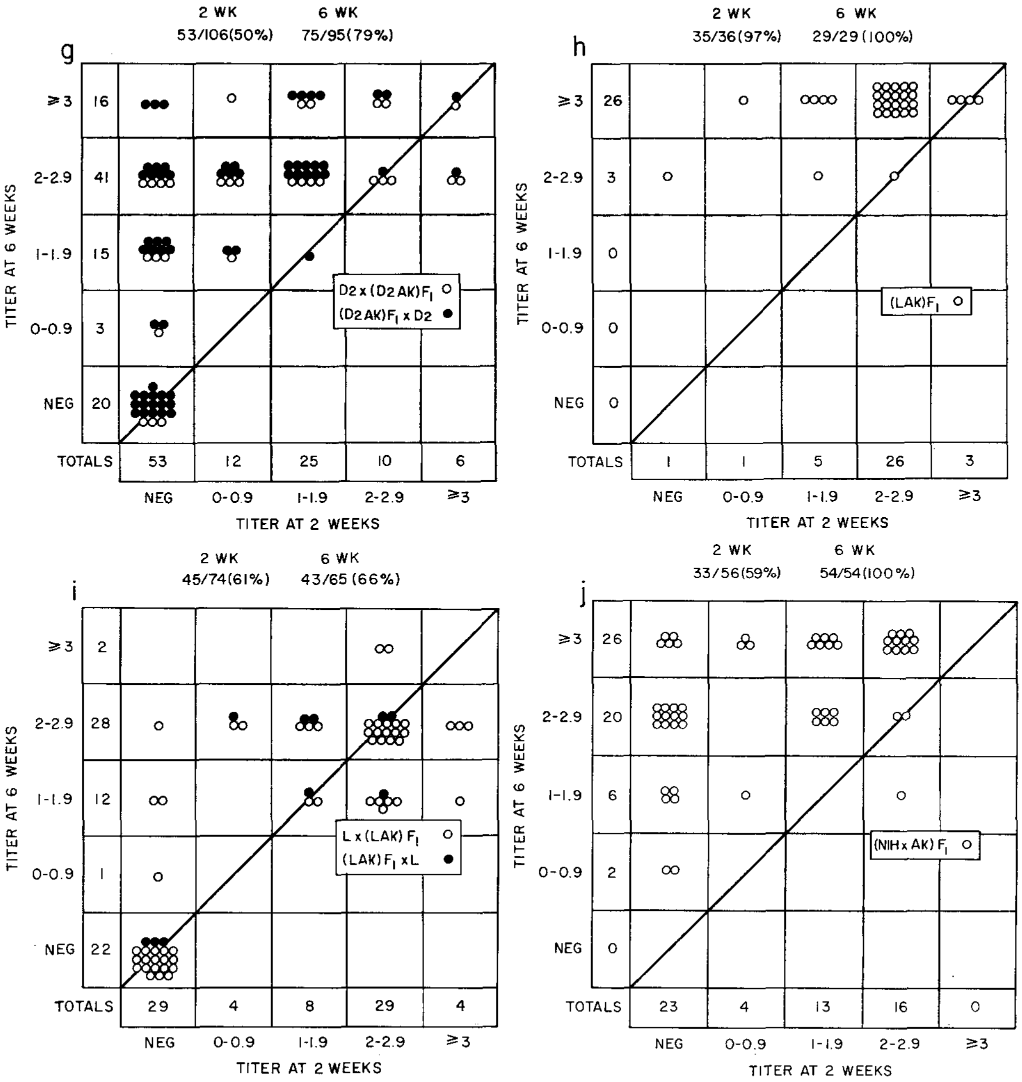


FIG. 1. Quantitation of MLV in tail extracts of AKR hybrids. The circles show the relationship of the titers at 2 and 6 wk in individual mice. Mice tested at only one time point are included in the totals along the edges. Crosses in which the high-virus parent is the male are open circles, while crosses in which the high-virus parent is the female are given as solid circles. *(a)* AKR, ●; *(b)* (BR × AK)F$_1$, ○, and (AK × BR)F$_1$, ●; *(c)* BR × (BR × AK), ○, and (BR × AK) × BR, ●; *(d)* (BR × AK)F$_2$, ●; *(e)* NIH × (BR × AK), ○; *(f)* (D2 × AK)F$_1$, ○; *(g)* D2 × (D2 × AK), ○, and (D2 × AK) × D2, ●; *(h)* (L × AK)F$_1$, ○; *(i)* L × (L × AK), ○, and (L × AK) × L, ●; *(j)* (NIH × AK)F$_1$, ○.

mice, but the titers at 6 wk were essentially the same as those in the (BR × AK)F_1 and (L × AK)F_1 mice. Stated in genetic terms, the tests of F_1 mice showed that the inheritance of the AKR virus phenotype was dominant, highly penetrant, and contributed equally well by both sexes.

Tests of the first backcross (Bc1) and F_2 generations showed clear-cut segregation, which was not dependent on the maternal direction of the cross. The segregation ratios (approximately 3:1 in the Bc1 generation and 15:1 in the F_2 generation) are those expected for two independently segregating loci, either of which gives a positive phenotype. The results are incompatible with models in which presence of virus is determined by a single gene or requires the joint presence of two genes from AKR. For convenience, we will refer to the two loci indicated by the segregation data as "AKR virus-inducing loci" and designate them as "V_1" and "V_2"; this is not meant to imply their mechanism of action or to be a formal proposal for nomenclature. "V_1" will be used to refer to the locus on linkage group I, as described below.

