A MAJOR GENETIC LOCUS AFFECTING RESISTANCE TO INFECTION WITH MURINE LEUKEMIA VIRUSES

II. Apparent Identity to a Major Locus Described for Resistance to Friend Murine Leukemia Virus

By THEODORE PINCUS, M.D., WALLACE P. ROWE, M.D., and FRANK LILLY,* Ph.D.

(From the Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014, and the Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461)*

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Since the original description of the murine leukemia virus (MLV)[1] isolated by Friend (F-MLV) it has been apparent that susceptibility to this virus is limited to certain strains of mice (1). The basis of the host susceptibility patterns has been found to be at least partially genetic (2–5).

By study of two host-range variants of F-MLV, two genes controlling susceptibility have been identified. The F-MLV that has been carried only in DBA/2 mice, called F-S, is about 100-fold more efficient in inducing spleen-focus response in vivo in the DBA/2, Ha/ICR (Swiss), C3H, AKR, and NZB strains, than in the BALB/c and A strains (5). F-S MLV can be adapted to BALB/c mice with acquisition of a broadened host range. The adapted virus, called F-B, induces spleen foci in BALB/c and A strain mice as efficiently as in DBA/2 (5).

It has been shown that these patterns of susceptibility to F-S MLV are determined by a genetic locus called *Fv* (6) or *Fv-1* (7), at which the allele for resistance is dominant; its map location in the mouse genome is not known.

Certain mouse strains, including C57BL/6, C57BL/10, and C57BR mice, are highly resistant to both F-S and F-B viruses. This resistance is determined by a second genetic locus, *Fv-2*, at which the allele for susceptibility is dominant; *Fv-2* is in linkage group II (7) and is not linked to *Fv-1*. A mouse must be susceptible at *Fv-2* for the effect of *Fv-1* to be observed in the spleen-focus assay, since mice homozygous for resistance at *Fv-2* are totally refractory to focus formation (7).

In studies of the genetic control of susceptibility in vitro to N-tropic and B-tropic host-range variants of naturally occurring murine leukemia viruses (8), a gene system was described which appeared to resemble *Fv-1* in several respects: cells from all mouse strains known to be sensitive (*s*) at the *Fv-1* locus were found to show N-type susceptibility, while all strains known to be resistant (*r*) at *Fv-1* were B-type. Further, in crosses of susceptible and resistant mouse strains, resistance was dominant in both

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[1] *Abbreviations used in this paper:* F-MLV, Friend murine leukemia virus; ME, mouse embryo; MLV, murine leukemia virus; *r*, resistance; *s*, susceptibility.

the N-B system in vitro and the *Fv-1* system in vivo. These findings suggested that the N-B gene system might be identical to *Fv-1*.

The identity of the N-type and *Fv-1* sensitivity could be established in reciprocal experiments involving the viruses and mouse strains used to define the *Fv-1* and N-B gene systems. The demonstration of identity or close linkage of these loci would require that: (*a*) F-S virus, which is about 100 times more infectious in vivo in *Fv-1*^{s} than in *Fv-1*^{r} mouse strains (7), should be N-tropic, i.e., grow at least 100 times more efficiently in vitro on N-type than B-type mouse embryo (ME) cells; (*b*) the F-B virus, which is equally infectious in *Fv-1*^{s} and in *Fv-1*^{r} mice (7), should be NB-tropic, i.e., grow as efficiently in vitro on B- as on N-type ME cells; (*c*) the F-S virus (if N-tropic) should show 100-fold lower plaquing efficiency on mouse embryo (ME) cells of (N × B) type F_1 hybrids, while the F-B virus (if NB-tropic) should plaque with equal efficiency on hybrid ME cells as on ME cells derived from inbred mice; (*d*) the D2-R^S strain, a partially congenic DBA/2 strain carrying the *Fv-1*^{r} allele from C57BL/6 (7) should be resistant to N-tropic viruses, and the partially congenic D2-R^B strain, which carries the *Fv-1*^{s} allele, should be sensitive to N-tropic viruses; (*e*) mice known to show N-type sensitivity in vitro but with unknown *Fv-1* sensitivity in vivo should carry the *Fv-1*^{s} allele. This report presents experiments to test these predictions.

