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Suppression and Reappearance of N-Tropic L Virus Production in Somatic Cell Hybrids after Introduction and Loss of Chromosomes Carrying Fv-1^{b}*

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Abstract—*Production of the N-tropic L virus was studied in segregating populations of hybrid somatic cells. The hybrids were derived from crosses between A9HT, a highly malignant L-cell variant, and normal C57Bl lymphocytes or fibroblasts. The presence of the chromosome(s) 4, derived from the normal partner was assessed by the Gpd-1^{a} marker. Chromosome 4 is known to carry the Fv-1^{b} locus. Virus production was suppressed in hybrids of the Fv-1^{nn} × Fv-1^{bb} type but continued at the same level in Fv-1^{nn} × Fv-1^{nn} crosses. In segregating Fv-1^{nn} × Fv-1^{bb} hybrid cell populations, the loss of chromosome(s) 4 carrying the Fv-1^{b} locus resulted in the reappearance of N-tropic L virus production. This indicates that the Fv-1 restriction can operate in cells that carry integrated proviral DNA.*

The hybrids between the malignant and normal cells initially produced few tumors, but selection for tumorigenicity in vivo *favored variants that had lost the chromosome(s) 4 derived from the normal parent cell. This provided a convenient method of selecting against the chromosomes carrying Fv-1^{b}. The loss of the Gpd-1^{a} marker of C57Bl origin was regularly accompanied by the resumption of virus production. These results confirm the role of the chromosome(s) 4 derived from the normal parent cell in the suppression of malignancy in somatic cell hybrids.*

INTRODUCTION

THE Fv-1 locus exerts an important influence on most endogenous mouse C-type viruses. N-tropic viruses are restricted by the Fv-1^{b} allele and B-tropic viruses are restricted by the Fv-1^{n} allele [1]. Restriction is dominant: it is closely similar in homozygous and heterozygous cells that carry the restrictive allele in a single or double dose [2]. Jolicoeur and Baltimore [3] and Sveda and Soeiro [4] have proposed that the mechanism of Fv-1 restriction works by influencing the integration of the exogenous proviral DNA copy into the cell genome.

In a previous study [5], we measured the production of the L virus in the L-cells themselves and in hybrids between these cells and various partners. The 'L virus' has C-type morphology [6], bands at 1.16–1.17 g/cm^{3} in sucrose gradients [7, 8] and contains RNA-dependent DNA polymerase [9]. This virus is N-tropic, like certain endogenous mouse viruses [5, 10], and forms individual syncytial cells in the XC-test [5]. Unlike the endogenous C-type viruses, it can be neutralized by mouse antisera to Moloney leukemia virus [11].

Virus production continued at the same level when L cells (A9 and A9HT sublines) were fused with Fv-1^{n}Fv-1^{n} cells (*nn* for short), but was suppressed in crosses with cells of the *nb* or *bb* type. This suggested that the Fv-1 restriction could operate in cells that already carried integrated proviral DNA. If these findings are to be reconciled in those of Jolicoeur and Baltimore [3] and Sveda and Soeiro [4], one must suppose that Fv-1 restriction can act at more than one level of virus replication.

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In the present study this problem was studied further in segregating somatic cell hybrids of the Fv-1^{nn} × Fv-1^{bb} type, derived from crosses between A9HT, A highly malignant L-cell variant, and normal C57Bl lymphocytes or fibroblasts. Initially, N-tropic virus production was suppressed in all hybrids. Clonal sublines were studied throughout the process of gradual chromosome loss that takes place during serial passage *in vitro*. They were tested periodically for N-tropic virus production and for the retention of the Fv-1^b-carrying chromosome(s) number 4, assessed by the presence of the linked glucose-6-phosphate dehydrogenase-1 (Gpd-1) locus [12, 13].

The hybrids were also examined for tumorigenicity in syngeneic recipients. It had previously been shown [14, 15] that hybrids between these highly malignant cells and normal lymphocytes or fibroblasts were initially non-tumorigenic, but could reacquire tumorigenicity on continued cultivation *in vitro*.

MATERIALS AND METHODS

Cells

A9HT is a highly malignant subline of the 8-azaguanine-resistant L-cell variant A9 [16]. It lacks HGPRT and cannot grow on HAT medium. The fusion of A9HT cells with normal C57Bl lymphocytes and the selection of hybrid clones has been described previously [15].

