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Unstable Resistance of G Mouse Fibroblasts to Ecotropic Murine Leukemia Virus Infection

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G mouse cells were resistant to N- and NB-tropic Friend leukemia viruses and to B-tropic WN1802B. Though the cells were resistant to focus formation by the Moloney isolate of murine sarcoma virus, they were relatively sensitive to helper component murine leukemia virus. To amphotropic murine leukemia virus and to focus formation by amphotropic murine sarcoma virus, G mouse cells were fully permissive. When the cell lines were established starting from the individual embryos, most cell lines were not resistant to the murine leukemia viruses. Only one resistant line was established. Cloning of this cell line indicated that the resistant cells constantly segregated sensitive cells during the culture; i.e., the G mouse cell cultures were probably always mixtures of sensitive and resistant cells. Among the sensitive cell clones, some were devoid of *Fv-1* restriction. Such dually permissive cells, and also feral mouse-derived SC-1 cells, retained glucose-6-phosphate dehydrogenase-1 and apparently normal number 4 chromosomes. The loss of *Fv-1* restriction in these mouse cells was not brought about by any gross structural changes in the vicinity of *Fv-1* on number 4 chromosomes.

Suzuki and Matsubara found that a portion of a non-inbred ddY mouse colony at Shizuoka Laboratory Animal Center, Japan, did not respond to Friend leukemia virus. From this colony, a resistant mouse strain, G strain, was established (19). Since resistance was inherited independently of *Fv-1* and *Fv-2*, a new locus, *Fv-4*, was assigned for resistance in G mice (18).

The resistance of G mice to viral leukemia was due to a complete repression of the growth of ecotropic murine leukemia viruses ($MuLV_E$) of N or NB tropism (8, 18). Subsequently, it was shown that the cultured fibroblasts of G mice were resistant to N-, B-, or NB-tropic $MuLV_E$'s (8), but the in vitro resistance was much lower than the in vivo one (24). Since both in vivo and in vitro resistances affected the replication of $MuLV_E$, probably the expression of *Fv-4* was quite weak in vitro.

To analyze the weak expression of *Fv-4* in vitro, we established cell lines of G mouse embryo tissues, and examined their clones with respect to MuLV sensitivity, karyotype, glucose-6-phosphate dehydrogenase-1 (G6PD-1), and cell morphology. Our experiments suggested that the $MuLV_E$ resistance of G mouse cells was unstable and tended to be lost during culture owing to a segregation of sensitive cells from resistant cells.

MATERIALS AND METHODS

Virus. $MuLV_E$'s were N-tropic Friend leukemia virus produced from FV131 cells; NB-tropic Friend leukemia virus, which was a 20% homogenate of the spleens of infected BALB/c mice (12); B-tropic WN1802B (6) propagated in YH-7 cells; N-tropic murine sarcoma virus (MuSV) obtained from S^+L^- C182 cells (1) infected with the N-tropic Friend leukemia virus; and NB-tropic Moloney isolate of MuSV-MuLV complex (originally obtained from N. Ida, Toyo Kogyo Hospital, Hiroshima, Japan, who obtained the virus from J. B. Moloney) in YH-7 cells. Amphotropic virus of feral mouse origin (5) and NZB mouse-derived xenotropic virus (9) were obtained from A. Ishimoto, National Institute of Allergy and Infectious Diseases, and propagated in mink cells. Amphotropic MuSV was obtained by infecting S^+L^- mink cells with the amphotropic MuLV.

The $MuLV_E$'s were titrated by the standard UV-XC assay (16), and the amphotropic and xenotropic viruses by the UV-S^+L^- mink assay method (7). MuSV was titrated by counting foci 4 to 5 days after infection, and the cultures were further submitted to UV-XC assay or UV-S^+L^- mink assay depending upon the helper viruses. In our assay condition, dose response of MuSV focus formation was two-hit; i.e., secondary infection was necessary for focus formation. For calculating MuSV titer, the number of foci per dish was simply multiplied by the dilution factor. Comparison of MuSV sensitivities was made whenever possible in the same dilution level of the virus; if foci were absent

in the dilution, virus titer was calculated from the focus count obtained at the highest dilution in which foci were present. Though this MuSV titration was not precise, it allowed us to estimate MuSV and MuLV sensitivities of the cells with the same plates; since dose response of focus formation was two-hit, generally the MuSV resistance calculated will be a high estimation.

Cell. N-type DR cell line and B-type YH-7 cell line were established, respectively, from DDD-*Fv*^{r} (11) and C57BL/6 embryo tissues. The feral mouse-derived SC-1 cells (5) and S^+L^- mink cells established by P. Peebles were obtained from A. Ishimoto. Culture medium consisted of 9 parts of Eagle minimal essential medium (Nissui Co.) and 1 part of fetal calf serum inactivated by heating at 56°C for 30 min.

