JOURNAL OF VIROLOGY, July 1982, p. 1-7
0022-538X/82/070001-07$02.00/0

Vol. 43, No. 1

Unique Features of Retrovirus Expression in F/St Mice

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Received 18 January 1982/Accepted 30 March 1982

F/St mice are unique in producing high levels of both ecotropic and xenotropic murine leukemia virus. The high ecotropic virus phenotype is determined by three or more V (virus-inducing) loci. A single locus for inducibility of xenotropic murine leukemia virus was mapped to chromosome 1 close to, but possibly not allelic to, *Bxv-1*. Although the high ecotropic virus phenotype is phenotypically dominant, the high xenotropic virus phenotype was recessive in all crosses tested. Suppression of xenotropic murine leukemia virus is governed by a single gene which is not linked to the xenotropic V locus.

Cells from several inbred strains of mice have been shown to produce high levels of murine leukemia viruses (MuLV) from early in life. Some strains, such as AKR, C58, PL, SL, and C3H/Fg, express high titers of ecotropic MuLV, whereas NZB mice express high titers of xenotropic virus (1, 2, 17). Genetic studies of these strains demonstrated that the high virus phenotypes are determined by one or more virus-inducing loci (V loci) which segregate with Mendelian ratios as dominant traits in crosses with permissive strains (1, 2, 17). It was also shown that for ecotropic MuLV (17), and possibly for xenotropic MuLV (8), the V loci are associated with genetic information coding for infectious virus.

Recently it was found that F/St mice are unique among inbred strains in expressing high levels of both ecotropic and xenotropic MuLV (H. C. Morse III and J. W. Hartley, Abstr. 4th Int. Cong. Immunol., abstr. 9.1.07, 1980; R. A. Yetter, J. W. Hartley, C. A. Kozak, and H. C. Morse III, Fed. Proc. **140**:785, 1981). In the current study, mice from genetic crosses with F/St were evaluated for expression of ecotropic and xenotropic viruses. The high ecotropic MuLV phenotype is shown to be governed by multiple independently assorting V loci which are dominantly expressed in all crosses. However, the high xenotropic virus phenotype of F/St behaved as a recessive trait in all F_1 hybrids tested.

MATERIALS AND METHODS

Abbreviations. Abbreviations used in this paper include: MiLu, mink lung cells; MuLV, murine leukemia virus; V loci, virus-inducing loci; and XenCSA, xenotropic MuLV envelope-related cell surface antigens.

Mice. F/St mice were obtained from the Laboratory Animals Centre of the Medical Research Council, Carshalton, Surrey, England. The mice are now bred in our laboratory and the laboratories of Michael Potter (National Cancer Institute) and Carl Hansen (Division of Research Resources, National Institutes of Health, Bethesda, Md.). AKR/J, BALB/cJ, C57BL/6J, SWR/J, C57L/J, and SEA/GnJ mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. NFS/N mice were from the colonies of the National Institutes of Health.

Virus assays. Suspensions of lymphocytes from spleen, thymus, bone marrow, and lymph nodes were tested by infectious center assays of mitomycin C-treated (25 μg/ml for 30 min at 37°C) cells, as previously described (6, 11). Cells producing ecotropic virus were detected by the XC plaque assay in SC-1 cells; titers were expressed as PFU per 10^7 lymphoid cells. Xenotropic virus-producing cells were assayed by focus induction in S^+L^- mink lung cells (15) or by MuLV fluorescent-antibody focus-induction tests in mink lung cells (MiLu), ATCC CCL64; titers are expressed as focus-forming units per 10^7 lymphoid cells. Tissue culture and virus assay procedures have been described in detail previously (6, 11).

Tests for spontaneous production of ecotropic virus in tail tissue were performed by XC plaque assays of tissue cultures established from tail biopsies (9) or of SC-1 cells infected with tissue extracts.