Secondary embyro fibroblasts of C57Bl origin were fused with A9HT cells with the help of polyethyleneglycol as described by Davidson and Gerald [17]. A9HT cells resistant to 20 μg of thioguanine were mixed with C57Bl embryo fibroblasts in equal amounts and cocultivated over night in Petri dishes. After carefully removing all medium, PEG 6.000 was added in a 10 g/10 ml solution, left for 2 min, and then carefully diluted with medium. Medium was then changed and after 24 hr replaced by selective HAT medium [18]. The resulting hybrids were isolated from individual Petri dishes.

Hybridity was confirmed by the total chromosome number, the presence of biarmed marker chromosomes from the A9HT parent and the simultaneous presence of H-2^k (from A9HT) and H-2^b (from the normal C57Bl parent) (Table 1).

Cloned hybrids were kept frozen in liquid nitrogen and thawed shortly before the actual experiments. All lines were carried for a maximum of 6 *in vitro* passages in Eagle's

Table 1. *Chromosome constitution and H-2 antigens of hybrid clones*

	Total chromosome No.		No. biarmed chromosomes		
	Mode	Range	Mode	Range	H-2 type†
Parent cells					
A9HT	53	50–53	25	25–27	H-2^k
Normal lymph node cell of C57Bl	40	40	—	—	H-2^b
Embryo fibroblasts	40	40	—	—	H-2^b
A9HT × C57Bl lymphocyte hybrids*					
Clone 1	89; 91	85–96	24	22–28	H-2^k, H-2^b
2	88	76–94	24; 25	21–28	H-2^k, H-2^b
3, line A	92; 93	83–94	25	19–28	H-2^k, H-2^b
line B	86	70–89	26; 27	23–30	
4	93; 95	85–107	—	22–29	H-2^k, H-2^b
5, line A	78	72–82	26	23–30	H-2^k, H-2^b
line B	79	69–97	23	20–28	
6					H-2^k, H-2^b
7, line A	93; 95	87–104	24	22–30	
line B					
A9HT × C57Bl fibroblast hybrids					
Clone 1	90	87–97	22	17–26	H-2^k, H-2^b
3	90	84–94	23	19–26	H-2^k, H-2^b
4	92	90–100	25	20–28	H-2^k, H-2^b

*Unless otherwise indicated, all data are taken from a previous publication (Wiener *et al.* [15]).
†Quantitative adsorption tests.

Minimum Essential Medium with Earle's salts, supplemented with 5% fetal calf serum. Table 1 summarizes the properties of hybrids.

Chromosome preparations

Metaphase spreads were produced by the air drying technique of Rothfels and Siminovitch [19].

Tumorigenicity in vivo

Cloned hybrid lines were inoculated into newborn H-2^kH-2^b mice of the following F_1 hybrid types: C3H × C57Bl, C3H × ABY, C3H × C57L and CBA × C57Bl. The animals were given 400 r of total body irradiation. Subcutaneous inocula of $2–5 \times 10^5$ cells per mouse were given. Some of the tumors produced were passaged further in adult syngeneic mice. All inoculated mice were kept under observation for 3 months.

Highly malignant sublines selected by growth *in vivo* were reestablished *in vitro* and were retested for tumorigenicity, Gpd-1 and virus production.

Enzyme assays

Autosomal glucose-6-phosphate dehydrogenase (Gpd-1) (also known as glucose dehydrogenase: E.C. 1.1.1.47) was studied by horizontal starch gel electrophoresis by a method based on that of Ruddle *et al.* [12]. Samples were prepared by lysing washed cell pellets, each containing about 10^7 cells, in an equal volume of distilled water. The cells were then subjected to two 10-sec bursts of sonication from a Probe sonicator (the sample being surrounded by ice) and centrifuged at 20,000 ***g*** for 20 min. The clear supernatant was used for electrophoresis, the sample being applied on inserts of 3 mm Whatman chromatography paper. The electrophoretic system was that described by Ruddle *et al.* [12], the buffer being Tris/EDTA borate, pH 8.6. The routine stain consisted of galactose-6-phosphate (300 mg), NADP (35 mg), MTT (25 mg), PMS (1.5 mg) in 100 ml of 0.5 M Tris buffer (pH 8.0) containing 0.01 M $MgCl_2$. Results were checked with glucose-6-phosphate as substrate, and, in the later experiments, the enzyme was also stained with glucose as substrate and NAD as co-enzyme [20].

Samples prepared as above were also tested for dipeptidase (Dip-1) and soluble malic enzyme (Mod-1) by minor modifications of routine methods [21, 22].

