GENETIC INTERACTIONS IN THE SPONTANEOUS PRODUCTION OF ENDOGENOUS MURINE LEUKEMIA VIRUS IN LOW LEUKEMIC MOUSE STRAINS*

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The appearance of infectious murine leukemia virus (MuLV) in uninfected mice is thought to result from the activation of genetically transmitted provirus(es) and the subsequent horizontal spread of virus throughout the individual mouse (1–3). The frequency of cells that release MuLV after induction in vitro is not identical for all strains of mice (4–7). In the case of proviruses of high inducibility, this induction frequency is controlled by genes identical to or tightly linked to structural MuLV elements (4, 8). Moreover, in high leukemic strains these genetic regions are the main determinants of spontaneous virus expression in vivo (1).

Recently, we identified (7) genes of low leukemic strains of mice that interact in certain F_1 hybrids and thereby increase the frequency of MuLV-producing cells after induction in vitro with halogenated pyrimidines. BALB/c and related strains of mice, i.e., A/J, CBA/J, CeH/He, and SEC, carry dominant (+) alleles at the locus *Inc-1* and recessive (−) alleles at the locus *Inb-1*, whereas C57BL/6 and related mice, i.e., C57BL/10 and C57BR, carry dominant alleles at the locus *Inb-1* and recessive alleles at the locus *Inc-1*. Cells from mice of the $Inc\text{-}1^+$, $Inb\text{-}1^+$ genotype, e.g., (BALB/c × B6)F_1 or various CXB recombinant inbred mice, show 10–50-fold higher levels of ecotropic virus induction than cells of either parental strain. From a comparison of the strain distribution pattern of *In* loci to that of ecotropic virus-specific nucleotide sequences (N. Jenkins, personal communication; J. Horowitz and R. Risser, unpublished observations), it seems likely that *In* loci are linked to ecotropic proviruses. In the present study, we explore the in vivo consequences of inheritance of these *In* loci and demonstrate that they influence the pattern of spontaneous virus expression.

A major consideration in the spread of MuLV throughout the mice is the *Fv-1* gene, a locus with alleles *n* and *b* that inhibit the replication of MuLV of the opposite tropism (9–11). With the exception of virus from B10.BR/Li mice (12), MuLV recovered from tissue culture cells induced with halogenated pyrimidines have invariably proved to be N-tropic (2) and, thus, severely limited in their ability to spread in mice of the $Fv\text{-}1^{b/b}$ genotype, such as BALB/c and B6. Both N- and B-tropic MuLV have been recovered from tissues of these mice, however, and evidence supports the notion the B-tropic MuLV are derived in part from the same sequences as N-tropic MuLV (13). Genetic and molecular studies (14–18) of recombinants between N- and B-tropic MuLV as well as NB-tropic viruses derived from B-tropic MuLV

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indicate that the principal viral gene product concerned with tropism is p30 and that the conversion of B- to NB-tropism might be the result of as simple an event as a base substitution. There is also evidence (19, 20) that the generation of B-tropic MuLV in vivo might in some instances involve recombination with xenotropic MuLV. It is not known whether other host genes regulate the generation of B-tropic MuLV in vivo.

To determine whether the genes that determine in vitro virus induction phenotype in low leukemic mice are also the principal determinants of spontaneous virus production in vivo, we examined low leukemic mice for MuLV expression as a function of age. These studies (7) demonstrate that viral loci of similar in vitro induction phenotypes (*Inc-1* and *Inb-1*) have different patterns of virus expression in the mouse and that interactions occur between different viral loci in vivo as well as in vitro.

Materials and Methods

Mice. BALBc/By and C57BL6/By were purchased from The Jackson Laboratory, Bar Harbor, ME. CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ, and CXBK breeding pairs and retired breeders were obtained from Dr. Donald Bailey's colony at The Jackson Laboratory. All F_1 and backcross progeny were bred at McArdle Laboratory under standard breeding conditions.

Infectious Center Assay. Spleens were removed from the appropriate mice and were teased apart on a sterile 2-cm square wire mesh grid with sterile forceps. After centrifugation, the cells were resuspended in minimum essential medium containing 10% fetal bovine serum and 20 μg/ml mitomycin-C (4 ml/spleen) and shaken in a water bath at 37°C for 30 min, or they were exposed to 4,000 rad in a ^{137}Cs irradator. The concentration of nucleated cells per tube was determined, and 5×10^7 cells were added to preseeded (2×10^5 SC-1 cells/60-mm plate) monolayers in duplicate (21). After 5 d of incubation, one of the plates was developed in the UV-XC plaque assay (22), and the other culture was used to prepare a viral isolate. Cells and supernatant media were harvested, disrupted, and spun at 2,000 *g* for 15 min, and the supernatant media were stored at −60°C. In some cases virus isolates were amplified by growth on SC-1 cells for 2–3 cell passages.

