Proc. Natl. Acad. Sci. USA
Vol. 75, No. 9, pp. 4553–4557, September 1978
Microbiology

Serological characterization of B-tropic viruses of C57BL mice: Possible origin by recombination of endogenous N-tropic and xenotropic viruses

(N-, B-tropism/C type RNA viruses/recombination/competition radioimmunoassays)

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Communicated by Henry S. Kaplan, June 23, 1978

ABSTRACT The serological properties of the *gag* gene products p15 and p12 of N- and B-tropic viruses of C57BL mice have been examined. Although these viruses were serologically identical by competition assays for proteins gp71 and p30, they were readily distinguishable in competition assays for proteins p15 and p12. Two isolates of N-tropic viruses had p12s serologically indistinguishable from AKR murine leukemia virus p12, while two B-tropic isolates had distinctly different p12s. The latter p12s were serologically indistinguishable from the p12 purified from the B-tropic radiation leukemia virus (RadLV)-/VL-3. Moreover, this p12 was indistinguishable from the p12 of the endogenous C57BL/Ka xenotropic virus. Similarly, the p15s of the B-tropic viruses were serologically distinct from the AKR murine leukemia virus type of p15, as was the p15 of one C57BL N-tropic virus, while another N-tropic isolate had a p15 identical to the AKR murine leukemia virus p15. These results are interpreted to suggest that the endogenous N-tropic virus of C57BL mice undergoes recombination with the endogenous, xenotropic virus and that this mechanism is involved in the generation of B-tropic viruses in C57BL mice.

Various strains of mice have been shown to genetically transmit ecotropic C type viruses (1–4). Although the site of virus integration may vary among mouse strains (2, 4), these viruses are very similar by hybridization and serological assays (5, 6). However, among the ecotropic viruses isolated from mice, two classes, N- and B-tropic, can be readily distinguished by host range characterization (7, 8). The relative restriction for replication on various mouse cells has been shown to be due to a single gene, *Fv-1* (9, 10), although the mechanism of restriction is not known. The isolation of B-tropic viruses from mice has been somewhat of an enigma, because in strains such as C57BL/6 and BALB/c there is only a single viral locus that is thought to code for an inducible N-tropic virus (4, 6). Only one instance of an inducible B-tropic virus has been detected (in a substrain of B10.BR mice), and this B-tropic locus appears to have been obtained by germ-line integration of a maternally transmitted virus (B. Moll, J. Hartley, and W. Rowe, personal communication).

In spite of the lack of evidence for genetic transmission of B-tropic viruses in most strains, many tumors in strains such as C57BL/6 actively replicate B-tropic virus (11–13). This observation is consistent with the ability of the *Fv-1* gene to actively suppress the infectious amplification of N-tropic virus expression *in vivo*. For this reason, the origin of B-tropic viruses in such mice and, moreover, in all *Fv-1* strains capable of restricting the replication of the endogenous N-tropic virus is of considerable interest. Previous studies have shown that differences in the virion proteins p15, p30, and gp70 are detectable by polyacrylamide gel electrophoresis (14, 15). An analysis of a variety of potential recombinant BALB/c viruses suggested that the N-, B-tropism was linked to the p30 differences. Similarly, we have previously shown that in C57BL/Ka mice the N- and B-tropic viruses can be distinguished on the basis of serological differences in the viral p12 protein (16). In the present experiments we have extended these observations and show that certain B-tropic viruses from both C57BL/6 and C57BL/Ka strains of mice have *gag* gene products similar to those of the endogenous, xenotropic virus. These data, in turn, suggest that the B-tropic viruses of C57BL mice arise by recombination between the endogenous ecotropic and xenotropic viruses.

MATERIALS AND METHODS

Viruses and Cells. WN1802N and WN1802B (N- and B-tropic viruses isolated from the spleen of a BALB/c mouse), as well as B6-7(N) and B6-7(B) (N- and B-tropic viruses isolated from a C57BL/6 mouse), were obtained from Janet Hartley (National Institutes of Health). The isolation of BL/Ka(N), BL/Ka(B), and BL/Ka(X) (N-tropic, B-tropic, and xenotropic, respectively) viruses from C57BL/Ka mice has been described (17). SC-1 wild-mouse cells (18) productively infected with these viruses were used as sources of virus for purification by banding in sucrose density gradients. AKR murine leukemia virus (MuLV) was obtained from an established line of AKR mouse embryo cells that had spontaneously initiated virus synthesis. Radiation leukemia virus (RadLV)/VL-3 was obtained from the BL-VL-3 line of lymphoma cells established from RadLV-induced lymphomas in C57BL/Ka mice (19). BALB-virus:2 (a xenotropic virus of the α or inducible subclass), and AT-124 (a xenotropic virus of the β or uninducible subclass) were obtained from productively infected mink lung cells. Cells were grown in Eagle's minimum essential medium, McCoy's 5A, or RPMI 1640 supplemented with 10% fetal calf serum.

