EXPRESSION OF XENOTROPIC MURINE LEUKEMIA VIRUSES AS CELL-SURFACE gp70 IN GENETIC CROSSES BETWEEN STRAINS DBA/2 AND C57BL/6*

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Rabbit antisera raised against rabbit corneal (SIRC) cells infected with xenotropic murine leukemia virus (MuLV)[1] contain antibodies specific for antigens related to the major glycoproteins (gp70) of the xenotropic MuLV envelope (1). These antigens appear to be normal constituents of the cell surface of lymphocytes of all inbred mice and have been termed XenCSA-xenotropic MuLV envelope-related cell-surface antigens (1).

The amount of XenCSA expressed on lymphocytes from various inbred strains differs greatly. Some strains, such as NZB, NZW, and DBA/2, express high levels of XenCSA on both thymocytes and spleen cells whereas others, such as C57BL/6, NFS, and MA, express comparatively low levels of the antigen on both types of cells (1). To determine if quantitative expression of XenCSA is under genetic control, we have evaluated XenCSA levels in 24 recombinant inbred (RI) strains derived from crosses between DBA/2 and C57BL/6 (2-7) as well as backcrosses of F_1s to both parental strains. The results indicate that in this cross, *Fv-1* or a closely linked gene on chromosome 4 has a major influence on XenCSA levels.

Materials and Methods

Mice. The BXD RI strains were derived by brother-sister mating beginning with the F_2 generation obtained by crossing C57BL/6J (B6) and DBA/2J (D2). All strains had attained an expected degree of genetic fixation of 0.99 or greater when XenCSA testing was initiated. D2 and B6 mice were obtained from the Animal Resources colonies of The Jackson Laboratory, Bar Harbor, Maine. Breedings of reciprocal F_1s and reciprocal backcrosses of $B6D2F_1$ female by D2 and B6 male mice were performed at The Jackson Laboratory. All mice were from 6 to 16 wk of age when tested for XenCSA and production of infectious virus.

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[1] *Abbreviations used in this paper*: B or B6, C57BL/6; cM, centimorgans, D or D2, DBA/2; EMEM, Eagles' minimal essential medium; FA, fluorescent antibody; FCS, fetal calf serum; FMF, flow microfluorometry; MuLV, murine leukemia virus(es); Mup, major urinary protein; RI, recombinant inbred; S + L-, sarcoma virus positive, leukemia virus negative; XenCSA, xenotropic MuLV envelope-related cell-surface antigens.

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Typing for XenCSA. Single-cell suspensions were prepared from thymuses and spleens as previously described (1). Cells were incubated with fluorescein-labeled $F(ab')_2$ prepared from IgG purified from serum of rabbit 283 (1). Assays for fluorescence levels on lymphocytes were performed by flow microfluorometry (FMF) by using a FACS 1 or 11 (Becton Dickenson Electronics Laboratory, Mountain View, Calif.) as described in reference 1.

Typing of Chromosome 4 Markers. Mice were tested for hexose 6-phosphate dehydrogenase (*Gpd-1*) and major urinary protein (*Mup-1*) polymorphisms by established methods (6). Typing of the Friend virus-1 locus (*Fv-1*) was done by utilizing the standard UV-XC procedure (8).

Tissue Culture. Indicator cells for detection of xenotropic MuLVs were the S+L − mink lung cell line of Peebles (9) and mink lung cell line ATCC CCL64 (10) grown and maintained in the Dulbecco-Vogt modification of Eagle's minimal essential medium (EMEM). Indicator cells for detection of ecotropic MuLVs were SC-1 cells (11) grown in 10% heated fetal calf serum (FCS) in McCoy 5A medium; for virus assays in this cell line, the maintenance medium was 5% heated FCS in EMEM. Secondary mouse embryo cultures (ME) were grown and maintained in 10% heated FCS in EMEM.

Virus Assays. Suspensions of spleen cells were prepared for infectious center assays in tissue culture as previously described (1, 12). The methods employed in fluorescent antibody (FA) and S+L− infectious center assays of mitomycin C-treated (25 μg/ml, 30 min) lymphoid cells are detailed in references 1 and 12. The XC plaque assay of SC-1 cells cocultivated with lymphocytes was performed as described (12).

In all infectivity assays, recipient cultures were used as subconfluent cultures on the day after planting; the cells were pretreated with 25 μg of DEAE-dextran per ml for 1 h, followed by one wash with medium, or were infected in polybrene-containing (Abbott Laboratories, North Chicago, Ill. 16 μg/ml) medium.

Results

XenCSA Expression on Lymphocytes of BXD RI Lines. In earlier studies, we demonstrated that both thymocytes and spleen cells of D2 mice express high levels of XenCSA as detected by FMF by using fluorescein-labeled rabbit antibodies (1). By comparison, B6 mice express very low levels of this antigen on thymocytes and only slightly higher levels on spleen cells. The patterns of fluorescence observed on spleen cells of these two inbred strains are shown at the top of Fig. 1. Assays of $B6D2F_1$ and F_2 mice showed that XenCSA expression on cells from both lymphoid compartments was intermediate between parental levels in F_1s (see Fig. 1 for spleen cells) and that 25% of 20 F_2s (data not shown) had high XenCSA levels. These results suggested that XenCSA expression in this cross was strongly influenced by a single semidominant gene, and prompted our investigation of the BXD RI strains.

