Expression of Murine Leukemia Viruses in the Highly Lymphomatous BXH-2 Recombinant Inbred Mouse Strain

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Among 12 recombinant inbred strains of mice derived from crossing two strains, C57BL/6J and C3H/HeJ, which have a low incidence of neoplastic disease, one strain (BXH-2) has been found to have a high incidence of lymphoma, of non-T-cell origin, at an early age. The BXH-2 strain carries the *Fv-1^b* allele and spontaneously expresses a B-tropic murine leukemia virus beginning at as early as 10 days of gestation and continuing throughout their life. No significant differences in ecotropic virus titers were observed at any age tested (16 to 17 days of gestation through 7 months), whereas xenotropic virus was first detected in lymphoid tissues of 2-month-old mice and virus titers increased with age. Dual tropic virus(es), which induced cytopathic changes on mink lung cells, was isolated from BXH-2 lymphomatous tissues. Unlike AKR mink lung focus-forming virus (N-tropic recombinant), BXH-2 dual tropic virus is B tropic and induces cytopathic changes in mouse fibroblast cultures as well. The BXH-2 mouse provides a model system for studying the role of replication-competent viruses in spontaneously occurring leukemias of non-T-cell lineage and neurological disease.

Recombinant inbred (RI) mouse strains constitute a powerful tool for genetic analysis and have been especially useful in analyzing the genetic basis of tumor susceptibility (36). The RI lines are derived by brother-sister inbreeding beginning with the F₂ generation from crosses of two standard inbred strains. Among 12 RI strains derived from crossing C57BL/6J and C3H/HeJ mice (two strains with a low lymphoma incidence), one strain (BXH-2 RI mice) was found to have a high lymphoma incidence (89% by 7 months of age). In contrast, the incidence of spontaneous lymphoma in the other 11 BXH RI strains, and including the two parental strains, was less than 20% by 2 years of age.

The RI strains are particularly useful, since: (i) multiple independent RI strains were derived without selection; (ii) each set of RI strains represents a stable segregant population; (iii) unlinked genes were randomized in the F₂ generation and were equally likely to be fixed in the recombinant phases; (iv) BXH RI strains have been typed for more than 80 markers on 14 chromosomes, and all additional information received is cumulative; and (v) a disease model (BXH-2 RI mice) and 11 genotypically distinct but related BXH RI strains, as well as the parental strains, are available for comparative biochemical and genetic analysis. BXH RI mice should be particularly useful for studying the genetic basis of murine leukemogenesis.

In the experiments described here we have characterized the BXH RI strains, in particular, BXH-2 mice, with respect to properties previously associated with a high lymphoma incidence in other inbred strains of mice. These properties include the following: (i) major *H-2* histocompatibility type, (ii) spontaneous production of ecotropic and xenotropic murine leukemia virus (MuLV) beginning early in life, and (iii) production of recombinant mink cell focus-forming (MCF) viruses by lymphomatous tissue. In addition, we have begun to characterize the type of lymphoma found in BXH-2 mice and the target cell(s) involved.

MATERIALS AND METHODS

Mice. RI strains are derived from the F₂ generation of the crosses between two different strains by brother-sister mating. The BXH-2 RI strain was derived from C57BL/6J × C3H/HeJ crosses by one of us (B.A.T.). The BXH-2 strain carries the *H-2^k* allele of C3H/HeJ and, in this respect, resembles other strains with a high leukemia incidence, such as AKR/J, C58/J, and HRS/J. However, we have no evidence that *H-2^k* confers susceptibility in this strain; other BXH strains that carry the *H-2^k* allele are not leukemic.

Cells. The cloned wild mouse embryo cell line SC-1 was maintained on Eagle minimal essential medium supplemented with 2 mM L-glutamine, 10% heat-inactivated (56°C for 30 min) fetal bovine serum, and antibiotics (100 U of penicillin and 100 µg of streptomycin). Other cell lines included the rabbit corneal

(SIRC) and mink lung (CCL-64) cell lines. These cultures were maintained in Dulbecco modified basal medium supplemented with heat-inactivated fetal bovine serum and antibiotics.