Tests for induction of xenotropic virus from tail tissue cultures were performed as previously described (8). Briefly, tissue cultures prepared from tail biopsies from 4- to 7-week-old mice were treated with 20 μg of 5-iododeoxyuridine per ml for 48 h and cocultivated with 2×10^5 MiLu cells. Harvests of culture fluid and cells were prepared at 12 to 14 days and tested by S^+L^- focus assay and fluorescent-antibody antigen induction in MiLu cells.

Mouse embryo cultures were used to type for alleles at *Fv-1* as previously described (4, 16). F/St was found to carry the *nr* allele at this locus.

Assays for XenCSA. Antigens cross-reactive with the gp70s of xenotropic MuLV, termed XenCSA, have been shown to be normal constituents of the cell surfaces of lymphocytes from all strains of mice (1, 11). XenCSA are detected by antibodies in sera from a rabbit immunized with SIRC (rabbit) cells infected with NZB xenotropic MuLV. These antibodies also

react with MCF gp70s and, to a much lesser extent, with ecotropic gp70s (11). Quantitative assays of XenCSA levels on lymphocytes were performed by flow microfluorometry with fluorescein-labeled rabbit antibody and a FACS II (Becton Dickinson FACS Systems, Mountain View, Calif.) instrument with methods previously described in detail (1, 11). Since levels of fluorescence did not vary extensively within each population of lymphocytes, mean fluorescence values (11) are given for each population.

Assays for isoenzymes and alloantigens. Extracts prepared from kidneys or erythrocytes and urine samples were assayed in starch gels or cellulose acetate for expression of allelic products at the following loci: *Pep-3* (chromosome 1), *Idh-1* (chromosome 1), *Mod-1* (chromosome 9), *Es-1* (chromosome 8), *Pgm-1* (chromosome 5), *Mup-1* (chromosome 4), *Es-3* (chromosome 11), *Hbb* (chromosome 7), *Gpi-1* (chromosome 7), and *Gpd-1* (chromosome 4). Assay methods for the first nine of these markers have been described (14). *Gpd-1* products were assayed by a modification of the procedure in reference 14. The product of the *a* allele of *Ly9* expressed on lymphocytes was assayed by flow microfluorimetry using a fluorescein-labeled hybridoma specific for Lgp100 (10), which is now called Ly9.1 (3). F/St has been typed as $Pep\text{-}3^b$, $Idh\text{-}1^a$, $Mod\text{-}1^a$, $Es\text{-}1^b$, $Pgm\text{-}1^b$, $Gpi\text{-}1^a$, $Gpd\text{-}1^b$, $Mup\text{-}1^a$, and Hbb^s in our laboratory.

RESULTS

Changes in expression of infectious ecotropic MuLV with age. Thymocytes and spleen cells from F/St mice 1 to 20 weeks of age were tested in infectious center assays for expression of infectious ecotropic MuLV (Table 1). High levels of virus were detected in both tissues from all mice at all ages tested. The proportion of virus-producing cells was consistently higher in spleen than in thymus. The frequency of virus-positive cells from thymuses of 1-week-old mice was 30- to 100-fold less than in older mice, whereas spleen cell levels in young mice were only slightly lower than in adult mice. The frequency of virus-positive cells in lymph node and bone marrow was approximately the same as in spleen cells (data not shown). Host-range assays of viruses obtained from the spleens and thymuses of two mice showed that all four isolates were N-tropic.

Inheritance of ecotropic MuLV. High expression of ecotropic MuLV was detected in F_1 hybrids of F/St with AKR, BALB/c, C57BL/6, NFS, SWR, C57L, and C3H/Bi mice (Table 2). Male F_1 hybrids of NFS (an ecotropic virus-negative strain) and F/St were crossed with NFS females, and tail tissues from the progeny were tested for ecotropic MuLV. Assays for spontaneous virus production were performed on tail tissue cultures (21 mice) or on extracts from tail biopsies (68 mice). All tail cultures and 63 of 68 tail extracts were positive for ecotropic MuLV for a total of 84 of 89 mice (94.2%). The average virus titer in 2% tail extracts from virus-positive mice was approximately $10^{2.5}$, which is about 1.5 log lower than titers from tail extracts of AKR mice. These results indicate that the high ecotropic virus phenotype of F/St is determined by at least three or four independently assorting V loci.

