

# RELATIONSHIP OF INFECTIOUS MURINE LEUKEMIA VIRUS AND VIRUS-RELATED ANTIGENS IN GENETIC CROSSES BETWEEN AKR AND THE *Fv-1* COMPATIBLE STRAIN C57L\*

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It is now well established that viruses of the murine leukemia virus (MuLV)<sup>1</sup> group are present in all mice (1, 2) and that viral genetic sequences are present as chromosomal DNA (3–5). The expression of this genetic material, as virus particles or viral antigens, differs markedly between strains of mice, both with regard to the age at which the expression occurs (6–8) and the spectrum of biologic indicators of this expression (7–10). The genetic basis of these differences in expression pattern has been the subject of much interest, and has been studied in a variety of genetic crosses, using a variety of biologic indicators (11–15). While much information on the genetic patterns has been obtained, all such studies have suffered from one (or both) of two drawbacks: Either the animals have been scored for only one or two of the many parameters of virus expression, or the genotype has been inferred from the phenotypic ratios in a single segregating generation rather than being confirmed by progeny testing.

A particularly helpful step in understanding the complex patterns of MuLV expression was the recognition that there are several classes of MuLV, differing markedly in their ability to spread from one cell to another. Mouse-tropic strains, represented by the classical Gross-AKR viruses, can infect mouse cells; these viruses are of two host range types, N tropic and B tropic (16) and the efficiency with which they infect mouse cells depends on the *Fv-1* genotype of the cells (see review 17). The hereditary transmission of Gross-AKR-type virus in AKR mice (*Fv-1<sup>a</sup>*) is determined by two dominant unlinked loci, *Akv-1* (chromosome 7) and *Akv-2* (chromosome ?) (7), and the *Fv-1<sup>b</sup>* allele has a marked suppressive influence (genetically dominant) on the expression of these viruses in the progeny of crosses between AKR and *Fv-1<sup>b</sup>* mouse strains (8). Other MuLV strains are xenotropic (9, 10, 18), being almost completely unable to establish exogenous infection of mouse cells, regardless of the *Fv-1* type.

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<sup>1</sup> Abbreviations used in this paper: AK, AKR; anti-NTD, (W/Fu × BN)F<sub>1</sub> anti-W/Fu(C58NT)D; Bc-1 (2, 3), first (second, third) backcross; CL, C57L; GSA, gross leukemia cell surface antigen; IF, immunofluorescence; IFA, immunofluorescence absorption; MuLV, murine leukemia virus; PBS, phosphate-buffered saline.

In mice carrying mouse-tropic virus, we have a working model according to which induction of genetically transmitted virus occurs in some cells, the virus disseminates to infect other cells, and a broad array of viral antigens are expressed as part of the viral replicative process (19). Involvement of sufficient numbers of cells to register a positive result in an assay for virus or viral antigens depends both on the frequency of spontaneous induction and on the *Fv-1* type in relation to the virus-host range type.

Antigen expression in the absence of infectious Gross-AKR-type MuLV ("noninfectious antigen expression") is different in several important respects. Since cell-to-cell spread of infection is presumably not a factor, a detectable level of antigen expression depends only on the number of cells undergoing spontaneous induction, and is not affected by *Fv-1* type. Antigen synthesis resulting from spontaneous induction of viral gene functions could be associated with synthesis of C-type particles representing xenotropic MuLV, defective mouse-tropic MuLV, or other unknown viruses of the MuLV group, or could occur in the absence of virus particle synthesis if only a portion of the viral genome is transcribed. The NZB mouse exemplifies the former (9, 18), while the 129 mouse may exemplify the latter mechanism (11, 20, 21).

Inheritance of p30, the major internal protein of MuLV (identified by group-specific antigenic reactivity), has been studied in systems where Gross-AKR-type virus is represented, and also in systems where it is not represented. In virus-positive systems (crosses involving AKR) the presence (12) and amount (14) of p30 was highly concordant with the presence of virus. In virus-negative systems (denoting those in which Gross-AKR-type virus was either not present or not expressed) p30 expression has been reported as recessive in one study [mouse strain B10.D2(58N); reference 13] but dominant in another (mouse strain NIH Swiss) (H. Ikeda and W. P. Rowe, unpublished observation).

Study of genetic control over expression of the MuLV-associated antigen  $G_{IX}$  has been carried out mainly in a virus-negative system, mouse strain 129 (11, 22) in which (as in other  $G_{IX}^+$  virus-negative mouse strains) the  $G_{IX}$  antigen is expressed predominantly on thymocytes. In crosses involving this strain,  $G_{IX}$  expression is determined by two genes, one semidominant (*Gv-1*) and one dominant (*Gv-2*). Both *Gv-1* and *Gv-2* are required for expression, and the genotype is fully penetrant.

The main purpose of the study which follows was to examine the qualitative and quantitative interrelationships of  $G_{IX}$ , p30, and MuLV expression in the virus-positive, antigen-positive system represented by segregants derived from AKR and C57L (a virus-negative, *Fv-1*-compatible strain) progenitor mice.