Immunofluorescence and XC Plaque Assays. "Hitness" determinations and end-point titrations of viruses on mouse NIH-3T3 cells (*Fv-1*^{nn}), C57BL/Ka cells (*Fv-1*^{bb}), and (BL/Ka × NIH) F_1 cells (*Fv-1*^{nb}) were carried out by the MuLV immunofluorescence assay as described by Declève *et al.* (20). Hitness and end-point titer were also ascertained by the XC plaque assay (21), using NIH-3T3 cells and BALB/c embryo fibroblasts (*Fv-1*^{bb}).

Radioimmunoassays. Double-antibody competition radioimmunoassays for viral structural proteins have been described (18, 22). Purified proteins were labeled with ^{125}I by the chloramine-T procedure (23). The assays for AKR-MuLV gp71,

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Abbreviations: RadLV, radiation leukemia virus(es); MuLV, murine leukemia virus(es).

p30, p15, and p12 used monospecific antisera raised in rabbits against these proteins. The assay for RadLV/VL-3 p12 utilized a rabbit antiserum prepared against disrupted RadLV/VL-3 virus (19). The second antibody used in each assay was raised in goats against rabbit immunoglobulin. Briefly, 2- or 3-fold dilutions of the respective disrupted viruses were incubated with 2–4 × 10^4 cpm of ^{125}I-labeled protein and limiting dilutions of the homologous rabbit antiserum. Immunoprecipitates were collected by centrifugation following addition of the antiglobulin. In each case, the limiting dilution of rabbit antiserum to be used (that which precipitated 50% of the respective labeled antigen) was determined by previous titration.

RESULTS

To examine the serological properties of N- and B-tropic viruses in C57BL mice, two independent sets of virus isolates were examined. One set of N- and B-tropic viruses was isolated from C57BL/6 mice and kindly provided to us by J. Hartley. The second set of N- and B-tropic viruses was isolated from C57BL/Ka mice and each was purified by limiting dilution techniques (16). The replication kinetics and end-point titers of the C57BL/Ka viruses in fibroblasts are shown in Fig. 1 and Table 1, respectively. As expected, the B-tropic BL/Ka(B) virus readily infects C57BL/Ka fibroblasts (*Fv-1*^{bb}) and displays one-hit kinetics (24) as determined by the immunofluorescence assay. Conversely, this virus is much less infectious on NIH-3T3 cells (*Fv-1*^{nn}) and (BL/Ka × NIH)F_1 cells(*Fv-1*^{nb}), showing a two-hit kinetic pattern (24) (Fig. 1*B*). The N-tropic BL/Ka(N) virus is considerably more infectious on NIH-3T3 cells than on C57BL/Ka or (BL/Ka × NIH)F_1 fibroblasts and shows one-hit kinetics on the former and two-hit kinetics on the latter cell

Table 1. End-point titers for BL/Ka(N) and BL/Ka(B) in fibroblasts

Cell line	Virus titer* BL/Ka(N)	BL/Ka(B)
NIH-3T3 (*Fv-1*^{nn})	7.5 × 10^7	1 × 10^1
C57BL/Ka (*Fv-1*^{bb})	2.5 × 10^3	2.5 × 10^6
(BL/Ka × NIH)F_1(*Fv-1*^{nb})	2.5 × 10^3	2.5 × 10^2
Mink cells	0	0

* End-point titers were determined for each virus by immunofluorescence assay four passages after infection. The numbers given represent infectious particles per ml.

