STUDIES OF GENETIC TRANSMISSION OF MURINE LEUKEMIA VIRUS BY AKR MICE

II. Crosses with *Fv-1*^{b} Strains of Mice

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Studies of the transmission of murine leukemia virus (MLV)[1] to hybrids between the high-virus AKR strain and low-virus mouse strains selected for being sensitive to infection with the AKR MLV showed that transmission of virus followed mendelian segregation patterns (1). The AKR strain appeared to be contributing two unlinked chromosomal loci, each of which was capable of inducing detectable levels of MLV by 2–6 wk of age.

To approach the question of whether these AKR loci are genetic elements of the virus itself or are genes which promote the expression of virus located elsewhere, it is necessary to use viral genetic markers. If the chromosomal loci are viral genomes, in crosses between AKR and low-virus mice carrying a genetically distinct endogenous MLV (which is not expressed in early life), the virus in the progeny should be AKR type. If the chromosomal loci are expression genes, then the virus-positive hybrids should show virus of both parental types.

The only genetic marker for naturally occurring MLV is the host range type (2). The naturally occurring MLV strains which can be detected by tissue culture techniques fall into two host range categories, N-tropic or B-tropic. The sensitivity of mouse cells in tissue culture to these viruses is determined by the *Fv-1* gene of the mouse (3–5). Cells of the *Fv-1*^{n} genotype are sensitive to N-tropic virus and relatively restrictive for B-tropic viruses, while *Fv-1*^{b} cells show the reciprocal pattern. Heterozygotes (*Fv-1*^{nb}) are restrictive for both N- and B-tropic viruses, the *Fv-1* alleles being dominant for resistance. The restriction produced by *Fv-1* is not absolute, and is overcome by increasing the multiplicity of infection (unpublished data). *Fv-1*^{n} mouse strains appear to carry only the N-tropic viral genome, while *Fv-1*^{b} strains generally carry B-tropic or both N- and B-tropic genomes. While it is known that *Fv-1* exerts its restrictive effect in vivo on Friend virus (3, 6) and murine sarcoma virus (2) infection, there is no information on its effect on endogenous MLV infection.

Host range variants introduce an additional element of complexity into genetic

[1] *Abbreviations used in this paper:* AK, AKR/J mice; Bc1, first backcross; BL, C57BL/10J mice; BR, C57BR/cdJ mice; C, BALB/cN mice; D2, DBA/2J mice; IdU, 5-iododeoxyuridine; METC, mouse embryo tissue culture; MLV, murine leukemia virus; NIH, National Institutes of Health.

studies of their transmission, since it is necessary to include another segregating gene (*Fv-1*) in the crosses. This is required in order to introduce both viral genomes into the progeny, and to produce the appropriate *Fv-1* genotypes favorable to expression of one or the other of the two host range variants in the segregating generations. A further complication that must be envisioned is that the *Fv-1* locus could be linked to or identical with one of the virus-inducing loci; this would produce a strong correlation between presence of a particular host range variant and *Fv-1* type.

The studies reported here are of hybrids between AKR (which is $Fv\text{-}1^n$ and carries N-tropic MLV) and $Fv\text{-}1^b$ mouse strains which are known to carry B-tropic MLV. They deal with the effect of *Fv-1* type on the expression (penetrance) of the N-tropic AKR MLV, and with the use of the host range marker for attempting to test for chromosomal integration of the AKR MLV genome.

Materials and Methods

Mice.—The parental strains and the abbreviations used are as follows: AKR/J (AK), BALB/cN (C), C57BR/cdJ (BR), C57BL/10J (BL), B10.BR/Sn, B10.D2 (old)/Lw, DBA/2J (D2), and National Institutes of Health (NIH) Swiss. Their genotypes for the genes of importance in MLV infection were listed in the preceding paper (1). The characteristics of the endogenous MLV infection of normal mice of the $Fv\text{-}1^n$ strains have been presented (1, 7); it should be stressed that AKR and the other $Fv\text{-}1^n$ mice have never yielded B-tropic MLV. The comparable testing of the $Fv\text{-}1^b$ strains is summarized in Table I. BALB/c mice show a high incidence of both N-tropic and B-tropic MLV after the age of 4–6 months; however, during the first 3 months of life they have been consistently negative for virus; similar patterns in BALB/c mice have been observed by Peters et al.[2] C57BL/10 mice also yielded both host range types of MLV, but at a much lower frequency. B10.D2 (old) mice showed a high level of infection, relatively early in life, but exclusively with B-tropic virus. The limited testing of B10.BR mice gave negative results; however, T. Pincus (personal communication) has obtained several isolations of B-tropic MLV from older B10.BR mice, and B10.BR embryo tissue cultures frequently show spontaneous plaque formation caused by emergence of B-tropic MLV (4). Thus, all of the $Fv\text{-}1^b$ strains used for crossing with AKR carry endogenous MLV, but during the period of life on which the present studies are concentrated they are either virus negative or manifest only a distinctly different host range type of virus from that carried by AKR.

Virus Testing.—Hybrid mice were routinely tested for virus in tail tissue at 6–12 wk of age (usually 8 wk); many of the mice were also tested at 2 wk, and a small number were tested again at 4–9 months. The procedures for preparing tail extracts and for doing plaque assays on them have been described (1). All specimens were tested in NIH mouse embryo tissue culture (METC); 543 specimens were also tested in BALB METC for detection of B-tropic virus. When specimens were tested in both types of culture, they were considered to contain only N-tropic virus if they induced plaques only in NIH METC, or if they titered more than 10-fold higher in NIH than in BALB METC, this being the expected plaquing ratio for N-tropic virus. A number of specimens giving this pattern were blind passaged to BALB METC, and no B-tropic virus was detected. If a specimen gave comparable numbers

[2] Peters, R. L., J. W. Hartley, G. J. Spahn, L. S. Rabstein, C. E. Whitmire, H. C. Turner, and R. J. Huebner. 1972. Prevalence of the group-specific (gs) antigen and infectious virus expressions of the murine C-type RNA viruses during the life span of BALB/cCr mice. Submitted for publication.

of plaques on both types of METC, the specimen was passaged in both cell types, and harvests from these were tested for plaquing ratio in NIH and BALB METC. The few specimens that gave this pattern were found by this procedure to contain both N- and B-tropic virus.