### Materials and Methods

*Mice.* The parental strains, AKR (AK) and C57L (CL) were obtained from The Jackson Laboratory, Bar Harbor, Maine, or from our colonies, which were originally established from Jackson breeders. The naming of crosses in the text follows the convention that the strain of the mother is given first. Virologic and other relevant data for these two strains and for the  $F_1$  hybrid are summarized in Table I.

Backcross segregants were tested for MuLV (XC test on tail biopsy at 5-6 wk of age); for  $G_{IX}$  (cytotoxicity test on thymocytes sampled at 5-7 wk of age); and for p30 (immunofluorescence absorption [IFA] test on spleens removed surgically at 8-10 wk of age). Most segregants were also tested for *H-2* (cytotoxicity test on lymph node cells) and *Gpd-1* (vertical starch gel electrophoresis on kidney).

#### *Antisera*

"ANTI-NTD." Anti-NTD is a common abbreviation for the antiserum (W/Fu  $\times$  BN) $F_1$  anti-W/Fu(C58NT)D originally described by Geering et al. (30). W/Fu and BN are inbred rat strains,

TABLE I  
*MuLV Expression and Other Relevant Data for AK, CL, and (CL × AK)F<sub>1</sub>*

	AK	CL	(CL × AK)F <sub>1</sub>	References
Gross-AKR MuLV infectivity	++++	-	++++	7
Xenotropic MuLV	+	+		23, 24
p30 (spleen)	++	-*	++	25, See text
G <sub>IX</sub> (thymus)	++	-	+	11, See text
MuLV sequences in cell DNA				
"Gross-AKR-unique sequences"	+	-		4, 5
"Common sequences"	++	++		
<i>Akv</i> genotype				
	V <sub>1</sub>	-		7, 8
	V <sub>2</sub>	-		
G <sub>IX</sub> genotype				
	Gv-1 (?)	-		26, See text
	Gv-2 (?)	Gv-2		
<i>Fv-1</i>	n	n		27, 28
<i>H-2</i>	k	b		
<i>Gpi-1</i>	a	a		
<i>Gpd-1</i>	b	a		

\* Although the phenotype of the CL parent is given here as V<sup>-</sup>p30<sup>-</sup>G<sub>IX</sub><sup>-</sup>, viral antigen is detectable in CL mice by the sensitive radioimmunoassay (25, 29) and the amount of antigen may well increase with age (6).

and leukemia W/Fu(C58NT)D is a W/Fu rat leukemia induced by wild-type MuLV. This antiserum was used for G<sub>IX</sub> typing (see below).

**RABBIT ANTI-MuLV p30.** The rabbit antiserum to p30 of MuLV-Rauscher (p30 fraction rechromatographed in guanidine hydrochloride gel filtration) was kindly provided by Doctors E. Fleisner and W. D. Hardy, Jr., Memorial Sloan-Kettering Cancer Center, New York.

In the indirect immunofluorescence (IF) test, this antiserum gives bright diffuse cytoplasmic fluorescence of acetone-fixed MuLV-infected cells (31) (titer approximately 1:1,000); in confirmation of the specificity of the antiserum, this IF activity was specifically absorbed by p30 fraction of MuLV-Rauscher (32); the IF reaction was also blocked by goat antiserum to p30 of MuLV-Rauscher (serum kindly provided by Doctors M. Strand and J. T. August of Albert Einstein College of Medicine, Bronx, N. Y.).

**G<sub>IX</sub> Typing of Thymocytes.** Cytotoxicity assay for G<sub>IX</sub> on thymocytes was done according to Stockert et al. (11). Briefly, equal volumes (50 μl) of anti-NTD serum (serially diluted), complement (selected rabbit serum 1:15), and cells (5 × 10<sup>6</sup> thymocytes/ml) are mixed and incubated at 37°C for 45 min; viability counts (percent cells lysed) are then made in the presence of trypan blue. Rat natural antibody to mouse thymocytes was removed from the anti-NTD serum by absorption with spleen cells and thymocytes of G<sub>IX</sub><sup>-</sup> mouse strains, always including CL.

The anti-NTD serum was used at dilutions of 1:20 and 1:40; with this amount of serum the proportion of thymocytes lysed in the cytotoxicity assays is 80-90% for AK mice [G<sub>IX</sub>(++)]; 40-50% for (CL × AK)F<sub>1</sub> or G<sub>IX</sub>-positive heterozygous segregants [G<sub>IX</sub>(+)] and less than 10% for CL or G<sub>IX</sub>-negative backcross segregants [G<sub>IX</sub>(-)]. The intermediate reading for the hybrid (40-50%) is attributable to the relative weakness of the cytotoxicity reaction with thymocytes showing only hemiexpression of antigen; it does not signify a mixture of G<sub>IX</sub><sup>+</sup> and G<sub>IX</sub><sup>-</sup> cells.