Virus assays. To assess the occurrence of virus in lymphoid tissues of BXH-2 mice, the infectious-center assay was used (23). Mice from different age groups were killed and cell suspensions were prepared separately from each of the organs and tumors. Single cell suspensions of spleen, lymph nodes, and thymus were obtained by mincing whole tissues on 60-mesh wire screen platforms. Single cell suspensions were obtained by expelling fragments through progressively smaller needles down to 25 gauge. Lymphoid cells were pretreated with mitomycin C (25 μ g for 30 min) before infection of the indicator cells (21).

Lymphoid cells were tested for the production of ecotropic virus by the XC plaque assay (31). SC-1 cells seeded 24 h earlier at a density of 2×10^5 cells per 60-mm tissue culture plate in Eagle minimal essential medium with 5% fetal bovine serum and 2 μ g of Polybrene (Aldrich Chemical Co.) per ml were infected with serial dilutions of lymphoid cells. Four to five days after infection, the SC-1 cells were UV irradiated and overlaid with 10^6 XC cells. Media were changed after 2 days, and 2 days later the cells were fixed and stained. The number of plaques was determined microscopically, and the virus titer was expressed as PFU per 10^6 cells. Tropism of the ecotropic isolate (13) was determined by infecting secondary embryo cultures of SWR/J (*Fv-1*ⁿ) or BALB/cJ (*Fv-1*^b) mice with lymphoid cells or with supernatant fluids from BXH-2 embryo cultures, followed by UV irradiation and overlaying with XC cells.

Lymphoid cells were also tested for the production of xenotropic and MCF-like virus on mink lung and SIRC cells, as described by Kawashima et al. (21). The presence of virus was determined by the RNA-directed DNA polymerase assay, as well as by the indirect fluorescent-antibody assay.

Reverse transcriptase assays were performed as previously described (22). Briefly, the supernatant fluids were concentrated by centrifugation and assayed for RNA-directed DNA polymerase activity in a reaction buffer consisting of 0.05 M Tris (pH 7.8)–0.002 M dithiothreitol–0.06 M KCl–0.08 M MnCl₂–0.01% Triton X-100–20 μ l of virus. After 10 min at 4°C, a template (0.02 absorbance [260 nm] units of polyriboadenylate-oligodeoxythymidylate–6 μ M [³H]TTP [6,600 cpm pmol⁻¹]) was added to make the final reaction mixture 100 μ l. The reaction mixture was incubated for 60 min at 37°C, and the reaction was terminated with yeast tRNA (100 μ g/ml) and cold 10% trichloroacetic acid containing 20 mM sodium pyrophosphate. Results were expressed as counts per minute of radioactive nucleotide incorporated into acid-insoluble product minus counts in reactions without template.

Xenotropic virus was also detected by the immunofluorescence assay (15). Mink lung cell cover slip cultures were infected with BXH-2 lymphoid cells. Cover slips were fixed after 10 days, incubated with goat antiserum prepared against Tween-ether-disrupted Moloney MuLV (supplied by the Research Resources Biological Carcinogenesis Branch of the

National Cancer Institute), and stained with fluorescein isothiocyanate-conjugated rabbit anti-goat antiserum. Controls for each fluorescence test included cells that were incubated with normal goat serum and conjugated serum or with fluorescein isothiocyanate antiglobulin alone; the controls were consistently negative.

For isolation of a dual tropic virus(es) the highest dilution of lymphoid cells which were RNA-directed DNA polymerase or fluorescent-antibody positive was carried through two cycles of limiting-dilution purification in mink lung and SC-1 cells. Cultures were scored for cytopathic changes 10 days after infection. Mink lung cultures with no observable cytopathic changes were transferred for an additional passage before being discontinued.

Determination of TdT. Purification and assay of terminal deoxynucleotidyl transferase (TdT) from normal and neoplastic lymphoid tissues were performed exactly as described by Pazmino and Ihle (25).

RESULTS

Pathological evaluation. The lymphoma incidence in BXH-2 mice is shown in Table 1. Eighty-nine percent of the total BXH-2 population was diagnosed as having lymphoma, with a mean survival time of 214 ± 59 days. The sex distribution of cases showed no significant difference between sexes (85% male and 90% female, Table 1). In addition, a lower-limb paralysis was found in conjunction with lymphoma in some mice of this strain. In contrast to lymphoma development, paralysis occurred more often in female (26%) than in male (4%) mice (Table 1).