Fv-1 Typing. The tropism of virus isolates was determined by titration of the isolate on *Fv-1*$^{n/n}$ (SWR mouse embryo), *Fv-1*$^{b/b}$ (BALB/c mouse embryo), or, occasionally, *Fv-1*$^{n/b}$ (SWR × BALB/c mouse embryo) cells. Cells were treated with 25 μg/ml of DEAE dextran, and 0.2 ml of the viral isolate or its appropriate dilution was added to each plate. 6 d later, plates were developed in the UV-XC plaque assay (22). N-tropic viruses had a 100-fold higher titer on *Fv-1*$^{n/n}$ cells than on *Fv-1*$^{b/b}$ cells, whereas B-tropic viruses had a 30–60-fold high titer on *Fv-1*$^{b/b}$ cells than on *Fv-1*$^{n/n}$ cells. In general, cells from the *Fv-1*$^{n/b}$ heterozygote were used only when it appeared that a mixture of N- and B-tropic viruses were present. In all the viral isolates that were tested, no NB-tropic MuLV were recovered, but many isolates contained mixtures of N- and B-tropic MuLV. Cloned N-tropic (WN1802N) and B-tropic (WN1802B) viruses were included in all tests as controls.

Results

Patterns of Virus Production in BALB/c, C57BL/6, and CBF$_1$ Mice. The pattern of spontaneous ecotropic virus expression for mice of a given genotype was deduced from data that answered three questions as a function of mouse age. The three questions that were asked are: (*a*) do spleen cells of the mouse produce ecotropic MuLV?; (*b*) how much MuLV is produced?; and (*c*) what tropism is the majority of the recovered virus?

In presenting the answers to the first two questions, individual mice of an age class have been classified into five groups to facilitate graphic presentation of the data.

Mice are classed as those with 0, 1–5(+), 6–50(++), 51–250(+++), and >250(++++) virus-producing cells per 5×10^7 spleen cells (Fig. 1). The data presented in Fig. 1 indicate that not all mice of a particular strain show identical numbers of virus-positive cells, and thus the pattern of virus expression in a particular strain might be best described as the probability of finding virus-positive individuals within a strain that show a given titer of MuLV at a particular age. In addition, virus isolates recovered from MuLV-positive cultures have also been typed for their *Fv-1* tropism, and this parameter was found to differ from strain to strain (Tables I and II). The observation that different inbred strains show different patterns of virus expression indicates that this phenotype is under genetic control.

BALB/c and B6 are low virus strains in comparison with the viremic strain AKR. As previously shown (7), embryo cultures from both BALB/c and B6 show low levels of virus production after I uridine (IUdR) treatment (Table I). However, these two strains differ markedly in their in vivo patterns of virus expression (Fig. 1, Table I).

Phenotype of B6(Inb-1$^{+/+}$) Mice. About 11% of B6 mice are positive for ecotropic MuLV (Table III) from 1–13 mo of age, and <9% ever show appreciable (6 plaques per 5×10^7 spleen cells) virus titers. Neither the percentage of mice that produce

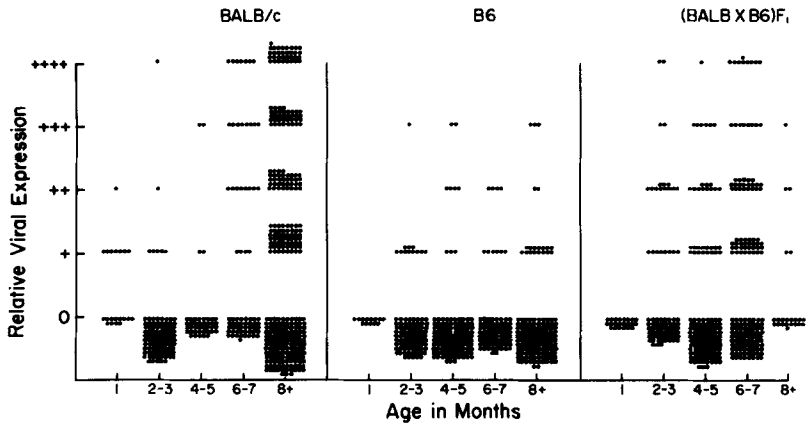

FIG. 1. Ecotropic MuLV expression as a function of age in BALB/c, B6, and (BALB × B6)F_1 mice. Individual mice (each dot) of an age class are classified into five groups, those with 0, 1–5 (+), 6–50 (++), 51–250 (+++), and >250 (++++) virus-producing cells per 5×10^7 spleen cells.