Cloning. Small numbers of cells were seeded in 60-mm petri dishes. When the colonies became macroscopically visible after 7 to 10 days of incubation, colonies were picked by the use of a stainless-steel cylinder in the manner described by Parker (14). MuLV sensitivities were tested about 2 weeks after the isolation, when enough of the cells were first available.

Determination of cell types with respect to MuLV$_E$ sensitivity. DR and YH-7 cell lines were used, respectively, as the standard N- and B-type established cell lines. Sensitivity of G mouse cell clones to the N- or B-tropic MuLV was expressed by the ratio of the virus titer in the G mouse cells to the virus titer in the sensitive reference cells, i.e., DR cells for N-tropic virus and YH-7 cells for B-tropic virus. The ratio was called N or B ratio, respectively. For determination of *Fv-1* type, B ratio was divided by N ratio to obtain B/N ratio. The B/N ratio was 0.01 to 0.001 for the reference N-type DR cells, 100 to 1,000 for B-type YH-7 cells, and 0.5 to 1.0 for the dually permissive SC-1 cells.

Typing of the G mouse cell clones was done in the following manner. G type (resistant type): N ratio was lower than 0.1%, and formation of discrete MuSV-transformed foci was absent. N type: N ratio was higher than 5%, and B/N ratio was lower than 0.01. D type (dually permissive type): N ratio was higher than 5%, and B/N ratio was higher than 0.5. The intermediates were expressed as G/N (intermediate between G and N type), N/D, G/N, etc.

For secondary cultures, C3Hf and BALB/c mouse cells were used as the standard N- and B-type cells, respectively. B/N ratio was calculated in a similar manner to that described above.

Karyotype and isozyme. Karyotype analysis was done according to the method described by Paul (15), and G banding was done by the method described by Wang and Fedoroff (25). G6PD-1 was analyzed by the method described by Nichols and Ruddle (10).

RESULTS

MuLV sensitivities of secondary cultures of G mouse cells. G mouse cells were resistant to N- and NB-tropic Friend leukemia viruses and to B-tropic WN1802B (Table 1). They were resistant to the focus formation of Moloney MuSV. In this assay, G mouse cells were almost fully permissive to the helper component of MuSV-MuLV complex. However, not all the G mouse secondary cultures were as sensitive as the cells used in this assay; Moloney MuLV sensitivity of some G mouse secondary cultures was only 4% of that of sensitive mouse cells

TABLE 1. *MuLV sensitivities of G and other mouse embryo secondary cultures*

Virus	Virus sensitivity relative to sensitive cells (%)[a]				
	C3Hf	DBA/2	BALB/c	DDD	G
Ecotropic virus					
Moloney MuSV-MuLV (NB-tropic) MuSV	100	38	17	110	2 (0.1-58)[b]
MuLV	100	100	30	110	100 (4-130)[b]
NB-tropic Friend leukemia virus	100	ND	140	130	4
N-tropic Friend leukemia virus	100	320	0.1	140	9
B-tropic WN1802B	0.7	1	100	14	1
B/N[c]	0.007	0.003	1000	0.1	0.1
Amphotropic virus (wild mouse derived)					
MuSV	100	50	<0.01	ND	120
MuLV	100	97	0.1	ND	110
Xenotropic MuLV (NZB mouse derived)	r	r	r	r	r

[a] Figures indicate relative MuLV sensitivities. Except for WN1802B, the sensitivities relative to the C3Hf mouse embryo cells are indicated (%); for WN1802B, BALB/c mouse embryo cells were used as reference. MuLV$_E$'s were titrated by the UV-XC assay, and amphotropic and xenotropic MuLV's by the UV-S^+L^- mink assay. ND, Not done. r, Completely resistant when infected with 10^4 PFU (titer on S^+L^- mink cells) of xenotropic MuLV.

[b] Range of the variation among 19 cultures obtained from individual G mouse embryos.

[c] Relative sensitivity to B-tropic virus was divided by the sensitivity to N-tropic virus.

(range of the variation among 19 cultures obtained from individual G embryos is indicated in parentheses in Table 1), and resistant G mouse cell clones were unambiguously resistant to Moloney MuLV (vide infra). It was also noticed that, even when the plaquing efficiency in G mouse cultures was comparable to efficiency in sensitive mouse cells, the plaques in G mouse cells were much smaller (24). In most G mouse cell cultures infected with Moloney MuSV, many transformed cells appeared scattered, and only a few discrete foci appeared even at the highest virus dose (about 10^3 focus-forming units per dish). Poor development of foci in G mouse cells may partly be due to inefficient propagation of the virus.

To amphotropic MuLV and also to focus formation by amphotropic MuSV, the G mouse cells were fully permissive. None of the cells tested were sensitive to the xenotropic virus.

It should be noticed that the restriction of B-tropic MuLV in G and DDD mouse cells was relatively weak. The B/N ratio was about 0.1 for G and DDD, and about 0.005 for C3Hf and DBA/2 (Table 1).