Changes in expression of xenotropic MuLV

TABLE 1. Ontogeny of MuLV in thymocytes and spleen cells of F/St mice[a]

Age (wk)	Ecotropic MuLV (log_{10} PFU/10^7 cells)		Xenotropic MuLV					
			MiLu S^+L (log_{10} FFU/10^7 cells)		MiLu (log_{10} IFA FFU/10^7 cells)[b]		XenCSA (mean fluorescence)	
	Thymus	Spleen	Thymus	Spleen	Thymus	Spleen	Thymus	Spleen
1	1.9 [3/3]	4.1 [3/3]	1.3 [1/3]	0.0 [0/3]	1.6 [3/3]	1.4 [3/3]	59	200
2	2.7 [3/3]	5.4 [3/3]	2.6 [2/3]	0.8 [1/3]	3.2 [2/3]	2.4 [3/3]	79	245
4	3.6 (2.8–4.2) [6/6]	4.2 (3.6–5.2) [6/6]	3.5 (3–4.4) [5/6]	1.3 (0.4–2.2) [5/6]	>3.0 [4/4]	2.3 [6/6]	467	426
8	3.8 [2/2]	4.6 [2/2]	3.8 [2/2]	2.6 [2/2]	ND	ND	2,630	416
12	3.7 [2/2]	4.6 [2/2]	3.9 [2/2]	3.0 [2/2]	ND	ND	2,951	398
16	4.0 [2/2]	4.6 [2/2]	3.8 [2/2]	2.9 [2/2]	ND	ND	ND	ND
20	3.8 [4/4]	4.5 [4/4]	3.8 [3/3]	2.6 (1.3–3.6) [4/4]	3.9 [1/1]	2.6 [1/1]	2,454	630

[a] Figures for ecotropic MuLV, MiLu S^+L^- xenotropic MuLV, and XenCSA assays are mean values for two to six mice; range of virus titers is given in parentheses when it exceeded 1 log_{10}. Brackets indicate number of positive mice/total number of mice tested. FFU, Focus-forming units; ND, not done.

[b] Approximate values. IFA, Immunofluorescent antibody.

TABLE 2. Expression of ecotropic and xenotropic MuLV in F_1 mice[a]

Mouse strain	Ecotropic MuLV (log_{10} PFU/10^7 cells)		Xenotropic MuLV					
			MiLu S^+L^- (log_{10} FFU/10^7 cells)		MiLu (log_{10} IFA FFU/10^7 cells)		XenCSA (mean fluorescence)	
	Thymus	Spleen	Thymus	Spleen	Thymus	Spleen	Thymus	Spleen
F/St	3.8	4.6	3.8	2.6	≧4.0	≧2.0	2,951	398
(AKR × F)F_1	4.9	4.6	0	1.9	<0.9	2.0	145	200
(BALB/c × F)F_1	2.6	4.4	0	0	<0.9	1.0	42	78
(C57BL/6 × F)F_1	1.7	3.6	0	0	<0.9	1.0	89	105
(NFS × F)F_1	3.4	4.5	0	0.8	<0.9	1.9	50	166
(F × SWR)F_1	2.5	4.6	0	0	<0.9	2.0	59	155
(SWR × F)F_1	4.0	4.6	0	0	<0.9	1.4	65	162
(C57L × F)F_1	4.1	4.5	0	0	1.2	1.4	81	132
(C3H/Bi × F)F_1	3.7	4.5	0	1.5	1.0	2.6	72	170
(SEA × F)F_1	ND	ND	0	0	<0.9	1.0	65	162

[a] Figures indicate the mean value for two to six mice, 6 to 17 weeks of age. The range of ecotropic and xenotropic virus titers for individual animals of each cross was less than 1 log except for (NFS × F)F_1, in which thymus values for ecotropic virus ranged from $10^{2.8}$ to $10^{4.4}$ (six mice tested). See Table 1, footnote *a*, for abbreviations and other information.

with age. Three assays were employed to monitor the expression of xenotropic MuLV: (i) infectious center assays on mink S^+L^- cells; (ii) infectious center assays on mink lung cells; and (iii) flow microfluorimetry analyses of XenCSA expression on thymic and splenic lymphocytes (Table 1, Fig. 1).