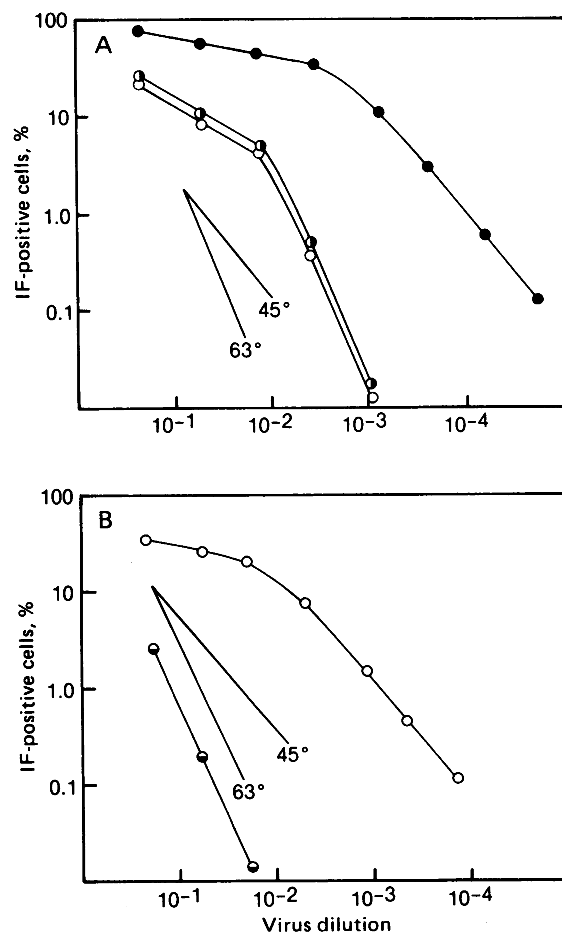

FIG. 1. (*A*) Immunofluorescence (IF) titration patterns for BL/Ka(N) on NIH-3T3 cells (●), C57BL/Ka cells (○), and (BL/Ka × NIH)F_1 cells (◑). Theoretical slopes for one-hit kinetics (45°) and for two-hit kinetics (63°) are shown for comparison. (*B*) Immunofluorescence titration patterns for BL/Ka(B) on C57BL/Ka cells (○) and (BL/Ka × NIH)F_1 cells (⊖). No response was obtained on NIH-3T3 cells. As in *A*, theoretical one- and two-hit slopes are also given.