Variable MuLV$_E$ resistance of G mouse cell cultures. When different culture lots of G mouse cells, each of which was derived from a litter of embryos, were tested for MuLV$_E$ sensi-

TABLE 2. *Sensitivity to N-tropic Friend leukemia virus of G and DDD mouse embryo secondary cultures derived from individual embryos*

Mouse strain	Litter no.	Days of gestation	Virus sensitivity relative to standard DDD (%)[a]
G	1	13	0.2, 0.9, 4.5, 5.0, 5.6, 6.0, 10.0
	2	16	0.5, 0.5, 1.9, 1.9, 5.7, 5.7, 47.6
	3	18	0.7, 1.6, 10.0, 14.5, 22.6
DDD	1	20	76, 83, 110, 162, 340, 400, 476

[a] Plaquing efficiency in the cells tested relative to the efficiency in a standard DDD mouse embryo culture. The standard DDD mouse embryo culture was a stock of the cells derived from a litter.

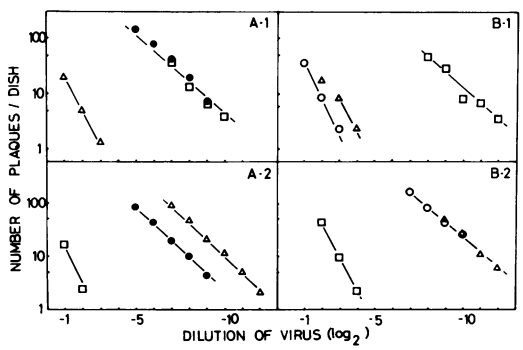

FIG. 1. *Titrations of B-tropic WN1802B (A-1, B-1) and N-tropic Friend leukemia virus (A-2, B-2) in dually permissive and typical N-type clones of G mouse cells by the standard UV-XC assay.* (●) *G66m4 (dually permissive cells);* (○) *G33kl (N-type cells);* (△) *DR cells (reference N-type cells);* (□) *YH-7 cells (reference B-type cells).*

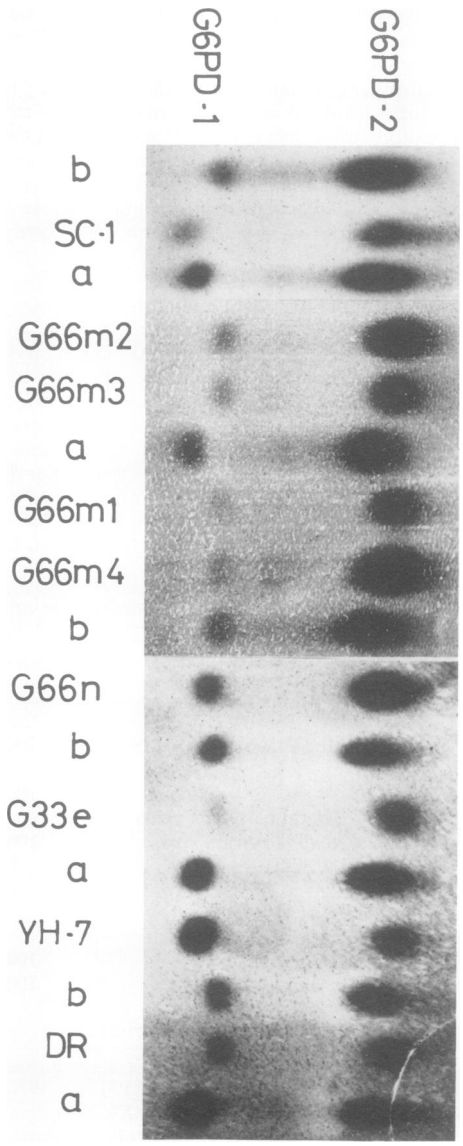

FIG. 2. *G6PD-1 starch gel electrophoresis. a and b are references for Gpd-1$^{a/a}$ and Gpd-1$^{b/b}$, respectively (samples were obtained from the kidney tissues of reference mice).*