The age-dependent changes in xenotropic MuLV from thymus and spleen registering in the S^+L^- assay (Table 1) differ from those of infectious ecotropic MuLV in several respects. First, virus levels were low in tissues of 1-week-old mice, but the proportions of virus-positive mice and the levels of virus detected in both tissues increased markedly in older mice. Second, "adult" levels of virus were not present in thymus until 4 weeks of age or in spleen until between 8 and 12 weeks of age. Finally, at all age points and for every mouse tested, higher levels of virus were detected in thymocytes than in spleen cells. The fluorescent-antibody assay for xenotropic MuLV tended to be slightly more sensitive than the S^+L^- assays of cells from younger mice; thymocytes and spleen cells from all animals except one 2-week-old mouse produced virus detectable by this assay. As with the S^+L^- assay, the frequency of thymocytes and spleen cells producing infectious virus increased with age.

Thymocytes and spleen cells of F/St mice were also assayed for their expression of XenCSA (Table 1, Fig. 1). Previous studies of NZB (1, 13) and other strains of mice (1, 11, 12) demonstrated that expression of this class of antigen on lymphocytes was not coordinate with expression of infectious xenotropic virus. It is therefore not surprising that changes with age in XenCSA expression on thymocytes and spleen cells differed from the patterns of virus expression revealed by infectious center assays. For mice 4 weeks of age or less, XenCSA levels on thymocytes were lower than or equal to the levels detected on spleen cells. Beyond this age, XenCSA levels on thymocytes were approximately 10-fold higher than on spleen cells. The difference in XenCSA levels on thymocytes and spleen cells of adult mice is of similar magnitude to the difference in the levels of infectious xenotropic MuLV produced by these tissues (Table 1). XenCSA levels on thymus in the range observed with F/St thymocytes have previously been seen only with AKR thymuses producing high levels of mink cell focus-inducing virus (5; H. Morse and J. Hartley, unpublished data).

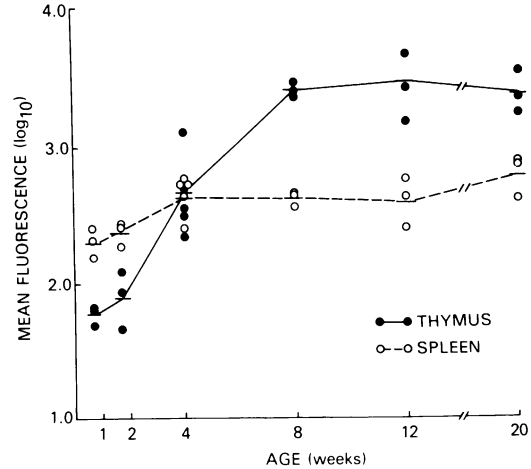

FIG. 1. Changes with age in XenCSA expression on thymocytes and spleen cells of F/St mice.

However, no mink cell focus-inducing MuLV has been detected in any assays of F/St tissues, including tests of two spontaneous thymic lymphomas.