TABLE I

Summary of Phenotypes Associated with Inc-1 and Inb-1 Genes

Genotype	Strains examined	In vitro virus induction*	In vivo virus expression
Inb-1$^{+/+}$	B6, CXBD, CXBG	0.4	10% of mice show low titer of N- or B-tropic MuLV throughout life (n = 1122)
Inc-1$^{+/+}$	BALB/c, CXBH	0.1	18% of mice show low virus at 1–5 mo; 52% mice show higher titer of N-tropic MuLV at 6–8 mo (n = 1076)
Inc-1$^{+/}$, *Inb-1*$^{+/}$	CBF_1, CXBE, CXBI, CXBJ, CXBK	10.4	37% of mice express N- or B-tropic MuLV throughout life (n = 2274)

* Units are syncytia per 2.5×10^5 induced embryo cells. Data were pooled from all experiments with mice of that genotype. For *Inb-1*$^{+/+}$ mice, n = 135; for *Inc-1*$^{+/+}$, n = 183; for *Inc-1*$^{+/}$, *Inb-1*$^{+/}$, n = 168.

TABLE II

Fv-1 Tropism of Induced and Naturally Occurring MuLV Isolates

Strain	Age at isolation of MuLV (number of N-tropic isolates/B-tropic isolates)				
	1	2–3	4–5	6–7	8+
			mo		
BALB/c (*Inc-1*$^{+/+}$)			1/0	12/3	59/9
CXBH (*Inc-1*$^{+/+}$)	0/2	1/0	6/4	20/3	34/7
B6, CXBD, CXBG (*Inb-1*$^{+/+}$)		1/0	3/3	3/3	6/4
CBF_1 (*Inc-1*$^{+/-}$, *Inb-1*$^{+/-}$)	1/0	5/10	3/3	8/22	1/0
CXBE (*Inc-1*$^{+/+}$, *Inb-1*$^{+/+}$)		51/10	8/7	18/4	2/3
CXBI (*Inc-1*$^{+/+}$, *Inb-1*$^{+/+}$)		14/2	17/8	11/7	10/22
CXBK (*Inc-1*$^{+/+}$, *Inb-1*$^{+/+}$)	1/0	10/2	24/3	6/1	6/7
CXBJ (*Inc-1*$^{+/+}$, *Inb-1*$^{+/+}$)	1/0	3/0	7/3	3/2	4/4
Inc-1$^{+/-}$, *Inb-1*$^{+/-}$ (BALB × D), (BALB × G) (H × G), (H × D), (B6 × H)	1/0	10/3	11/7	4/6	1/1
Inc-1$^{+/+}$, *Inb-1*$^{+/-}$ (BALB × E), (BALB × I) (E × H), (I × H), (K × H)		4/1	17/3	9/5	2/3
Inc-1$^{+/-}$, *Inb-1*$^{+/+}$ (B6 × E), (B6 × I) (B6 × K), (G × E) (G × K), (D × E), (J × D)		23/5	9/7	4/1	0/3
B6 × CBF_1		3/3		5/4	19/30
BALB × CBF_1		5/1			
CBF_2 IUdR-induced embryo cultures	48/0				

MuLV nor the mean titer of virus recovered from positive cultures significantly changes as a function of age in B6 mice (t tests at $P = 0.05$). Approximately equal numbers of N-tropic and B-tropic isolates were recovered from *Inb-1*$^{+}$ mice (Table II), and this pattern does not statistically change throughout life. Thus, the pattern of virus expression in B6 mice might be characterized as a low level of N- or B-tropic MuLV production from a low percentage of individuals throughout life (Table I).

Phenotype of BALB/c (Inc-1$^{+/+}$*) Mice.* 12% of BALB/c mice are positive for virus early in life (1–5 mo) and <3% show appreciable titer (>6 plaques/5 × 10^7 spleen cells). Early in life, BALB/c and B6 mice resemble each other both in terms of percentage of virus-positive mice and mean titer of the virus-positive mice as determined by the t test at $P = 0.05$. However, from 6–14 mo, infectious virus can be recovered from 53% of BALB/c mice with a mean titer of 157 plaques, whereas at this same time period only 15% of B6 mice were positive for virus with a mean titer of 9 plaques. At 6–14 mo, both the percentages of mice positive for virus and the mean