The patterns of fluorescence observed in FMF assays of these strains varied greatly from strain to strain but were consistent within any one strain. Some strains, exemplified by BXD RI strains 2 and 14 in Fig. 1, had patterns quite similar to those of the progenitor strains. Others, however, resembled the F_1 pattern but were shifted to the right or left indicating greater or lesser levels, respectively, of fluorescent staining. It was therefore not possible to classify the BXD RI strains as being D2- or B6-like on the basis of fluorescence profiles.

As an alternative approach to comparing XenCSA levels among strains, we have employed the mean fluorescence value as a measure of the total fluorescence exhibited by cells from a tissue (1). This value is obtained by summing the products of each channel number times the number of cells in that channel and dividing by the total number of cells counted.

Prior work indicated that mean fluorescence values obtained from repeated FMF assays of thymocytes or spleen cells from a single strain were lognormally distributed.

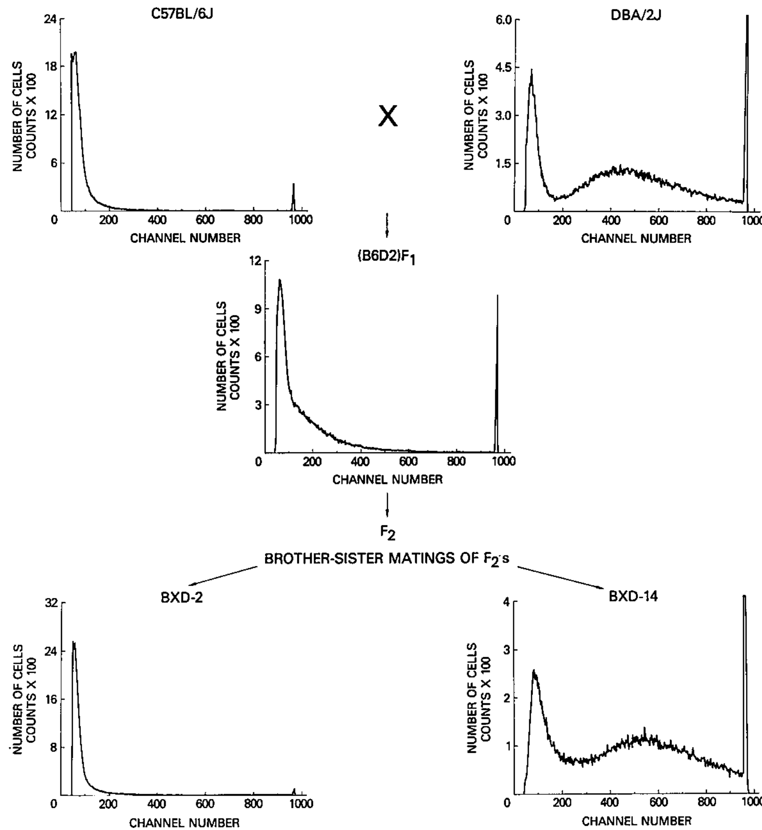

FIG. 1. Fluorescence-activated cell sorter (FACS) analyses of spleen cells from BXD RI lines. Fluorescence profiles of spleen cells from the BXD RI progenitor strains (DBA/2 and C57BL/6), $B6D2F_1$ mice, and two RI strains, BXD-2 and 14. Cells were incubated with fluorescein-labeled $F(ab')_2$ from R283 antibodies, washed, and analyzed by FMF by using the FACS.

This suggested first, that the geometric mean of these values should be used to characterize the level of antigen expression, and second, that geometric means could be used to compare the differences in total fluorescence detected on cells from various strains (1).

The results of FMF analyses of thymocytes and spleen cells from the 24 BXD RI strains are presented in Fig. 2 and Table I. The geometric means of the mean fluorescence values determined in multiple (6–10) assays of thymocytes and spleen cells of the progenitor strains are given on the far left side of the figure. The stippled areas indicate the range of ± 2 SD for each of the parental means. The geometric mean and the limits of two SDs are given for the mean fluorescence values assessed in tests of each RI strain. For each strain, except for BXD-20, 22 and 30, we tested at least two mice of both sexes; no male-female differences were detected in any strain.