The lymphomas which developed were characterized by splenomegaly and sometimes hepatomegaly, with generalized but primarily visceral lymphadenopathy. The thymus appeared

TABLE 1. Pathological evaluation of BXH-2 mice necropsied for all causes, lymphoma, and paralysis

Diagnosis ^a	No. of mice	Survival (days $\pm$ SD)
All records, since 1977	88	212 $\pm$ 62
With leukemia, total	78 (89) ^b	214 $\pm$ 59
All causes, females	61	208 $\pm$ 65
Leukemia, females	55 (90)	211 $\pm$ 60
Leukemia and paralysis, females	16 (26)	221 $\pm$ 91
All causes, males	27	220 $\pm$ 54
Leukemia, males	23 (85)	221 $\pm$ 56
Leukemia and paralysis, males	1 (4)	238

^a Moribund mice were sacrificed for autopsy and histological examination.

^b Number in parentheses is percentage of mice that developed lymphoma per number of mice in each group.

to be uninvolved or was only secondarily affected; however, the parathymic lymph nodes were generally enlarged. Depending on the stage of disease, the number of nucleated cells in the peripheral blood ranged from 10,000 to 70,000 cells per mm³, containing primarily monocytoid cells with the occasional appearance of granulocytic cells. Within the spinal cord of paralyzed mice, areas of extensive focal demyelination along with degeneration of the neurofibrils and endoplasmic reticulum were observed by electron microscopy.

To further define the cell type(s) transformed in lymphomatous BXH-2 mice, we looked for the presence of the T-cell antigen Thy-1 by immunofluorescence and the specific thymocyte enzyme marker TdT in leukemic BXH-2 tissue. TdT activity is consistently detectable in thymocytes and bone marrow of a variety of mouse strains, but not in other tissues of normal mice or in tumorous tissues of mice with nonthymic lymphoma (25). Furthermore, TdT activity is detectable in peripheral tissues of leukemic AKR mice or in radiation-induced thymomas of C57BL/6 mice (25); both of these strains are known to produce a T-cell leukemia.

The levels of TdT activity in thymocytes of age-matched C57BL/6J, C3H/HeJ, AKR/J, and BXH-2 mice were compared with those obtained from BXH-2 lymphomatous tissues. In nonleukemic thymocytes, TdT activity was found in all four strains tested (204 to 1,035 U/10⁸ cells; Table 2). However, unlike the lymphomatous spleen of AKR/J mice, enzyme activity was not detected in the lymphomatous spleens or nodes of BXH-2 mice (<0.5 U/10⁸ cells; Table 2). Also, tumor cells from BXH-2 mice did not express the Thy-1 T-cell antigen, as determined by indirect immunofluorescence (data not shown) with goat anti-mouse brain (C3H)-adsorbed antiserum (Litton Bionetics, Kensington, Md.),

TABLE 2. *TdT enzyme activity in normal and lymphomatous mouse tissues*

Cells tested ^a	Strain	TdT enzyme activity (U/10 ⁸ viable cells)
Normal thymocytes	BXH-2	863
	C3He/J	986
	C57BL/6J	1,035
	AKR/J	204
Lymphomatous spleen	BXH-2	<0.5
	AKR/J	163
Lymphomatous node	BXH-2	<0.5

^a Thymuses or lymphomatous tissues from different mice (four from each strain) were suspended in extraction buffer followed by chromatography on phosphocellulose, as described previously (25).

whereas leukemic tissues from thymus and spleen of AKR mice were positive (approximately 90% of the cells) for Thy-1 antigen. These observations therefore suggest that the target cell transformed in the BXH-2 lymphoma is not a TdT-expressing T-cell.