TABLE III
Percentage of Mice Producing MuLV

Strain	Age	
	1–5 mo	6–15 mo
B6	21/184 (11%)	31/220 (15%)
CXBD	15/156 (6%)	17/193 (9%)
CXBG	12/223 (5%)	15/146 (10%)
Total	48/563 (9%)	63/559 (11%)
BALB/c	18/156 (12%)	187/351 (53%)
CXBH	74/344 (22%)	112/225 (50%)
Total	92/500 (18%)	299/576 (52%)
CBF_1	57/220 (26%)	72/169 (42%)
CXBE	121/303 (40%)	54/224 (29%)
CXBI	130/254 (51%)	121/181 (67%)
CXBJ	27/162 (17%)	42/182 (23%)
CXBK	129/339 (38%)	77/240 (32%)
Total	464/1278 (36%)	366/996 (37%)

titers of virus are statistically different ($P = 0.05$) for BALB/c and B6 mice. An additional parameter that distinguishes these strains is the time-course of appearance of B-tropic MuLV. Although B6 produces equal amounts of N- and B-tropic MuLV throughout life, BALB/c produces predominantly N-tropic MuLV from 1–14 mo (85% of the isolates examined [$n = 83$] are N-tropic). Therefore, the pattern of expression of endogenous MuLV is different in B6 and BALB/c mice.

Phenotype of CBF_1 ($Inb\text{-}1^{+/-}$, $Inc\text{-}1^{+/-}$) Mice. The F_1 hybrid between these strains exhibits yet a different pattern of virus expression. More F_1 mice (26%) were found to be virus positive at an earlier age (1–5 mo) compared with BALB/c and B6. Statistical analysis indicates that the percentage of virus-positive CBF_1 mice is significantly different from the percentages of virus-positive BALB/c and B6 mice at this time interval. However, the mean titers of recovered virus from positive mice in these populations are not significantly different in early life ($P = 0.05$). These results suggest the frequency of expression of MuLV is greater in the CBF_1 strain than in either parental strain early in life. However, the mean titer of virus in spleens of positive mice is similar in these three strains early in life.

Early in life (1–5 mo) viruses recovered from CBF_1 mice are predominantly N-tropic (75%, $n = 12$). This frequency is similar to the frequency of N-tropic MuLV observed in old (6–14 mo) BALB/c mice. Later in life (6–16 mo), the proportion of N-tropic viruses decreases, and B-tropic MuLV (61%, $n = 23$) are predominantly recovered. The frequency of B-tropic MuLV recovered from older CBF_1 is similar to the frequency of B-tropic MuLV observed in B6 mice (44%) throughout life.

In conclusion, the pattern of virus expression observed in CBF_1 mice is complex. From 1–5 mo of age, there is a higher frequency of MuLV-positive mice than either BALB/c or B6, and the tropism of viruses recovered from CBF_1 mice resembles those recovered from BALB/c mice. Although the frequency of virus-positive mice increases slightly with age in CBF_1 mice, the recovery of B-tropic MuLV increases dramatically and resembles the percentage of B-tropic MuLV recovered from B6 mice throughout

life. The simplest model to explain these results is that the expression of MuLV is activated with greater frequency in young CBF_1 mice than in young BALB/c or B6 mice, and thus one may propose that genes of BALB/c and B6 interact to determine virus expression.

To determine whether the genes that determine these in vivo patterns of expression correspond to *Inc-1* and *Inb-1*, which were previously shown to enhance virus induction in vitro (7), we examined the pattern of virus appearance in CXB recombinant inbred strains.

Patterns of Virus Expression in the CXB Recombinant Inbred Strains. The patterns of spontaneous virus expression in the seven BALB/c × B6 recombinant inbred strains have been examined as a function of age (Fig. 2 and Table III). CXBD and CXBG ($Inb\text{-}1^{+/+}$ mice) both resemble B6 in terms of virus expression in vivo (Fig. 2). These mice have a low frequency of spontaneous virus expression throughout life, with an average of 8% of the population ($n = 718$) expressing virus (Fig. 2). In those individuals that do yield MuLV, the titer is usually low, and the mean titers of virus-positive mice in these populations are not statistically different from those of the B6 population. Because of the low number of isolates from these $Inb\text{-}1^{+/+}$ strains, results from *Fv-1* typing of virus pools recovered from B6, CXBD, and CXBG mice have been combined (Table II) and demonstrate that $Inb\text{-}1^{+/+}$ mice produce N- and B-tropic MuLV in equal proportions. Each individual strain shows approximately equal numbers of N- and B-tropic isolates (data not shown).