The quantitative data (Fig. 1) give further support to a multiple independent gene model, in that 6-wk virus titers in the low and intermediate categories, and delayed appearance of virus, which were infrequent in the F_1 mice, were common in the segregating generations. This would be expected if presence of both V loci tended to lead to earlier and higher titer of virus than either one alone.

Comparison of virus titers in the different backcrosses again suggested that there was more often a delay in development of titer in the hybrids with DBA as compared with those with C57BR and C57L, as was seen in the F_1 generation. The C57L backcross mice were unique in that, although titers were as high as in the C57BR hybrids at 2 wk, in many mice the titer remained the same or had declined somewhat in the 6-wk test. Since this pattern was not seen in the F_1 generation, it suggests that C57L mice may have one or more recessive genes, not shared by C57BR or DBA, which partially inhibit production or spread of virus in mature mice.

Second Backcross Generation of C57BR Hybrids.—To obtain further information on the number of AKR genes leading to appearance of infectious virus, and to obtain each of these genes in isolation from the others, second-backcross generation mice were studied. 29 unselected BR × (BR × AK) mice were mated with C57BR; many of the males were also given NIH partners, and in a few cases C57L and DBA also. Females having a litter by a C57BR male were generally remated with an NIH male. The offspring of these crosses were tested for virus in the tail at 2 and 6 wk.

19 families with more than 10 offspring were obtained. Table IV shows the results of the 6-wk virus testing and their interpretation in terms of the number of AKR virus-inducing genes carried by the Bc1 parent. Three families showed 3:1 ratios of virus to no virus; 12 families had ratios compatible with 1:1; and three families were virus negative. One family, No. 3-3, gave a pattern compatible with either a 3:1 or 1:1 segregation ratio. Interpreted in terms of the

TABLE IV

Virus Testing of Progeny of BR × (BR × AK) Mice Mated with Low-Virus $Fv\text{-}1^n$ Mice