Induction of MLV by 5-Iododeoxyuridine (IdU).—This procedure was carried out as described in the preceding paper (1). In addition to the routine assay for *in situ* zones of infection, the majority of IdU-treated cultures were cocultivated with NIH and BALB METC, and the harvests tested for N-tropic and B-tropic virus by plaque titrations in NIH and BALB METC.

Fv-1 Typing.—Determination of *Fv-1* type in vivo was done by the Friend virus spleen focus assay of Axelrad and Steeves (8). These tests were done on mice segregating for $Fv\text{-}1^n$ and $Fv\text{-}1^{nb}$. The F-S (N-tropic) line of Friend virus, obtained from Dr. Frank Lilly, was used as an extract of infected DBA/2 spleen. Hybrid mice on whom tail testing had been completed were inoculated intraperitoneally with 100–200 spleen focus-forming units (as determined by titration in DBA/2 mice inoculated intraperitoneally); inoculated DBA/2 and (BALB/c × DBA/2)F_1 or (C57L × A)F_1 mice were included as $Fv\text{-}1^n$ and $Fv\text{-}1^{nb}$ controls, respectively. Spleen focus counts were made 9 days after inoculation.

TABLE I

Summary of Tests of Four $Fv\text{-}1^b$ Inbred Mouse Strains for N- and B-Tropic MLV

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	4–6	7–12	13–16
BALB/c	Tail	0/25		1/3	4/22	0/25		0/3	9/22
	Pool‡	0/5		2/3	11/22	0/5		1/3	4/22
	Spleen	0/14	5/25	40/62	4/7	0/6	0/14	13/47	3/7
C57BL/10	Tail	0/10		0/30		0/10		0/30	
	Pool	0/10		1/29		0/10		1/29	
B10.BR	Tail		0/6	0/5			0/6	0/5	
B10.D2	Tail	0/44	0/5	0/4		23/45	2/5	4/4	
(old)	Pool			0/4				4/4	

* Tests were of 0.2–0.4 ml of 2% tail extract or of 5 or 10% extract of viscera.

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

In vitro typing of hybrid METC was done on strain combinations segregating for $Fv\text{-}1^{nb}$ and $Fv\text{-}1^b$. On the day after cell planting, primary cultures were treated with diethylaminoethyl (DEAE)-dextran by the usual procedure, and two dishes were challenged with 300 plaque-forming units of a B-tropic MLV (WN1802B strain). One dish was infected with Moloney MLV, which is NB-tropic and not affected by *Fv-1*, to verify that the cultures did not have some nonspecific resistance to plaque induction. Plaques were developed by the UV-XC procedure (9).

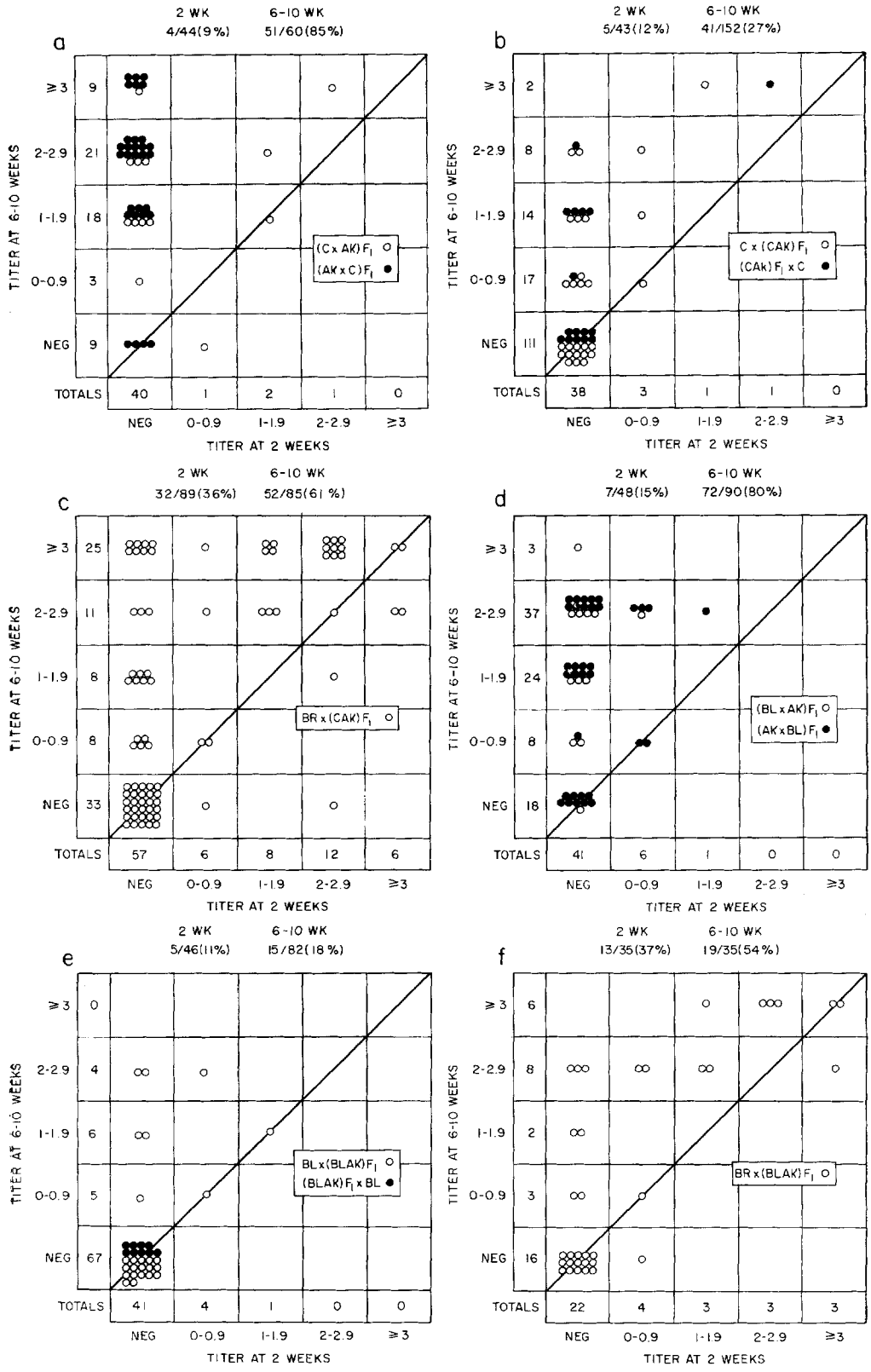
Nomenclature.—In referring to the classes of backcrosses involving various *Fv-1* combinations and AKR ancestry, terminology such as n × (b × AK) will be used. This means that an $Fv\text{-}1^n$ female was mated to an F_1 of a cross between an $Fv\text{-}1^b$ female and an AKR male. Since AKR is $Fv\text{-}1^n$, this cross would segregate for $Fv\text{-}1^n$ and $Fv\text{-}1^{nb}$. A b × (b × AK) cross