Anti-NTD contains antibodies to several MuLV-associated antigens in addition to G<sub>IX</sub> (30). The specificity of anti-NTD for G<sub>IX</sub> in the cytotoxicity assay was reconfirmed in this study by showing

(a) that 129 thymocytes ( $G_{IX}^+/\text{MuLV}^-$ ) remove all activity for young AK thymocytes ( $G_{IX}^+/\text{MuLV}^+$ ), and (b) that AK thymocytes remove all activity for 129 thymocytes.

**IFA Test for MuLV p30 antigen.** The method of Hilgers et al. (33) with modifications described elsewhere (31) was used. Briefly, the standard test cell was leukemia E $\delta$ G2 (a transplanted C57BL/6 leukemia induced by passage A Gross virus). E $\delta$ G2 cells were incubated for 20 min at 37°C on microscope slides and then fixed with acetone. These slides were stored at -70°C.

In typing spleens for presence of p30, a 20% wt/vol homogenate of spleen was prepared in phosphate-buffered saline (PBS) and then sonicated for 3-5 s with a Biosonik IV, Will Scientific, Inc., Rochester, N. Y. After centrifugation at 2,000 g for 30 min at 4°C, the supernate ("antigen") was stored at -70°C.

20  $\mu$ l of antigen (seriallyly diluted) was mixed with an equal volume of anti-p30 serum (at a predetermined critical dilution in the region of 1:250), incubated overnight at 4°C, and tested for residual IF activity. For this purpose, the antigen-antibody preparations were applied for 1 h at 37°C to the acetone-fixed E $\delta$ G2 cells; the slides were then washed with PBS, and further reacted with fluorescein-conjugated goat antirabbit IgG (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) for 1 h at 37°C. After removal of excess conjugate, the slides were counterstained with 0.5% Evan's-Blue, and examined for intensity of IF staining. The antigen titer is expressed as the highest dilution giving substantial reduction of IF activity; the base-line figures are 8 or higher for AK or (CL  $\times$  AK) $F_1$  spleen [p30(++)]; 1-4 for NIH Swiss [p30(+)], and <1 for CL spleen [p30(-)].

The IFA test as described above is strictly a test for the presence of the group-specific antigen of p30, the major internal component of MuLV.

**Virus Testing.** At 6 wk of age, mice were tested for MuLV in tail tissue, using the XC plaque assay as described previously (34). In the earlier stages of the study, virus assays were done in secondary cultures of NIH Swiss mouse embryo, while later assays, representing half of the first backcross (Bc-1) generation and all of the Bc-2 and Bc-3 mice, were done in the SC-1 clonal line of feral mouse embryo cells (35). The SC-1 cell line is about 10-fold more sensitive than NIH mouse embryo for detection of naturally occurring MuLV; however, the proportion of Bc-1 mice positive for virus was not significantly affected by the cell type used for testing (72% positive in NIH, and 76% in SC-1 cells).

## Results

**Bc-1 generation.** A total of 180 [CL  $\times$  (CL  $\times$  AK)] backcross mice were studied, representing successive litters of 9 CL females mated with 10 (CL  $\times$  AK) $F_1$  males. Table II shows the results of typing these 180 backcross mice for

TABLE II  
*Typing of 180 (CL  $\times$  [CL  $\times$  AK]) Backcross Mice for MuLV (V), p30, and  $G_{IX}$*

V (tail)*	Phenotype		No. of mice
	p30* (spleen)	$G_{IX}$ (thymus)	
+	+	+	97
+	+	-	34
+	-	+	0
+	-	-	3
-	+	+	7
-	+	-	10
-	-	+	2
-	-	-	27
Total			180

\* + includes both p30(+++) and p30(+) phenotypes.

MuLV in the tail ( $V^+$  vs.  $V^-$ ), p30 in spleen ( $p30^+$  vs.  $p30^-$ ), and  $G_{IX}$  on thymocytes ( $G_{IX}^+$  vs.  $G_{IX}^-$ ). The data will first be considered as they stand, and the conclusions later amplified (below) in the light of progeny testing of individual members of this backcross population.

Overall, 74% (134/180) were positive for MuLV ( $V^+$ ), 82% (148/180) for p30, and 59% (106/180) for  $G_{IX}$ . These data confirm the existence in AK of at least two unlinked loci determining appearance of infectious MuLV early in life ("V loci") (7), and they suggest that there are at least three discrete loci determining p30. Comment on the apparent  $G_{IX}$  segregation figures recorded in Table II will be deferred until progeny testing (Tables III and V) has been considered, because progeny testing is especially relevant to interpretation in the case of  $G_{IX}$ .

The three markers show strong associations with each other both qualitatively and quantitatively (Fig. 1). The phenotypes for p30 (spleen) ranged from high [++ as in AK and the (CL  $\times$  AK) $F_1$  hybrid], through intermediate (+ as in DBA/2 or NIH Swiss mice), to negative (-), evidently reflecting MuLV titers more than gene dosage; i.e., higher titers of MuLV tended to be associated with higher titer of p30 (see Fig. 1).