The CXBH strain ($Inc\text{-}1^{+/+}$ mice) resembles BALB/c in terms of spontaneous virus expression in vivo. 22% of CXBH mice are positive for virus early in life, and the percentage increases to ~50% late in life, with many of the virus-positive mice having a higher titer of virus ($\bar{x} = 203$) in their spleens and other tissues. Virus recovered from 6–15-mo-old CXBH mice was predominantly N-tropic, and the proportion of N-tropic virus recovered from BALB/c and CXBH mice is statistically the same ($P = 0.05$).

Embryo cells from the recombinant inbred strains CXBE, CXBI, CXBJ, and CXBK mice have approximately 10–50-fold more virus-positive cells after IUdR treatment than cultures prepared from either parental strain and have been shown in genetic crosses to carry both *Inc-1* and *Inb-1* (Table I). CXBE, CXBI, and CXBK mice resemble CBF_1 hybrid mice in that all of these strains have more virus-positive individuals (38%) (range 17–51) at an early age (1–5 mo) compared with either parental strain (Table III). Later in life (6–12 mo), the percentage of virus-positive mice in the CXBI strain increases (51–67%), whereas this percentage decreases slightly in CXBE and CXBK mice (39–30%). 80% of the recovered viruses from these mice at 1–5 mo of age were N-tropic ($n = 171$) (Table II). The percentage of N-tropic MuLV recovered in these mice early in life statistically resembled the percentages of N-tropic MuLV recovered late in life in the BALB/c and early in life in the CBF_1 populations. However, virus recovered from older (6–15 mo) mice of these strains was an equal mixture of N- and B-tropic MuLV (54% N-tropic, $n = 110$) (Table II). The frequency of B-tropic MuLV expressed late in life in CXBE, CXBI, and CXBK is statistically indistinguishable from the frequency of B-tropic MuLV observed in CBF_1 mice late in life and B6 mice throughout life.

CXBJ mice have a lower percentage of virus-positive mice than the E, I, or K strains. However, 50% of the virus-positive CXBJ mice show appreciable titers of

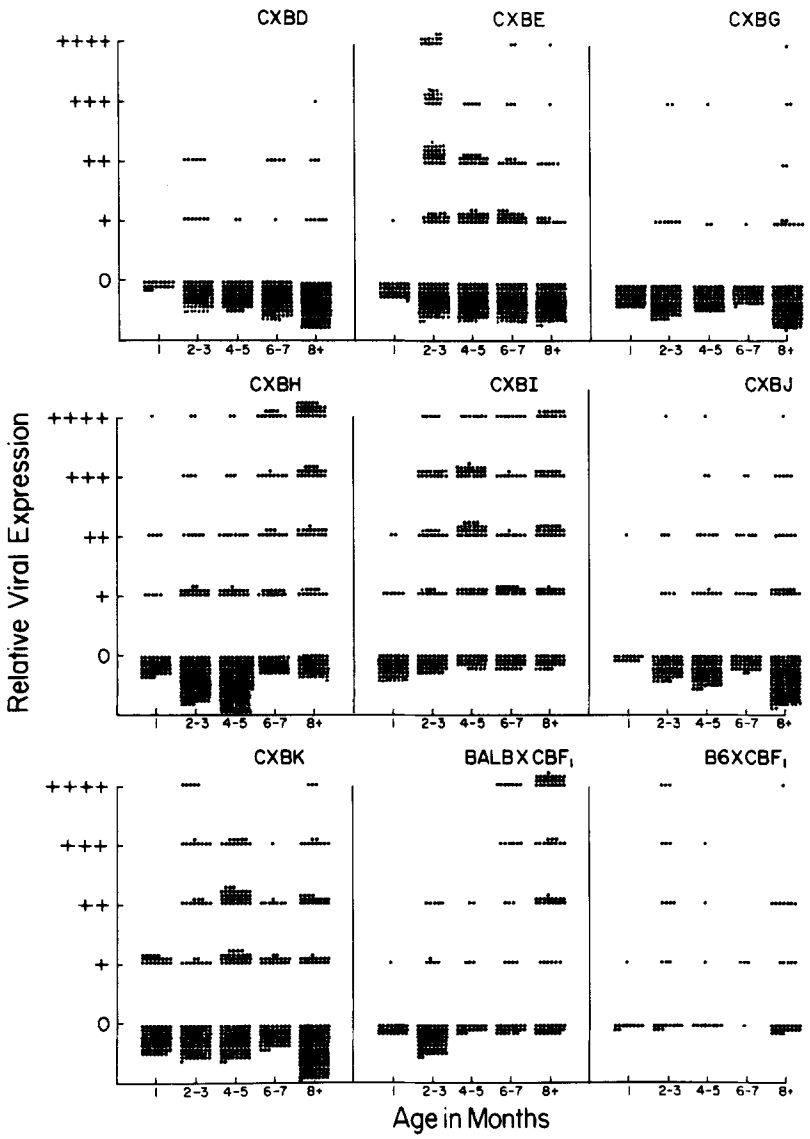

FIG. 2. Ecotropic MuLV expression as a function of age in the CXB recombinant inbred lines BALB × CBF_1 and B6 × CBF_1 backcross mice.