TABLE II

Presence of N-Tropic MLV in Tail Extracts of Hybrids between Fv-1^b Mice and AKR

Generation	Strain	2-wk tests			6–10-wk tests		
		No. with virus/total	%	Median titer of positive mice*	No. with virus/total	%	Median titer of positive mice*
				log_{10}			log_{10}
F_1	(C × AK)F_1	4/13	31	Neg.	20/24	83	1.7
	(AK × C)F_1	0/31	0	Neg.	31/36	86	2.6
	(BL × AK)F_1	1/13	8	Neg.	32/36	89	2.2
	(AK × BL)F_1	6/35	17	Neg.	40/54	74	2.2
	(B10.BR × AK)F_1	13/24	54	0.0	24/24	100	2.7
	(AK × B10.BR)F_1	2/20	10	Neg.	20/20	100	2.9
	(B10.D2 × AK)F_1	0/7	0	Neg.	16/19	84	2.2
	(AK × B10.D2)F_1	1/7	14	Neg.	19/20	95	2.1
	Total	27/150	18		202/233	87	
1st backcross to parental, *Fv-1^b*, low-virus strain	C × (C × AK)	4/26	15	Neg.	31/102	30	1.0
	(C × AK) × C	1/17	6	Neg.	10/50	20	1.4
	BL × (BL × AK)	3/28	11	Neg.	9/27	33	1.4
	(BL × AK) × BL	0/9	0		6/61	10	(0.6)
	Total	8/80	10		56/240	23	
Cross to nonparental, *Fv-1^b*, low-virus strain	C × (B10.D2 × AK)				3/32	9	(0.8)
	C × (AK × B10.D2)				4/26	15	(0.5)
	Total				7/58	12	
Cross to *Fv-1^n* low-virus strain	BR × (C × AK)	32/89	36	0.6	52/85	61	2.9
	BR × (BL × AK)	13/35	37	0.6	19/35	54	2.6
	D2 × (BL × AK)	9/50	18	0.2	19/49	39	2.1
	BR × (B10.D2 × AK)				13/35	37	2.5
	Total	54/174	31		103/204	50	
1st backcross to AKR	AK × (BL × AK)	17/25	68	1.4	24/24	100	3.0
	(BL × AK) × AK	11/26	42	1.3	9/14	64	3.1
	Total	28/51	55		33/38	87	

* Positive mice are those which had virus at either time point. A mouse which was negative at 2 wk and positive at 6–10 wk is included for calculating the median at 2 wk.


a
2 WK 4/44(9%)
6-10 WK 51/60(85%)
TITER AT 6-10 WEEKS
≥3 9
2-2.9 21
1-1.9 18
0-0.9 3
NEG 9
TOTALS 40 1 2 1 0
(C x AK)F1 ○
(AK x C)F1 ●
NEG 0-0.9 1-1.9 2-2.9 ≥3
TITER AT 2 WEEKS
b
2 WK 5/43(12%)
6-10 WK 41/152(27%)
≥3 2
2-2.9 8
1-1.9 14
0-0.9 17
NEG 111
TOTALS 38 3 1 1 0
C x (CAK)F1 ○
(CAK)F1 x C ●
c
2 WK 32/89(36%)
6-10 WK 52/85(61%)
≥3 25
2-2.9 11
1-1.9 8
0-0.9 8
NEG 33
TOTALS 57 6 8 12 6
BR x (CAK)F1 ○
d
2 WK 7/48(15%)
6-10 WK 72/90(80%)
≥3 3
2-2.9 37
1-1.9 24
0-0.9 8
NEG 18
TOTALS 41 6 1 0 0
(BL x AK)F1 ○
(AK x BL)F1 ●
e
2 WK 5/46(11%)
6-10 WK 15/82(18%)
≥3 0
2-2.9 4
1-1.9 6
0-0.9 5
NEG 67
TOTALS 41 4 1 0 0
BL x (BLAK)F1 ○
(BLAK)F1 x BL ●
f
2 WK 13/35(37%)
6-10 WK 19/35(54%)
≥3 6
2-2.9 8
1-1.9 2
0-0.9 3
NEG 16
TOTALS 22 4 3 3 3
BR x (BLAK)F1 ○