Virus assay

Culture supernatants of 2×10^5 cells, seeded in 5 ml plates 24 hr earlier, served as the virus source. All media were passed through a Millipore® filter (pore size 0.45 μm) and kept frozen at −90°C.

JLS-V9 test

Moloney virus-determined cell surface antigen (MCSA) was induced as described previously [23]. JLS-V9 cells were seeded into milk dilution bottles and 24 hr later treated with 100 μg/ml DEAE–dextran. Cells were then infected with 1 ml of undiluted virus and virus diluted 1:100. Infected and control cultures were subcultured at a 1 : 10 reduction ratio twice a week and tested for MCSA by membrane immunofluorescence every week for a total of 5 weeks. The sera were produced in A × C57Bl or A × C57L F_1 mice, by 3–6 immunizations with Moloney lymphoma (YAC) cells, given 6000 rad of radiation.

XC-test

This was done according to the method of Rowe *et al.* [24]. CBA ($Fv\text{-}1^{nn}$) and C57Bl ($Fv\text{-}1^{bb}$) secondary mouse embryo cultures were seeded in 60 mm Petri dishes (Falcon, Los Angeles, Calif.) at a density of 2×10^5 cells/dish. Twenty-four hr later, cultures were treated with 100 μg/ml DEAE–dextran and infected as described previously [25]. Six days later cultures were u.v. irradiated and overlayed with XC cells. Individual syncytial cells were scored at a 100 × magnification.

RESULTS

Enzyme patterns

Hybrid clones and sublines selected *in vivo* were tested for Dip-1 and Mod-1 since these enzymes occur in different isozymic forms in C3H and C57Bl mice. Mod-1 is determined by a locus on chromosome 9 and, in hybrid cells, it shows the characteristic 5-banded hybrid pattern of a tetrameric enzyme (Fig. 1a). This characteristic enzyme pattern was seen in every hybrid clone and subline selected *in vivo* thus confirming their hybridity. In addition to Mod-1, clone 1, clone 2, clone 4 and their sublines clone 2-H7A7, clone 4-H7A10 and clone 4-H7A12 were tested for Dip-1 (Fig. 1b). Dip-1 is determined by a locus on chromosome 1. In all hybrids tested, enzyme contributions from both parental cell lines were present.

Figure 2 illustrates the Gpd-1 enzyme patterns. The A9HT cells had a strong band of Gpd-1 activity corresponding to the 'b' phenotype seen in tissues from C3H mice. The C57Bl lymphocytes had only very weak activity for Gpd-1, which appeared as a faint band corresponding in mobility to the 'a' phenotype of C57Bl tissues. Some difficulty was encountered in scoring the hybrids as the A9HT 'b' component was stronger than the C57Bl 'a' component in all cases. The presence or absence of the 'a' component was therefore assessed by the presence of the 'ab' heteromeric band rather than the 3-banded pattern expected for a dimeric enzyme in a hybrid. Each sample was tested on at least 2 occasions and the gels were scored by two independent observers who had no knowledge of the identity of the samples. One sample (clone 7) was scored once as 'b' and twice as 'ab'. Otherwise repeated tests always gave the same result, and these are shown in Table 2.

Virus production

Since the L virus does not induce plaques but individual syncytial cells in the XC-tests it was assayed by the induction of the Moloney virus determined cell surface antigen (designated MCSA) on JLS-V9 cells. MCSA was stained by indirect membrane immunofluorescence, using syngeneic mouse anti-Moloney lymphoma sera.

As shown in Table 2, only one of the A9HT/C57Bl hybrid clones (No. 3) produced detectable infectious virus. This is in agreement with our previous finding [5] that fusion of a Fv-1^{nn} cell with a Fv-1^{bb} cell leads to suppression of N-tropic virus production, while fusion with a Fv-1^{nn} partner has no effect. The virus-producing clone 3 was also unique in lacking the C57Bl-derived Gpd-1^a. When the sublines selected by 4–12 passages *in vivo* from the hybrid clones were explanted and then tested, they were found to produce virus. The virus resembled the original A9HT associated L virus by a number of criteria. In the XC test, it induced individual syncytial cells rather than the plaques produced by MLV. It was infectious for Fv-1^{nn}, but not for Fv-1^{bb}, embryo cells. It was able to induce MCSA in JLS-V9 cells, in contrast to endogenous eco- and xenotropic C-type viruses (Fenyö *et al.*, manuscript in preparation). The virus produced by hybrid clone 3 also showed the same characteristics. The close resemblence to the virus associated with the parental A9HT cell made it unlikely that an accidental virus has been picked up during *in vitro* or *in vivo* passage. The results thus indicate that production of the parental L virus has been switched on.