BR × (BR × AK) parent					No. of virus- positive progeny/No. tested 6-wk tests					
Inferred virus genotype	Mouse No.	Sex	Genotype		Total*		By *Hbb*‡		By color§	
			c	*Hbb*	No.	%	*d/s*	*s/s*	*c/c*	+/*c*
V_1/− V_2/−	1-8	M	+/*c*	*d/s*	34/48	71			5/9	1/3
	2-1	M	+/*c*		17/21	81			4/4	3/5
	2-5	M	+/+	*s/s*	15/20	75				
V_1/− V_2/− or V_1/− −/−	3-3	M	+/*c*	*d/s*	24/40	60	6/10	6/7	1/11	11/12
V_1/− −/−	1-4‖	F	+/*c*	*d/s*	5/16	31			3/6	0/4
	1-2¶	M	+/*c*	*d/s*	19/36	53	9/14	3/11	2/4	5/7
	2-4	M	+/*c*	*s/s*	22/47	47			14/20	4/20
	4-5	M	+/*c*	*d/s*	12/32	38	3/5	0/2	8/16	1/9
−/− V_2/−	3-5	M	+/*c*		27/57	47	2/3	0/4	10/19	13/22
	4-1	M	+/*c*	*d/s*	22/56	39	5/11	6/10	6/22	5/13
	4-2	M	+/*c*		25/65	38	5/9	4/7	8/24	8/25
	4-3	M	+/*c*	*d/s*	22/51	43	3/3	3/7	7/17	9/24
V_1/− −/− or −/− V_2/−	1-7	F	+/+	*s/s*	6/13	46				
	2-7	M	+/+		5/11	45				
	5-1	F	+/*c*		2/11	18			0/4	2/7
	5-4	M	+/+		9/16	56				
−/− −/−	1-1	M	+/+	*s/s*	0/40	0				
	1-3	M	+/+	*s/s*	0/31	0				
	1-5	F	+/+	*s/s*	0/18	0				

---

* Matings by C57BR, NIH, C57L, and DBA.

‡ Matings by C57BR and C57L.

§ Matings by NIH.

‖ Linkage to the *c* locus in this family was established by progeny testing. A virus-positive Bc2 male, No. 62-3-2 (from a mating with BR) was mated to NIH females; all 21 progeny were +/*c*. Three males of this generation were mated to NIH females. Of their progeny, 39 (75%) of 52 +/*c* mice were virus positive, as compared to 13 (37%) of 35 *c/c* progeny ($P = 0.001$). Mouse No. 62-3-2 is thus a recombinant between *c* and V_1. These results estimate V_1 to be 30 map units from the *c* locus.

¶ Linkage to *c* was also established in this family by progeny testing. Three +/*c* male offspring of NIH × 1-2, selected for being virus positive, were mated with NIH females. In the progeny, 16 of 31 +/*c* mice (52%), and 6 of 30 *c/c* mice (20%) were virus positive ($P = 0.02$).

number of AKR virus-inducing loci carried by the Bc1 parent, these results indicate that three or four mice carried two loci, 12 or 13 carried one, and 3 carried none. This is in full agreement with the two-locus model inferred from the data in the preceding section, which predicts that the 19 families would show two, one, and zero locus inheritance in a ratio of 4.8:9.5:4.8.

Localization of One Virus-Inducing Locus on Linkage Group I.—Our attention was drawn to linkage group I by the observation that white (BR × AK)F_2 mice were consistently positive for virus at 2 wk of age. To investigate this for possible linkage, NIH × (BR × AK) hybrids were produced to test for association between virus and albino (the *c* locus), and BR × (BR × AK) mice were typed for ***Hbb*** and/or progeny tested for presence of the albino gene from AKR; the ***Hbb*** locus, which is closely linked with *c* on linkage group I, determines whether the hemoglobin migrates as a single or diffuse, multiple band pattern on electrophoresis (21). The results, shown in Table V, indicate that Bc1 mice which had received the ***c-Hbb*** region from AKR were consistently more likely to be virus positive than those without the AKR linkage group I markers.