Determinants of xenotropic MuLV expression. (i) regulation of endogenous xenotropic MuLV. F/St mice were bred to a number of inbred strains, and the F_1 hybrids were tested for expression of infectious xenotropic MuLV and XenCSA (Table 2). The inbred strains used in these crosses inclded those which are inducible (AKR, BALB/c, C57BL/6, C57L, C3H/Bi) and noninducible (NFS, SWR, SEA) for xenotropic MuLV. BALB/c, AKR, and C57L strains regularly exhibited spontaneous production of low levels of infectious xenotropic virus in lymphocytes of young mice; young C57BL/6 and C3H/Bi mice were not tested. Surprisingly, thymocytes from all the F_1 hybrids were almost completely suppressed for expression of infectious xenotropic virus. In the case of SWR F_1 mice, suppression of infectious xenotropic MuLV was observed if SWR was either the maternal or the paternal parent of the cross with F/St. Trace to low levels of infected spleen cells were detected in some animals. In spite of the low levels of spontaneous expression, induction of virus from F_1 spleen cells and tail cultures with 5-iododeoxyuridine was quite efficient (data not shown). XenCSA levels on F_1 thymocytes were reduced about 30-fold from the level of F/St thymocytes and were comparable to XenCSA levels determined for thymocytes of the second parents (11). XenCSA levels on F_1 spleen cells were also reduced in comparison to the levels of F/St spleen cells, but the suppression was less dramatic than that observed with F_1 versus F/St thymocytes.

To determine the genetic basis for the suppression of xenotropic virus expression observed in F_1 mice, (C57L × F)F_1 and (NFS × F)F_1 female mice were backcrossed to F/St mice, and their progeny were tested for expression of infectious xenotropic virus and XenCSA (Fig. 2). Evaluations of thymocytes from mice in both backcrosses revealed the expression of two

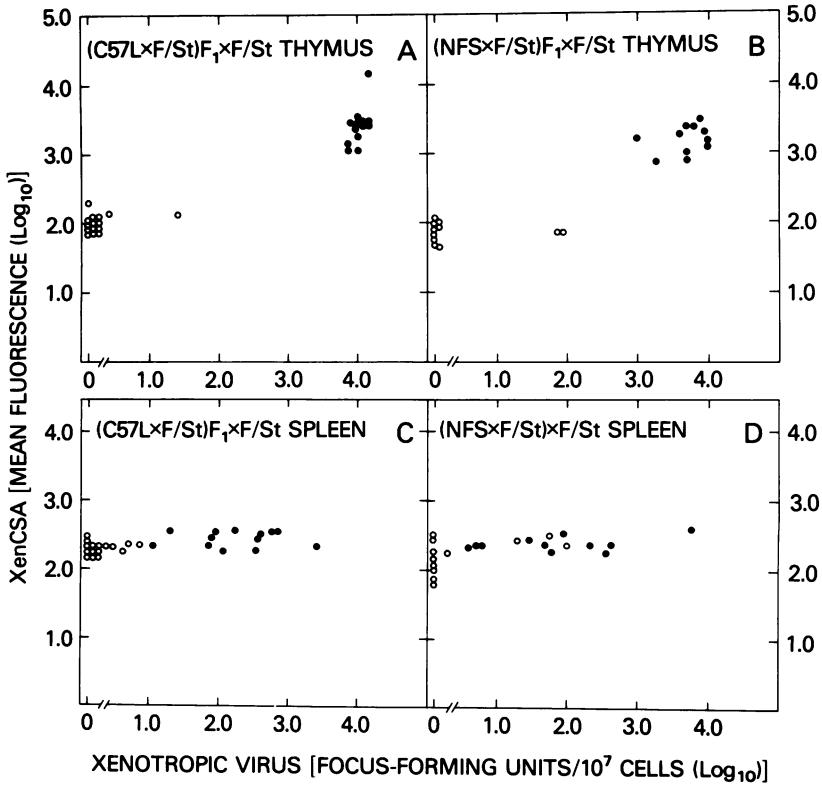

FIG. 2. Expression of infectious xenotropic virus and XenCSA by thymocytes (A and B) and spleen cells (C and D) of (C57L × F)F_1 × F/St mice (A, C) and (NFS × F)F_1 × F/St mice (B, D). For convenience in comparing spleen and thymus values in the same mice, (i) points for animals having the high virus-high XenCSA phenotype in thymus are shown as closed circles and the points for animals having the low virus-low XenCSA phenotype in thymus are shown as open circles, and (ii) XenCSA mean fluorescence values are given as $\log_{10}$.

