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Mouse Strain Resistant to N-, B-, and NB-Tropic Murine Leukemia Viruses

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Mouse strain G was studied for its susceptibility to various strains of murine leukemia and sarcoma viruses. Both N- and NB-tropic Friend leukemia viruses neither induced splenomegaly nor grew efficiently in strain G mice. Using the XC test, cultured embryo cells were found to be resistant, but not absolutely, to all the tested viruses, N-tropic AKR virus, N- and NB-tropic Friend leukemia viruses, NB-tropic Rauscher leukemia virus, B-tropic WN1802B virus, NB-tropic Moloney leukemia and sarcoma viruses, and N-tropic Kirsten sarcoma virus, although the resistance to Moloney leukemia and sarcoma viruses is sometimes not as strong as that for other viruses. Thus, the strain G mice are unique among mouse strains because they show resistance that is not related to the N-B tropism of murine leukemia viruses.

Host genes affect innate resistance to murine leukemia viruses (MuLV's) in mice (5). The *fv-1* locus affects the growth of most ecotropic MuLV. Mice having the *Fv-1*^{nn} genotype are susceptible to N-tropic MuLV but resistant to B-tropic MuLV. Those with *Fv-1*^{bb} have the reciprocal pattern of susceptibility. *Fv-1*^{nb} hybrid mice are resistant to both N- and B-tropic viruses. However, the locus does not affect growth of NB-tropic virus; the virus grows equally well in mice with any *Fv-1* genotype. These *Fv-1* phenotypes are also expressed on cultured mouse cells (4-7, 9-11). The *Fv-2* locus is specifically concerned with focus formation on spleen surface and rapid development of splenomegaly after infection of Friend (F-MuLV) and Rauscher leukemia viruses. The locus does not seem to affect growth of MuLV, except for spleen focus-forming virus (SFFV) of the F-MuLV complex. Since F-MuLV is a complex of SFFV and lymphatic leukemia virus (LLV), the splenomegaly induction by F-MuLV is dually controlled by *Fv-1* and *Fv-2* (4, 6, 8, 14).

Suzuki and Matsubara (16) had established the G strain from a closed colony of random-bred ddYS mice by selecting mice that are genetically resistant to splenomegaly induction by N-tropic F-MuLV. Genetic cross experiments between G and susceptible strains indicated that a new gene, *Fv-4*, controlled the resistance in G mice (15). Here we report on the growth of F-MuLV in G mice and the susceptibility of cultured embryo cells to various strains of MuLV and murine sarcoma viruses (MuSV).

MATERIALS AND METHODS

Mice. Strains DDD (*Fv-1*^{nn}*Fv-2*^{ss} genotype) and C57BL/6 (*Fv-1*^{bb}*Fv-2*^{rr}) were described previously (6). The history of G strain was described previously (16), but its *Fv-1 Fv-2* genotypes are not yet known. Strain G mice resulting from more than 10 brother-sister matings were used. Mice of these three strains were produced in our laboratory. Both males and females were used at the age of 4 to 5 weeks. Random-bred female ddYS mice were purchased from the Shizuoka Laboratory Animal Center, Hamamatsu, Japan. They were from a colony that is highly susceptible to F-MuLV and used for virus assays when 4 to 5 weeks old.

Virus. F-MuLV was prepared from the spleens of infected mice as described previously (6, 8). WN1802B virus (12) was from the culture fluid of infected embryo cells of BALB/c mice. AKR virus was prepared from the spleens of 5-week-old AKR mice. The spleens were pooled and homogenized in phosphate-buffered saline containing 0.25% gelatin to make a 5% (weight/volume) suspension. The homogenate was cleared by centrifugation at 5,000 $\times$ *g* for 20 min in the cold. Similarly, Rauscher MuLV was prepared from the spleens of infected DDD or BALB/c mice. Moloney MuLV was isolated from Moloney MuSV and propagated in YH-7, an established cultured cell line of C578BL/6 (18). One of two lots of Kirsten MuSV was from the culture fluid of transformed BALB/3T3 cells which had been obtained through the courtesy of Y. Ikawa, Cancer Institute, Tokyo. This virus was grown in NRK cells, and the culture fluid of the infected NRK cells was also used. Three lots of Moloney MuSV were used: an extract of tumors produced in DDD mice, the culture fluid of YH-7 infected with the virus that had been passed in vitro in mouse cells, and the culture fluid of a virus-producing cell line

(RBT-2,C2) of rat brain tumor (3). The RBT cell line was obtained through the courtesy of N. Ida, Toyo Kogyo Hospital, Hiroshima, Japan. All the viruses were stored in a Revco refrigerator at −80°C until used. The culture medium was Eagle minimum essential medium (Nissui Co. Ltd., Chiba, Japan) supplemented with heat-inactivated calf or fetal calf serum (10%).