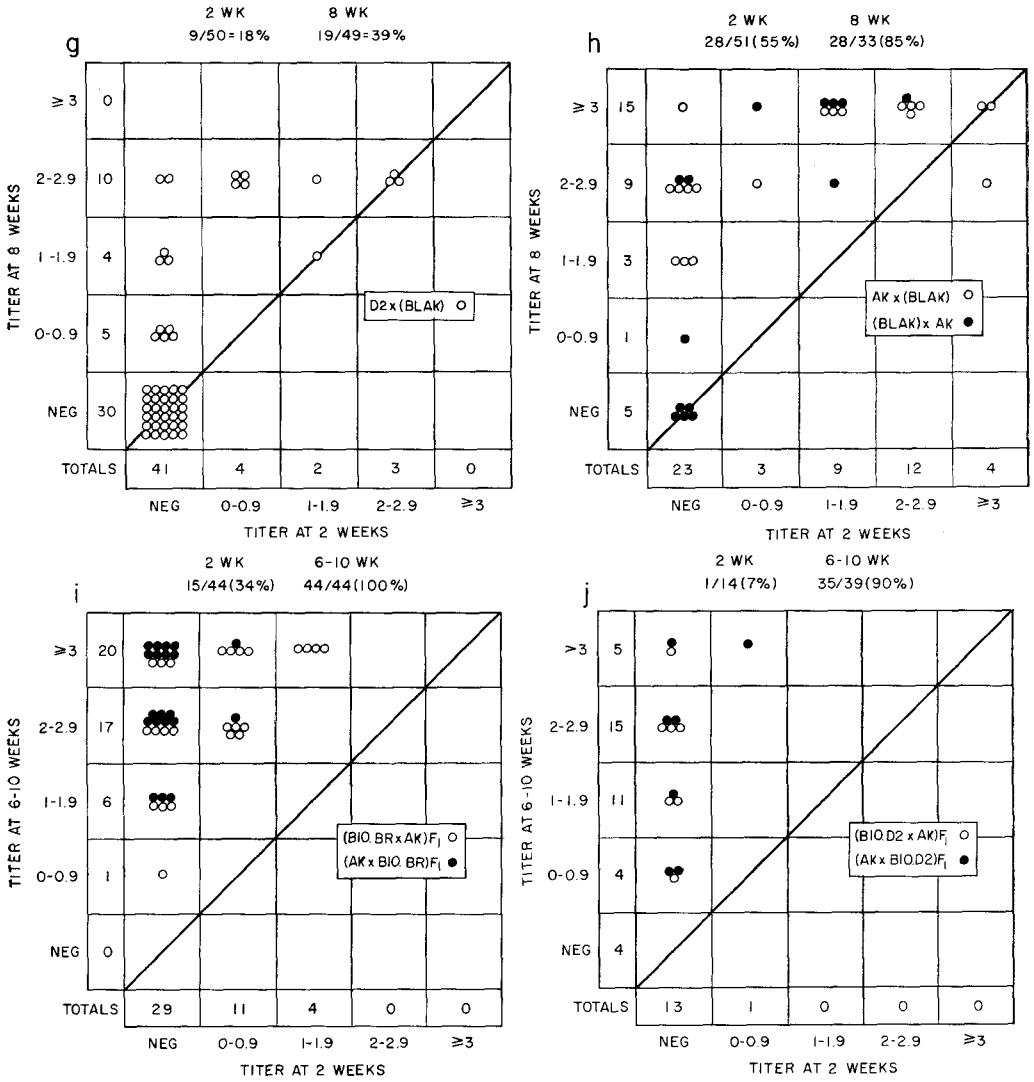


FIG. 1. Frequency distributions of the MLV titers in tail extracts of hybrid mice. The circles show the relationship of the titers at 2 and 6–12 wk in individual mice. Mice tested at only one time point are included in the totals along the edges. (*a*) (C × AK)F_1, ○, and (AK × C)F_1, ●; (*b*) C × (C × AK), ○, and (C × AK) × C, ●; (*c*) BR × (C × AK), ○; (*d*) (BL × AK)F_1, ○, and (AK × BL)F_1, ●; (*e*) BL × (BL × AK), ○, and (BL × AK) × BL, ●; (*f*) BR × (BL × AK), ○; (*g*) D2 × (BL × AK), ○; (*h*) AK × (BL × AK); ○, and (BL × AK) × AK, ●; (*i*) (B10.BR × AK)F_1, ○, and (AK × B10.BR)F_1, ●; (*j*) (B10.D2 × AK) F_1, ○, and (AK × B10.D2)F_1, ●.

would segregate for *Fv-1*^{nb} and *Fv-1*^{b}. As before (1), the two AKR loci which cause appearance of virus early in life in hybrids with *Fv-1*^{n} mice will be referred to as "virus-inducing loci" V_1 and V_2, of which V_1 is on linkage group I.

RESULTS

N-Tropic Virus in Hybrids of Fv-1^{b} *Mice with AKR.*—As will be described later, essentially all viruses isolated from F_1 and first-backcross (Bc1) mice from crosses of *Fv-1*^{b} mice with AKR were N-tropic, i.e., compatible with being derived from the AKR ancestor. Consequently, this and the following two sections will deal only with the testing for N-tropic virus. The results of testing for N-tropic virus in F_1 and various backcross combinations are shown in Table II and Fig. 1.