Materials and Methods

Mice.—Inbred strains and F_1 hybrids were obtained as described previously (8). The partially congenic strains D2-R^S and D2-R^B were developed for sensitivity or resistance at the *Fv-1* and *Fv-2* loci. The D2-R^S strain is homozygous for resistance at *Fv-1* and for sensitivity at *Fv-2*; the D2-R^B strain is homozygous for sensitivity at *Fv-1* and resistance at *Fv-2* (7). The inbred strains used to develop these partially congenic strains were C57BL/6J (B6), a B-type strain, and DBA/2J (D2), an N-type strain; each congenic line originated from the progeny of a (B6 × D2)F_1 × D2 backcross female and D2 male (7).

Tissue Cultures.—Mouse embryo fibroblast cultures were prepared from inbred and congenic strains and F_1 hybrids as described (8).

Viruses.—The F-S variant F-MLV has been maintained by in vivo passage biweekly in DBA/2 mice. The F-B variant was developed after repeated passage in BALB/c mice and has been similarly maintained in the BALB/c strain. 10% spleen extracts were prepared for testing in vitro and in vivo, with slow centrifugation to remove particulate matter from the supernatant.

Virus Titrations.—Virus preparations were titrated in vitro as described (8). Titrations in vivo were performed utilizing the spleen-focus assay (9); susceptible mice showed enlarged spleens with many foci when killed 9 days after virus infection (9).

RESULTS

Titration of the F-S and F-B Passage Lines of F-MLV on ME Cells of Inbred Strains and F_1 Hybrids.—The two F-MLV passage lines were titrated in vitro on ME cells of various inbred strains (Table I). Titers of the F-B variant were similar on cells of all strains, while titers of the F-S variant were 100–1000 times

greater on N-type than on B-type ME cells (8). These findings indicate that the F-S variant is N-tropic and the F-B variant NB-tropic.

Plaquing efficiency of the two variants on ME cells of the C57BL/6N and C57BR/cdJ strains, both homozygous for *Fv-2*^{r}, was reduced slightly or not at all from that seen on ME cells of the *Fv-2*^{s} strains tested. Thus, there is little or no apparent effect of *Fv-2* in tissue culture fibroblasts, in contrast to the spleen-focus assay in vivo (5, 7).

In testing the two variants on ME cells of (N × B)F_1 hybrids, a 100-fold reduction in titer from the sensitive N-type parent to the level of the insensitive B-type parent was seen with the F-S variant, with no appreciable change in titer

TABLE I

Titer (Log_{10}) of Two Variants of Friend Murine Leukemia Virus on Embryo Cells of Various Inbred Mouse Strains

N-B type	Cells	Viruses	
		F-S	F-B
N	NIH	5.5	5.6
	DBA/2N	5.8	5.4
	CBA/J	5.9	5.5
	C3H/HeN	5.6	5.3
	C57L/J	5.7	5.5
	129/J	5.5	5.8
	NZB/N	5.2	5.0
	C57BR/J	4.8	4.8
B	BALB/c	3.1	5.5
	A/J	2.8	5.1
	C57BL/6	2.2	5.3
	B10.BR/J	2.9	5.5

of the F-B variant (Table II). These findings confirm the tropisms noted for the two variants, as N-tropic MLV types show this reduction on F_1 hybrid cells due to the dominance of resistance, while NB-tropic types grow equally well on ME cells of F_1 hybrids and inbred mice (8). The results also show that the broad host range of F-B virus is not due to its being a mixture of N-tropic and B-tropic variants.

Testing of Naturally Occurring Viruses on ME Cells of Partially Congenic Mice.—ME cells were prepared from the partially congenic mice, D2-R^B (*Fv-1*^{s}) and D2-R^S (*Fv-1*^{r}). Three N-tropic and three B-tropic viruses were titered on these cultures (Table III). The D2-R^B cells showed N-type sensitivity, and D2-R^S B-type susceptibility, indicating the association of the *Fv-1*^{s} allele with N-type susceptibility and the *Fv-1*^{r} allele with B-type susceptibility.