Only one Gpd-1^a negative subline, clone 6-H7D4 failed to produce virus. One other exceptional subline, clone 2-H7A7, released infectious virus but contained Gpd-1^a. The remaining sublines produced virus and lacked Gpd-1^a.

Growth of hybrid clones in vivo

Table 3 shows the tumorigenicity as measured by the take incidence of the hybrid clones in syngeneic newborn irradiated recipients. Of the A9HT × C57Bl lymphocyte hybrids, clones 1 and 2 produced few tumors (take incidence 30 and 16%, respectively). This confirms previous findings with these clones [15]. Two sublines of each of clone 3 and 5 were tested, one poorly tumorigenic (take incidences 10 and 0%) and one highly tumorigenic (take incidences 61 and 100%). Clones 6 and7 were moderately tumorigenic with take incidences of 57 and 44%.

Of the A9HT × C57Bl fibroblast hybrids, clone 4 was low and clones 1 and 3 highly tumorigenic.

The majority of tumors arising from hybrid clones with low or moderate take incidence failed to grow upon further passage, for example, clones 1, 6 and 7 of the A9HT × lymphocyte cross and clone 4 of the A9HT × fibroblast cross. From clones which initially gave high take incidence, tumor lines could easily be established. Table 4 shows the take incidences of malignant sublines.

Relationship between infectious virus production, tumorigenicity and the absence of C57Bl-derived Gpd-1^a

Fusion of high malignant cells with normal cells results in low malignant hybrids, as a rule. Upon *in vivo* passage, occasional tumors may arise from such low malignant hybrids. The tumors have invariably lost a number of chromosomes. Therefore selection for malignancy appeared to be an efficient way to isolate variants that have lost certain chromosomes. This was particularly useful in the present study, since Jonasson *et al.* [26] previously found that the chromosome pair 4, derived from the normal parent, is regularly eliminated in the high malignant segregants that are isolated from malignancy-suppressed hybrids.