TABLE 3. *MuLV sensitivity of G mouse cell clones*

Parent[a]	Clone	Chromosomes		Moloney MuSV-MuLV		N[f]	B[g]	N/B[h]	Cell type[i]
		Modal no.[b]	S.M.[c]	Foci[d]	Plaque[e]				
G6	G61	71	38	39	5	100	2	0.02	N/D
	G62	—[j]	—	21	6	34	0.8	0.02	N/D
	G63	—	—	7	115	15	2	0.13	N/D
	G64	—	—	20	61	13	25	1.9	D
	G65	—	—	46	170	210	61	0.3	N/D
	G66	—	—	400	770	280	55	0.2	N/D
	G67	—	—	250	28	45	5	0.12	N/D
	G68	—	—	90	16	10	7	0.7	D
	G69	82	24	29	86	20	39	1.5	D
G66	G66a	—	—	53	23	4	1.6	0.4	N/D/G
	G66b	—	—	27	40	5	10	2	D
	G66c	—	—	18	44	16	8	0.5	D
	G66d	—	—	18	7.4	0.8	1.2	1.5	D/G
	G66e	—	—	41	23	22	7	0.3	N/D
	G66f	—	—	105	45	7	20	2.9	D
	G66g	—	—	11	23	12	26	2.2	D
	G66h	—	—	16	38	40	24	0.6	D
	G66i	—	—	20	19	8	1.2	0.15	N/D
	G66j	—	—	32	41	13	16	1.3	D
	G66k	—	—	33	28	7	6	0.9	D
	G661	—	—	-	18	-	3	—	—
	G66m	—	—	50	11	26	48	1.9	D
	G66n	—	—	200	15	28	34	1.2	D
G66m	G66ml	78	4	89	86	3.2	9.5	2.9	D
	G66m2	79	2	360	62	32	18	0.6	D
	G66m3	96	2	140	25	4	7.5	1.9	D/G
	G66m4	—	—	88	43	140	158	1.1	D
	G66m5	160	14	53	93	20	5	0.25	N/D
	G66m6	—	—	380	—	133	7.2	0.05	N/D
G3	G31	—	—	<0.1	0.1	<0.1	<0.1	—	G
	G32	66	0	<0.1	10	<0.1	<0.1	—	G
	G33	—	—	<0.1	15	<0.1	<0.1	—	G
	G34	61	0	<0.1	19	<0.1	<0.1	—	G
	G35	65	0	11	35	10	<0.1	0.01	N
	G36	—	—	<0.1	1.3	<0.1	<0.1	—	G
	G37	56	0	30	127	220	0.1	0.0005	N
	G38	60	19	14	63	19	0.03	0.002	N
G33	G33a	—	—	+[k]	1.1	<0.1	<0.1	—	G
	G33b	—	—	+	6.3	<0.1	<0.1	—	G
	G33c	—	—	7.2	5.4	0.3	0.2	0.7	G/D
	G33d	—	—	44	15	5	0.2	0.04	N/D
	G33e	62	97	<0.1	0.01	<0.1	<0.1	—	G
	G33f	—	—	+	3.7	<0.1	0.1	—	
	G33g	—	—	50	—	19	0.1	0.005	N
	G33h	—	—	—	—	7	0.04	0.006	N
	G33i	—	—	—	—	16	0.02	0.001	N
	G33k	64	0	93	—	66	0.02	0.003	N
	G331	—	—	+	10	0.6	0.03	0.05	N/D/G
G33k	G33k1	64	2	57	10	212	0.01	0.00005	N
	G33k2	—	—	10	58	25	5.7	0.23	N/D
	G33k3	—	—	4.4	32	8.2	0.3	0.04	N/D
	G33k4	63	8	233	—	296	0.03	0.0001	N

TABLE 3.—*cont'd.*

Parent[a]	Clone	Chromosomes		Moloney MuSV-MuLV		N[f]	B[g]	N/B[h]	Cell type[i]
		Modal no.[b]	S.M.[c]	Foci[d]	Plaque[e]				
G33e	G33e1	62	96	0.6	7	0.14	0.01	0.07	N/G
	G33e2	61	96	12	25	20	0.01	0.0005	N
	G33e3	—	—	133	—	99	0.06	0.0006	N
	G33e4	60	100	<0.1	3.9	0.01	<0.003	—	G

[a] Parent from which the clones were derived. G31, G32, etc., were clones of G3, and G33a, G33b, etc., were subclones of G33.
[b] Modal chromosome number.
[c] Frequency of cells that had metacentric chromosomes (%).
[d] MuSV focus titer relative to DR cells (%).
[e] MuLV plaque titer relative to DR cells (%).
[f] N-tropic Friend leukemia virus titer relative to DR cells (%).
[g] B-tropic WN1802B titer relative to YH-7 cells (%). Generally, DR or YH-7 cells gave about 20-fold higher titer than the DDD or BALB/c primary cultures when infected with N- or B-tropic virus, respectively.
[h] Values in column "B" were divided by the values in column "N."
[i] Cell type with respect to MuLV sensitivity.
[j] —, Not done.
[k] MuSV transformation was detected, but no discrete foci were present.

tivities, the sensitivity to N-tropic Friend leukemia virus of G mouse cells relative to standard DDD mouse cells was 0.6, 1.4, 1.6, 2.7, and 6.2% for six lots. In cultures derived from individual embryos, the variation was much larger, i.e., from 0.2 to 48% (Table 2). The variation could not have been due to a genetic heterogeneity, because the three litters were obtained from mothers inbred for 15 to 16 generations of brother-sister matings. Actually, *Gpd-1* locus was homozygous (vide infra).