Assay of BXH RI strains for production of ecotropic and xenotropic MuLV's. Supernatant fluids from BXH-2 mouse embryo fibroblasts were assayed for ecotropic and xenotropic MuLV content (Table 3). BXH-2 embryo fibroblasts were found to spontaneously produce an XC-positive ecotropic, but not a xenotropic, MuLV. This ecotropic virus showed a titration pattern on SWR/J (*Fv-1*ⁿ) and BALB/J (*Fv-1*^b) mouse cells which was characteristic of a B-tropic virus (Table 3). In addition, tumor extracts from BXH-2 mice, as well as brain and spinal cord tissues from paralyzed BXH-2 mice, were also found to produce an XC-positive B-tropic virus (Table 3). In contrast to BXH-2 mice, the spontaneous release of ecotropic virus was not observed in embryo cultures of the other 11 BXH RI strains (data not shown). In the parental strains (C3H/HeJ and C57BL/6J), N-tropic and xenotropic viruses are readily ex-

TABLE 3. *Tropism of BXH-2 ecotropic MuLV*

Cell line	XC plaque assay	
	Supernatant from embryo cultures ^a (PFU/ml)	Tumor infectious center (PFU/10 ⁶ cells) ^b
SWR/J (<i>Fv-1</i> ⁿ) mouse embryo fibroblasts	4 × 10 ^{1c}	14
BALB/cJ (<i>Fv-1</i> ^b) mouse embryo fibroblasts	9.8 × 10 ⁴	7.8 × 10 ⁵
SC-1 (<i>Fv-1</i> ⁿ) mouse embryo fibroblasts	6.0 × 10 ⁴	8.1 × 10 ⁵
CCL64 mink lung cells ^d	<0.2	<0.2

^a Supernatant fluids from embryo cultures were clarified, and 0.5 ml of 10-fold dilutions was inoculated into the various embryo cell lines. Four days after infection, the plates were exposed to UV irradiation and overlaid with 10⁶ XC cells. Plates were stained 3 to 4 days later and examined for plaques microscopically.

^b The murine and mink lung cell cultures were infected with mitomycin C-treated spleen cells from a lymphomatous BXH-2 mice. The XC assay was as described in footnote a.

^c Titers represent the average from three different assays.

^d Mink lung cells infected with supernatant fluids from embryo cultures or spleen cells were UV irradiated after 4 days and overlaid with 2 × 10⁵ SC-1 cells. Four days later, the cultures were exposed to UV irradiation a second time and overlaid with XC cells. Plates were stained 4 days later and examined for plaques.

pressed, either spontaneously or by induction in vitro (17, 35), whereas B-tropic virus is only occasionally expressed in aged C57BL/6 mice (24). However, the expression of C57BL/6 endogenous MuLV can be significantly accelerated by gamma irradiation (9, 17).

No specific difference ($P \leq 0.01$, Student's *t* test) was observed by infectious-center assay in the number of cells producing ectropic MuLV (70 to 100% positive cells) among cells of 14- to 16-day-old embryos and cells from 7-month-old BXH-2 mice. In contrast to ecotropic virus production, xenotropic virus expression was not observed in supernatant fluids from BXH-2 embryo cultures assayed on CCL64 mink lung cells (Table 3) or by cocultivating BXH-2 embryo fibroblast with mink lung cells. However, beginning at 2 months of age, xenotropic virus expression could be detected by sedimentable DNA polymerase activity or infectious-center assay after cocultivation of mitomycin C-treated BXH-2 lymphoid cells on mink lung cells. Furthermore, a steady increase in the number of lymphoid cells producing xenotropic virus was found with increasing age (Table 4). Although xenotropic virus levels are consistently higher in spleens of BXH-2 mice, we do not find significant differences in xenotropic virus expression among lymphoid tissues, as is observed in strains with a high incidence of thymic lymphomas (21). Since MuLV antigens show cross-reactivity, the possibility exists that we may have detected some ecotropic virus which entered the mink cells as a pseudotype or that residual mouse cells

which survived mytomycin treatment had been carried along with the mink cells. However, we were not able to detect XC-positive virus by infecting SC-1 cells with cell-free supernatants from virus-positive mink cells.

Expression of MCF virus by BXH-2 leukemic mice. Because of the association between the appearance of MCF viruses and spontaneous lymphoma development in other strains with a high leukemia incidence, such as AKR and C58 (14), lymphoid tissues from BXH-2 lymphomatous mice were examined for the presence of MCF-like viruses. To select for MCF virus, lymphomatous BXH-2 lymphoid cells were cocultivated with CCL64 mink lung cells, and supernatant fluids from virus-positive cultures (assayed by sedimentable DNA polymerase activity) were used to infect fresh mink lung cultures. By the second passage, after virus infection, foci were observed on confluent mink lung cells. The proportion of cells giving rise to foci was low (<1%). Virus from cell lines that showed morphological alterations were then freed of any contaminating ecotropic virus by limiting dilution on CCL64 mink lung cells.