distinct phenotypes with approximately equal numbers of mice being represented in both sets; thymocytes from 24 of 51 mice expressed high levels of S^+L^- virus and XenCSA, whereas thymocytes from the remaining 27 mice expressed low levels of both xenotropic MuLV determinants. In the cross with NFS, F/St is the only strain that could contribute to expression of infectious xenotropic MuLV. In the cross with C57L, both parental strains could produce infectious xenotropic MuLV, but the distribution patterns (50% high, 50% low xenotropic MuLV) suggest that a single locus controlled expression of infectious xenotropic MuLV from either locus. Similar results were obtained in studies of 28 backcross mice obtained from crosses between strains AKR and F/St (data not shown). These results indicate that a single gene controlled the expression of xenotropic virus and XenCSA.

The mice in these studies were also evaluated for segregation at loci on chromosome 1 (*Pep-3*, *Idh-1*, *Ly9*), chromosome 4 (*b*, *Gpd-1*), chromosome 5 (*Pgm-1*), chromosome 7 (*Gpi-1*), chromosome 8 (*Es-1*), chromosome 9 (*d*, *Mod-1*), and chromosome 14 (*s*). There were no correlations between alleles at these loci and the high and low phenotypes for xenotropic virus and XenCSA.

(ii) Inheritance of xenotropic MuLV. Previous studies demonstrated that inducibility of xenotropic MuLV for at least five inbred strains is governed by a single locus, termed *Bxv-1*, on chromosome 1. As the locus governing spontaneous expression of xenotropic MuLV in F/St is not linked to *Pep-3* or *Ly9*, it was of interest to determine whether an inducibility locus for xenotropic virus in F/St was located on this chromosome. To evaluate this possibility, first and second backcrosses of F/St to SEA, a xenotropic virus induction-negative strain, were tested for virus inducibility and alleles at *Pep-3* (Table 3). Xenotropic MuLV was detected in 64 of the 132 cultures, indicating that inducibility was governed by a single gene. Among these mice there were 13 dissociations between alleles at *Pep-3* and virus induction, giving an estimated map distance of 9.8 ± 3 centimorgans.

Tumors of F/St mice. A total of 81 mice have been followed for over 2 years for the development of tumors. Of these animals, 14 developed lymphomas, 7 of which were thymic in origin. Tumors developed between 12 and 28 months of age.

TABLE 3. Recombination between virus inducibility and *Pep-3* in first and second backcrosses between SEA and F/St mice

Mice	No. of animals	Inheritance of the allele of the induction-positive strain[a]	
		Virus induction	*Pep-3*
First backcross: SEA × (SEA × F)F_1			
Nonrecombinant	26	+	+
	34	−	−
Recombinant	2	+	−
	3	−	+
Second backcross: SEA × {SEA × [(SEA × F)F_1]}			
Nonrecombinant	32	+	+
	27	−	−
Recombinant	4	+	−
	4	−	+

[a] Percentage recombinant (± standard error): first backcross, 5/65 = 7.7 ± 3; second backcross, 8/67 = 11.9 ± 4; combined, 13/132 = 9.8 ± 3.

DISCUSSION

The results of the studies of F/St mice reported here are remarkable in several respects. First, F/St is the only inbred strain that produces high levels of infectious ecotropic as well as xenotropic MuLV (Table 1). Second, the high ecotropic virus phenotype of the strain is governed by three or more independently assorting loci. Third, the high xenotropic virus phenotype of these mice is almost completely recessive in all crosses examined. Finally, the chromosome 1 xenotropic V locus of F/St may not be allelic to that detected in other inbred strains.