For DDD mouse embryo cells in a litter, the sensitivity to N-tropic Friend leukemia virus relative to the standard DDD mouse cells varied from 76 to 480% (Table 2). About 200-fold variation in G mouse embryo cultures far exceeded the 6-fold variation in DDD mouse embryo cultures.

Establishment of G mouse cell lines. We established cell lines starting from the individual embryos of a G mouse mother inbred for 13 generations. After three to four months of culture, we obtained one resistant, two N-type, and two cell lines sensitive to both N- and B-tropic MuLV's almost equally (H. Yoshikura, *in* Y. Ikawa, ed., *Genetic Aspects of Friend Virus and Friend Cells*, in press). A dually permissive G-6 and a resistant G-3 line were studied in detail.

Dually permissive G-6 line. (i) MuLV sensitivities. Because the G-6 line might have been a mixture of N- and B-type cells (though it derived from a single embryo), the cells were repeatedly cloned. After three successive clonings, we still obtained clones permissive to both N- and B-tropic $MuLV_E$'s (Table 3). In Fig. 1, titration curves of N- and B-tropic $MuLV_E$'s in a three-times-cloned G66m4 are shown together with the control titration curves in N-type DR and B-type YH-7 cells. The G66m4 showed single-hit titration kinetics for both viruses, and the level of the sensitivities was comparable to those of the respective sensitive host cells. Though feral mouse-derived dually permissive SC-1 cells were sensitive to the xenotropic MuLV (4), dually permissive G-6 clones were completely resistant to the xenotropic MuLV (data not shown).

(ii) G6PD-1. *Gpd-1* locus, which controls the isozyme pattern of G6PD-1, is located only 1 map unit away from the *Fv-1* locus in the number 4 chromosome, which controls susceptibility to N- or B-tropic $MuLV_E$'s (17, 20). Since *Fv-1*-controlled restriction is dominant (6), dual sensitivity may quite well be brought about by a deletion of this region. Unexpectedly, all the dually permissive G mouse cell clones, G66m2, G66m3, G66m4, and G66n, and also the feral mouse-derived SC-1 cells, retained the marker isozyme (Fig. 2). G mouse cells were of $Gpd\text{-}1^{b/b}$, and SC-1 cells were $Gpd\text{-}1^{a/a}$.

(iii) Karyotype analysis. By G banding (25), we examined the number 4 chromosomes where *Fv-1* is located. The dually permissive G66m2 and G66m5, whose respective modal chromosome numbers were 79 and 160, had two and four number 4 chromosomes, respectively (Fig. 3A). For SC-1 cells, the whole set of chromosomes were classified by identifying with the G banding. Among 90 chromosomes, nearly one-third of them could not be classified owing to extensive chromosomal aberrations. Two number 4 chromosomes were identified, and there were four number 5 and four number 8 chromosomes, which were respectively assigned for