virus, which is not the case with young BALB/c mice or B6 mice. Moreover, the tropism pattern of the viruses recovered from CXBJ mice resembles the tropism pattern of virus recovered from other *Inc-1*$^+$, *Inb-1*$^+$ mice (Table II). For these reasons, it is likely that the CXBJ strain is the low extreme of *Inc-1*$^+$, *Inb-1*$^+$ mice.

Thus, the in vivo phenotypes of these strains correlate with their previously defined in vitro *Inc-1* and *Inb-1* genotypes determined by IUdR induction of tissue culture cells. It seems likely that the correlation of in vivo phenotype with *Inc-1* and *Inb-1* genotype is not fortuitous but reflects the consequence of inheritance of alleles at these two loci. Therefore, we may conclude that *Inc-1* and *Inb-1* influence not only the

quantity of MuLV in IUdR-treated embryo cultures in vitro, but also the pattern of the spontaneous appearance of MuLV in vivo.

Patterns of Virus Expression in CBF_1 Mice Backcrossed to BALB/c and B6. The B6 × CBF_1 backcross population exhibits a CBF_1-like phenotype throughout life and is not statistically different from the CBF_1 population in terms of percentage or mean titer of virus-positive mice ($P = 0.05$). The BALB/c × CBF_1 population exhibits a more complex pattern of virus expression. Later in life, the BALB × CBF_1 backcross mice have a high percentage of virus-positive mice (66%). The tropism patterns of viruses recovered from BALB/c × CBF_1 and CBF_1 mice are similar in that predominantly N-tropic MuLV was recovered from 2–3 mo mice and predominantly B-tropic MuLV was recovered from 6–20 mo old mice (Table II). These results indicate that the genetic interaction that occurs in CBF_1 mice also occurs in some of the BALB/c × CBF_1 and B6 × CBF_1 mice.

Patterns of Virus Expression in Hybrids of the CXB Recombinant Inbred Strains with BALB/c and B6. To further confirm the interaction between *Inb-1* and *Inc-1* and to demonstrate the dominant nature of these genes, the recombinant inbred strains were crossed to the parental or other recombinant inbred strains, and the spontaneous expression and tropism patterns of MuLV were followed. Table IV presents the patterns of spontaneous virus expression in various hybrid mice classified according to their inheritance of *Inc-1*$^+$ and *Inb-1*$^+$, as determined by induction of MuLV in vitro by halogenated prymidines (7). (*Inc-1*$^+$ × *Inb-1*$^+$)F_1 mice are heterozygous at both loci; (*Inc-1*$^+$ × *Inc-1*$^+$, *Inb-1*$^+$)F_1 mice are homozygous at *Inc-1*$^+$ and heterozygous at *Inb-1*$^+$; and (*Inb-1*$^+$ × *Inb-1*$^+$, *Inc-1*$^+$)F_1 mice are homozygous at *Inb-1*$^+$ but heterozygous at *Inc-1*$^+$. The CBF_1 pattern is seen in all these classes of mice both in terms of the percentage of mice that are virus positive and the percentages of N-tropic MuLV recovered. These results indicate that the gene dosage of *Inc-1* or *Inb-1* does not significantly modify the pattern of spontaneous virus expression.

The results presented in this paper demonstrate that all strains examined in this study produce more N- than B-tropic MuLV early in life (1–5 mo). The occurrence of B-tropic MuLV in these strains is influenced by two factors, the presence of *Inb-1* and/or the age of the mouse. In mice that carry *Inb-1* in addition to *Inc-1*, B-tropic MuLV appears as early as 2–3 mo of age and becomes more prevalent by 6–15 mo of age. No correlation was found between the number of syncytia observed with spleen cells from an individual and the *Fv-1* tropism of that pool of viruses (data not shown).