XenCSA levels on thymocytes of the RI strains (Fig. 2a) present a spectrum of values from those in the lower range of B6 to a few exceeding the upper confidence limits for D2 values. FMF studies of spleen cells from these strains (Fig. 2b) yielded a similar range of values, but with several differences: first, for every RI strain, spleen cells had higher mean fluorescence values than thymocytes and the range of values

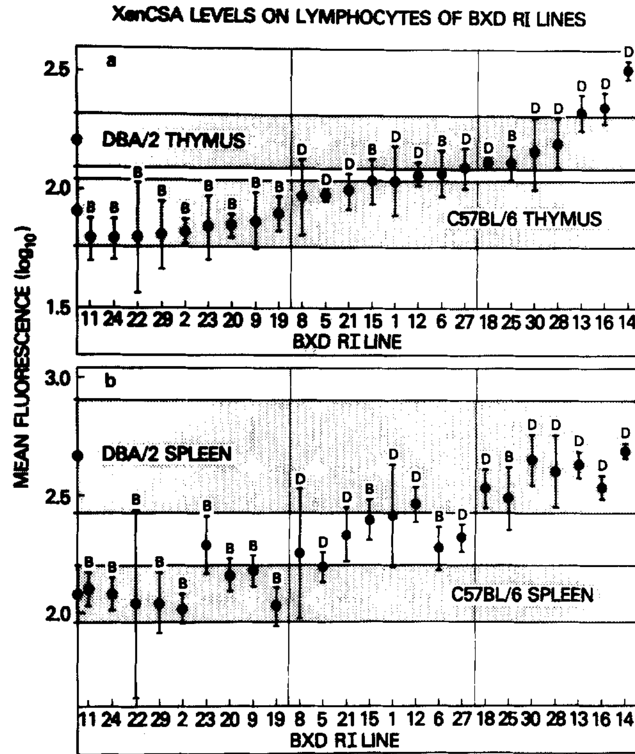

FIG. 2. FMF analyses of XenCSA levels on thymocytes (a) and spleen cells (b) from 24 BXD RI strains. Stippled areas indicate the geometric means ± 2 SD for mean fluorescence values obtained with cells from the parental strains, DBA/2 and C57BL/6. Points and bars indicate the geometric means ± 1 SD determined by repeated assays of cells from the BXD RI strains designated on the ordinate. The BXD RI strains are arranged from left to right, in the order of increasing XenCSA levels on thymocytes. The letters B and D above the fluorescence values for each tissue are used as generic symbols for alleles for *Fv-1* inherited from B6 and D2, respectively.

for spleen cells was higher than that for thymocytes; second, none of the values for spleen cells exceeded the range of confidence limits for D2 mice.

If, as suggested by the preliminary studies of F_1 and F_2 mice, a single gene determines the level of XenCSA expressed on lymphocytes of mice in crosses between B6 and D2, it would be expected that XenCSA levels detected in the BXD RI strains should fall into two distinct groups, those which are B6- and those which are D2-like. The continuum of XenCSA levels detected on thymocytes and spleen cells of the BXD RI strains was clearly not in keeping with this prediction. From Fig. 2, it appears that there are three rather than two groupings of strains: the first nine strains appear to be B6-like in having mean fluorescence values for thymocytes and spleen cells in the range of B6; the last seven strains appear to be D2-like in having values for both tissues in or above the range of D2; the remaining eight strains in the middle of the figure are not easily classified because mean fluorescence values for thymocytes and/or spleen cells lie between the ranges of the progenitor strains.

To facilitate further analyses of these strains, two criteria were employed to designate the fluorescence values of an individual tissue as being B6- or D2-like. First, tissues with the geometric mean fluorescence value in the range of one parental strain were classified as being like that strain; in Table I, these values are doubly underlined

TABLE I
XenCSA Levels and Chromosome 4 Markers of BXD RI Strains

BXD Strain	Gpd-1		Fv-1		XenCSA* Thymus	XenCSA* Spleen		b	No. of mice tested
1	b		n		111	261		b	6
2	a		b		67	104		+	6
5	b		n	(x)‡	96	156		+	5
6	a		b		118	189		+	7
8	b		n	(x)	95	180		+	4
9	a		b		74	151	(x)	b	4
11	a		b		62	126		+	6
12	b		n		114	291	(x)	+	6
13	b		n		216	430		+	5
14	b		n		328	493	(x)	+	5
15	a		b		109	250	(x)	b	6
16	b		n		227	345	(x)	+	5
18	b		n		131	340	(x)	+	6
19	a		b		80	107		+	5
20	a		b		71	143		+	2
21	b		n	[x]§	101	215	[x]	b	6
22	a		b		63	110		+	2
23	a		b		70	194		+	4
24	a		b		62	120	(x)	b	6
25	a		b	(x)	133	308		b	6
27	a	x‖	n		126	208		b	7
28	b		n		160	405		b	4
29	a		b		65	110		+	7
30	b		n		146	451		b	3
DBA/2	b		n		160	464		b	10
C57BL/6	a		b		80	120		+	7
$B6D2F_1$	a/b		b/n		102	167		+/b	9
$D2B6F_1$	b/a		n/b		67	121		b/+	9

* Figures indicate geometric mean fluorescence values obtained by repeated assays of each tissue. Means falling within the ranges of the progenitor strains are doubly underlined with solid lines for those in the range of D2 and dotted lines for those in the range of B6. Tissues with the majority of individual mean values in the range of the progenitor strains are singly underlined.

‡ (x), crossovers between *Fv-1* and *b* previously known to exist (7). Placement on either side of XenCSA values was made as suggested by the data.

§ [x], crossovers not known to exist between *Fv-1* and *b* (7) but suggested by the data.