Virus assay. (i) F-MuLV. F-MuLV was assayed by two methods: splenomegaly induction in vivo and XC plaque formation in vitro. The spleens of mice to be tested were individually homogenized in cold phosphate-buffered saline containing 0.25% gelatin, and the homogenates were centrifuged for 10 min at 1,000 × *g* in the cold. The virus in the supernatants was titrated by injecting 0.2 ml of each decimal dilution into ddYS mice and scoring splenomegaly (more than 0.5 g) occurring within 3 weeks after inoculation. For each dilution three mice were used. The mean infective dose was defined as the dose that produced splenomegaly in 50% of the inoculated mice. This method is considered to assay the complex of SFFV and LLV because of the following reasons. Our F-MuLV preparations are composed of SFFV and LLV (14). SFFV is always accompanied by LLV and has never been freed from LLV. In contrast, LLV free from LLV can be isolated from F-MuLV, but LLV alone cannot induce splenomegaly within 3 weeks after infection. The spleen extracts were also assayed using the XC test as described below. The viral infectivity detected by this method was considered to be due to LLV, because SFFV free of LLV is not available, and so we do not know whether SFFV alone induces XC plaques.

(ii) MuLV. MuLV was assayed either on embryo cells or on S^+L^--C-182 cells using the XC test as described previously (1, 6, 13). Virus titers were expressed as PFU.

RESULTS

Growth of F-MuLV in strain G mice. In the previous study, the genetic resistance of strain G mice to F-MuLV was determined on the basis of spleen weight of infected mice. The virus growth in them was not taken into account (15, 16). Since in some strains of mice F-MuLV complex or LLV may grow without inducing splenomegaly (6), virus growth in strain G mice was studied here.

DDD and G mice were intraperitoneally inoculated with F-MuLV. The inoculum of N-tropic F-MuLV contained a 6.3×10^2 mean infective dose of F-MuLV complex and 5.0×10^3 PFU of LLV, and that of NB-tropic F-MuLV contained a 3.2×10^3 mean infective dose of the complex and 1.6×10^4 PFU of LLV. Two mice of each strain were killed at weekly intervals. The spleens were weighed, examined for foci, and individually homogenized in cold phosphate-buffered saline containing 0.25% gelatin. The homogenates were cleared by centrifugation and immediately assayed by two methods: splenomegaly induction method for F-MuLV complex (SFFV plus LLV) and XC test on C-182 cells for LLV (Table 1).

TABLE 1. *Spleen enlargement and virus growth in F-MuLV-infected mice*[a]

Tropism of virus	Days after infection	DDD			G		
		Spleen (g)	SFFV + LLV (ID_{50}/spleen, × 10^4)	LLV (PFU/spleen, × 10^5)	Spleen (g)	SFFV + LLV (ID_{50}/spleen, × 10^4)	LLV (PFU/spleen, × 10^5)
N	7	1.00	>16	2.1	0.18	–[b]	–[c]
		1.22	>16	4.1	0.14	–	–
	15	1.95	>79	34	0.18	–	–
		1.97	>4.0	25	0.15	–	0.00008
	21	2.50			0.18	–	–
		2.58			0.20	–	–
	29				0.17	–	–
					0.15	–	–
NB	7	0.52	16	2.4	0.22	–	–
		1.17	7.9	13	0.18	–	–
	14	1.53	4.0	1.2	0.17	–	–
		2.03	4.0	2.8	0.17	–	0.00018
	21	2.55			0.23	–	–
		2.13			0.17	–	–
	28	2.06			0.26	–	0.00008
					0.25	–	–

[a] The mice were killed on the indicated days after infection. The spleen extracts were made individually and used for virus assay. The SFFV + LLV complex was titrated by the splenomegaly induction method, whereas LLV was titrated by the XC test on C-182 cells.

[b] –, Less than 10 mean infective doses (ID_{50}'s).

[c] –, Less than 7.5 PFU.

On day 7 of infection of N-tropic F-MuLV, DDD mice had spleens weighing 1 g. The spleens contained more than a 1.6×10^5 mean infective dose of F-MuLV complex and more than 2×10^5 PFU of LLV. In contrast, the spleens of strain G mice weighed less than 0.2 g and were free of spleen foci. Neither F-MuLV complex nor LLV was detected. On day 15, similar results were obtained, except that a trace amount of LLV was found in the spleen of one strain G mouse. The infected strain G mice were further tested on days 21 and 29. The spleens were small, and no infectious virus was detected. NB-tropic F-MuLV gave results similar to those of N-tropic F-MuLV. The almost complete suppression of NB-tropic F-MuLV in G mice was not expected, because as far as known, mouse strains have either the *Fv-1*^{nn} or *Fv-1*^{bb} genotype and the NB-tropic virus overcomes the *Fv-1* barrier (9).