Discussion

Previously, we showed (7) that BALB/c and B6 mice contain two genetic elements, *Inc-1* and *Inb-1*, respectively, which enhance the production of MuLV after IUdR treatment of hybrid embryo cultures. In the present study, evidence supports the conclusion that these same two genes act in vivo to determine the pattern of MuLV expression in spleen cells. Therefore, it seems clear that the enhanced virus induciton observed in IUdR-treated embryo cultures also occurs spontaneously. However, the situation is more complex in vivo because the interaction of these viral loci occurs in an age-dependent fashion, and the elements themselves produce nonequivalent phenotypes (Table I).

Our results with BALB/c mice are very similar to those of Peters et al. (23, 24), who found the same pattern of late appearance of N-tropic MuLV in these mice.

TABLE IV
Percentage of Mice Producing MuLV

F_1 hybrid	Age	
	1–5 mo	6–12 mo
Inb-1$^{+/-}$, *Inc-1*$^{+/-}$		
(B6 × CXBH)F_1	30/110 (27%)	21/46 (46%)
(CXBD × CXBH)F_1	26/63 (41%)	8/31 (58%)
(CXBG × CXBH)F_1	46/166 (28%)	9/36 (25%)
(BALB × CXBD)F_1	5/16 (31%)	6/11 (55%)
(CXBG × BALB)F_1	7/44 (16%)	3/11 (27%)
Total	114/399 (29%)	47/135 (35%)
Inc-1$^{+/-}$, *Inb-1*$^{+/+}$ × *Inc-1*$^{+}$		
(CXBE × CXBH)F_1	9/63 (14%)	3/15 (20%)
(CXBI × CXBH)F_1	26/52 (50%)	19/32 (50%)
(CXBJ × CXBH)F_1	10/28 (36%)	7/21 (33%)
(CXBK × CXBH)F_1	26/80 (33%)	2/7 (29%)
(CXBE × BALB)F_1	34/65 (52%)	13/36 (36%)
(CXBI × BALB)F_1	13/25 (52%)	6/14 (43%)
(CXBJ × BALB)F_1	7/22 (32%)	3/8 (38%)
(CXBK × BALB)F_1	8/23 (35%)	0/2 (0%)
Total	133/358 (37%)	53/135 (39%)
Inb-1$^{+/+}$, *Inc-1*$^{+/-}$ *Inb-1*$^{+}$		
(B6 × CXBE)F_1	21/36 (58%)	8/16 (50%)
(B6 × CXBI)F_1	12/24 (50%)	20/41 (49%)
(B6 × CXBJ)F_1	21/53 (40%)	7/24 (29%)
(B6 × CXBK)F_1	45/74 (61%)	14/24 (50%)
(CXBD × CXBE)F_1	17/41 (41%)	1/7 (14%)
(CXBJ × CXBD)F_1	2/22 (9%)	
(CXBE × CXBG)F_1	23/52 (44%)	3/7 (43%)
(CXBG × CXBK)F_1	9/17 (53%)	
Total	150/319 (47%)	53/119 (45%)

They also observed that older BALB/c mice (18–33 mo of age) produce predominantly B-tropic MuLV. Although we have not examined virus production in BALB/c mice older than 14 mo, we have observed that in 18-mo-old BALB/c × CBF_1 mice the frequency of virus-positive mice is high (72%), and 90% of the viruses isolated are B-tropic. Odaka (25) and Ihle and colleagues (26, 27) observed low levels of virus or viral antigen production in B6 mice. These authors also demonstrated that both N- and B-tropic MuLV could be recovered from these mice.

Because BALB/c and B6 mice show essentially the same IUdR induction phenotype, one might predict that they would show the same in vivo phenotypes of spontaneous virus expression. However, they show quite different phenotypes, as we and others have demonstrated. BALB/c mice express considerable virus late in life, whereas B6 mice are uniformly low for virus production. This result indicates that the two strains differ either in the induction of MuLV and/or in the regulation of virus spread throughout the animal. If multiple genes were involved in virus spread, then one might expect that such genes would segregate in recombinant inbred strains and be phenotypically expressed in hybrid mice. Clearly, (BALB/c × B6)F_1 hybrid mice

do not suppress virus expression at 1–5 mo of age; indeed, they show higher virus expression early in life than either parent. The concordant segregation of spontaneous virus expression in vivo with in vitro induction phenotype and proviral sequences (Jenkins and Copeland, personal communication; Horowitz and Risser, unpublished observations) indicates that these chromosomal regions are the major determinants of MuLV expression in low leukemic mouse strains. Thus, if separate regulatory genes (aside from *Fv-1*) exist that control the spread of virus in these strains, it is likely that such loci are closely linked to viral sequences.