The virus isolated in this manner showed a one-hit dose response on mink lung cells and readily replicated on SIRC (rabbit corneal cells), SC-1 (*Fv-1*^a), and BALB/cJ (*Fv-1*^b) mouse cells, but not on SWR/J (*FV-1*^a) cells (Table 5). SC-1 and BALB/cJ embryo cultures infected with the MCF virus were positive for sedimentable DNA polymerase activity but did not produce plaques when exposed to UV irradiation and overlaid with XC cells. Thus, the BXH-2 MCF virus appears to be XC negative and B tropic.

In later passages, mink cells infected with BXH-2 MCF virus no longer reached confluency, but became vacuolated and finally degenerated (Fig. 1b). In addition, unlike the AKR MCF virus (14), but similar to Moloney MCF (37), the BXH-2 dual tropic virus induced cytopathic changes on SC-1 mouse cells. The degenerative changes were observed to occur at an advanced stage of infection; cell growth was retarded, followed by vacuolization and cell death (Fig. 1c). The virus released from degenerating SC-1 cells induced characteristic foci when put back onto mink lung cells.

That the BXH-2 MCF virus is truly a recombinant between ecotropic and xenotropic viruses is supported by the following facts: (i) the tropism of the MCF virus is B tropic (Table 3); (ii) in interference tests, the MCF virus is inhibited by both B-tropic and xenotropic viruses but not by amphotropic virus (Table 5); (iii) the tryptic peptides of the *gag* protein p30 are identical to those of other B-tropic viruses, whereas tryptic peptide analysis of the envelope glycoprotein

TABLE 4. Expression of ecotropic and xenotropic viruses with age in tissues of BXH-2 mice

Age of BXH-2 mice (mo)	Virus assay ^a (infectious centers [$\log_{10}$] per 10 ⁶ cells, ecotropic/xenotropic)		
	Thymus	Spleen	Nodes
1	5.7/0.0 ^b	5.9/0.0	5.7/0.0
2	5.8/1.8	5.8/2.0	5.9/ND ^c
3	5.8/2.0	5.8/2.3	5.9/2.1
4	6.0/2.9	5.7/ND	5.7/3.2
5	5.6/3.0	5.8/3.5	5.7/ND
6	6.2/3.3	5.7/3.8	5.9/3.1

^a Ecotropic and xenotropic viruses were assayed by the infectious-center assay. For ecotropic virus SC-1 cells were infected with serial dilutions of mitomycin C-treated lymphoid cells. After 5 days, the infected cultures were UV irradiated and overlaid with XC cells. Four days later, syncytial plaques were counted (10). For xenotropic virus mitomycin C-treated single cells prepared from the different lymphoid tissues were plated onto mink lung cells and assayed 10 days later by indirect immunofluorescence, as described in the text.

^b Viral titers represent the average of 10 mice.

^c ND, Not done.

TABLE 5. *Tropism and interference properties of BXH-2 MCF virus*

Virus dilution	<i>Fv-1</i> tropism of BXH-2 MCF virus		Interference assay (no. of foci per 500 infectious centers in cultures preinfected with MuLV) ^a		
	BALB/cJ (uninfected)	SWR/J (uninfected)	BALB/cJ (B-tropic MuLV)	CCL64 (xenotropic MuLV)	CCL64 (amphotropic MuLV)
10 ⁰	112	0	0	1	100
10 ⁻¹	10	0	0	0	8
10 ⁻²	1	0	0	0	0
10 ⁻³	0	0	0	0	0

^a Cultures containing 2×10^5 uninfected BALB/cJ (*Fv-1^b*), SWR/J (*Fv-1^b*), BALB/cJ preinfected with B-tropic MuLV, or CCL64 mink cells preinfected with NZB xenotropic or 4070A amphotropic virus were infected in parallel with serial dilutions of BXH-2 MCF virus. Six days after infection, 500 mitomycin C-treated (25 μ g for 30 min) cells from each culture were seeded in duplicate onto 2×10^5 mink cells per 60-mm dish. Cultures were scored for focus formation 10 to 12 days after seeding.