‖ x, established crossover between *Fv-1* and *Gpd-1* (7).

with solid (D2) or dotted lines (B6). Second, tissues with the majority of individual mean fluorescence values in the range of one parental strain were defined as being like that strain; in Table I, tissues defined by this criterion only are singly underlined

with a solid (D2) or dotted (B6) line. To summarize, by using these two criteria, it was possible to classify both thymocytes and spleen cells of 20 of the 24 BXD RI strains and one of the tissues of the remaining four strains as being B6- or D2-like. Nonetheless, it is evident from the data presented in Fig. 1 and Table I that the strains designated as being like the progenitor strains do not form two homogeneous groups. This suggests that although one locus may have a predominant effect on XenCSA expression, other genetic (e.g. residual heterozygosity, mutation, additional genes) or nongenetic factors affect the final phenotype.

Assignment of a Gene Influencing XenCSA Expression to Chromosome 4. The BXD RI strains have been characterized for alleles at 50 loci on 14 of the 20 mouse chromosomes (2–7; B. A. Taylor et al., unpublished observations). The pattern of inheritance of the XenCSA phenotypes described above was found to exhibit a significant ($P < 0.001$) association with the pattern of inheritance of alleles at the *Fv-1* locus (Fig. 2, Table I). In Fig. 2, the letters B and D, placed above the fluorescence values for each strain, are used as generic symbols for the *Fv-1* alleles inherited from B6 ($Fv\text{-}1^b$) and D2 ($Fv\text{-}1^n$), respectively. In Table I, these alleles are listed along with those of two other chromosome 4 markers, *Gpd-1* and *b*. The location of previously documented crossovers among these loci is indicated by (x) including one recombinant between *Gpd-1* and *Fv-1* (BXD-27) and 10 recombinants between *Fv-1* and *b* (6).

These data strongly suggest that *Fv-1* or a closely linked gene exerts a major influence on XenCSA expression. From Fig. 2 it can be seen that all nine strains readily classified as B6-like carry the *Fv-1* allele of B6 and that six of the seven D2-like strains carry the D2 allele at this locus. This indication of a close association between $Fv\text{-}1^n$ and high expression of XenCSA and $Fv\text{-}1^b$ and low expression of the antigen was observed with 20 of the 24 RI strains (Table I; $P < 0.01$). A much lower association was detected between XenCSA phenotypes and alleles at the *b* locus (eight discrepancies) indicating tighter linkage with *Fv-1* than *b*. The data obtained with BXD-27 suggest that the locus affecting XenCSA expression is centromeric to *Gpd-1* and segregates with *Fv-1*; this strain carried the *Gpd-1* allele from B6, the *Fv-1* allele of D2 and is high for XenCSA expression (Table I).

There are four apparent dissociations between alleles at *Fv-1* and XenCSA phenotypes-BXD-5, 8, 21, and 25. In three strains, BXD-5, 8 and 25, crossovers between *Fv-1* and *b* were previously known to exist and the location of each of these crossovers has provisionally been indicated by an (x) between the *Fv-1* allele and the XenCSA phenotype. No crossovers were expected between *Fv-1* and *b* for the fourth strain, BXD-21. For a gene regulating XenCSA expression to be localized between *Fv-1* and *b* it would be necessary to postulate that BXD-21 is a double recombinant on chromosome 4. The fixation of a double recombinant within a region of 32 centimorgans (cM) is not unexpected in an RI strain since the two crossovers may have occurred in different generations. In Table I, the postulated locations of these crossovers are indicated by [x].

It should be noted that the gene order on chromosome 4 (*b-Fv-1-Gpd-1*) is different from that shown on current linkage maps (14) (*b-Gpd-1-Fv-1*), but is consistent with the gene order suggested by the *Fv-1-Gpd-1* recombinant mouse identified by Rowe and Sato (13), an *Fv-1 Gpd-1* recombinant defined in our backcross studies (see below), and that suggested by our analyses of BXD-27.