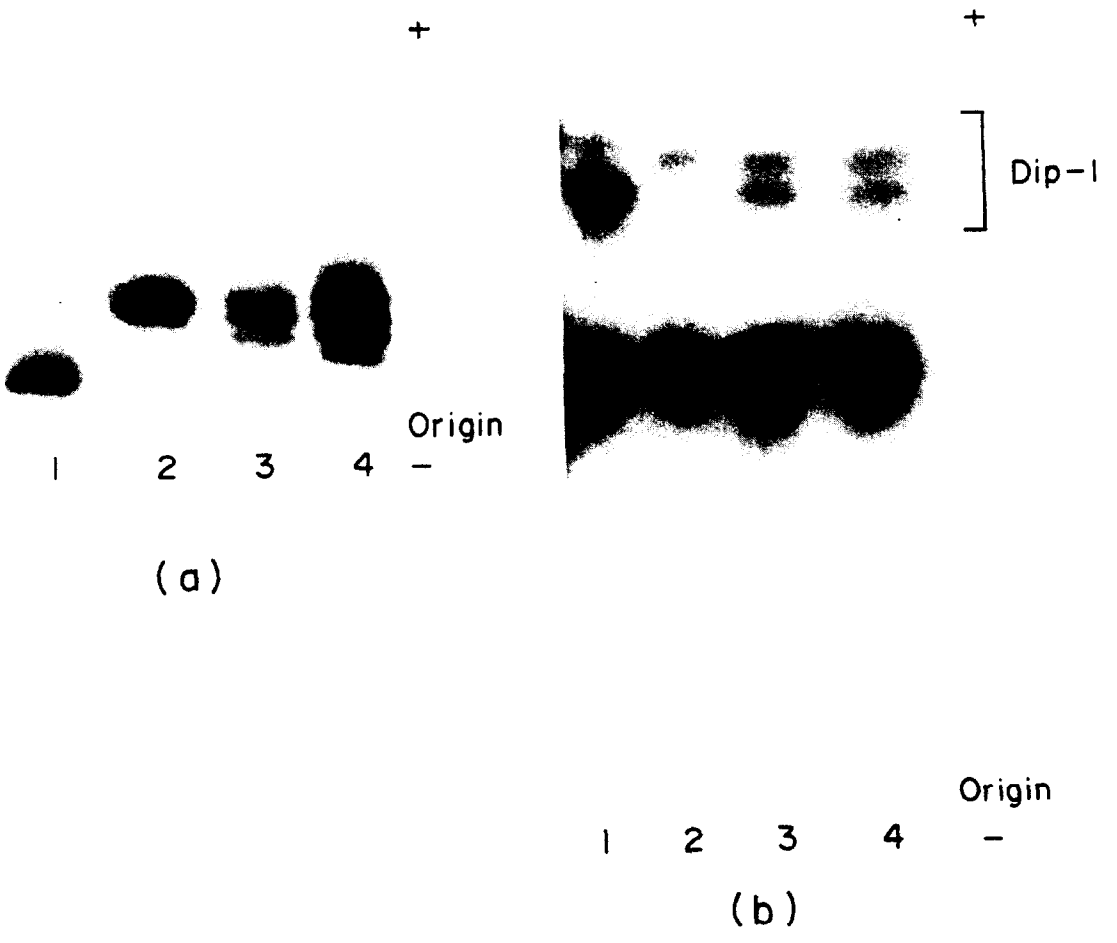

Fig. 1. Photograph of starch gel stained for (a) malic enzyme (Mod-1) and (b) dipeptidase (Dip-1). The sample in channel 1 is of C57Bl origin, channel 2 is A9HT and channels 3 and 4 are different in vivo selected high tumorigenic hybrids. The hybrids have both parental components.

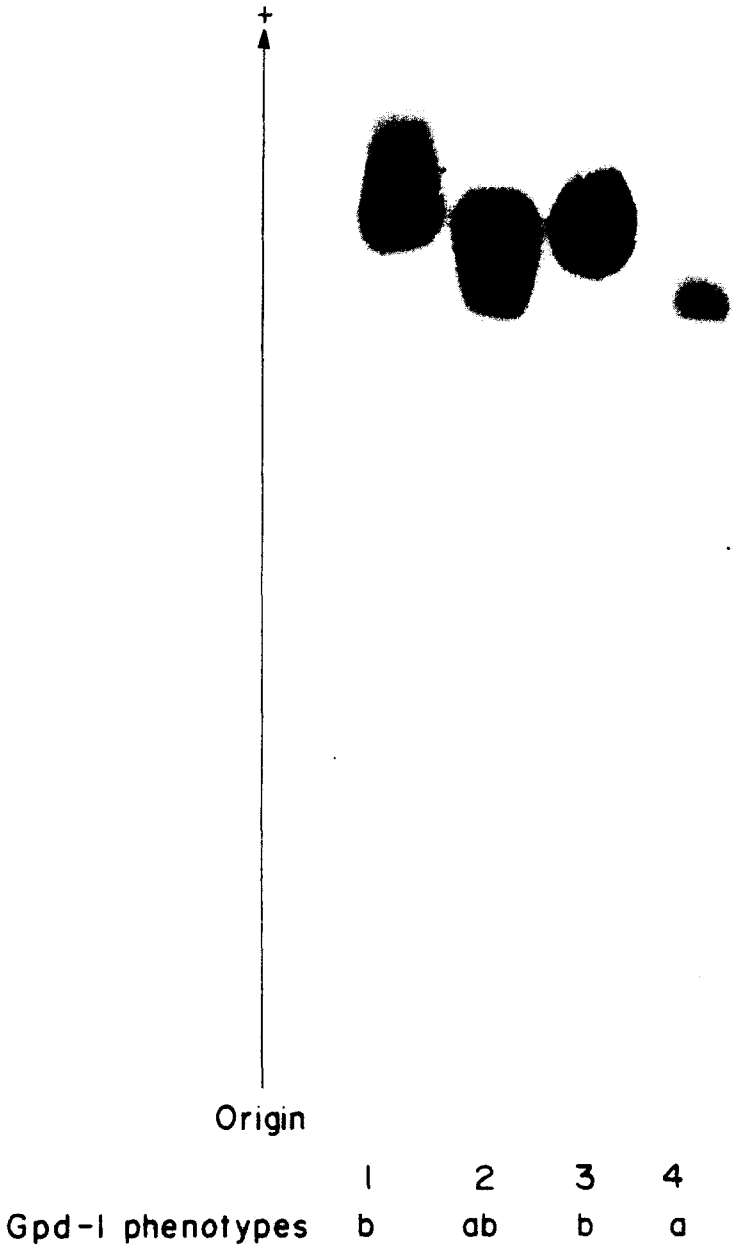

Fig. 2. Photograph of starch gel stained for autosomal glucose-6-phosphate dehydrogenase (Gpd-1). The sample in channel 1 is a high tumorigenic subline selected in vivo *(A9HT-C57Bly clone 1-H7A8), channel 2 is a hybrid (clone 1), channel 3 is A9HT and channel 4 is mouse embryo fibroblasts of C57Bl origin. The hybrid (channel 2) has both parental components, whereas its subline selected* in vivo *has only the A9HT component.*