Virus was detected in the large majority of F_1 mice, but in contrast to crosses of AKR with *Fv-1*^{n} mice (1), its appearance was markedly delayed and the titers attained by 6–10 wk were generally 10-fold lower. Hybrids with B10.BR (*H-2*^{k}) showed a higher incidence of virus at 2 wk of age, were uniformly positive at 6–10 wk, and had somewhat higher virus titers than the other (b × AK)F_1 hybrids. There was no difference in these parameters between hybrids with *H-2*^{b} or *H-2*^{d} strains. There was a significantly greater incidence of virus in 2-wk old (B10.BR × AK)F_1 mice than in the reciprocal hybrid; however, the majority of the positives showed only trace amounts of virus, and the biological significance of this finding is not clear.

Backcrosses to *Fv-1*^{b} mice (b × [b × AK] backcrosses) gave positive tests for virus in about 20% of animals. This is in marked contrast to the approximately 75% seen in n × (n × AK) backcrosses (1). The b × (b × AK) hybrids are presumably segregants for three genetic loci of importance for development of the virus-positive phenotype: *Fv-1*, V_1, and V_2. Half of these Bc1 hybrids are *Fv-1*^{b}, and half *Fv-1*^{nb}; both of these genotypes are restrictive for N-tropic virus, but tissue culture studies indicate that *Fv-1*^{b} cells are even more restrictive than *Fv-1*^{nb} cells (4). Although three-fourths of the b × (b × AK) mice presumably received one or both genes for virus induction, only one-sixth of these potentially virus-positive mice have the F_1 genotype (V_1/—, V_2/—, *Fv-1*^{nb}). The others would have only one V gene with *Fv-1*^{nb}, or one or both V genes with *Fv-1*^{b}; all of these genotypes would presumably result in a lower probability of virus being expressed to detectable levels than the F_1 genotype. Thus, the low segregation ratios in the Bc1 mice may be interpretable primarily in terms of the inhibitory effect of *Fv-1*.

Evidence further indicating the importance of *Fv-1* in determining virus phenotype is seen in the n × (b × AK) crosses in Table II. The proportion of virus-positive mice in these crosses was about twice that in the comparable backcrosses to *Fv-1*^{b} strains.

Backcrosses of (BL × AK)F_1 mice to AKR gave a higher proportion of 2-wk positives, as well as higher virus titers in the positive mice than in the F_1 animals. This also is explicable in terms of the three segregating gene model, since

half of the Bc1 mice would be *Fv-1*^{n}, and all mice would have two, three, or four copies of the AKR virus-inducing genes.

There was a consistent indication of a maternal influence in the backcrosses. In all three instances where comparisons were made, there was a higher incidence of virus in mice born of F_1 fathers than of F_1 mothers. It is probable that the F_1 mothers, having endogenous virus as an antigenic stimulus, were producing some antibody which was partially suppressive for virus expression in their offspring.

Virus Expression in Fv-1^n *and Fv-1*^{nb} *Segregants in n* × (*b* × *AK*) *Crosses.*—A direct test of the hypothesis that *Fv-1* is the major determinant of virus appearance was provided by *Fv-1* typing. n × (b × AK) mice were tail tested at 2 and

TABLE III

Relationship of Virus Expression to Fv-1 Type in n × (*b* × *AK*) *Mice*

Strain	Time of virus test	*Fv-1* type*						
		Fv-1^n			*Fv-1*^{nb}			
		No. positive for virus/ total	%	Median titer of positive‡ mice	No. positive for virus/ total	%	Median titer of positive‡ mice	P§
	wk			log_{10}			log_{10}	
BR × (C × AK)	2	13/25	52	1.4	8/43	19	Neg.	0.01
	8	18/25	72	3.2	21/43	49	3.0	0.1
D2 × (BL × AK)	2	8/27	30	0.3	1/20	5	Neg.	0.07
	8	15/27	56	2.3	4/20	20	0.8	0.03
AK × (BL × AK) and (BL × AK) × AK	2	10/11	91	2.3	3/16	19	Neg.	<0.001
	8	11/11	100	3.3	11/16	69	2.6	0.1

* *Fv-1* type as determined by spleen focus response to challenge with F-S virus.

‡ Positive mice are those which had virus at either time point.

§ *P* value (two-tail) comparing proportions of *Fv-1*^{n} and *Fv-1*^{nb} mice positive for virus.

8 wk and then challenged with F-S virus; their *Fv-1* genotype was determined from their spleen focus response 9 days after challenge. Backcrosses to both AKR and to low-virus *Fv-1*^{n} mice (C57BR and DBA) were studied. Table III shows the correlation of virus tests with *Fv-1* type.

As expected, virus was detected significantly more often, and was higher in titer, in *Fv-1*^{n} than in *Fv-1*^{nb} segregants. Also, the *Fv-1*^{n} mice showed the expected tendency to be positive by 2 wk, while the *Fv-1*^{nb} mice tended to become positive after 2 wk; thus, of the 44 virus-positive *Fv-1*^{n} mice in Table III, 31 (70%) were positive by 2 wk, while of 36 virus-positive *Fv-1*^{nb} mice, only 12 (33%) were positive at 2 wk ($P < 0.01$). In the BR × (C × AK) cross, the segregation ratio in the *Fv-1*^{n} mice was the same (approximately 75% virus positive) as in n × (n × AK) mice (1). The lower proportion of positives in the

D2 × (BL × AK) mice may relate to the relative resistance of the *Fv-1* alleles in both DBA and C57BL mice (4).