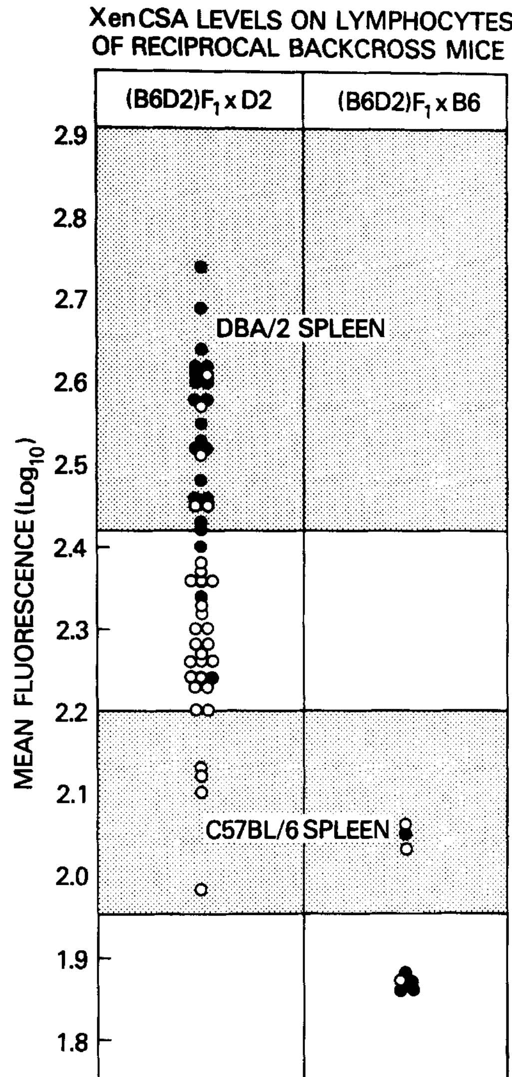

FIG. 3. FMF analyses of XenCSA levels on spleen cells from 62 progeny of crosses between B6D2F$_1$ and D2 (left) or B6 (right) mice. Stippled areas indicate the geometric means ± 2 SD for mean fluorescence values obtained with cells from the progenitor strains. Points indicate the mean fluorescence values for spleen cells from individual backcross mice. Solid circles indicate mice homozygous and open circles mice heterozygous at *Gpd-1*.

Evaluations of F$_1$s Backcrossed to D2 and B6. To confirm the linkage of a gene affecting XenCSA expression to *Fv-1* and *Gpd-1* suggested by the studies of BXD RI mice, we tested XenCSA levels on spleen cells from 62 progeny of crosses between B6D2F$_1$ mice and both parental strains (Fig. 3). The data presented in this figure represent the mean fluorescence values for spleen cells from each of the mice tested. As in Fig. 2, the stippled areas indicate the ranges of plus or minus two SD from the mean for D2 and B6 spleen cells.

In the backcross to D2, the mean fluorescence values form a near continuum from

low B6-like values to intermediate D2-like levels. 24 of the 54 mice had values in the range of D2, 6 were in the range of B6, and the rest lay between the ranges of the parental strains. In the backcross to B6, only eight mice were tested, but two distinct sets of values are evident: three within the range of B6, and five below this range.

All mice were also tested for alleles at the *Gpd-1* locus and 28 were tested for alleles of *Fv-1*. In Fig. 3, mice homozygous at the *Gpd-1* locus in both directions of the cross are indicated by solid circles and heterozygotes by open circles. As in the analyses of the BXD strains, there was a strong association between high expression of XenCSA and homozygosity for the $Gpd\text{-}1^b$ allele of D2 in the $B6D2F_1 \times$ D2 backcross mice. 28 of these mice were also tested for alleles of *Fv-1*. In all except one case, typing for *Fv-1* was consistent with the alleles determined for *Gpd-1*. This mouse typed $Gpd\text{-}1^a\text{-}Fv\text{-}1^n$-XenCSA-high-*b* which, as with BXD-27, emphasizes the close relationship between $Fv\text{-}1^n$ and high expression of XenCSA. One of the mice with XenCSA expression below the range of D2 which typed $Gpd\text{-}1^b\text{-}Fv\text{-}1^n$-XenCSA-low- + was progeny tested by mating with D2; all six progeny were high for XenCSA expression indicating that this mouse did not represent a dissociation between $Fv\text{-}1^n$ and high XenCSA phenotype. Two other mice with apparent discrepancies between *Fv-1* typing and XenCSA expression were sacrificed at the time of testing for XenCSA and were not available for further study.

In the B6 direction of this cross, two of three mice heterozygous at *Gpd-1* had higher XenCSA levels than four of the five homozygotes at this locus. These mice were not typed for *Fv-1* and the mice showing apparent dissociations between XenCSA phenotype and *Gpd-1* alleles were not available for progeny testing.

To summarize, the linkage studies derived from evaluations of the BXD RI strains and reciprocal backcross mice, it appears that a gene on chromosome 4 exerts a major influence on XenCSA levels. Without confirmation of the observations by progeny testing, there were four dissociations between *Fv-1* and XenCSA phenotype among the 24 BXD strains and two dissociations among 28 $B6D2F_1 \times$ D2 mice. If XenCSA expression is affected by a gene distinct from *Fv-1*, the estimated map distance between *Fv-1* and this gene was calculated to be 7 cM (15). We would stress at this point that until formal evidence of recombination between *Fv-1* and this putative locus is obtained, the locations of crossovers suggested by the data in Table I and indicated by apparent dissociations between *Fv-1* and XenCSA phenotype in the backcross studies should only be considered as tentative and not indicative of established gene order on chromosome 4.

As a final point, it is noteworthy that in the D2 direction of this cross, the majority of fluorescence values for presumptive heterozygotes at *Gpd-1* fall between the ranges for D2 and B6, whereas in the B6 direction of the cross, the heterozygotes group in the range of B6. This observation may be related to the finding (Table I) that reciprocal F_1 hybrids differ in their levels of XenCSA expression. As noted previously (Fig. 1), $B6D2F_1$ mice express XenCSA at levels intermediate between the parental strains. By comparison, XenCSA levels of $D2B6F_1$ lymphocytes are indistinguishable from those of B6.