(gp70) shows peptides in common with both ecotropic and xenotropic viruses (J. D. Gautsch and J. H. Elder, personal communication); and (iv) attempts to dissociate ecotropic and xenotropic virus by limiting-dilution purification have failed (12).

***H-2* genotypes of BXH RI strains.** The relationship among leukemogenesis, immune responsiveness, and major *H-2* histocompatibility type has been investigated extensively (33). Congenic mice differing at the *H-2* locus have provided an especially useful tool with which to evaluate this problem. Recently, Boyse et al. (4) and Johnson et al. (19) reported that congenic AKR mice carrying the *H-2^b* haplotype had a reduced and delayed leukemia incidence compared with AKR mice carrying the *H-2^k* haplotype. In addition, they present evidence which suggests that the significantly higher leukemia incidence in *H-2^k* mice may be due to their inability, compared with *H-2^b* mice, to mount an effective immune response against the leukemia-inducing agent.

Since the parental strains of the BXH RI strains were C57BL/6J and C3H/HeJ, which carry the *H-2^b* and *H-2^k* haplotypes, respectively, we have typed the BXH RI strains (in particular, BXH-2 mice) with respect to their major histocompatibility type (32). The results of this analysis are shown in Table 6. Six of the BXH RI strains carried the *H-2^b* haplotype of the C57BL/6J parent and six carried that of the C3H/HeJ parent. The equal distribution of histocompatibility types in the BXH RI strains was expected due to the completely random segregation of chromosomes which occurs during the inbreeding of the RI strains. Additionally, we found that the high lymphomatous BXH-2 strain carried the *H-2^k* haplotype of the C3H/

HeJ parent. Whether the acquisition of the *H-2^k* haplotype by the BXH-2 strain was an important event in the genesis of this highly lymphomatous strain remains to be determined by future experiments.

DISCUSSION

The natural occurrence of lymphomas in C3H/HeJ and C57BL/6J mice and their F₁ hybrids is low. A majority of lymphocytic neoplasms which do occur in these strains appear after 1 year of age and are observed more frequently in females than in males (38). The host genetic factors affecting the incidence and development of neoplasia in these strains may include virus inducibility and infectivity loci, cell susceptibility to transformation, and immune responsiveness. Strains C3H/HeJ and C57BL/6J have been shown to differ in these parameters (16).

Among 12 RI strains of mice derived from crossing two strains, C57BL/6J and C3H/HeJ, which have a low incidence of neoplastic disease, BXH-2 RI strain mice have been found to have a high incidence of lymphoma at an early age. The lymphomas which develop around 7 months of age are in some cases associated with hind-limb paralysis. The lymphomatous cells do not express the T-cell markers, TdT, or Thy-1, an observation consistent with the notion that the lymphoma cell type in BXH-2 mice is not of T-cell origin. These early lymphomas are not characteristic of the BXH-2 line at an intermediate stage of inbreeding (F₁₂). Also, BXH-2 mice were virus negative at the F₁₁ and F₁₃ generations when their *Fv-1* tropism (*Fv-1^b*) was determined. However, by the F₂₅ generation of inbreeding, these mice were virus positive and showed a high incidence of lymphoma. The virus

FIG. 1. Changes induced by BXH-2 MCF virus. (a) Uninfected mink lung cell line CCL64. (b) Vacuolization of mink lung cells infected with BXH-2 MCF virus. (c) Degeneration of SC-1 cells infected with BXH-2 MCF virus. Phase-contrast micrographs; X10.

TABLE 6. *Fv-1* and *H-2* genotypes of BXH RI strains

Strain	<i>Fv-1</i> genotype ^a	<i>H-2</i> haplotype ^b
C57BL/6J	B	B
C3H/HeJ	N	K
BXH		
2	B	K
3	B	K
4	B	B
6	N	K
7	B	K
8	B	B
9	B	B
10	N	B
11	B	B
12	B	K
14	B	K
19	N	B

^a *Fv-1* typing was done by the standard UV XC procedure. Secondary embryo cultures prepared in duplicate from the various BXH strains were inoculated with N- and B-tropic MuLV's at multiplicities of 10^{-1} through 10^{-4} PFU/ml. Plates were UV irradiated 4 to 5 days postinfection and overlaid with 10^6 XC cells. Four days later, the plates were stained and plaques were counted microscopically. The BXH strains are typed as *Fv-1*ⁿ or *Fv-1*^b depending on whether the cells are permissive for N-tropic or B-tropic virus, respectively.