Determination of Segregation Ratios by IdU Activation of Embryo Tissue Cultures.—Tests of tissue extracts for presence of virus, as done in the present studies, almost certainly require two independent events for a positive result. These are (*a*) spontaneous activation of a certain number of cells to produce infectious virus, and (*b*) amplification of the amount of virus by spread of infection. The ability of the *Fv-1* gene to restrict initiation of infection in vitro suggests that its inhibitory effect in vivo is on the second of these steps. To further evaluate the role of *Fv-1* it was desirable to study the first of these steps in isolation from the second.

The ability of the halogenated pyrimidines IdU and 5-bromodeoxyuridine to activate production of infectious virus by AKR cells in tissue culture (10) pro-

TABLE IV

IdU Induction of Infectious MLV in Cultures of b × (b × AK) Embryos in Relation to Fv-1 Type

Strain	No. of embryos positive after IdU/No. tested		
	Total	By *Fv-1* type	
		nb	*bb*
C × (B10.D2 × AK)	21/31 (68%)	11/16 (69%)	10/15 (67%)
C × (AK × B10.D2)	6/7 (86%)	2/2	4/5
(BL × AK) × BL	10/11 (91%)		
Total	37/49 (76%)	13/18 (72%)	14/20 (70%)

vides a technique for doing this. By treating cells with IdU and overlaying with mouse cells of appropriate host range sensitivity, the effect of the *Fv-1* type of the IdU-treated cells on spread and consequent detection of virus can be eliminated. Only if a gene system affects the activation event or the infectivity characteristics of the virus induced would it affect the results obtained. While infectious virus is readily induced by IdU in AKR cell cultures, little or no infectious virus is induced in cells from BALB/c (11), C57BL, or B10.D2 (unpublished data). Thus, the IdU induction characteristics of these strains tend to parallel the virologic findings in vivo, and provide a comparable basis for segregation analysis.

Tests of three lots each of (C × AK)F_1 and (AK × C)F_1 embryos showed that their capacity to be activated to produce infectious virus by IdU treatment was essentially the same as AKR embryos. Under the standard treatment conditions, primary AKR embryo cell cultures regularly showed 300–400 virus-producing cells per dish, and the (C × AK)F_1 and (AK × C)F_1 cultures, 200–300 per dish. From these results it was apparent that ability of virus to be acti-

vated was transmitted by AKR males as well as females, was fully dominant, and was not markedly affected by the *Fv-1^{nb}* genotype.

For determination of segregation ratios in the Bc1 generation, the three crosses which showed the most marked *Fv-1*-induced suppression of virus expression (Table II) were selected; these were C × (B10.D2 × AK), C × (AK × B10.D2), and (BL × AK) × BL, in which only 8–15% of mice were positive at

TABLE V

Tests of Hybrids between Fv-1^b Mice and AKR for N-Tropic and B-Tropic MLV Strains

Generation	Age	No. tested	Virus isolation result			
			N-tropic only	N-tropic and B-tropic	B-tropic only	No virus
	months					
F_1*	2–3	211	185	2‡	0	24
	4–7	27	20	0	0	7
	8–9	10	10	0	0	0
b × F_1	2–3	220	48	0	0	172
	4–7	8	4	0	0	4
	8–9	38	7	3§	7‖	21
n × F_1	2–3	29	15	0	0	14
Total	2–3	460	248	2	0	210
	4–7	35	24	0	0	11
	8–9	48	17	3	7	21
Grand total		543	289¶	5	7	242

* The strain combinations and the number of specimens tested are as follows. F_1:(C × AK), 34; (AK × C), 45; (BL × AK), 45; (AK × BL), 42; (B10.BR × AK), 22; (AK × B10.BR), 20; (B10.D2 × AK), 20; (AK × B10.D2), 20. b × F_1: C × (C × AK), 83; (C × AK) × C, 32; BL × (BL × AK), 42; (BL × AK) × BL, 51; C × (B10.D2 × AK), 32; C × (AK × B10.D2), 26. n × F_1: BR × (C × AK), 7; BR × (BL × AK), 8; BR × (B10.D2 × AK), 14.

‡ 1 (C × AK)F_1; 1 (AK × BL)F_1.

§ All C × (C × AK)F_1.

‖ 4 C × (C × AK) and 3 BL × (BL × AK) mice.

¶ 93 of these specimens gave plaques on BALB-ME cultures, but the titer was ≥ 10-fold less than the titer on NIH-ME, which is the pattern expected for N-tropic virus. It is possible that some of these 93 contained a small amount of B-tropic virus. The other 196 specimens gave plaques only on NIH-METC, or were negative on blind passage in BALB-METC.

6–10 wk. Individual embryos were trypsinized and grown as primary cultures. On the day after planting, two cultures were treated with IdU and tested for virus-producing cells *in situ* as described. In the case of the C × (B10.D2 × AK) and C × (AK × B10.D2) cultures, replicate dishes were infected with a B-tropic MLV strain to determine the *Fv-1* genotype of the embryo. The results (Table IV) indicate: (*a*) three-fourths of the embryos showed activable virus, i.e., the same segregation ratio (3:1) as was observed in n × (n × AK) hybrids in vivo and after IdU treatment in vitro (1), and (*b*) that the *Fv-1* type (*Fv-1^{nb}*

or *Fv-1*^{b}) did not affect the proportion of embryos yielding virus. From these results, three inferences can be made. First, the 3:1 segregation ratio indicates that inducibility of infectious virus by IdU results from the presence of either of two unlinked AKR loci; these almost certainly are the two V loci identified by the in vivo studies (1). Second, the *Fv-1* locus does not affect the activation process. Third, *Fv-1* is not identical with, nor closely linked to, either of the two V loci.