To reconfirm the results obtained with NB-tropic F-MuLV, strain G was compared with C57BL/6, which is the most resistant to F-MuLV among inbred strains so far tested. Since the growth of SFFV is always accompanied by LLV, the assay of the SFFV plus LLV complex was omitted. The same dose of NB-tropic F-MuLV as used above was inoculated intraperitoneally into C57BL/6 and G mice. They were killed on days 5 and 11 of infection. The spleens were examined, and the LLV contents were determined (Table 2). In contrast to DDD, mice of both strains did not develop splenomegaly; the spleens weighed less than 0.3 g and were free of spleen foci. As expected from NB-tropism of the inoculated virus, large amounts of LLV were found in C57BL/6 mice. In contrast, LLV was not detected in the spleens of G mice.

Susceptibility of cultured embryo cells to MuLV. To determine whether the resistance of strain G mice is also operative in vitro and to determine their *Fv-1* genotype, cultured embryo cells were infected with MuLV having a known tropism. The previous genetic cross experiment in vivo suggested an *Fv-1*^{nn} genotype for strain G mice (15), but this genotype cannot account for the data shown in Tables 1 and 2.

For comparison, various mouse strains and (BALB/c × DBA/2)F_1 hybrids were used to prepare embryo cells. NZB was included here, because according to a report (9), NZB embryo cells are less susceptible to N-tropic virus than were embryo cells from other *Fv-1*^{nn} strains and show some resistance to NB-tropic virus.

Embryo cells were plated in plastic dishes and infected on the next day with N-tropic F-MuLV, B-tropic WN1802B, and NB-tropic F-MuLV. After 5 to 6 days of cultivation, the cells were submitted to XC testing (Table 3). Cells of

TABLE 2. *LLV growth in mice infected with NB-tropic F-MuLV*[a]

Days after infection	C57BL/6		G	
	Spleen (g)	LLV (PFU/spleen, × 10^3)	Spleen (g)	LLV (PFU/spleen, × 10^3)
5	0.16	3.8	0.28	–[b]
	0.12	>7.5	0.15	–
11	0.30	>7.5	0.20	–
	0.28	>7.5	0.22	–

[a] See footnote *a* of Table 1.
[b] –, Less than 12.5 PFU/spleen.

TABLE 3. *Titration of N-, B-, and NB-tropic viruses on cultured embryo cells*[a]

Cells	*Fv-1* genotype	Virus titer (PFU/ml)		
		N-tropic F-MuLV (×10^4)	B-tropic WN1802B (×10^3)	NB-tropic F-MuLV (×10^5)
DDD	*nn*	7.0	0.095	2.8
NZB	*nn*	4.8	<0.003	1.1
(BALB/c × DBA/2)F_1	*nb*	0.035	0.18	2.1
BALB/c	*bb*	0.018	3.6	4.5
G	?	0.025	<0.003	0.003

[a] Stock preparation of each virus was simultaneously assayed on embryo cells of indicated mouse strains by XC test.

DDD and NZB were susceptible to N-tropic virus, resistant to B-tropic virus, and susceptible to NB-tropic virus. (BALB/c × DBA/2) F_1 hybrid cells were resistant to both N- and B-tropic viruses but susceptible to NB-tropic virus. Cells of BALB/c were resistant to N-tropic virus but susceptible to both B- and NB-tropic viruses. These results are as expected from the *Fv-1* genotype of the mice (5, 9). In contrast, cells of G mice were resistant to all of the tested viruses. N-tropic F-MuLV gave titers of 4×10^4 to 7×10^4 PFU on *Fv-1*^{nn} cells, as compared with 0.025×10^4 on G cells. Similarly, both B- and NB-tropic viruses were titrated about 10^3-fold less efficiently on G cells than on susceptible cells.

Whether G mice are resistant to other MuLV or MuSV was studied in vitro. Development of foci by MuSV on G cells was too poor to count accurately. Therefore, XC testing was done on MuSV-infected cells. Embryo cells of DDD, (BALB/c × DDD)F_1, BALB/c, and G were simultaneously infected with stock preparations of each MuLV or MuSV described in Table 4 and subjected to the XC test. These viruses were either from culture fluid of infected mouse or rat cells or were prepared from infected mice. Since the tested viruses were N- or NB-tropic,

TABLE 4. *Resistance of G embryo cells to MuLV and MuSV*[a]