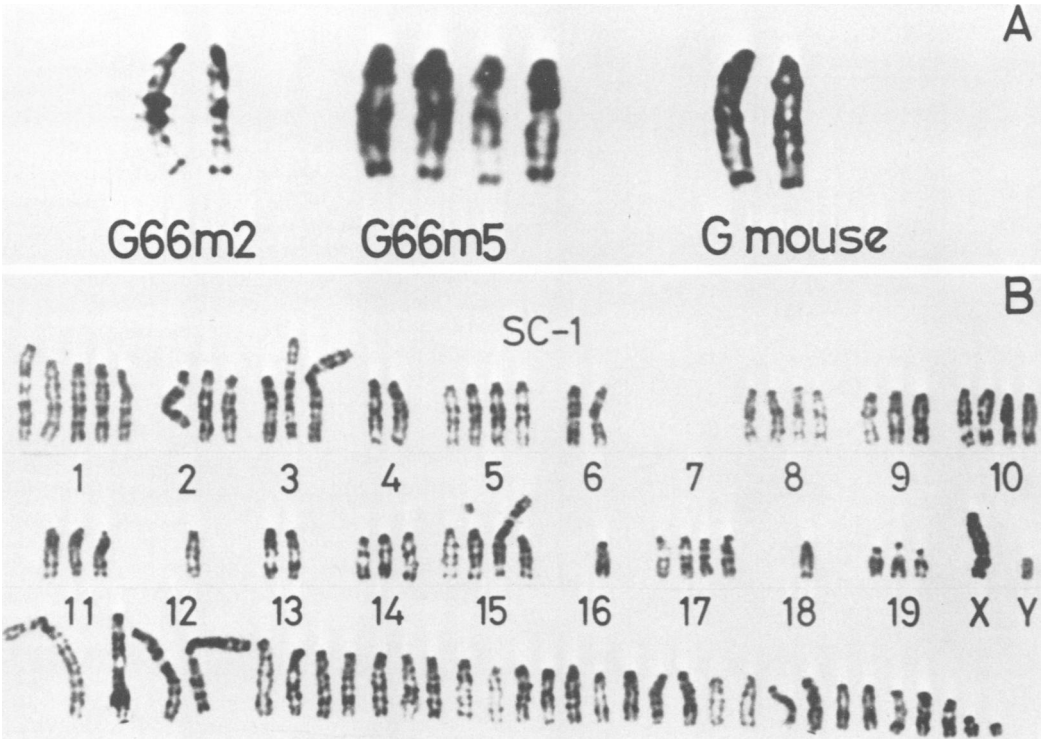

FIG. 3. *Karyotypes of G6 clones and SC-1 cells by the G banding. (A) Number 4 chromosomes; (B) classification of whole chromosomes of SC-1 cells. Figures indicate the chromosome numbers. It was not possible to classify the bottom 31 chromosomes owing to extensive aberrations.*

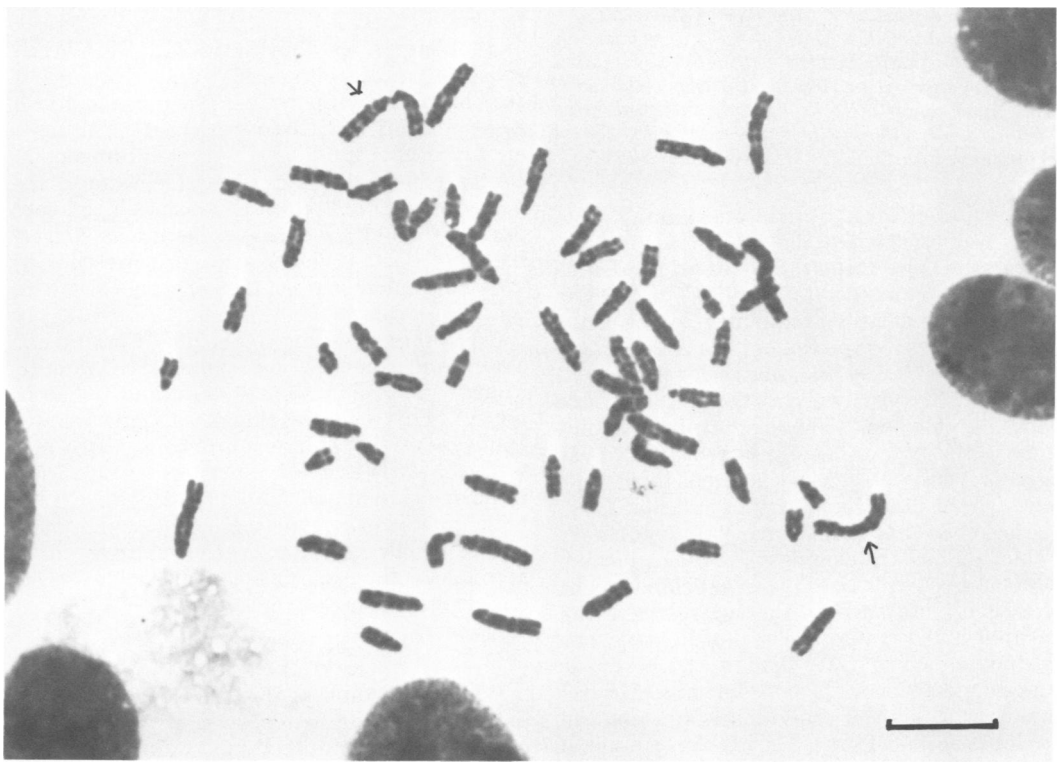

FIG. 4. *Large submetacentric marker chromosome indicated by arrows in G33e-derived clone, G33e4.*

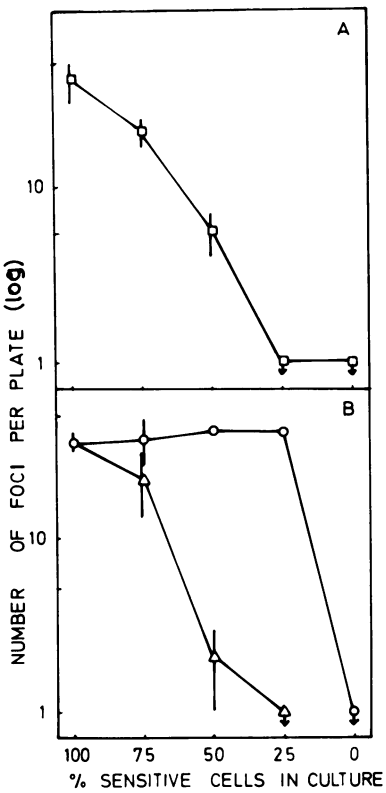

FIG. 5. *Reconstitution experiments. (A) G33e (resistant cells) and G33k (sensitive cells) were mixed in various ratios and infected with Moloney MuSV-MuLV complex. (B) C3H2K (sensitive cells) were mixed with mink cells (completely resistant cells)* (△) *and YH-7 cells (partially resistant cells)* (○) *in various ratios and infected with N-tropic MuSV.*

those required for ecotropic and amphotropic MuLV replications (2) (Fig. 3B).