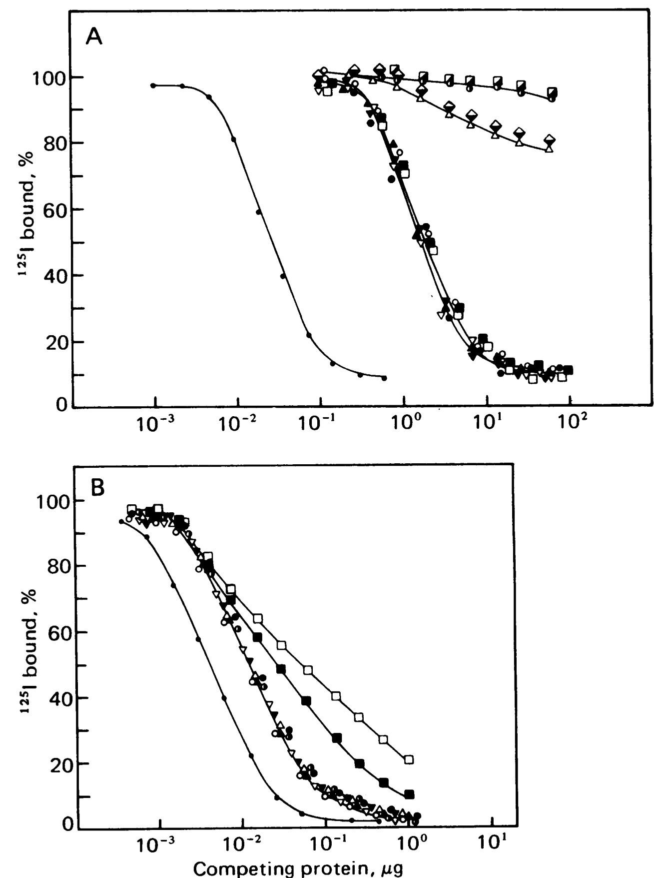

FIG. 2. (*A*) Homologous double-antibody competition radioimmunoassay for AKR-MuLV gp71. A monospecific rabbit antiserum to AKR-MuLV gp71 was used to precipitate ^{125}I-labeled AKR-MuLV gp71. The anti-globulin was raised in goats against rabbit IgG. Purified AKR-MuLV gp71 (•) is shown in comparison with the following band-purified, disrupted virions: BL/Ka(N) (▼), BL/Ka(B) (▽), B6-7(N) (●), B6-7(B) (○), BL/Ka(X) (♦), AT-124 (◪); NZB-MuX (◑), BALB:virus-2 (△), WN1802N (■), WN1802B (□), AKR-MuLV (▲). (*B*) Homologous double-antibody competition radioimmunoassay for AKR-MuLV p30. A monospecific rabbit antiserum to AKR-MuLV p30 was used to precipitate ^{125}I-labeled AKR-MuLV p30. Purified AKR-MuLV p30 (•) is shown in comparison with the following disrupted virions whose symbols are given in *A*: BL/Ka(N), BL/Ka(B), B6-7(N), B6-7(B), NZB-MuX, BALB:virus-2, WN1802N, WN1802B, and AKR-MuLV.

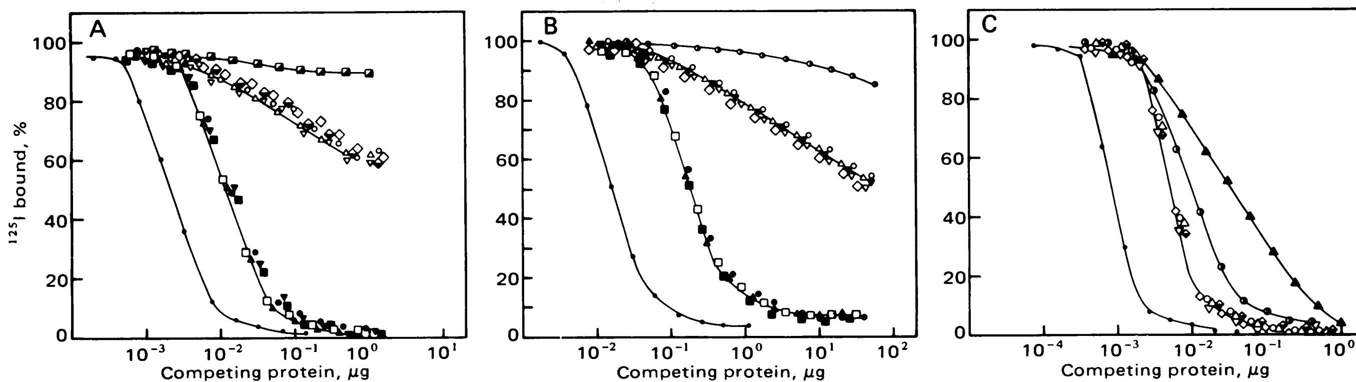

FIG. 3. (A) Homologous double-antibody competition radioimmunoassay for AKR-MuLV p12. A monospecific rabbit antiserum to AKR-MuLV p12 was used to precipitate ^{125}I-labeled AKR-MuLV p12. Purified AKR-MuLV p12 (•) is shown in comparison with disrupted RadLV/VL-3 virions (◇) and those of the following viruses whose symbols are given in Figure 2A: BL/Ka(N), BL/Ka(B), B6-7(N), B6-7(B), BL/Ka(X), BALB:virus-2, AT-124, WN1802N, WN1802B, and AKR-MuLV. (B) Homologous double-antibody competition radioimmunoassay for AKR-MuLV p15. A monospecific antiserum to AKR-MuLV p15 was used to precipitate ^{125}I-labeled AKR-MuLV p15. Purified AKR-MuLV p15 (•) is shown in comparison with disrupted RadLV/VL-3 virions (◇) and those of the following viruses whose symbols are given in Fig. 2A: BL/Ka(N), BL/Ka(B), B6-7(N), B6-7(B), NZB-MuX, BALB:virus-2, WN1802N, WN1802B, and AKR-MuLV. (C) Homologous double-antibody competition radioimmunoassay for RadLV/VL-3 p12. A rabbit antiserum raised against disrupted RadLV/VL-3 virions was used to precipitate ^{125}I-labeled RadLV/VL-3 p12. Purified RadLV/VL-3 p12 (•) is shown in comparison with disrupted RadLV/VL-3 virions (◇) and those of the following viruses whose symbols are given in Fig. 2A: BL/Ka(B), B6-7(B), NZB-MuX, BALB:virus-2, BL/Ka(X), and AKR-MuLV.