Virus (tropism)	Source of virus[b]	PFU/ml determined on:				DDD/G[d]
		DDD	F_1[c]	BALB/c	G	
MuLV						
AKR(N)	s(m)	7.4×10^2		15	8	99
		9.2×10^2		10	10	92
Rauscher (NB)	s(m)	1.3×10^4			17	765
		1.8×10^4		5.0×10^4	1.3×10^2	138
Moloney (NB)	c(m)	1.1×10^6			2.5×10^4	44
		1.2×10^6			1.5×10^5	8
		1.9×10^5	4.8×10^5	2.9×10^5	1.0×10^3	185
MuSV						
Kirsten (N)	c(m)	6.0×10^3			27	220
	c(r)	2.5×10^3		10	10	250
Moloney (NB)	c(m)	2.5×10^4			1.9×10^3	13
		1.7×10^4	5.3×10^4	1.7×10^4	38	450
	t(m)	4.6×10^6			4.5×10^5	10
		1.4×10^6	4.4×10^6	1.7×10^6	2.5×10^3	560
	c(r)	1.3×10^5			1.3×10^4	10
		1.5×10^5	3.6×10^5	1.4×10^5	4.0×10^2	362

[a] See footnote *a* of Table 3.
[b] Abbreviations: s, Spleen; c, culture fluid; t, tumor; m, mouse; r, rat.
[c] (BALB/c × DDD)F_1.
[d] DDD/G represents ratios of the virus titers obtained with DDD cells to those obtained with G cells.

DDD cells with the *Fv-1*^{nn} genotype are susceptible to all the viruses. N-tropic viruses grow poorly in BALB/c cells with the *Fv-1*^{bb} genotype, whereas NB-tropic viruses grow equally well on DDD, F_1, and BALB/c cells. DDD cells gave higher titers to all the viruses than did G cells; i.e., ratios of titer obtained with DDD to that obtained with G cells (DDD/G ratio) were always more than 1 (Table 4). AKR and Rauscher MuLV gave DDD/G ratios of 92 to 765, but ratios obtained with Moloney MuLV varied from 8 to 185. Lower DDD/G ratios were again obtained with Moloney MuSV. The ratios of Kirsten MuSV were more than 220, whereas those of Moloney MuSV ranged from 10 to 560. In general, plaques produced on G cells were smaller than on DDD cells.

MuLV shows a one-hit pattern in plaque formation on *Fv-1*-susceptible embryo cells and a multi-hit pattern on *Fv-1*-resistant cells (10). Most viruses used in the above experiments produced so few plaques on G cells even after inoculation of the largest dose that the precise hit pattern could not be determined. Only Moloney viruses produced enough numbers of plaques. The pattern was of the one-hit type.

DISCUSSION

F-MuLV induces spleen foci or splenomegaly within a short period after infection. Most mouse strains are susceptible, but some strains, such as C57BL/6, are resistant to the virus (2). This strain difference in susceptibility is due to host genes, especially *Fv-1* and *Fv-2* (6). Genetic studies on strain G mice, a recently established F-MuLV-resistant mouse line, indicated that another gene, *Fv-4*, is also concerned with the resistance to F-MuLV (15).

Strain G mice develop neither spleen foci nor splenomegaly after infection of N- or NB-tropic F-MuLV (Table 1) (15). In this respect, strain G mice are similar to C57BL/6. However, LLV of NB-tropic F-MuLV grew well in C57BL/6 but not in strain G mice (Table 2). This indicates that no development of splenomegaly in C57BL/6 is due to the *Fv-2*^{rr}, whereas the resistance of strain G mice can be principally ascribed to the poor virus growth.

A major gene locus that controls growth of MuLV in mice is *Fv-1*. According to the *Fv-1* type, mouse strains resist either N- or B-tropic MuLV. NB-tropic MuLV is not suppressed by the locus. The resistance of strain G mice is unusual, because they show resistance to N-, B-, and NB-tropic viruses in vivo and in vitro (Tables 1, 3, 4). To the best of our knowledge, such a mouse strain has never been reported. The linkage study seems to negate the possibility that the resistance gene of strain G mice, *Fv-4*, is a new allele of *Fv-1* and *Fv-2* (15). This consideration leads to the hypothesis that *Fv-4* affects a broader range of MuLV than *Fv-1* does and hence masks the *Fv-1* phenotype in G mice.

Because DNA synthesis of host cells is neces-

sary for the growth of MuLV (17), poor cell growth may lead to reduced formation of XC plaques; however, the growth of G embryo cells was comparable to that of cells of other mouse strains.

Problems to be solved are: (i) why Moloney viruses are sometimes not so strongly inhibited in G mice as the other viruses (Table 4) and (ii) whether such a wide variation in the degree of inhibition is limited to Moloney viruses. The variation may be ascribed to uncontrollable conditions of cell culture, but N- and NB-tropic F-MuLV were always inhibited strongly in vivo and in vitro. In any case, mouse strain G is a unique strain that is resistant to MuLV with any N-B tropism.

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