The incidence of the  $G_{IX}^+$  phenotype was higher among segregants with high MuLV and p30 (++) levels (Fig. 1). However, the quantitative level of expression of  $G_{IX}$  on thymocytes of the segregants was always apparently equal to that of the (CL  $\times$  AK) $F_1$  hybrid [ $G_{IX}(+)$ ], regardless of the MuLV and p30 titers. This was confirmed by the following test: Thymocytes of  $G_{IX}^+$  backcross segregants were compared with thymocytes of (CL  $\times$  AK) $F_1$  mice in quantitative absorption tests for  $G_{IX}$  antigen (cytotoxicity assay with anti-NTD serum and 129 thymocytes; see reference 11). The absorption capacity of both thymocyte populations was the same, and equalled half the absorption capacity of AK thymocytes, the

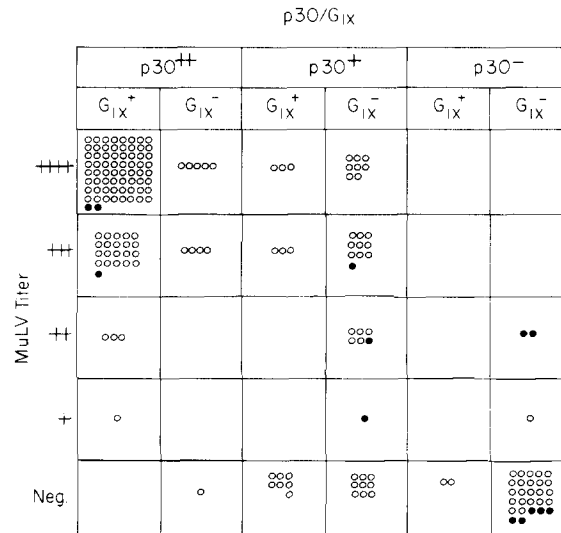


FIG. 1. Concordance for the three MuLV markers (V, p30, and  $G_{IX}$ ) among the 180 Bc-1 segregants. Closed circle: Family CL $\eta$  number 40 (see Table VII). MuLV titer (log 10): +++++,  $\geq 3$  in SC-1 or  $\geq 2$  in NIH-ME; +++, 2-2.9 in SC-1 or 1-1.9 in NIH-ME; ++, 1-1.9 in SC-1 or 0-0.9 in NIH-ME; +, 0-0.9 in SC-1; and -, negative in SC-1 or NIH-ME.

parental homozygous strain. Thus, expression of  $G_{IX}$  on thymocytes, unlike expression of p30 in spleen, was not quantitatively related to the MuLV titer of individual segregants.

All but 3 of the 135  $V^+$  mice were  $p30^+$  (Table II); one of these three ( $V^+p30^-$ ) discrepant mice yielded  $V^+p30^+$  segregants on progeny testing ( $\delta$  160 in Table III) suggesting that in fact these discrepant mice had given false negative scores for p30. 17 mice were  $V^-p30^+$ ; essentially all of these showed the intermediate p30 phenotype [ $p30(+)$ ]. Among  $V^-$  mice, the expression of  $G_{IX}$  was generally associated with expression of p30; only 2 of 32  $p30^-$  segregants were  $G_{IX}^+$  (Table II).

*Progeny Testing.* The positive correlations among  $V$ , p30, and  $G_{IX}$  in the Bc-1 generation could be interpreted as genetic linkage, interdependent manifestations of MuLV activation and spread, or both. Also, some of the discordance between the three markers could be due to reduced penetrance or test errors. To assess these factors it was necessary to carry out progeny tests. For this purpose

TABLE III  
Progeny testing of  $CL \times (CL \times AK)$  backcross mice mated to  $CL$  (representing a selected Bc-2 population)

Bc-1 phenotype	% Bc-1 mice with phenotype (See Table II)	Bc-1 mouse number	No. of progeny with given phenotype						Total % positive			Inferred genotype of parent			
			V+			V-			V	p30	$G_{IX}$	Akv	Akv $\dagger$		
			+/+	+/-	-/+	-/-	+/+	+/-						-/+	-/-
+ + +	54	♀ 34 ♀ 108	6	2		1			2	82	73	55	+ (2)§	+?	
+ + -	19	♂ 39 ♂ 42 ♂ 113 ♂ 153 ♂ 158 ♂ 159 ♀ 35 ♂ 132 ♂ 151	12	9				1	1	4	81	85	46	+ (2)	+
+ - -	2	♂ 160 ♀ 201	7	9					2	12	53	60	23	+ (1)	+?
- + +	4	♂ 95 ♀ 99 ♂ 101							7	4	11	0	50	32	+
- + -	6	♂ 127 ♂ 137							3	14	0	18	0		+
- - +	1	♀ 135 ♂ 154	1						3	4	20	0	26	11	+
- - -	15	♂ 128 ♂ 138 ♂ 139 ♂ 200							1	4	0	20	0		
			1	5						7	46	46	8	+ (1)	

\* Including  $p30(+)$  and  $p30(++)$  phenotypes.

† AKR locus giving  $V^-p30^+$  phenotype (See text).