Genetic analyses of other strains producing high levels of ecotropic MuLV have shown that the high virus phenotype of these strains is governed by from one (PL, SL) to three or possibly four independently assorting loci (C58; 17). Our determination of three or more independently assorting loci determining the high virus phenotype of F/St must be regarded as a minimum estimate, since (i) only a limited number of mice were tested and (ii) the few virus-negative mice identified were scored only for spontaneous expression; no attempt was made to identify the presence of additional loci with poor induction efficiencies. Furthermore, molecular hybridization techniques suggest that of all inbred mice, F/St has the greatest number of ecotropic MuLV integration sites. Southern blot hybridization of F/St cellular DNA reveals that at least 13 different restriction fragments carry sequences homologous to an ecotropic MuLV-specific genomic probe (M. D. Hoggan, personal communication). The presence of multiple V loci in high-virus mice is probably the result of germ line reintegrations of infectious virus (18).

The patterns of xenotropic MuLV expression

in tissues of F/St mice differ strikingly from the patterns observed in the only other strain which produces high levels of infectious xenotropic virus, NZB (1, 2, 13). First, the frequency of virus-producing cells in lymphoid tissues of NZB mice was shown to be bone marrow $\gg$ spleen > lymph node > thymus, whereas for F/St the order is thymus $\gg$ spleen = bone marrow and lymph node. Second, expression of xenotropic virus in tissues of mice in genetic crosses with F/St differs from that observed in similar crosses involving NZB mice. In the latter studies it was shown that two independently assorting dominant genes govern the expression of infectious xenotropic virus, with both V loci showing a high spontaneous rate of activation in NZB and its hybrids (1, 2); neither of these loci has been mapped. In the current study we demonstrated that F/St mice have a single induction locus on chromosome 1 and that spontaneous activation of virus (presumably from this locus) is extremely low, particularly in thymocytes, in mice from all F_1 hybrids tested (Table 2).

It should be noted that the chromosome 1 induction locus of F/St may not be allelic with *Bxv-1*, the xenotropic virus induction locus carried by at least five other inbred strains. The linkage estimate obtained from studies of the latter strains indicates that *Bxv-1* is 19.5 ± 2.2 centimorgans from *Pep-3* (7, 8; C. Kozak, unpublished data). By comparison, the current estimate for the distance of the F/St induction locus from *Pep-3* is 9.8 ± 3 centimorgans. By chi-squared analysis, the estimated map distances for *Bxv-1* and the F/St xenotropic V locus from *Pep-3* are significantly different at $P < 0.025$ ($\chi^2 = 7.47$). Further studies are in progress to define more precisely the F/St induction locus with respect to *Bxv-1* and to determine whether the high levels of virus detected in parental and backcross mice reflect expression of this or some other locus in F/St mice.

From the data in Fig. 2, it is evident that a single locus in at least two strains suppresses production of infectious xenotropic virus and XenCSA in hybrids with F/St mice. We do not know at present whether suppression of virus is determined by the same locus in all strains, nor do we know the location of these loci in the genomes of these strains. We have shown, however, that this locus in AKR is not at or near the xenotropic virus induction locus *Bxv-1*; 9 of 17 recombinants were detected between the locus governing virus expression and *Ly9*, which is in close proximity to *Bxv-1* (10; C. Kozak, B. J. Mathieson, and H. C. Morse, unpublished data).

The effect of the suppressive locus on virus expression by spleen cells is not as great as that observed with thymocytes (Fig. 2). Titers of infectious virus in spleen cells correlated well with titers of virus in thymocytes of mice in the cross with C57L but less well in the cross with NFS. By comparison, there were no significant differences among XenCSA levels on spleen cells from mice high or low for infectious virus in either cross. The basis for the differential effects of this locus on thymocytes and spleen cells is not known but may be related to the multiple cell types present in spleen as compared to those in thymus.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of Julia Chandler, Charles Shifler, and Nancy Wolford and the secretarial and editorial assistance of Virginia Shaw, Susan Grove, and the National Institute of Allergy and Infectious Diseases editorial staff. We thank Wallace P. Rowe for numerous helpful discussions.

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