Virus Production by BXD RI Strains. Two to four mice from all of the BXD RI strains except line 20, both progenitor strains and $B6D2F_1$ mice, were tested by focus-forming assays of spleen cells for spontaneous production of ecotropic and xenotropic MuLV (Table II). Ecotropic virus was assayed by XC focus formation on SC-1 cells

TABLE II

*Infectious Center Assays of Spontaneous MuLV Production by Spleen Cells of BXD RI Strains**

BXD RI Strain	No. tested	Ecotropic MuLV			Xenotropic MuLV			
					S+L−		FA	
		No. positive	XC Foci/10^7 cells	Tropism	No. positive	Foci/10^7 cells	No. positive	Foci/coverslip
1	3	2/3	8–6,000	N	0/3	0	2/3	+++
2	2	0/3	0	NA‡	1/2	2	1/1	++++
5	2	0/2	0	NA	2/2	5–6	2/2	++++
6	2	0/2	0	NA	2/2	2–4	1/1	++++
8	2	0/2	0	NA	0/2	0	2/2	+
9	2	1/2	2,000	B	0/2	0	2/2	+++
11	3	0/3	0	NA	0/3	0	3/3	++
12	3	2/3	64–84	N	2/3	1–2	2/3	++
13	3	0/3	0	NA	0/3	0	2/3	+
14	3	0/2	0	NA	3/3	67–105	1/1	++++
15	2	0/2	0	NA	1/2	8	1/1	++
16	2	1/2	2	ND	2/2	20–105	2/2	++++
18	2	2/2	300–5,000	N	0/2	0	2/2	++
19	2	0/2	0	NA	0/2	0	1/2	+
21	2	0/2	0	NA	2/2	1	1/1	++
22	2	0/2	0	NA	1/2	1	(2/2)	(+)
23	2	0/2	0	NA	2/2	1–3	2/2	+++
24	3	0/3	0	NA	0/3	0	3/3	+
25	4	2/4	2–4,000	N;B	2/4	1–2	4/4	++
27	2	0/2	0	NA	0/2	0	2/2	++
28	2	0/2	0	NA	2/2	3–4	2/2	+
29	3	2/3	>4,000	B	2/2	1	2/2	++
30	2	2/2	100–3,000	N	1/2	1	2/2	+
DBA/2J	10	1/10	200	ND	3/10	1–2	8/8	+++
C57BL/6J	9	1/9	125	N;B	0/9	0	7/9	++++
$B6D2F_1$	10	8/10	6–2,000	N	2/10	1–6	3/3	+++

* Assays for MuLV were performed as described in Materials and Methods. Numbers under foci indicate the range of values observed in individual assays of spleen cells from each strain.

‡ NA, not applicable; ND, not done.

cocultivated with spleen cells. Almost all isolates of ecotropic virus were tested to determine if they were N- or B-tropic. Spontaneous production of xenotropic MuLV was assessed in two systems; focus formation in mink S+L− (S+L−), and formation of foci (FA) detectable in mink lung cells with fluorescein-labeled anti-Moloney MuLV. In the latter assay, spleen cells from most strains produced very few foci on the primary (P_0) coverslips. However, after one passage in vitro (P_1) of the cultures, almost all coverslips had variable numbers of foci. Differences in the numbers of FA foci detected in P_0 as compared to P_1 cultures varied considerably. For instance, one culture with 1 FA focus in P_0 had more than 200 foci in the P_1 culture; by comparison, another culture with 1 focus in P_0 had only 1 focus observed in P_1. The basis for these apparent differences in the ability of these viruses to spread is unknown. With these considerations in mind, we have elected to report the results from P_1 cultures as levels

of FA virus production by the RI strains. The data from all three assay systems are presented in Table II.

Ecotropic MuLV was produced by 1 of 10 D2 spleens, 8 of 9 F_1 spleens and 1 of the 9 B6 spleens tested. 7 of the 23 BXD strains also spontaneously produced ecotropic MuLV. Four of the virus-producing strains were $Fv\text{-}1^n$ and three were $Fv\text{-}1^b$. All of the virus isolates from the $Fv\text{-}1^n$ lines were N-tropic. One of the two isolates from BXD-25 ($Fv\text{-}1^b$) was B-tropic and the other was N-tropic. The other $Fv\text{-}1^b$ BXD strains expressing ecotropic MuLV (BXD-9 and 29) produced B-tropic virus. There was no constant relationship between XenCSA phenotype or alleles of *Fv-1* and production of ecotropic MuLV in that 5 of 9 XenCSA-high; 2 of 14 XenCSA-low; 3 of 11 $Fv\text{-}1^b$, and 4 of 12 $Fv\text{-}1^n$ strains yielded virus from spleen cells.

Xenotropic MuLV registering in the S+L− assay was detected in some D2 and F_1 but not B6 spleen cells (Table II). 14 of the BXD RI strains produced virus detectable by this assay. 12 of these 14 strains produced low levels of virus in the range of D2 and F_1 mice. However, two strains (BXD-14, 16) produced more virus detectable by this assay than either parental strain or any inbred strain tested except NZB or NZW (2). Again, there was no definite correlation between alleles at *Fv-1* or XenCSA phenotype and virus production; 7 of 12 $Fv\text{-}1^n$; 6 of 11 $Fv\text{-}1^b$; 6 of 9 XenCSA-high, and 8 of 15 XenCSA-low strains produced virus.