Table 2. Gpd-1 pattern and production of infectious virus in hybrid clones and malignant sublines

Virus source*	Gpd-1 phenotype	No. of tests	Virus assay JLS-V9† undil.	JLS-V9† 10^{-2}	XC undil.	Tropism
Moloney Lymphoma (YAC)		4	+ + + +	+ +	Plaques	NB
A9HT	b	6	+ + +	+	Sync.‡	N
C57Bl lymphocyte	a		−		—	
C57Bl embryo	a		−		—	
CBAT6T6 embryo	b		−		—	
A9HT × C57Bl lymphocyte hybrids						
Clone 1	ab	4	−	−	—	
2	ab	3	−	−	—	—
3B	b	2	+ +	+	Sync.	N
4	ab	3	−	−	—	
5B	ab	3	−	−	—	
6B	ab(b ≫ a)	3	−	−	—	
7B	ab(b ≫ a)	4	−	−	—	
Sublines selected *in vivo*§						
Clone 1 —H7A8	b	2	+	+		N
—H7C4	b	2	+ +	−	Sync.	N
Clone 2 —H7A7	ab(b ≫ a)	3	+ +	+	Sync.	N
Clone 4 —H7A10	b	3	+	−	—	
—H7A12	b	3	+ + +	+	Sync.	N
Clone 6B—H7D4	b	4	−	−	—	
—H7F4	b	5‖	+	−	—	
—H7H4	b	2	+ +	+	Sync.	N
Clone 7B—H7D4	b	3	+ + +	+	Sync.	N
—H7F4	b	2	+ +	−	Sync.	N
—H7L4	b	1	+ +	−		
—H7O4	b	2	+ + +	+ +	Sync.	N
A9HT × C57Bl fibroblast hybrids						
Clone 1	ab	3	−	−	—	
Clone 3	ab	2	−	−	—	
Clone 4	ab	2	−	−	—	
Sublines selected *in vivo*§						
Clone 1 —H7A4	b	2	+ + +	+ +	Sync.	N
—H7B4	b	2	+ + +	+	Sync.	N
—H7D4	b	2	+ +	+	Sync.	N
Clone 3 —H7A4	b	2	+ + +	+		
—H7C4	b	2	+ +	+		
Clone 4 —H7A4	b	2	+ +	−		
—H7C4	b	1	+ +	−		
A9HT × CBAT6T6 fibroblast hybrids						
Clone 3		2	+ + +	+	Sync.	N
Clone 5		2	+ + +	+	Sync.	N

*Culture fluid harvested 24 hr after feeding 2×10^5 cells in 10 ml MEM with 5% FCS. YAC: Moloney lymphoma cell which produces the Moloney leukemia virus.

†Virus release was assessed by the ability of culture media to induce the Moloney cell surface antigen (MCSA) in JLS-V9 cells. The time necessary for the appearance of specific membrane immunofluorescence was a function of virus dilution. If a fluorescence index of 0.5 was produced within 7 days, the result was scored + + + +; within 7–14 days, + + +; within 14–21 days, + +; within 21–28 days, +. When the fluorescence index did not exceed 0.25 during the 5 weeks of observation, the result was indicated by −.

‡Sync.: individual syncytical cells. Since all C57Bl cultures were negative with the L virus, only results obtained on CBA cultures are shown.

§Designation of malignant sublines. H7 is the symbol for the C3H × C57Bl F_1 cross in which the highly tumorigenic subline was selected. A and B indicate independently selected sublines. The final figure gives the number of passages *in vivo*.

‖Two tests out of 5 were positive.