§ Number in parenthesis is the estimated number of  $V$  genes.

|| Confirmed by progeny testing (See Table V).

a number of Bc-1 mice, selected to represent each observed Bc-1 phenotype, were bred with CL, and their progeny (Bc-2 population) were scored for the same three markers. Similarly, representative mice of this Bc-2 generation were mated with CL to provide a Bc-3 population. Tables III and V present the total Bc-2 and Bc-3 results in relation to the parental phenotypes, with our interpretation of the genotypes of the parents (final columns of Tables III and V). It is seen that there are two classes of genes responsible for the virus-specific phenotypic markers; the two previously established V genes, which give rise to infectious mouse-tropic MuLV, and a gene or genes which induce p30 and/or  $G_{IX}$  antigen in the absence of such virus.

**EXPRESSION OF p30 AND  $G_{IX}$  ANTIGENS IN FAMILIES OF Bc PARENTS CARRYING V GENES ( $V^+$ ).** Here we are concerned with the progeny of  $V^+$  Bc segregants. In these  $V^+$  families, relationships among MuLV, p30, and  $G_{IX}$  phenotypes were essentially the same as in the  $V^+$  mice of the Bc-1 population (Table IV). That is, essentially all  $V^+$  offspring were p30<sup>+</sup> and the majority of these were p30(++) and the  $G_{IX}^+$  phenotype was found primarily in  $V^+$ p30(++) mice. Since  $V^+$ p30(+) parents generated p30(++) progeny in the next generation (Table IV), we conclude that the quantitative expression of p30 in segregants with V genes is mostly related to the titer of MuLV rather than to gene dosage.

The incidence of the  $G_{IX}^+$  phenotype in the  $V^+$  segregants was about 55% in both the Bc-2 and Bc-3 generations, and was the same in offspring of mice carrying one V gene as in mice carrying both V genes (Tables III and V). While this ratio might suggest that an independently segregating epistatic gene is required in addition to a V gene for  $G_{IX}$  expression, the following observations refute this interpretation: (a) The  $G_{IX}^+$  phenotype was no more common in the  $V^+$  offspring of  $V^+G_{IX}^+$  parents than of  $V^+G_{IX}^-$  parents. That is, among  $V^+$  mice,

TABLE IV  
*p30 and  $G_{IX}$  Phenotypes of " $V^+$  Families" (Progeny of  $V^+$ Bc Segregants Mated to CL)*

Generation	V <sup>+</sup> parent		Progeny (Bc-2 and Bc-3)					
	p30	No. tested	Virus	No. of segregants	Phenotypes of segregants, %			
					p30			$G_{IX}^+$
++	+	-						
Bc-1	++	5	+	62	85	13	2	55
			-	23	0	13	87	0
	+	6	+	46	80	20	0	76
			-	26	0	8	92	0
	-	2	+	17	65	29	16	41
			-	24	0	8	92	0
Bc-2	++	6	+	26	73	27	0	42
			-	31	0	26	74	0

TABLE V  
Progeny Testing of Selected Bc-2 Mice

c-2 phenotype			Bc-2 mouse number	Bc-1† parent number	No. of progeny with given phenotype								Total % positive			Inferred genotype of Bc-2 parent				
V	p30*				V+				V-‡				V	p30	G <sub>IX</sub>	A <sub>ku</sub>	A <sub>kup</sub>			
	p30*	G <sub>IX</sub>			p30/G <sub>IX</sub>		p30/G <sub>IX</sub>		p30/G <sub>IX</sub>		p30/G <sub>IX</sub>									
			+/+	+/-	-/+	-/-	+/+	+/-	-/+	-/-										
+	+	+	♀ 281	♂ 154	2				2				46	64	17	+	?			
			♀ 47	♂ 113					4											
			♀ 87	♂ 113					1											
			♂ 269	♂ 151	7	8					11									
+	+	-	♂ 91	♂ 113	1	1					1				40	45	20	+(1)	?	
			♂ 60	♀ 35	3	2					5									
			♂ 59	♀ 35																
-	+	+	♂ 157	♂ 95					4	4			4				0	50	21	+
			♀ 69	♀ 99							2		2							
			♂ 74	♀ 99					4	4			4							
			♂ 76	♀ 99					1	1			8							
-	+	-	♂ 119	♂ 39					6	2			5				0	43	23	+
			♀ 153	♂ 95							5		2							
			♂ 40	♀ 99					3	1			6							
			♂ 77	♀ 99									10							
			♂ 157	♂ 127									1							
			♀ 141	♀ 135					2		5		6							
			♀ 174	♂ 128									3		8					
-	-	+	♂ 41	♀ 99									1		19		0	0	4	?
			♂ 39	♀ 99											6					

Including p30(++) and p30(+) phenotypes.

For phenotype and progeny testing see Table III.

Mice with two generations of V<sup>-</sup> ancestry were assumed to be V<sup>-</sup>.

AKR locus giving V<sup>-</sup> p30<sup>+</sup> phenotype (see text).

the G<sub>IX</sub> phenotype of progeny was not predictable from the G<sub>IX</sub> phenotype of the Bc parent. (b) The G<sub>IX</sub><sup>+</sup> phenotype occurred mostly in p30(++) mice, and these tended also to be the segregants with the higher titers of MuLV (Fig. 1 and Table IV), indicating a stochastic element in the occurrence of the G<sub>IX</sub><sup>+</sup> phenotype. Thus, unless we postulate some unrecognized complex mode of genetic determination for G<sub>IX</sub>, the G<sub>IX</sub><sup>-</sup> phenotype seen in about half the V<sup>+</sup> segregants does not reflect the genotype.