Resistant G-3 line. (i) Segregation of sensitive cells from resistant cells. The G-3 line was cloned repeatedly. In the first cloning, three N-type and five G-type clones were isolated. One of the G-type clones, G33, was recloned. Five N-type, four G-type, one D/G-type, and one N/D/G-type clones were isolated. In the third cloning, a G-type G33e segregated two N-type together with one G-type and one N/G-type clone. On the other hand, N-type subclone G33k produced two N-type and three N/D-type clones (Table 3). Since the cloning was done by picking the colonies on a plate, the resistant clones might have been initiated from the aggregates of the sensitive and resistant cells. Fortunately, the resistant subclone G33e had a characteristic large submetacentric chromosome at a high frequency, whereas the other clones did not. All the clones derived from G33e, both resistant and sensitive, had the marker chromosome (Fig. 4, Table 3). This clearly shows that the resistant G33e was really a clone, and it segregated the sensitive cells together with the resistant ones.

In Fig. 1, titration curves of N-tropic and B-tropic $MuLV_E$'s in an N-type clone G33k1 are shown. The titration curves in G33k1 were almost identical to those in the N-type DR cells.

The situation above indicates that, if cultured for a long time, the resistant clones came to contain more and more sensitive cells. Actually, a resistant clone G33 showed less than 0.1% sensitivity to N-tropic Friend leukemia virus when first tested, but after a further 30 days of culture, the sensitivity went up to 47%. A serious question here is what proportion of the cells in the resistant culture was actually resistant. For rough estimation, we performed a reconstitution experiment. We mixed the resistant G33e and the sensitive G33k cells in different proportions and infected the cells with Moloney MuSV. Cultures consisting of 1 part sensitive and 3 parts resistant cells were as resistant as those consisting of the resistant cells alone (Fig. 5A). That is, the resistant phenotype was obtained even if the culture contained 25% sensitive cells. The value is a low estimation, since the resistant clonal cell culture itself contained sensitive cells in a certain proportion.

We performed a similar reconstitution experiment with a different combination of the cells, i.e., N-type C3H2K cells (22), B-type YH-7 cells, and mink cells resistant to the $MuLV_E$. C3H2K cells were mixed with either mink cells or YH-7 cells in various proportions. The mixed cultures were infected with N-tropic MuSV. The presence of 50% of mink cells in the culture significantly reduced the focus formation, whereas the presence of 75% of partially resistant YH-7 cells did not (Fig. 5B). This experiment may suggest that the resistant clones were mixtures consisting of completely resistant cells plus sensitive cells.

(ii) Cell morphology. The dually permissive clones and some N-type clones grew rapidly, losing contact inhibition (G66ml and G33k5 in Fig. 6). The resistant clonal cells grew slowly and were of filamentous morphology. However, typical N-type and G-type clones derived from the same parent (G33e2 and G33e4 in Fig. 6) both grew slowly and were of a similar morphology, indicating that the slow growth cannot account for the $MuLV_E$ resistance of the resistant clones.

DISCUSSION

When we established cell lines starting from individual G mouse embryos, most cell lines established were not restricted to $MuLV_E$ infection (except for *Fv-1* restriction). Only one re-