Table 3. *Tumorigenicity of hybrid clones*

Cell or Hybrid	Take incidence* (total No. of animals with progressive tumors/total No. inoculated)	Percentage takes	No. of tumor lines established *in vivo*/ No. of attempts
A9HT	57/64	89	
A9HT × C57Bl lymphocyte hybrids			
Clone 1	8/27	30	2/7
Clone 2	5/31	16	1/2
Clone 3, line A†	1/10	10	
line B	11/18	61	
Clone 4	14/26	54	1/2
Clone 5, line A†	12/12	100	1/1
line B	0/16	0	
Clone 6, line B	12/21	57	3/7
Clone 7, line A†	1/14	7	
line B	18/41	44	4/13
A9HT × C57Bl fibroblast hybrids			
Clone 1	9/9	—	
Clone 3	23/31	74	3/4
Clone 4	7/18	39	2/4

*Test animals were H-2 compatible newborn X-irradiated mice.
†Data taken from a previous publication (Wiener *et al.* [15]).

Table 5 shows that all hybrid clones—except one—contained Gpd-1^a, the marker for the C57Bl-derived chromosome 4 and that this was lost from hybrid cells able to grow as tumors. The regular disappearance of the C57Bl-derived chromosome 4 marker (Gpd-1^a) from the highly malignant sublines selected *in vivo* confirms that the chromosome(s) 4 derived from the normal cell participates in the suppression of the malignancy which occurs when normal cells are fused with tumor cells. This is in agreement with the previous findings of Jonasson *et al.* [26].

The fortunate coincidence that selection for *in vivo* tumorigenicity favours variants that have lost their normal parent-derived chromosome(s) 4, provides a technique to examine the effect of Fv-1^b loss on N-tropic virus production.

The Fv-1 gene was shown previously to be linked to Gpd-1 [13] consequently, the presence of Gpd-1^a indicates that Fv-1^b is also present. The C3H-derived Gpd-1^b, which is linked to Fv-1^n, was invariably present in all lines tested. None of the Gpd-1^a positive hybrids produced infectious virus, whereas the one Gpd-1^a negative hybrid (clone 3) did. All highly malignant sublines selected *in vivo*, except one, lacked Gpd-1^a and produced virus. One line, clone 2-H7A, still contained Gpd-1^a but nevertheless produced a high titer of virus. It seems likely that in this case the gene coding for the enzyme and the Fv-1^b allele have become dissociated or, alternatively, the hybrid cell population was heterogenous and still segregating chromosome 4.

In summary, since Fv-1^b is linked to Gpd-1^a, and since the former is known to suppress N-tropic virus production, selection for the highly tumorigenic phenotype is paralleled by the reappearance of infectious virus production. We consider this relationship coincidental, rather than causal, the selective loss of chromosome 4 being the common denominator.

DISCUSSION

The present study confirms that the Fv-1 locus governs the production of N-tropic virus in hybrid cells. Since the proviral DNA was introduced in an integrated form by one of the parent cells (A9HT), our findings imply that the Fv-1^b product suppresses some post-integrational event. On the other hand, Tennant *et al.* [27] found that when Fv-1^{nn} cells were fused with Fv-1^{bb} cells within 2 hr of infection with N or B-tropic virus, fewer N × B heterokaryons were producing virus protein than if the cells were

fused 6 hr after virus infection. These authors suggested that the Fv-1 gene product affected an early step in virus infection. Jolicoeur and Baltimore [3] and Sveda and Soeiro [4] also considered that the restrictive event precedes the integration of viral DNA. Recently, Hsu *et al.* [28] have succeeded in transfecting nonpermissive cells with DNA derived from virus-infected cells. They suggest that viral DNA can integrate with the genome of nonpermissive cells and also conclude that the restrictive step occurs earlier in virus infection.

However, all the above experiments have examined the events occurring after exogenous C-type virus infection, while our experiments deal with cells that already harbor integrated virus. The two different kinds of experimental approaches may be concerned with two different levels of Fv-1 gene action.

The present observation, made on a new series of hybrids, confirm previous findings [5] that introduction of Fv-1^b-carrying chromosomes into N-tropic virus-producing L-cell variants closes down virus production. On further cultivation of the hybrid cells, an excellent correlation was found between the loss of chromosome 4, either spontaneously (as in hybrid clone 3) or following selection for malignancy *in vivo* and the reappearance of N-tropic virus production. Taken together, these results clearly indicate that Fv-1 restriction can act at a post-integrational level, although