Attempts to Detect B-Tropic MLV in Fv-1^b*-AKR Hybrids.*—As indicated, a major objective of the present studies was to determine if the AKR V loci could lead to expression of B-tropic MLV in appropriate hybrids. An effect on activation of B-tropic MLV should be particularly evident in b × (b × AK) backcross mice, since the *Fv-1*^{b} segregants would preferentially express B-tropic virus, and all of the mice would suppress N-tropic virus.

As seen in Table V, B-tropic virus was rarely detected in any of the crosses; over 99% of the isolates from mice less than 7 months old were N-tropic. Only in b × (b × AK) mice 8–9 months of age was a significant incidence of B-tropic virus found, and this was roughly comparable to the incidence in similarly aged mice of the *Fv-1*^{b} parental strains.

In addition, 25 virus isolates from IdU-treated b × (b × AK) and b × (AK × b) embryo tissue cultures cocultivated with BALB METC were tested for N- and B-tropism. Despite the experimental design favoring detection of B-tropic virus, all of the isolates were N-tropic.

It can be concluded that the V loci of AKR have essentially no ability to promote activation of B-tropic MLV strains; this conclusion is compatible with the hypothesis that the V loci are the genomes of the N-tropic AKR MLV.

DISCUSSION

The *Fv-1* gene system was first defined in studies of the host range of Friend virus in vivo (3). Subsequent work showed that *Fv-1* also determines the sensitivity of mouse embryo cells in tissue culture to infection with the two host range types of naturally occurring MLV (4, 5). There have been no studies of the effect of *Fv-1* on naturally occurring MLV infection in vivo.

The data presented in this and the preceding paper establish clearly that the *Fv-1* gene system is of major importance in determining the virologic characteristics of offspring of crosses with AKR. First, F_1 mice from crosses of AKR with *Fv-1*^{b} strains consistently showed later appearance of detectable levels of virus, and lower titers of virus, than crosses with *Fv-1*^{n} mice. Second, backcrossing (b × AK)F_1 mice to *Fv-1*^{n} mice gave virus-positive offspring at twice the frequency observed with backcrosses to *Fv-1*^{b} mice. And third, *Fv-1* typing of n × (b × AK) mice showed that the *Fv-1*^{n} segregants were significantly more often virus-positive than *Fv-1*^{nb} segregants.

The IdU activation studies (Table IV) showed that this correlation of virus with *Fv-1* type in Bc1 mice was not due to linkage between *Fv-1* and one of the

two V loci. The IdU induction tests also indicated that *Fv-1* had no qualitative or quantitative effect on the inducibility of infectious virus. The majority of the IdU-treated Bc1 embryo cultures showed as many virus-producing cells as in IdU-treated (C × AK)F_1 cultures, and these in turn show almost as many as AKR cultures.

It cannot be assumed that the spontaneous induction rate is proportional to the rate obtained with IdU, but this may well be the case. A feasible model is that the AKR parent contributes two unlinked chromosomal loci which confer high frequency of spontaneous activation of synthesis of infectious N-tropic MLV, and also render the cells susceptible to virus activation by IdU in vitro. Although the spontaneous activation rate may be high when viewed in comparison with that in the low-virus parent, in absolute terms it is still very low. In the absence of spread from the spontaneously activated cells, the quantity of virus may not reach detectable levels. In *Fv-1^n* mice, secondary infection proceeds efficiently, but in *Fv-1^{nb}* or *Fv-1^b* mice secondary infection is inefficient, resulting in later appearance of virus, lower frequency of its detection, and lower titers in those mice which do become virus positive.

The marked effect of *Fv-1* on expression of virus in the present studies suggests that it might also be a major determinant of spontaneous leukemia in hybrids with AKR or other high-leukemia *Fv-1^n* strains. A search of the literature suggests that this is probably the case. In the reported studies of the incidence of lymphoma in F_1 hybrids between AKR or C58 and low-leukemic strains whose *Fv-1* type is now known, the incidence of leukemia was consistently higher in crosses with *Fv-1^n* strains (C3H, C57BR, DBA, and STOLI) than with *Fv-1^b* strains (A, BALB/c, and C57BL) (12–20). F_1 hybrids between AKR and RF (*Fv-1^n*) showed a lower incidence of leukemia than the other *Fv-1^n* crosses (21), which may correlate with the observation that the *Fv-1^n* allele of RF is less permissive than that in most other strains (4).

In the preceding paper (1), the quantitative data were interpreted as indicating that there were no major genes inhibitory to expression of endogenous MLV segregating in crosses between AKR and four *Fv-1^n* strains: C57BR, C57L, DBA, and NIH. This type of analysis is more complicated in the crosses with *Fv-1^b* strains, but it seems possible that the qualitative and quantitative data reported here can be interpreted in terms of *Fv-1* type, the number of V genes received from AKR, and possibly *H-2* type, without the necessity of postulating additional genetic influences. An effect of *H-2* was suggested by the greater penetrance of virus in (B10.Br × AK)F_1 mice (*H-2^k*) than in crosses with the *H-2* congenic strains, C57BL and B10.D2. The effect of *H-2* on virus expression in AKR hybrids will be reported in detail in a subsequent report.

One of the two chief objectives of the present studies, to utilize virus host range markers to critically test whether the two AKR V loci are MLV genomes or host genes which facilitate virus expression, was only partially achieved. The data provide support for the concept of chromosomal localization of the MLV

genome, but they do not suffice to prove this. The major evidence for this hypothesis is the finding that almost all of the viruses detected in the 2- and 6–12-wk tests of hybrid mice were N-tropic, and thus probably of AKR origin. This was particularly impressive in the crosses with B10.D2, a strain in which N-tropic virus has never been detected. Even in tests of hybrid mice as old as 8–9 months, it did not appear that inheritance of AKR genes had any marked effect on the spontaneous appearance of B-tropic virus. These findings indicate that the only alternative to the hypothesis of chromosomal location of the MLV genome is the less likely hypothesis that the V loci are genes whose products specifically activate N-tropic viruses, and are without effect on B-tropic virus genomes presumably in the same cells.