Whether the different in vivo phenotypes associated with the BALB/c and B6 ecotropic proviruses result from different locations of the proviruses in the mouse genome or differences within the proviral sequences has yet to be determined. Jaenish and colleagues (28) have proposed that the major determinants of viral expression in germ line integrated Moloney MuLV proviruses are chromosomal positions. An equally likely explanation is that mutations carried by proviruses are responsible for different phenotypes. In this regard, O'Rear and Temin (29) have observed that molecularly cloned spleen necrosis proviruses recovered from infected chicken cells each show distinct nucleotide sequence alterations. Whatever the molecular explanation for the different viral phenotypes of BALB/c or B6 mice, it seems clear that such phenotypes are by no means invariant traits but can be modified by interaction with other viral genes.

The genetic interaction we observed in mice carrying both *Inc-1* and *Inb-1* resulted in two distinct changes in virus expression. First, more virus is expressed earlier in life compared with the parental strains. We suspect this increased frequency of early virus appearance reflects a higher rate of spontaneous MuLV activation in cells carrying both genes and has the same molecular basis as the increased frequency of virus production in IUdr-treated embryo cultures carrying these genes. It remains to be determined whether the increase in virus induction in hybrid mice reflects recombinational events between the two different proviruses or the action of genes that regulate MuLV expression. If the early increase in virus expression in $Inc\text{-}1^+$, $Inb\text{-}1^+$ mice reflects recombination between proviral genes, it is an unusually efficient process because other examples of in vivo retrovirus recombinational events, such as the generation of dual-tropic MuLV (30–32) or B6 B-tropic MuLV (20), appear to be much less frequent.

The interaction we observed in $Inc\text{-}1^+$, $Inb\text{-}1^+$ mice also resulted in the earlier appearance of B-tropic MuLV at 6–12 mo of age. This earlier shift in tropism pattern, (N- to B-tropic MuLV) might be related to the proposed models for generation of B-tropic MuLV. Faller and Hopkins (15–17) and Rommelaere and co-workers (18) have shown that the difference between N- and NB-tropic MuLV might be due to subtle modifications of the p30 molecule (major core protein), and a single base change might be sufficient for this change. This change could be accomplished by mutation or recombination with other endogenous *gag*-like genes. Benade and co-workers (19, 20) have documented that some B-tropic viruses isolated from B6 mice have *gag* regions that code for proteins immunologically more similar to the xenotropic than ecotropic MuLV gene products. They suggest that B6 B-tropic viruses arise by recombination of endogenous ecotropic and xenotropic MuLV sequences. According to both of these models, mutation or recombination, B-tropic MuLV would be expected to occur more frequently if more virus was expressed. Clearly, more virus is

expressed earlier in mice that contain *Inc-1* and *Inb-1*, and B-tropic MuLV appears earlier in *Inc-1*$^+$, *Inb-1*$^+$ mice. Experiments are in progress to determine the origin of the B-tropic sequences in these viruses.

Summary

The spontaneous expression of ecotropic murine leukmia virus (MuLV) in spleen cells of BALB/c, C57BL/6 (B6), and derivative mice was examined as a function of age. The patterns of spontaneous virus induction in vivo correlate with the patterns of virus induction in vitro, which result from the action of two loci, *Inc-1* and *Inb-1* (7). Whereas mice carrying *Inc-1* or *Inb-1* have similar phenotypes in vitro, they have significantly different phenotypes in vivo. Mice of the *Inb-1*$^{+/+}$ genotype, e.g., B6, rarely expressed MuLV, and the titer of MuLV recovered from rare MuLV-positive mice of this genotype was usually low. Mice of the *Inc-1*$^{+/+}$ genotype, e.g., BALB/c, expressed low amounts of MuLV early in life, however, from 6–12 mo of age approximately one-half of the *Inc-1*$^{+/+}$ mice expressed virus, frequently of high titer. Equal numbers of N-tropic and B-tropic MuLV were recovered from *Inb-1*$^+$ mice, but predominantly N-tropic MuLV was recovered from *Inc-1*$^+$ mice. Strains that carry dominant (+) alleles at both *Inc-1* and *Inb-1* show higher titers of MuLV earlier in life than strains that carry only *Inc-1* or *Inb-1*. The presence of dominant alleles at both loci results in the appearance of predominantly N-tropic virus early in life. These results demonstrate that the principal determinants of spontaneous virus expression in these low leukemic strains of mice are the *In* loci or genes linked to them. A further inference that can be drawn from these studies is that the appearance of B-tropic virus is by no means a random process but rather results from predictable patterns of MuLV expression and alteration.

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