^b *H-2* typing was done by M. Cherry of this laboratory, using *H-2* alloantisera (33).

recovered from supernatant fluids taken from BXH-2 embryo cultures revealed a titration pattern on SWR/J (*Fv-1*ⁿ) and BALB/cJ (*Fv-1*^b) secondary embryo cultures characteristic of an XC-positive B-tropic virus. Similar titration patterns were observed with supernatant fluids from lymphomatous tissues established in culture or with tumor extracts from BXH-2 mice. Furthermore, brain and spinal cord tissue from paralyzed BXH-2 mice also expressed XC-positive B-tropic virus. In contrast to BXH-2, the spontaneous release of ecotropic virus was not observed in embryo cultures or in tissue suspensions from young mice (up to 4 months of age) of the other 11 BXH strains. However, by 8 months to 1 year of age, some mice from two other BXH strains (BXH-8 and -12) were found to release a low level (9 to 120 PFU/ml) of XC-positive virus (data not shown). In the parental strains (C3H/HeJ and C57BL/6J), N-tropic and xenotropic viruses are spontaneously released from tissues of older mice or chemically induced by a variety of procedures (17, 35) in vitro. Generally, the spontaneous expression of B-tropic viruses has been observed only in aged *Fv-1*^b mice, and the appearance of the B-tropic virus is usually correlated with the onset of neoplastic disease (9, 27). Although infectious

N-tropic and xenotropic MuLV's are often expressed in murine tissues and are readily inducible from mouse cells by a variety of procedures, the inducibility of B-tropic virus is rare. The induction of B-tropic virus from embryo cultures has been reported in two *H-2* congenic C57BL/10 strains (24) and from cells of SWR/J embryos (*Fv-1*ⁿ), in which it is believed to represent a distinct class of MuLV (29, 34). Also, infectious B-tropic virus is detected in spleens of 3-month-old leukemia-prone B10.Y congenic mice (6), whereas younger mice in this strain were found to be negative for infectious virus.

Evidence has recently been presented which suggests that B-tropic viruses arise by recombination within the *gag* region of N-tropic and xenotropic viruses (3, 8). By serological analysis, the BXH-2 B-tropic virus was found to have the *gag* gene p12 structural protein identical to that of an N-tropic AKR MuLV (L. E. Benade, personal communication), and the tryptic peptide maps of the viral p30 were shown to contain peptide markers characteristic of class III xenotropic virus (James Gautsch, personal communication).

Since in BXH-2 mice we have been unable to detect the expression of N-tropic virus, it is unlikely that the B-tropic virus, which is detected in high titer as early as 10 days of gestation, arose by recombination within this strain. However, the N-tropic virus may have been expressed at an early stage of inbreeding followed by recombination and amplification of the B-tropic virus in the permissive BXH-2 strain (*Fv-1*^b), with the eventual integration of the virus into the germ line of BXH-2 mice. Although the acquisition into the germ line of new viral loci is rare, the generation of new viral loci during brother-sister mating has recently been reported by Rowe and Kozak (30). It is also possible that the BXH-2 B-tropic virus may have been introduced by one of the parental strains during inbreeding and is not expressed due to the nature of the BXH-2 genetic background.

The high incidence of lymphoma and the spontaneous production of B-tropic MuLV were traits acquired simultaneously during inbreeding of the BXH-2 strain. BXH-2 mice were apparently heterozygous for virus production at the F₂₅ stage of inbreeding since about 50% (9 of 16) of the F₁ hybrid mice generated from crossing a BXH-2 male to C57BL/6J females produced ecotropic MuLV. Thus, C57BL/6J mice are permissive to BXH-2 B-tropic virus expression, but we do not know whether they are also leukemia prone. Nevertheless, 100% of the BXH-2 mice tested for virus production were viremic, presumably as a consequence of both vertical and

horizontal MuLV transmission. BXH-2 inbreeding is now at the F₄₁ generation, and the probability of the B-tropic MuLV being fixed within the germ line is >90%.