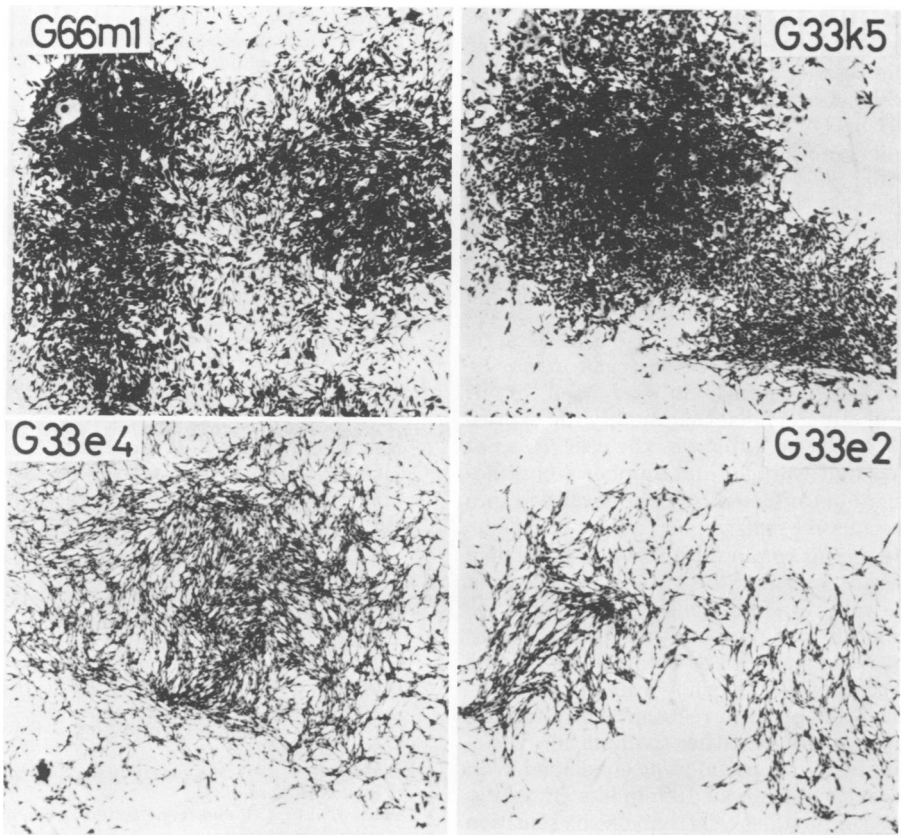

FIG. 6. *Colonies of G mouse cell clones developing after 7 days of culture. G66m1: D type; G33k5: N type; G33e4: G type; G33e2: N type.*

sistant line was established. The resistant cells constantly segregated sensitive cells. To maintain the resistance, constant clonings were required, or else all the cultures became MuLV$_E$ sensitive sooner or later. Thus, the G mouse cell cultures were always mixtures of sensitive and resistant cells, the proportion varying from one culture to another. The lower MuLV$_E$ resistance in cultured cells and its variability among culture lots can be attributed to this phenomenon.

The mechanism of the segregation of sensitive from resistant cells is intriguing, but remains obscure. No karyological changes specifically associated with the process have been detected. However, many cellular functions tend to be lost during the culture; for example, disappearance of melanin from pigmented cells, of chondroitin sulfate synthesis from cartilage, or of D-amino acid oxidase and glucose-6-phosphatase from kidney cells, etc. (see ref. 15 for review). The unstable MuLV$_E$ resistance of G mouse cells may be similar to these phenomena.

The *Fv-1* restriction is dominant both in genetic crosses (6) and in somatic hybrids (21). In somatic hybrids between mouse and hamster cells, the loss of number 4 chromosome and dual permissiveness were well correlated (2). Since we already know that *Fv-1* is located only 1 map unit away from *Gpd-1*, we expected to correlate the loss of *Fv-1* restriction in G-6 clones with that of G6PD-1 marker isozyme. However, all the dually permissive cells derived from G mouse, and also feral mouse-derived SC-1 cells, retained the isozyme and, apparently, normal number 4 chromosomes as well. This may have been brought about by a point mutation in *Fv-1* locus. But, since *Fv-1* restriction is dominant, to lose the restriction the cells have to undergo two identical mutations in the pair of chromosomes. If the mutation occurs randomly, the probability will be quite low. Thus, we cannot but think that the dual permissiveness was due neither to deletion nor to mutation of *Fv-1* locus. The dually permissive cells probably retained the normal structure of *Fv-1*, which was rendered nonfunctional by a mechanism of which we are ignorant.

Establishment of dually permissive mouse cells has been documented (3, 4). It is not known, however, whether it is possible with any mouse strains or not. Though G mouse fibroblasts, as

well as DDD mouse fibroblasts, were more sensitive to the N-tropic virus than to the B-tropic virus, the restriction of B-tropic virus in these mouse cells was weak. The B/N ratio was 0.005 for C3Hf or DBA/2 mouse cells, whereas for DDD and G mouse cells the ratio was about 0.1. In addition, SC-1 cells were obtained from feral mouse-derived N-type cells that were relatively sensitive to the B-tropic virus even before the clonings (4). It appears as if N-type cells, which have only low resistance to B-tropic virus, segregated typical N-type cells together with the dually permissive cells (see also the data by Hartley and Rowe [4]). The segregation may be due to either (i) changes in *Fv-1* itself, or (ii) changes in other gene(s) that modify the expression of *Fv-1*. If the latter was the case, it is not surprising that C6PD-1 and number 4 chromosomes were not affected by the conversion into dually permissive cells.

Finally, in this communication we did not deal with the mechanism of the $MuLV_E$ resistance of G mouse cells. Since G mouse cell cultures are always mixtures of resistant and sensitive cells, no definitive answer can be obtained concerning this point. However, as shown in the reconstitution experiments, the resistance of G mouse cells may be absolute rather than partial. Vesicular stomatitis virus pseudotype enveloped in an $MuLV_E$ coat plated about 10-fold less efficiently on the resistant clonal cells than on the sensitive clonal cells (preliminary data). Therefore, resistance may quite possibly be expressed at the level of viral penetration, and consequently in the cell membrane. If so, the segregation of the sensitive cells should have been accompanied by changes in the cellular membrane where the $MuLV_E$ receptors are located.

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