TABLE V

Correlation of Virus Positivity with Inheritance of Linkage Group I Markers from AKR in Bc1 Mice

Hybrid	Marker	No. with virus/No. in category		*P*
		Marker from AKR	Marker not from AKR	
BR × (BR × AK)	*c* (progeny testing)	15/16 (94%)	8/13 (62%)	0.09
	Hbb	22/27 (81%)	15/27 (56%)	0.08
	c or ***Hbb***	30/35 (86%)	18/33 (55%)	0.01
NIH × (BR × AK)	*c*	33/45 (73%)	18/35 (51%)	0.07

It should be kept in mind that linkage relationships are less easily discerned where two independently segregating genes give the same phenotype than in the usual one-gene system. In this type of two-gene system, the closest possible linkage of one of the two genes with a given marker would give only a 2:1 ratio between the proportions positive in mice with and without the marker.

The association with linkage group I was fully confirmed by the second backcross families (Table IV). Four of the families showing one-gene segregation ratios (Nos. 1-4, 2-4, 4-5, and 1-2), as well as the family with the intermediate segregation ratio (No. 3-3) showed clear-cut linkage to group I. Since BR × (BR × AK) mouse No. 2-4 is a recombinant between *c* and ***Hbb***, having the AKR *c* locus, it appears that the gene order is V_1-*c*-***Hbb***; however, it is possible that he is a double recombinant. The father of family 3-3 is a recombinant between *c* and V_1. Calculation of map distance from the data on both backcross generations gives an estimate that the gene is about 30 units from the *c-Hbb* region.

In contrast, four other one-locus families (Nos. 3-5, 4-1, 4-2, and 4-3) showed no association of virus with linkage group I, and presumably carry only the second locus (V_2). The remaining one-locus families either did not have the AKR linkage group I markers, or had too few progeny to evaluate for linkage.

There was no evidence of linkage with the other coat color markers (brown, dilute, and leaden). There was no close linkage with *H-2*, though distant linkage cannot yet be ruled out.

Activation of Infectious Virus in Tissue Cultures of Backcross Embryos by IdU.—Confirmation of the segregation ratios observed in vivo was obtained by studying the ability of IdU to activate infectious virus from individual backcross embryos in vitro. IdU treatment of primary cultures of AKR embryo cells regularly results in induction of infectious virus in about 0.1% of the cells (7). In contrast, cultures from low virus strains of mice rarely release detectable infectious virus after IdU treatment; this has been shown with BALB/c (9), C57BR, DBA, NIH, C57BL, and B10.D2 (J. W. Hartley and W. P. Rowe, unpublished data). Cultures from F_1 hybrids between AKR and BALB/c react like AKR cells (22). Studies were made of the proportion of backcross embryos in which infectious virus could be induced by IdU; the embryos were from the Bc1 generation and from the Bc3 generation of three of the one-locus families (Nos. 1-4, 1-2, and 4-1 in Table IV) which are considered to carry V_1 or V_2. Table VI shows the results.

TABLE VI

Activation of Infectious MLV by IdU Treatment of Individual Backcross Embryos in Tissue Culture

Generation	Parents				No. of embryos yielding virus/No. tested	
	Strain		Presumed genotype			
	Mother	Father	Mother	Father		
Bc1	BR	(BR × AK)F_1	–/– –/–	V_1/– V_2/–	24/29	Total Bc1: 31/37 (84%)
	(L × AK)F_1	L	V_1/– V_2/–	–/– –/–	7/8	
Bc3	NIH	Family 1-4 Bc2	–/– –/–	V_1/– –/–	9/18	Total Bc3: 16/37 (43%)
	NIH	Family 1-2 Bc2	–/– –/–	V_1/– –/–	4/8	
	NIH	Family 4-1 Bc2	–/– –/–	–/– V_2/–	3/11	

In full agreement with the in vivo results, Bc1 embryos gave results compatible with a 3:1 segregation ratio, while the one-locus families gave 1:1 ratios. Although the numbers tested are small, these findings provide an important confirmation of the tail test results, and indicate that the spontaneous development of virus in vivo and the inducibility of infectious virus by IdU in vitro are functions of the same genetic loci.