Whether the BXH-2 B-tropic virus is truly endogenous remains to be clarified. Studies are currently under way (in collaboration with N. Jenkins of this laboratory), using Southern blotting, restriction enzyme analysis, and hybridization with a viral DNA probe specific for ecotropic DNA sequences, to determine whether the BXH-2 B-tropic virus is present in the germ line of BXH-2 mice. It is also possible that a viremic mother can infect her offspring with virus at several stages of development (preimplantation stage, transplacental infection, or via the mother's milk). The frequency of transplacental infection from a viremic mother to the embryo is reportedly low (18). In comparison, viral transmission via the milk is highly effective, and nearly all newborn mice become infected with MuLV upon foster nursing by viremic mothers (18). However, the presence of B-tropic virus in embryonic tissues of all BXH-2 mice tested argues against milk transmission as the only means of virus spread.

The role of B-tropic viruses in BXH-2 tumorigenesis has still to be defined. BXH-2 mice carry the *Fv-1^b* (inherited from the C57BL/6J parent) and *H-2^k* (inherited from the C3H/HeJ parent) alleles. To assess the oncogenic potential of B-tropic MuLV isolated from BXH-2 mice, biologically cloned B-tropic viruses have been injected into newborns of another BXH RI strain (BXH-7) which was matched for the *Fv-1^b* and *H-2^k* genotypes as well as into newborns of the C57BL/6J strain (*Fv-1^b* *H-2^b*). The animals inoculated with virus are now viremic (determined by tail biopsy at 3 weeks post-inoculation) and are being maintained to determine the incidence of lymphoma.

Spontaneous lymphomas from BXH-2 mice also produce virus which is unrestricted for growth in cells of either murine or nonmurine origin. The BXH-2 dual tropic virus induces cytopathic changes in mink lung cells as well as in mouse embryo fibroblasts (SC-1 cells and mouse strains carrying the *Fv-1^b* allele). The proportion of cells giving rise to mink cell foci was low (<1%), whereas >70% of the lymphoid cells produced XC plaques on SC-1 and BALB/c fibroblasts.

It has been shown that MCF viruses fall into two classes with respect to their ability to induce lymphomas (5). The noninducing and lymphoma-inducing isolates can be distinguished by tryptic peptide analysis of their envelope glycoprotein and by their ability to replicate in the target organ (5). Based on peptide analysis, the

BXH-2 recombinant virus originally isolated from the thymus of a lymphomatous BXH-2 mouse falls into the non-lymphoma-inducing class (J. Elder, personal communication). Relative to N-tropic MCF viruses, the number of B-tropic MCF isolates is reportedly low. Two isolates are from pristane-induced tumors of BALB/c mice, and the ability of these viruses to induce tumors is uncertain (1, 5), whereas the B-tropic isolates from these BALB/c mice are oncogenic (20, 26). In contrast to the situation in BALB/c mice, two cloned dual tropic viruses isolated from a bone marrow cell line of irradiated C57BL/6J mice induced nonthymic lymphomas in this strain, whereas the B-tropic isolate was not oncogenic (10, 11). The involvement of other BXH-2 MCF isolates in BXH-2 lymphomas remains to be elucidated.

By analogy with AKR lymphoma, one might suppose that a combination of genes from C3H/HeJ and C57BL/6J contribute to lymphoma susceptibility in the BXH-2 strain. No evidence is as yet available which directly shows that high exotropic virus expression early in life is directly responsible for the high incidence of lymphomas in this strain. However, that viremia and the high lymphoma incidence are characteristics that were acquired simultaneously in the BXH-2 strain makes us suspect that the two are related, probably causally.

The BXH strains are important, since they present both a disease model and 11 genetically distinct but related controls to study the genetics of virus expression in relation to both tumor susceptibility and paralysis associated with neoplastic disease. The majority of spontaneously occurring tumors in mice which express high levels of ecotropic virus are of the T-lymphocyte lineage. Viruses able to induce leukemias of the B-cell lineage have primarily been replication defective (2), with the exception of Rauscher leukemia virus (28), which was originally isolated from a transplantable tumor (7). Therefore, the BXH-2 mouse provides a new model system for studying the role of replication-competent viruses in spontaneously occurring lymphomas of non-T-cell lineage and in neurological disease.

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