Xenotropic MuLV registering in the FA assay of P_1 cultures was detected in spleen from both parental strains, F_1s and all the BXD RI strains except for strain 22. For this strain, FA foci were detected in P_2 cultures and the level of virus production is given in parentheses. The levels of virus produced varied considerably among the strains. For purposes of comparison, the strains can be divided into four groups (+ to + + + +) based on the maximal numbers of FA foci in P_1 cultures: group 1 (+)-less than 100 foci per coverslip (BXD-8, 13, 19, 22, 24, 28, 30); group 2 (+ +)-5% or less foci per coverslip (BXD-12, 18, 21, 29); group 3 (+ + +)-50% or less foci per coverslip (BXD-1, 9, 23); and group 4 (+ + + +)-greater than 50% of cells per coverslip having foci (BXD-2, 5, 14, 16). In each of these groups, there is an approximately equal representation of alleles at the *Fv-1* locus and high and low XenCSA phenotypes.

Virus Production by B6D2F$_1$ × *D2 Mice.* Spleens of 27 mice in the B6D2F_1 × D2 backcross were tested for spontaneous production of ecotropic and xenotropic MuLV. Ecotropic virus was detected in cultures of spleen cells from five mice. Three were homozygous $Fv\text{-}1^{n/n}$ and two heterozygous $Fv\text{-}1^{n/b}$, three were XenCSA-high and two were low.

Spleens from seven mice produced xenotropic MuLV detectable in the S+L− assay. Five were homozygous $Fv\text{-}1^{n/n}$, two were $Fv\text{-}1^{n/b}$; five were typed as XenCSA-high, and two as XenCSA-low. As in the studies of the BXD RI strains, neither the *Fv-1* locus or XenCSA phenotype appeared to be related to spontaneous production by spleen cells of ecotropic MuLV or xenotropic MuLV detectable in the S+L− assay.

Xenotropic MuLV registering in the FA assay was detected in P_0 or P_1 cultures from all but two spleens. Employing the criteria noted above to classify individual mice as being + to + + + + for FA virus production, 13 were +, 7 were + +, 3 were + + +, and 4 were + + + +. Surprisingly, 10 of the 13 + mice were $Fv\text{-}1^{n/b}$ whereas only 4 of the 14 mice classified as + + to + + + + were typed as heterozygotes at this locus. ($\chi^2 = 4.7$; $P < 0.05$). Similarly, 9 of 13 + mice were XenCSA-low,

and 11 of the 14 + + to + + + + mice were XenCSA-high. These results, in contrast to those obtained with the BXD RI strains, suggest a relationship between high production of FA virus and expression of the n allele of *Fv-1* and high XenCSA expression.

Discussion

The results of this study demonstrate that expression of XenCSA on lymphocytes of mice derived from crosses between B6 and D2 is probably under multigenic control but that the major influence on XenCSA levels in these mice is exerted by a single semidominant gene. Based on segregation data gathered from analyses of BXD-RI strains (Figs. 1 and 2; Table I) and reciprocal backcrosses of B6D2 F_1 mice to the parental strains (Fig. 3), this gene was located on chromosome 4 at or in close proximity to the *Fv-1* locus.

These findings invite a number of interrelated questions. First, is this gene the (or a) structural gene coding for XenCSA, or one governing the extent to which xenotropic MuLV genomes are expressed? From available evidence, it seems most reasonable to suggest that the gene is regulatory. This view is based on the observations (*a*) that xenotropic MuLV genomes are probably present in all mice (16) and (*b*) that both parental strains in these crosses spontaneously produce infectious xenotropic MuLV and XenCSA but to different extents (Figs. 1 and 2, Table II). The demonstration that spontaneous production of xenotropic MuLV in reciprocal backcross mice correlates with the XenCSA phenotype suggests that this gene may affect expression of the entire xenotropic MuLV genome(s) rather than only that portion of the genome(s) coding for gp70. Further work is clearly required to clarify this issue, particularly in view of the observation that spontaneous production of xenotropic MuLV by the BXD RI strains does not correlate with high or low XenCSA phenotypes (Table II).

Second, is the gene regulating XenCSA expression truly linked to *Fv-1*? The alternative points of view regarding this question include the following possibilities: (*a*) *Fv-1* itself and not a separate gene regulates expression of XenCSA. This possibility remains open because conclusive evidence for recombination between *Fv-1* and the locus affecting XenCSA levels has not been established. In addition, although *Fv-1* is thought of primarily in terms of its effect on expression of ecotropic viruses, studies by Blank and Lilly of crosses between RF and AKR mice suggest that late expression of infectious xenotropic MuLV by AKR is restricted by the $Fv\text{-}1^{nr}$ allele of RF (17). Finally, XenCSA levels on lymphocytes of D2 mice partially congenic for the $Fv\text{-}1^{b}$ locus of BALB/c (D2.RS—kindly provided by Dr. F. Lilly, Albert Einstein College of Medicine, New York) were only 30–40% that of normal D2 mice. These results are in keeping with the backcross studies in this report indicating that a single locus affects both infectious xenotropic MuLV and XenCSA levels. (*b*) A gene closely linked to *Fv-1* has an effect on xenotropic MuLV similar to that of *Fv-1* on ecotropic viruses. There are multiple examples in the mouse of tightly linked genes with similar functions. One example, the *Gpd-1* and *Pgd* loci on chromosome 4 in proximity to *Fv-1*, suggests that tandem gene duplication has occurred in this region (6). In some instances such genes are very closely linked. For example recombination between *Car-1* and *Car-2* on chromosome 3 has never been detected (18). A similarly tight linkage between *Fv-1* and a putative second gene affecting XenCSA expression would render fruitless efforts