types (Fig. 1A). Table 1 gives the end-point titers for BL/Ka(N) and BL/Ka(B) on fibroblasts. BL/Ka(N) has a titer more than 4 logs greater on NIH-3T3 cells than on C57BL/Ka or (BL/Ka × NIH)F_1 fibroblasts. Conversely, the titer for BL/Ka(B) on C57BL/Ka cells is 4 logs greater than on (BL/Ka × NIH)F_1 cells and 5 logs greater than that on NIH-3T3 cells. Significantly, neither virus shows any detectable infectivity for mink cells, indicating the absence of infectious xenotropic particles in these virus preparations. Comparable data were obtained with the C57BL/6 and BALB/c N- and B-tropic viruses by the XC plaque assay (data not shown). These results demonstrate that the viruses have the expected biological differences characteristic of N- and B-tropic viruses.

The serological properties of the C57BL N- and B-tropic viruses were next examined and compared to those of other virus preparations by radioimmune competition assays for various virion components. The results of competition assays for the AKR-MuLV-type of gp71 are shown in Fig. 2A. In this assay all the C57BL N- and B-tropic viruses competed to the same degree and with the same slope as AKR-MuLV and were serologically distinguishable from the endogenous, xenotropic viruses, including BALB:virus-2, BL/Ka(X), NZB-MuX, and AT-124. For comparison we also examined the BALB/c N- and B-tropic viruses WN1802N and WN1802B, which similarly compete completely in the AKR-MuLV gp71 assay.

The results obtained with these viruses in competition assays for AKR p30 are shown in Fig. 2B. As expected, the C57BL viruses all competed equivalently to AKR-MuLV, as did the xenotropic viruses. Interestingly, however, there were distinct and reproducible differences obtained with the BALB/c N- and B-tropic viruses. The WN1802N virus competed very similarly to the other viruses, although the competition curve was slightly more protracted. The WN1802B virus, however, competed differently from the other viruses with a distinctly protracted slope of competition.

We next examined these viruses in competition assays for AKR-MuLV p12. As previously described (16), this assay is highly type specific and can readily detect serological differences between the C57BL/Ka N- and B-tropic viruses. As illustrated in Fig. 3A, the BL/Ka(N) virus competes completely and equivalently to AKR-MuLV in this assay. In contrast, BL/Ka(B) only weakly competes in this assay and is serologically quite distinguishable from the N-tropic virus. The results obtained with the C57BL/6 N- and B-tropic viruses were identical in that B6-7(N) competed completely and B6-7(B) virus competed only partially. The results obtained with the BALB/c-derived WN1802N and WN1802B are also shown for comparison in Fig. 3A. In distinct contrast to the C57BL viruses, both the N- and B-tropic viruses competed completely in this assay, as did the AKR-MuLV. Last, the AT-124 xenotropic virus did not compete in this assay, while the BALB:virus-2 and the C57BL/Ka xenotropic viruses competed only weakly. Interestingly, however, although the latter two viruses only partially competed in this assay, the extent and slope of competition were similar to those of the C57BL B-tropic viruses.

Among the *gag* gene products, p15 usually has the same degree of type specificity as p12 and can also be used to serologically discriminate among the various C type viruses. We therefore examined the above viruses in competition assays for AKR-MuLV p15. As illustrated in Fig. 3B, the B6-7(N) virus competed completely and equivalently to AKR-MuLV. Similarly, as expected from the p12 competitions, the BALB/c N- and B-tropic viruses competed identically to AKR-MuLV. In contrast, B6-7(B) and BL/Ka(B) competed incompletely and with a less pronounced slope in the AKR-MuLV p15 assay. Unexpectedly, BL/Ka(N) competed incompletely in this assay, similarly to the B-tropic viruses. Lastly, the NZB-MuX virus did not compete, while the BALB:virus-2 and BL/Ka(X) xenotropic viruses only partially competed in this assay. As with the p12, the partial competition seen with the B-tropic viruses was identical in slope and extent to the partial competition seen with the BALB:virus-2 and C57BL/Ka xenotropic viruses.