The chief reason for our caution in these interpretations is our lack of understanding of the natural history of B-tropic MLV. Unless the underlying assumption is true, i.e., that B-tropic MLV is a heritable cellular element analogous to N-tropic virus in AKR and BALB/c, we cannot make the above inferences. There are several observations which suggest that the biology of B-tropic virus may not be analogous to that of N-tropic virus. As shown in Table I, normal BALB/c mice yield both N-tropic and B-tropic MLV strains; however, when BALB/c embryo cells in tissue culture are induced with IdU, the trace amount of virus which may appear has thus far been exclusively N-tropic (11; unpublished data). Another unusual pattern is seen with B10.D2. When it was found that these mice show a high incidence of B-tropic virus relatively early in life, we assumed that they were a B-type analogue of AKR, and would be ideal for testing for independent segregation of viral markers in crosses with AKR. However, they differ from AKR in a number of important respects. First, F_1 hybrids of B10.D2 males with *Fv-1*^{b} females (BALB/c and C57BL/10) are only rarely virus-positive (1 positive of 35 F_1 mice tested at 12–14 wk). Second, B10.D2 embryo cultures are not induced by IdU; in 39 dishes of early passage tissue culture of cells from B10.D2 embryos or newborn mice, treated with IdU and tested for virus by the most sensitive virus detection procedures available, only one virus-producing cell was found among the 1.1×10^7 cells at risk (unpublished studies). Third, the tests of postnatal B10.D2 mice have shown significant variation between litters in proportion of animals positive for virus. These findings lead us to suspect that there may be a fundamental difference between the host-virus relationships of B-tropic and N-tropic MLV. A further point is worth noting: the high incidence of B-tropic MLV seems to be unique for B10.D2 (old); of 8 B10.D2/Sn (new) mice tested, only one yielded virus, and it was N-tropic.

In addition to the genetic influences on development and expression of MLV in tail extracts, the effect of a number of possibly complicating phenomena must be considered. Horizontal spread of virus, either through natural processes or by cross-contamination during tail testing could produce false positive results. Tests to date indicate that this is not a significant factor; when DBA mice were

foster nursed from birth on AKR mothers, generally with AKR litter contacts, none of 16 tested at 2 wk showed virus in the tail, and only 1 of 25 showed virus at 6 wk.

False negative results could result from maternal immunity. It has long been recognized that a maternal influence can suppress spontaneous leukemia (12–14). In the crosses of AKR with *Fv-1*^{n} mice (1), there were no differences in virologic findings between reciprocal matings, but in the crosses with *Fv-1*^{b} mice described here, there was a consistent tendency toward lower virus expression if the mother was the virus-positive parent. However, this is the opposite direction of the cross from that giving protection against leukemia. A maternal effect on virus expression was also indicated by observations, not presented here, that there was more heterogeneity between litters born of virus-positive mothers than in the reciprocal matings.

A major factor that must be considered is whether tail testing is an adequate sampling procedure, that is, whether different segregation patterns would have been obtained if spleen or thymus had been tested. The tests of normal animals (Table I and reference 1) indicate that tail testing gives results comparable to testing of lymphoid organs; also, limited testing of b × (b × AK) mice showed no significant discrepancies. It must also be kept in mind that negative tests for MLV infectivity are not absolute, but are a function of the sensitivity of the assay system. Not only could trace amounts of virus fail to be detected, as is certainly happening in the *Fv-1*^{b} crosses, but a qualitatively different virus could be present but not detected by present techniques. Whether tests for viral antigens in tissues of mice in the segregating generations will parallel the infectivity test results is currently under study.

Another class of nongenetic mechanism which must be considered is infection of germ cells, with consequent genetic or nongenetic introduction of virus into the offspring. The 3:1 segregation ratio in the Bc1 generation could be explained by a one-locus model in which half of the V-negative gametes in the F_1 parent were infected from without. This model seems to be ruled out by the Bc2 families described in the preceding paper (1); also, the finding of 3:1 segregation ratios in both n × (n × AK) and b × (b × AK) hybrids is against this, since spread of virus to germ cells should be much less efficient in the parents of the latter mice. Another variant of this idea is that, if the viral genomes are integrated in chromosomal loci, a cell containing the potentially infectious MLV genome in one locus could conceivably integrate a copy of that genome into the other locus in other cells, or even in the same cell. Cross-infection with integration of a DNA copy of the genome could accomplish this between different cells, while integration into a different locus in the same cell could occur by a DNA-RNA-DNA information transfer (22, 23) without infectious virus being involved. If this occurred in gametes, a one-locus family could revert to a two-locus family. This cannot be a frequent event, but may well have occurred at some time in the ancestry of the AKR mouse.

SUMMARY

The transmission of murine leukemia virus (MLV) to hybrids between AKR and *Fv-1*^{b} mice was studied in order to evaluate the effect of the *Fv-1* gene on endogenous MLV infection and to attempt to determine if the genetic loci contributed by AKR carry viral genetic determinants. *Fv-1* was shown to have a marked suppressive effect on time of appearance of detectable infectious virus and on the titers attained in vivo, but did not affect the ability of the cells to produce virus in vitro after induction with 5-iododeoxyuridine.

The host range type of the virus detected in the hybrid mice was almost always of the type carried by AKR, although the low-virus *Fv-1*^{b} parents carry the genome of a different host range type. This finding provides strong, but not conclusive, evidence that the virus-inducing loci of AKR contain MLV genetic determinants.

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