EXPRESSION OF p30 AND G<sub>IX</sub> ANTIGENS IN FAMILIES OF Bc MICE WITH "NONINFECTIOUS" p30/G<sub>IX</sub> GENES (Bc PARENTAL PHENOTYPE V<sup>-</sup>p30<sup>+</sup>). As described above, analysis of the Bc-1 generation revealed a class of segregants that express p30 alone, or p30 and G<sub>IX</sub>, but did not produce detectable virus. Progeny testing (Tables III and V) confirmed that there is indeed a genotype which expresses p30 and G<sub>IX</sub> in the absence of detectable mouse-tropic MuLV.

In addition to the usual tests for MuLV in tail extracts, shown in Tables III and V, virus isolation tests were carried out on spleen extracts from each of 12 progeny of ♀ 99 (V<sup>-</sup>p30<sup>+</sup>G<sub>IX</sub><sup>+</sup>; Table III) with consistently negative results even though 8 of these 12 mice were p30<sup>+</sup>. Also, pooled embryos from matings of V<sup>-</sup>p30<sup>+</sup>G<sub>IX</sub><sup>+</sup> progeny of ♀ 99 with NIH Swiss mice did not yield MuLV when grown in tissue culture and tested for 5-iododeoxy-uridine inducibility of AK-type virus (1). Further analysis of this phenotype (V<sup>-</sup>p30<sup>+</sup>G<sub>IX</sub><sup>+</sup>) was hindered by



low fertility of the relevant mice after the Bc-3 generation.

All such  $V^-p30^+$  mice in the Bc-2 and Bc-3 generations of these families were of the  $p30(+)$ , not  $p30(++)$ , phenotype. Again,  $G_{IX}$  expression (with a possible exception; see below) was restricted to  $p30^+$  segregants. The segregation patterns in the Bc-2 and Bc-3 generations are compatible with there being a single gene which is fully penetrant for expression of p30 antigen, and 50% penetrant for  $G_{IX}$  antigen expression. As with the  $V^+$  families, this ratio might suggest another gene segregating independently of this p30 gene and required for expression of  $G_{IX}$ . But again progeny testing showed no difference in expression of the two antigens in offspring of  $p30^+G_{IX}^+$  parents as compared with  $p30^+G_{IX}^-$  parents (Table V).

In general, progeny testing of mice discordant for p30 and  $G_{IX}$  has given no definitive evidence that these phenotypes are genetically separable. Most  $p30^+G_{IX}^-$  mice gave some  $p30^+G_{IX}^+$  progeny, as did one mouse ( $\text{♀}$  135 in Table III) that was scored  $p30^-G_{IX}^+$ . The phenotype  $V^-p30^-G_{IX}^+$  has not been propagable; among 56 progeny of 4 such segregants (Bc-1  $\text{♀}$  135 and  $\text{♂}$  154 in Table III and Bc-2  $\text{♂}$  39 and  $\text{♂}$  41 in Table V) only one  $V^-p30^-G_{IX}^+$  segregant was obtained; obviously no conclusion can be drawn regarding this single apparent exception. It may be that there is a further locus inducing  $p30^+$  alone, with low penetrance, because among 38 total progeny from  $\text{♂}$  127 and  $\text{♂}$  128 (Table III and V), 8 were  $p30^+$  and none  $G_{IX}^+$ .

*Linkage Analysis with Other Markers.* None of the three viral markers was associated with *Gpd-1*-type (chromosome 4), *H-2*-type (chromosome 17) or the coat color loci *b* (chromosome 4), and *ln* (chromosome 1). Since AK and CL mice do not differ at the *Gpi-1* locus, which is linked to *Akv-1* (36) and therefore serves as a marker for *Akv-1* in segregation tests, we were not able to score for *Gpi-1* type to determine which single V-gene segregants were likely to be carrying *Akv-1* in distinction to *Akv-2*.

## Discussion

*Interpretation of the Segregation Data.* The first notable feature of this study of phenotypes in the backcross  $CL \times (CL \times AK)$  is the high degree of concordance among the three markers, V (virus in tail extracts), p30 (viral protein in spleen), and  $G_{IX}$  (expressed on the surface of thymocytes). However, the data indicate a more complex genetic basis than we had anticipated for the expression of MuLV antigens in AK mice. Thus Ak outcrosses yield stably heritable antigen-positive genotypes of both (a) virus-positive and (b) virus-negative types. Of the eight phenotypes that could possibly be derived from three sets of alternative markers (V, p30, and  $G_{IX}$ ), seven were observed in the Bc-1 generation (Table II) but only three were certainly perpetuated on progeny testing, and can thereby be ascribed to actual genotypes; these are the genotypes giving rise to the two parental phenotypes  $V^+p30^+G_{IX}^+$  and  $V^-p30^-G_{IX}^-$ , and the third genotype yielding the phenotype  $V^-p30^+G_{IX}^+$  (Table VI). We propose to call the AK locus giving the  $V^-p30^+$  phenotype *Akvp*, the initials *vp* standing for "viral protein;" this designation is used because a viral protein is the phenotypic basis for its recognition, and is not meant to imply that the locus is expressed as viral protein synthesis in the absence of virion production.