to dissociate these loci in further backcross studies; and (*c*) the localization of a gene affecting XenCSA expression to chromosome 4 reflects quasilinkage rather than true linkage. This possibility deserves exploration in view of the studies of Ikeda et al. and Stockert et al. on segregation of *Fv-1* and another marker for a subset of gp70 molecules, G_{IX}. The salient features of their investigations are as follows. In crosses between G_{IX}-positive and G_{IX}-negative strains which differed at alleles of *Fv-1*, one of the two genes governing G_{IX} expression, *Gv-1*, was found to be linked to *Gpd-1* (19). However, when linkage with *Gpd-1* was assessed in crosses between strains bearing the same allele at *Fv-1*, there was no linkage of *Gv-1* and *Gpd-1* (20).

The basis for this phenomenon has been ascribed to quasilinkage, a term used to describe recombination data from linkage tests in which two phenotypes do not assort independently, as Mendel's second law would require, even though the relevant genes are located on different chromosomes and therefore cannot be truly linked (20, 21). The same explanation might be offered for the current demonstration of apparent linkage between *Fv-1* and a gene affecting gp70 expression in crosses between the *Fv-1* disparate strains B6 and D2. As a current point of comparison, it should be noted that the apparent map distances simulated by quasilinkage, 19 for the *Gv-1*:*Gpd-1* association (19) and 35 for a *Gv-1*:*H-2* association (22), are significantly greater than the estimate of 7 cM for the interval between *Fv-1* and the locus affecting XenCSA levels suggested by our study. For this reason, we regard quasilinkage as an unlikely explanation of the association between XenCSA levels and *Fv-1*.

Finally, although the question is probably premature, it seems worthwhile asking how many genes might affect XenCSA expression. From the results of this study, it is apparent that more than one factor (or gene) influences XenCSA phenotypes. This view is drawn primarily from the observation of the continuum of XenCSA levels expressed on lymphocytes of the BXD RI strains (Fig. 2) rather than two distinct groupings of levels which would be expected if only one gene were important.

A second influence on XenCSA expression is also suggested by two separate yet potentially interrelated observations: (*a*) differences in XenCSA levels on lymphocytes of reciprocal hybrid mice (Table I); and (*b*) the failure of the single mouse from the backcross studies to score appropriately on progeny testing. Although these results might reflect technical problems, the same explanation could be offered for both findings, i.e., suppression of phenotype due to antigenic modulation by maternally transferred antibodies. This possibility was previously raised (and dealt with in more detail) in studies demonstrating low penetrance of G_{IX} in genetic crosses between strains AKR and C57L (23) and is strengthened by the observation of spontaneous production of antibodies against G_{IX} (24) and XenCSA (J. Longstreth, T. Chused, and H. Morse, unpublished observations) by some strains of mice.

Studies of xenotropic MuLV expression in genetic crosses between NZB and SWR (25, 26) or NFS (T. Chused, H. Morse, and J. Hartley, unpublished observations) have demonstrated that two loci can independently affect spontaneous release of virus from adult spleen cells—these loci have not been mapped. In addition, other investigations have recently established linkage on chromosome 1 for genes governing iododeoxyuridine-inducibility of xenotropic MuLV in strains C57BL/10J and BALB/cAnN (16). It is not known if any of these loci are related to the major gene affecting XenCSA levels or other genes, suggested by this study, which influence XenCSA expression in crosses between B6 and D2 mice.

To summarize this discussion, our results define a semidominant gene, which exerts the predominant influence on XenCSA expression in crosses between B6 and D2 mice. Further studies are clearly required to distinguish between (*a*) quasilinkage or true linkage to chromosome 4 markers, and (*b*) pleiotropic effects of the *Fv-1* locus or the effects of an independent gene. Investigations designed to answer these questions should enhance our understanding of these viruses with regard to their role in generation of recombinant viruses (27, 28), their importance in tumorigenesis (12, 27) and their participation in development of autoimmune diseases (24, 29, 30).

Summary

Flow microfluorometry was used to assess levels of xenotropic murine leukemia virus envelope-related cell-surface antigens (XenCSA) expressed on lymphocytes of mice derived from crosses between C57BL/6 (B6) and DBA/2 (D2); 24 recombinant inbred strains (BXD RIs) and 62 backcross mice were studied. The results suggest that XenCSA expression is affected by more than one gene but that the predominant influence is exerted by a single semidominant gene apparently located on chromosome 4 at or in close proximity to the *Fv-1* locus. Studies of spontaneous virus production in $B6D2F_1 \times D2$ mice suggest that this locus may also affect production by spleen cells of xenotropic MuLV registering in a fluorescent antibody assay of mink lung cells.

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