DISCUSSION

The data presented here are completely compatible with a model in which the high-virus characteristics of the AKR mouse are the result of its carrying two unlinked chromosomal loci, both of which have the capacity to induce virus synthesis early in life. One of the loci is located 25–30 map units from the albino locus on linkage group I, while the linkage of the other is not known.

Our results are in full agreement with the Bc1 and F_2 segregation data which Taylor et al. (14) observed in their studies of gs antigen in the spleen of hybrids between AKR and C57L; however, our data are incompatible with their findings in the F_2-derived inbred lines. This discrepancy may indicate that different genetic loci are being followed in the two studies, or may be a reflection of the different tissues studied and the difference in techniques used.

While the data fit a two-locus model very well, and are incompatible with a single-locus model, it cannot be rigorously excluded that more than two virus-inducing loci are segregating. If there were one locus with high penetrance and several other loci with low penetrance, the over-all segregation ratios might mimic a two-locus pattern. Further studies of the Bc2 families will be needed to clarify this point. Isolation of the virus-inducing loci in the backcross families will also be important for determining the linkage of the second locus, for testing for possible genetic differences between the MLV strains induced by the different loci, for testing for allelism with comparable loci in other high-virus mice, and for clarifying the basis of the quantitative differences observed in mice in the F_1, Bc1, and F_2 generations (Fig. 1).

The type of genetic system under study here should be thought of in a different frame of reference than most classical genetic systems. It is likely that we are not dealing with the usual type of cross involving the presence or absence of genetic material capable of making the functional gene product under study, since two of the four low-virus parental strains studied here show a low incidence of endogenous MLV infection. Rather, the high- and low-virus parental strains differ in the probability that the genetic material will be expressed. An appropriate analogy might be the high efficiency yielder and low efficiency yielder adenovirus type 2-SV40 hybrid virus particles (23). Both of these viruses contain the complete SV40 genome integrated into a defective Ad.2 genome, but they differ by a factor of 10^4 to 10^5 in the probability that an infected cell will produce SV40 virus.

A further difference from classical genetic systems is that the end result of the expression of the genetic element being studied is production of infectious virus, which can convert other cells to a positive phenotype independently of their own genetic makeup and regulation. This carries the implication that a positive phenotype, instead of resulting from the normal functioning of the locus in a large number of cells, could be the result of the misfunctioning (mutation, misreading, excision, etc.) of the locus in a very small number of cells, or even in a single cell. This concept also applies to genetic studies of MLV antigen expression if the crosses involve a high-virus parent.

In the crosses studied here there are no known virus genetic markers which would differentiate whether the MLV found in the various hybrids is derived from the AKR or the low-virus parent. Consequently, the studies reported here do not bear on the question of whether the two chromosomal loci detected are the viral genomes themselves, or expression genes which increase the probability of activation of virus determinants elsewhere in the cell. Attempts to utilize a virus host range marker for this purpose will be described in the companion paper (22).

It has long been known that spontaneous leukemogenesis in the mouse is influenced by a large number of genetic factors (24, 25). In two respects, the studies reported here are of importance toward unraveling these genetic complexities. First, identification of two loci for expression of infectious virus affords a degree of control over this fundamental, early step in the disease process. By introducing a known set of virus-inducing genes into various genetic backgrounds, the genes affecting the later stages of the pathogenetic process may be amenable to identification and analysis. Second, the ability to monitor mice sequentially for degree of virus activity provides a means of evaluating the role of the early virologic events in determining the risk of developing leukemia. Questions such as whether leukemia incidence correlates with early appearance of virus, maximal titer attained, or the linkage group from which the virus derived can now be studied.