The segregation of virus-positive backcross lines confirms earlier studies (7, 12) showing that AK contains at least two unlinked loci (*Akv-1* and *Akv-2*) specifying virus positivity, and that appearance of virus is regularly accompanied by expression of p30 antigen (12). Because the  $G_{IX}^+$  thymocyte phenotype could not be genetically isolated from expression of p30 (either alone or with V, Table V) there is no evidence in our data for genes specifying  $G_{IX}$  independently of genes responsible for either complete AK virus ( $V^+$ ) or p30 expression.

From limited data, it appears so far that in  $V^-p30^+$  segregants  $G_{IX}$  is demonstrable in thymus but not spleen, as is the case in mice of strain 129, which similarly are  $V^-p30^+$ . On the other hand,  $G_{IX}$  was found also in the spleen of some  $V^+p30^+$  segregants, as is the case in AK, which is the prototype  $V^+p30^+G_{IX}^+$  mouse (these data are not included in this report). Thus, with regard to demonstrability of  $G_{IX}$ , it may prove to be the case; (a) that the thymus<sup>+</sup>/spleen<sup>+</sup>  $G_{IX}$  phenotype is characteristic of widespread MuLV dissemination as in AK, whereas (b) the thymus<sup>+</sup>/spleen<sup>-</sup>  $G_{IX}$  phenotype characterizes "noninfectious" MuLV expression as in 129 mice.

In regard to the quantity of antigen expressed in the different types of segregant, p30 and  $G_{IX}$  differ. In  $V^+$  segregants: (a) the level of p30 in spleen was generally high (+ +), (b) p30 levels tended to be positively correlated with MuLV levels, and (c) there was no tendency for high or low levels to be perpetuated on progeny testing (Table IV). Thus, we consider the splenic level of p30 in  $V^+$  mice to be phenotypic, i.e., secondary to the extent of viral replication. On the other hand, all  $V^-p30^+$  mice were low in p30 [p30(+)], which is consistent with lack of cell-to-cell spread of virus in these mice.

In contrast to p30, the degree of  $G_{IX}$  expression on thymocytes of  $G_{IX}^+$  segregants was constant, being the same in  $V^+$  as compared with  $V^-$  segregants and in  $V^+$  mice with high or low MuLV titers. Apparently, the number of non-allelic  $G_{IX}$ -inducing genes carried by each segregant did not affect the amount of  $G_{IX}$  expressed on thymocytes, since (CL × AK) $F_1$  mice, which are heterozygous for all three of the  $G_{IX}$ -inducing genes carried by AK (*Akv-1*, *Akv-2*, and *Akvp*), had

TABLE VI  
*The Three Backcross Genotypes that are Confirmed by Progeny Testing*

Category	Genotype		Phenotype			Estimated incidence (%) in 180 Bc-1 mice*
	<i>Akv-1</i> and/or <i>Akv-2</i>	<i>Akvp</i>	V	p30	$G_{IX}$	
1	+	+ or -	+	+	+	136 (75.6)
2	-	+	-	+	+ } - ‡	19 (10.6)
3	-	-	-	-	-	25 (13.9)

\* Because progeny testing reveals that a minority of segregants of category 3 phenotype can generate offspring of category 1 and 2, the incidence figures (last column) are minimal for categories 1 and 2, and maximal for category 3.

‡ It is uncertain whether this represents a discrete genotype (see text).

the same level of  $G_{IX}$  expression (50% of AK level) as  $G_{IX}^+$  segregants in the Bc-1 and later generations which bore only one or two of these genes. It is not known whether homozygosity for any one of the three loci individually would induce the maximal  $G_{IX}$  phenotype of the AKR mouse.

*Suppression of MuLV Phenotypes; Possible Mechanisms.* In a number of instances the phenotypes of Bc segregants were not propagated on progeny testing; this generally entailed the production of positive offspring by a negative parent.

The question of the negative phenotypes which score positive on progeny testing is of considerable importance in two connections. Firstly, it has obvious theoretical implications, and secondly it brings to light the hazards of interpreting segregation data that depend on direct scoring alone, in the case of markers related to leukemia virus and its components.

While some of the discrepancies could be due to technical errors, we suspect that the majority are the result of occasional suppression of the phenotypes by maternal transfer of antibody. Maternal transmission of resistance is a factor that has long been recognized in crosses involving leukemia incidence. This phenomenon was originally recognized (37, 38) as causing a lowered incidence of leukemia: (a) in the hybrid offspring of certain crosses as compared with progeny of reciprocal crosses, and (b) in mice foster nursed on mothers of the former crosses. It was later seen to be associated with reduced levels of the MuLV-associated antigen Gross leukemia cell surface antigen (GSA) in the blood (39), and more recently as suppression of infectious virus.<sup>2</sup> In the present study, when the Bc-1 families, which represent progeny of nine ♀ ♀ CL mice, are considered separately, we find evidence of maternal resistance in at least one of them (Table VII).