Table 4. *Tumorigenicity of lines selected* in vivo*

Cell type	*In vivo* passage No.		Take incidence†	Percentage takes
A9HT × C57Bl lymphocyte hybrids				
Clone 1 —H7A	4–9		13/14	93
—H7C	1–4		8/8	—
		Total	21/22	95
Clone 2 —H7A	3–13		22/22	100
Clone 4 —H7A	1–14		33/34	97
Clone 5A—H7A	1–2		4/4	—
Clone 6 —H7D	1–4		10/10	100
—H7F	1–4		6/8	—
—H7H	1–4		5/8	—
		Total	21/26	81
Clone 7 —H7D	1–4		4/7	—
—H7F	1–4		7/8	—
—H7L	1–4		8/8	—
—H7O	1–4		6/8	—
		Total	25/31	81
A9HT × C57Bl fibroblast hybrids				
Clone 1 —H7A	1–4		8/8	—
—H7B	1–4		8/8	—
—H7C	1–4		8/8	—
—H7D	1–4		8/8	—
		Total	32/32	100
Clone 3 —H7A	1–4		8/8	—
—H7C	1–4		7/8	—
—H7D	1–5		10/10	100
		Total	25/26	96
Clone 4 —H7A	1–4		7/8	—
—H7C	1–4		8/8	—
		Total	15/16	94

*For designation of *in vivo* selected sublines see Table 2.
†For definition see Table 3.

this does not exclude an action on other, pre-integrational events.

There were two exceptional hybrid cell lines that did not fit the rule. In one, the C57Bl-derived Gpd-1^a was lost, but virus production was not regained. In the other, the enzyme locus was still present, but virus production was nevertheless resumed. In these cases, dissociation of the isozyme marker from the Fv-1 locus by chromosome breakage might have occurred or there might have been heterogeneity in the segregating cell population. These exceptions do not invalidate the general conclusion drawn from a total of 28 hybrid lines and segregants derived from them.

Selection *in vivo* for tumorigenicity was correlated with the loss of chromosome 4 and the reappearance of virus production. The question can be asked whether there is any causal relationship between virus production and tumorigenicity. We do not believe that there is. The L virus is non-oncogenic in all systems so far tested. In our previous studies we found no

Table 5. Virus production, tumorigenicity and the Gpd-1 isozyme marker

Cell type	Virus production	Gpd-1^{a}*	Take incidence (%)†
A9HT	+	−	89
C57Bl lymphocyte	−	+	—
C57Bl embryo	−	+	—
A9HT × C57Bl lymphocyte hybrids			
Clone 1	−	+	30
Clone 2	−	+	16
Clone 3 B	+	−	61
Clone 4	−	+	54
Clone 5 B	−	+	0
Clone 6 B	−	+	57
Clone 7 B	−	+	44
Sublines selected *in vivo*			
Clone 1 —H7A8	+	−	93
—H7C4	+	−	(100)
Clone 2 —H7A7	+	+	100
Clone 4 —H7A10	+	−	97
Clone 6 B—H7D4	−	−	100
—H7F4	+	−	(75)
—H7H4	+	−	(63)
Clone 7 B—H7D4	+	−	(60)
—H7F4	+	−	(87)
—H7L4	+	−	(100)
—H7O4	+	−	(75)
A9HT × C57Bl fibroblast hybrids			
Clone 1	−	+	(100)
Clone 3	−	+	(75)
Clone 4	−	+	(50)
Sublines selected *in vivo*			
Clone 1 —H7A	+	−	(100)
—H7B	+	−	(100)
—H7C	+	−	(100)
—H7D	+	−	(100)
Clone 3 —H7A	+	−	(100)
—H7C	+	−	(87)
—H7D	+	−	(100)
Clone 4 —H7A	+	−	(87)
—H7C	+	−	(100)

*Presence of 'a' band indicates the presence of C57Bl-derived chromosome 4.
†Wherever less than 10 mice inoculated figures appear in parentheses (cp. Table 3).

systematic difference between poorly tumorigenic or highly malignant L-cell variants with regard to virus production [29], nor in hybrid cells of varying tumorigenicity [30, 31], provided that Fv-1 restriction was avoided.

Jonasson *et al.* [26] previously found that both copies of chromosomes 4 derived from the normal parent cell were regularly lost when malignant segregants were selected *in vivo* from hybrids between tumor and normal cells in which malignancy was suppressed. This indicated that some genetic element capable of suppressing malignancy was located on chromosome 4. Its linkage with Fv-1 may well be purely accidental. The fact that this linkage exists, however, provides a convenient method of selecting against the chromosomes 4 derived from the normal parent cell, and hence of studying the action of the Fv-1 gene. Conversely, the regular resumption of virus production accompanying the loss of the C57Bl-derived Gpd-1^a marker provides additional confirmation of the conclusion of Jonasson *et al.* [26] concerning the role played by the chromosome 4 derived from the normal parent cell in suppressing malignancy.

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