To further examine the serological differences among the C57BL N- and B-tropic viruses, we next examined the above viruses in competition assays for the p12 of the B-tropic RadLV/VL-3 virus. RadLV/VL-3 virus is the thymotropic, leukemogenic C57BL/Ka virus, which has an *in vivo* B-tropism and preferentially replicates in thymocytes (20). Although the virus has a unique gp71, the p12 is serologically identical to that of C57BL/Ka B-tropic virus (20), as illustrated in Fig. 3C. The results obtained with the C57BL/6 B-tropic B6-7(B) virus are also shown. This virus competed completely and equivalently

in this assay to the BL/Ka(B) virus and RadLV/VL-3. Interestingly, the BL/Ka(X) and BALB:virus-2 xenotropic viruses also competed completely and equivalently to the B-tropic viruses and the RadLV/VL-3 virus. In contrast, AKR-MuLV, although competing significantly, competed with a less pronounced slope and was serologically distinguishable from the other viruses. These results show that the C57BL B-tropic viral p12s are serologically indistinguishable from those of the BALB:virus-2 or C57BL/Ka xenotropic viruses, while the N-tropic viral p12s are indistinguishable from the AKR-MuLV p12.

DISCUSSION

The results of our serological analysis of N- and B-tropic viruses are summarized in Table 2. The most striking results are the p12 differences demonstrable in two independent N- and B-tropic isolates from C57BL/6 and C57BL/Ka strains of mice. The N-tropic viruses clearly have a p12 serologically identical to AKR-MuLV, whereas the B-tropic viruses have a p12 serologically identical to the B-tropic RadLV/VL-3 p12. Although we do not have available the assay for the C57BL/Ka xenotropic p12, the serological homology of the C57BL/Ka xenotropic virus in the RadLV/VL-3 p12 assay strongly suggests that the B-tropic viruses, RadLV/VL-3, and the xenotropic virus all have serologically identical p12s. The competition assay for AKR-MuLV p15 also detected serological differences among these viruses. The C57BL/6 N-tropic virus had a p15 identical to AKR-MuLV p15, whereas the C57BL/Ka N- and B-tropic viruses and the C57BL/6 B-tropic virus had a serologically distinct p15. Although the assays for the RadLV/VL-3 p15 and the xenotropic p15 are not available, the degree of competition and the p12 data also suggest that these p15s would be serologically identical.

In spite of the readily apparent differences in the p15s and p12s of the C57BL viruses, the WN1802N and WN1802B BALB/c viruses had p12s and p15s identical to those of AKR-MuLV. In fact, the only detectable difference between these viruses or relative to the other viruses was in the competition assays for p30. The latter result was unexpected because we have not generally seen any differences among a variety of viruses in this assay. Whether the differences detectable in competition assays are related to the differences detected by polyacrylamide gel electrophoresis (14, 15) is unknown.

The origin of the serologically distinct C57BL ecotropic viruses is not known. One possibility is the existence of multiple loci in C57BL mice for ecotropic viruses. This possibility seems unlikely, because by nucleic acid hybridization of backcrossed NIH × (NIH × C57BL/6) mice there appears to be only one locus for an AKR-MuLV type virus (4). Second, the B-tropic virus has never been directly detected in 5-iododeoxyuridine-treated C57BL tissue culture cell lines, although the N-tropic is readily inducible (13). Because of these observations it appears that the B-tropic virus is not endogenous but rather arises *in vivo* or *in vitro* by a mechanism of genetic mutation or recombination. The possibility that the B-tropic virus arises by recombination is particularly relevant because C type viruses are known to undergo recombination at a relatively high frequency (25, 26). This type of mechanism seems to be especially prevalent in AKR thymomas and generates a diverse group of viruses (27, 28). Our data are consistent with a mechanism involving recombination and suggest that in C57BL mice such recombination involves the endogenous N-tropic and inducible xenotropic virus. In these studies the potential recombinant viruses would include the BL/Ka(N) virus with the non-AKR-MuLV p15 as well as the C57BL B-tropic viruses with the non-AKR-MuLV p15s and p12s.