In Table VII the phenotypes of the 180 Bc-1 mice are recorded in the nine family groups. The progeny of one of the CL mothers (♀ 40) has a significantly lower frequency of expression of  $G_{IX}$  ( $P = 0.002$ ) and of p30 ( $P < 0.001$ ), a somewhat lower frequency of virus detection, and a tendency toward lower virus titers (see Fig. 1) than the rest of the Bc-1 mice. Five of the progeny-tested Bc-1 segregants recorded in Table III came from this family (♂♂ 158, 159, 160, and 200 and ♀ 201, selected for mating because of their discrepant phenotypes). All except one of these (♀ 201) yielded Bc-2 progeny expressing one or more markers for which they themselves were negative, suggesting that their phenotypes in respect to these markers had been suppressed. Similarly, maternal resistance may be responsible for some of the  $V^{-}p30^{+}$  phenotypes in the progeny of  $V^{+}$  Bc-2 females (Table V, ♀♀ 281, 47, and 87).

Secondly, it is now well established that adult mice may respond immunologically to certain endogenous virus components (40–44); however, to date no naturally occurring neutralizing antibody against Gross-AK-type viruses has been reported. Of the three virus-related antigens scored in the present study, (V, p30, and  $G_{IX}$ ), the one that most commonly gave a discrepant-negative phenotype, revealed by progeny testing, was  $G_{IX}$ . From the data in Table VII,  $G_{IX}$  also appeared to be the most subject to phenotypic suppression by the maternal resistance factor. This tempts us to speculate that the low penetrance

<sup>2</sup> Rowe, W. P. Manuscript in preparation.

TABLE VII  
Family Data for the 180 Bc-1 Segregants\*

Parent no.		No. of progeny	G <sub>IX</sub> <sup>+</sup>	V <sup>+</sup>	p30 <sup>+</sup>
CL♀	(CL × AK)F <sub>1</sub> ♂				
			%	%	%
51	111	11	91	91	91
2	12	17	76	76	88
1	10 + 13	20	70	85	95
45	110	20	70	85	90
3	11	33	64	82	91
4	10	29	59	59	76
5	13	14	50	64	79
39	108	25	36	72	76
40	109	11‡	9	55	36
Total 180					

\* The families of the 9 CL females have been listed in order of decreasing incidence of G<sub>IX</sub><sup>+</sup> progeny. Most segregants were typed for *H-2*; there were no significant familial differences in occurrence of *H-2* homozygotes vs. *H-2* heterozygotes.

‡ Of the 11 mice of this "lowest score" family, 4 ♂♂ were progeny tested, and all yielded segregants that were positive for one or more of the three markers for which the tested male parents were negative (the one progeny-tested female did not), see text.

of G<sub>IX</sub> in this study may represent antigenic modulation, a phenomenon well recognized for another thymocyte membrane antigen, thymus-leukemia (45, 46). The MuLV-associated antigen GSA is also said to be susceptible to antigenic modulation (47).

### Summary

In a further genetic study of murine leukemia virus (MuLV) and its components we examined the backcross C57L × (C57L × AKR). This population was selected because strains AKR and C57L are both *Fv-1<sup>a</sup>*, and the restriction which the *Fv-1<sup>b</sup>* allele imposes on the output of virus was thereby obviated. The segregants were scored for three characters: (a) infectious Gross-AKR-type MuLV (V), in the tail; (b) group-specific antigen indicative of p30 internal viral protein, in spleen; and (c) G<sub>IX</sub> antigen, now thought to be indicative of gp69/71 viral envelope glycoprotein, on thymocytes. Our conclusions are: (a) It is confirmed that the AKR mouse has two unlinked chromosomal genes, *Akv-1* and *Akv-2*, each of which can independently give rise to the life-long high output of MuLV that is characteristic of AKR mice. (b) Of the eight phenotypes that could possibly be derived from segregation of the three pairs of independent alternative traits, seven were observed, but on progeny testing only three were shown to reflect stably heritable genotypes; these were V<sup>+</sup>p30<sup>+</sup>G<sub>IX</sub><sup>+</sup> and V<sup>-</sup>p30<sup>-</sup>G<sub>IX</sub><sup>-</sup> (the parental types) and V<sup>-</sup>p30<sup>+</sup>G<sub>IX</sub><sup>+</sup>. A third, newly identified AKR gene, designated *Akvp*, segregating independently of *Akv-1* and *Akv-2*, also determines expression of p30 and G<sub>IX</sub> but in this case independently of XC-detectable MuLV. (c) The four remaining observed phenotypes, which did not breed true on

progeny testing, involved mostly antigen-negative parents yielding antigen-positive progeny; it is likely that these discrepancies represented suppression of phenotype by a maternal resistance factor.

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