It was striking that for the most part the virologic patterns in the various hybrids were very similar, both qualitatively and quantitatively. This implies that there are no major undefined genetic influences, from either dominant or recessive genes, inhibiting virus activation or spread in the four *Fv-1*^{n} strains studied here. Minor effects were suggested by the slight delay in virus development in DBA and NIH hybrids, and in the failure to attain maximal virus titers seen in the L × (L × AK) backcross mice. The latter could represent an effect of the *H-2*^{b} allele, which has been shown to confer partial resistance to viral leukemogenesis (26–28). However, there was clearly no significant effect of *H-2* type on the virologic pattern of the F_1 hybrids.

The lack of correlation of virologic status with the dilute locus, which is closely linked with *Fv-2* (29), indicates that *Fv-2* is not one of the V loci. *Fv-2* type also appeared to be without effect on the expression of endogenous virus as judged by the similarity of the virologic findings in Bc1 mice of the C57BR and C57L crosses (half *Fv-2*^{r}/*Fv-2*^{r} and half *Fv-2*^{s}/*Fv-2*^{r}) to those in the DBA crosses (all *Fv-2*^{s}/*Fv-2*^{s}). In contrast, *Fv-1* has a marked influence, as will be shown in the companion paper (22).

SUMMARY

AKR mice, which regularly contain infectious murine leukemia virus, were mated with four *Fv-1*^{n} strains of mice which show little or no expression of virus. F_1, F_2, and first and second backcross generation hybrids were tested for virus in tail tissue at 2 and 6 wk of age. The segregation data indicate that the AKR mouse contains two unlinked, autosomal, chromosomal loci, either of which suffices to induce detectable levels of infectious virus in *Fv-1*^{n} progeny by 6 wk of age. One of the loci (tentatively referred to as V_1) is on linkage group I, 25–30 map units from the locus for albino; the gene order tentatively appears to be V_1-*c*-***Hbb***.

REFERENCES

1. Gross, L. 1951. Pathogenic properties, and "vertical" transmission of the mouse leukemia agent. *Proc. Soc. Exp. Biol. Med.* **78**:342.
2. Kaplan, H. S. 1967. On the natural history of the murine leukemias. Presidential address. *Cancer Res.* **27**:1325.
3. Geering, G., L. J. Old, and E. A. Boyse. 1966. Antigens of leukemias induced by naturally occurring murine leukemia virus: their relation to the antigens of Gross virus and other murine leukemia viruses. *J. Exp. Med.* **124**:753.
4. Huebner, R. J., and G. J. Todaro. 1969. Oncogenes of RNA tumor viruses as determinants of cancer. *Proc. Natl. Acad. Sci. U.S.A.* **64**:1087.
5. Kajima, M., and M. Pollard. 1968. Wide distribution of leukemia virus in strains of laboratory mice. *Nature (Lond.).* **218**:188.
6. Hartley, J. W., W. P. Rowe, W. I. Capps, and R. J. Huebner. 1969. Isolation of naturally occurring viruses of the murine leukemia virus group in tissue culture. *J. Virol.* **3**:126.
7. Lowy, D. R., W. P. Rowe, N. Teich, and J. W. Hartley. 1971. Murine leukemia virus: high-frequency activation in vitro by 5-iododeoxyuridine and 5-bromodeoxyuridine. *Science (Wash. D. C.).* **174**:155.
8. Rowe, W. P., J. W. Hartley, M. R. Lander, W. E. Pugh, and N. Teich. 1971. Noninfectious AKR mouse embryo cell lines in which each cell has the capacity to be activated to produce infectious murine leukemia virus. *Virology.* **46**:866.
9. Aaronson, S. A., G. J. Todaro, and E. M. Scolnick. 1971. Induction of murine C-type viruses from clonal lines of virus-free BALB/3T3 cells. *Science (Wash. D. C.).* **174**:157.
10. Huebner, R. J., G. J. Kelloff, P. S. Sarma, W. T. Lane, H. C. Turner, R. V. Gilden, S. Oroszlan, H. Meier, D. D. Myers, and R. L. Peters. 1970. Group-specific antigen expression during embryogenesis of the genome of the C-type RNA tumor virus: implications for ontogenesis and oncogenesis. *Proc. Natl. Acad. Sci. U.S.A.* **67**:366.
11. Abelev, G. I., and D. A. Elgort. 1970. Group-specific antigen of murine leukemia viruses in mice of low leukemic strains. *Int. J. Cancer.* **6**:145.
12. Stockert, E., L. J. Old, and E. A. Boyse. 1971. The G_{IX} system. A cell surface allo-antigen associated with murine leukemia virus; implications regarding chromosomal integration of the viral genome. *J. Exp. Med.* **133**:1334.