Assuming that the gene order for these viruses is 5′-p15-p12-p30-p10-*pol*-*env*-3′ as previously suggested (29), recombination at the 5′ end would be involved in three of the C57BL viruses. The BL/Ka(N) virus appears to have undergone recombination only in the p15 region, whereas the BL/Ka(B) and BL/6(B) viruses have undergone more extensive recombination involving minimally the p15 and p12 regions. Whether these recombinations extend into the p30 and p10 regions cannot presently be assessed because of the serological homology of the xenotropic and ecotropic proteins. In the future it may be possible to assess this region by tryptic peptide analysis. It is also apparent that, given the number of serological changes seen in this limited group of C57BL viruses, recombinational events may be extensive among the endogenous C57BL viruses. This could be due in part to the constitutive expression, in most tissues, of low levels of the inducible xenotropic virus as well as the *Fv-1* restriction for replication of endogenous, ecotropic virus.

Whether the serological differences we have detected among the C57BL viruses contribute to the N-, B-tropic host range of

Table 2. Summary of radioimmunoassay data for viral structural proteins

Virus	Mouse strain	Tropism	Serological type				
			gp71*	p30†	p15*	p12*	p12‡
BL/Ka(N)	C57BL/Ka	N-ecotropic	+	+	−	+	−
BL/Ka(B)	C57BL/Ka	B-ecotropic	+	+	−	−	+
B6-7(N)	C57BL/6	N-ecotropic	+	+	+	+	−
B6-7(B)	C57BL/6	B-ecotropic	+	+	−	−	+
WN1802N	BALB/c	N-ecotropic	+	±	+	+	NT
WN1802B	BALB/c	B-ecotropic	+	±	+	+	NT
RadLV/VL-3	C57BL/Ka	B-ecotropic§	−	NT	−	−	+
BALB:virus-2	BALB/c	Xenotropic	−	+	−	−	+
NZB-MuX	NZB	Xenotropic	−	+	−	NT	±
AT-124	NIH	Xenotropic	−	NT	NT	−	NT
BL/Ka(X)	C57BL/Ka	Xenotropic	−	NT	−	−	+
AKR-MuLV	AKR	N-ecotropic	+	+	+	+	−

NT, not tested.
* Gross/AKR-MuLV, N-tropic MuLV type of antigen.
† "Common," no type-specific distinctions made.
‡ C57BL B-tropic, C57BL/Ka xenotropic type of antigen.
§ RadLV/VL-3 is B-ecotropic *in vivo* and replicates preferentially in the thymus but grows poorly on fibroblasts *in vitro*.

the viruses is an important issue. The presence of two serologically distinct p15s in the C57BL/Ka and the C57BL/6 N-tropic virus strongly suggests that this protein is not involved. These data are therefore consistent with previous experiments with BALB/c viruses, which tended to exclude this protein (15). The serological differences in the p12s are more consistent with tropism for the C57BL viruses, although this difference was not seen with the BALB/c WN1802N and WN1802B viruses. One unlikely possibility to explain this difference is that the mechanism of restriction is different for C57BL and BALB/c viruses and therefore a consistent serological pattern would not be expected among these strains. The more likely possibility, however, is that p12 itself is not involved but rather only reflects more extensive changes that have occurred in the genome. Presumably as more isolates are obtained and other *gag* gene products are examined it may be possible to map the gene region involved in the tropism.

The results we have obtained indicate that C57BL viruses are quite different from the BALB/c viruses examined here or other BALB/c B-tropic isolates previously characterized (30). In particular, although it appears likely that C57BL B-tropic viruses arise by recombination, similar events are not readily obvious in the BALB/c B-tropic viruses. Several possibilities exist to explain these differences, including the possibility that both arise by recombination, although mechanistically the type of recombination is different. With the C57BL viruses, recombination from the 5′ end appears likely because changes in p15 have occurred without p12 changes, but the p12 changes are always accompanied by p15 changes. In contrast, BALB/c viruses might undergo intragenomic recombination, leaving the 5′ and 3′ ends intact. A second possibility is that BALB/c B-tropic viruses arise by mutation that mediates the same type of structural change obtained by recombination of the C57BL viruses. Lastly, the mechanism of tropism may be inherently different. Clearly these possibilities can only be examined by characterizing additional isolates. Nevertheless, our data demonstrate that serological differences exist among cloned isolates of C57BL ecotropic viruses that appear to arise by recombination. It should now be possible to use these viruses to further study the origin and biology of C type viruses.

This research was supported in part by the National Cancer Institute under Contract NO1-CO-75380, with Litton Bionetics, and in part by Research Grant CA-03352, with Stanford University School of Medicine.

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