13. Nowinski, R. C., L. J. Old, E. A. Boyse, E. deHarven, and G. Geering. 1968. Group-specific viral antigens in the milk and tissues of mice naturally infected with mammary tumor virus or Gross leukemia virus. *Virology.* **34:**617.
14. Taylor, B. A., H. Meier, and D. D. Myers. 1971. Host-gene control of C-type RNA tumor virus: inheritance of the group-specific antigen of murine leukemia virus. *Proc. Natl. Acad. Sci. U.S.A.* **68:**3190.
15. Aoki, T., E. A. Boyse, and L. J. Old. 1968. Wild-type Gross leukemia virus. II. Influence of immunogenetic factors on natural transmission and the consequences of infection. *J. Natl. Cancer Inst.* **41:**97.
16. Rowe, W. P., and T. Pincus. 1972. Quantitative studies of naturally occurring murine leukemia virus infection of AKR mice. *J. Exp. Med.* **135:**429.
17. Pincus, T., J. W. Hartley, and W. P. Rowe. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. *J. Exp. Med.* **133:**1219.
18. Pincus, T., W. P. Rowe, and F. Lilly. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to Friend murine leukemia virus. *J. Exp. Med.* **133:**1234.
19. Hartley, J. W., W. P. Rowe, and R. J. Huebner. 1970. Host-range restrictions of murine leukemia viruses in mouse embryo cell cultures. *J. Virol.* **5:**221.
20. Rowe, W. P., J. W. Hartley, and W. E. Pugh. 1970. Plaque assay techniques for murine leukemia viruses. *Virology.* **42:**1136.
21. Ranney, H. M., and S. Gluecksohn-Waelsch. 1955. Filter-paper electrophoresis of mouse hemoglobin: preliminary note. *Ann. Hum. Genet.* **19:**269.
22. Rowe, W. P., and J. W. Hartley. 1972. Studies of genetic transmission of murine leukemia virus by AKR mice. II. Crosses with *Fv-1*^{b} strains of mice. *J. Exp. Med.* **136:**1286.
23. Lewis, A. M., Jr., and W. P. Rowe. 1970. Isolation of two plaque variants from the adenovirus type 2-simian virus 40 hybrid population which differ in their efficiency in yielding simian virus 40. *J. Virol.* **5:**413.
24. MacDowell, E. C., and M. N. Richter. 1935. Mouse leukemia. IX. The role of heredity in spontaneous cases. *Arch. Pathol.* **20:**709.
25. Cole, R. K., and J. Furth. 1941. Experimental studies on the genetics of spontaneous leukemia in mice. *Cancer Res.* **1:**957.
26. Lilly, F., E. A. Boyse, and L. J. Old. 1964. Genetic basis of susceptibility to viral leukemogenesis. *Lancet.* **2:**1207.
27. Lilly, F. 1968. The effect of histocompatibility-2 type on resistance to the Friend leukemia virus in mice. *J. Exp. Med.* **127:**465.
28. Tennant, J. R., and G. D. Snell. 1968. The H-2 locus and viral leukemogenesis as studied in congenic strains of mice. *J. Natl. Cancer Inst.* **41:**597.
29. Lilly, F. 1970. *Fv-2*: identification and location of a second gene governing the spleen focus response to Friend leukemia virus in mice. *J. Natl. Cancer Inst.* **45:**163.