The pattern of virus growth on ME cells of the D2-R^B strain indicates the

correlation of N-type and *Fv-1* sensitivity. In addition, the finding of B-type sensitivity in the D2-R^S strain is suggestive of allelism or close linkage of the determinants of N- and B-type sensitivity. This strain originated from a backcross of a (C57BL/6 × DBA/2)F_1 × DBA/2 with further backcrossing to DBA/2 (7); thus the D2-R^S mouse derives most of its genetic material from DBA. If the N and B genes were on different linkage groups, D2-R^S should show the resistance pattern of (N × B)F_1 hybrids. As B-type sensitivity was found in D2-R^B, the possibility of allelism appears more likely.

Classification of the N-Type C57BR Strain as Fv-1.—Mice of the C57BR strain are homozygous for *Fv-2*^{r}, and therefore totally refractory to the induction of

TABLE II

Titer (Log_{10}) of Two Variants of Friend Murine Leukemia Virus on Embryo Cells of F_1 Hybrids of (N-Type × B-Type) Mouse Strains

N-B Type	Cells	Viruses	
		F-S	F-B
N	DBA/2N	5.8	5.4
B	BALB/cN	3.1	5.5
(N × N)F_1	(C57BR/cdJ × C57L/J)F_1	5.8	5.7
(B × B)F_1	(A/J × BALB/cN)F_1	3.0	5.4
(N × B)F_1	(C57BR/cdJ × BALB/cN)F_1	3.0	5.6
	(C57L/J × A/J)F_1	3.0	5.7
	(C57L/J × BALB/cN)F_1	3.0	5.6
(B × N)F_1	(BALB/cN × DBA/2N)F_1	2.5	5.2
	(BALB/cN × C57BR/cdJ)F_1	2.5	5.7

spleen foci by either F-S or F-B virus. Consequently, no direct method was available to test in vivo for their *Fv-1* type. Since the results in vitro indicated that C57BR mice are N-type (8), and thus presumably homozygous for *Fv-1*^{s}, it was of interest to verify this prediction by the indirect genetic method of progeny testing. As sensitivity is dominant at the *Fv-2* allele, the effect of resistance at *Fv-2* is not seen in heterozygotes. Thus, the cross (DBA/2 × C57BR)F_1 should possess the genotype *Fv-1*^{s}/*Fv-1*^{s} and *Fv-2*^{s}/*Fv-2*^{r} and therefore be strongly susceptible to focus formation by both F-S virus and F-B virus; the cross (D2-R^S × C57BR)F_1 should be *Fv-1*^{r}/*Fv-1*^{s} and *Fv-2*^{s}/*Fv-2*^{r} and be much more strongly susceptible to focus formation by F-B than by F-S virus; the cross (D2-R^B × C57BR)F_1 should be *Fv-1*^{s}/*Fv-1*^{s} and *Fv-2*^{r}/*Fv-2*^{r} and be refractory to focus formation by both viruses. These predictions were completely borne out, as shown in Table IV.

DISCUSSION

It should be emphasized that these studies were performed with two independently developed systems for study of virus-host interaction: (*a*) a system for testing F-MLV variants in vivo, with development of partially congenic lines to study genetic factors in this system (7); (*b*) a system for testing naturally occurring isolates in vitro (8, 10). In the experiments presented here, viruses

TABLE III

Titer (Log_{10}) of N-Tropic and B-Tropic MLV Types on Mouse Embryo Cells Prepared from Partially Congenic and Inbred Strains

N-B type	Cells	N-tropic viruses			B-tropic viruses		
		AKR-L1	BALB/c-S3N	BALB/c-U1	BALB/c-T1	BALB/c-T2	BALB/c-L1
N	C57L/J	4.9	4.7	3.5	0.5	0.9	0.8
	D2-R^B	4.7	3.9	3.1	<0.5	<0.5	<0.5
B	BALB/c	2.5	2.0	0.9	3.5	5.0	3.7
	D2-R^S	<1.5	<1.0	0.5	4.1	5.7	4.0

TABLE IV

Spleen Response at 9 Days to F-S and to F-B Viruses in Mice of the C57BR, DBA/2, D2-R^S, and D2-R^B Strains and Their F_1 Hybrids

Mouse strain	N-B type	Genotype		F-S virus 0.2 ml i.v.				F-B virus 0.2 ml i.v.	
				1:10 dilution		1:100 dilution		1:1000 dilution	
		Fv-1 type	Fv-2 type	Spleen wt.	Foci	Spleen wt.	Foci	Spleen wt.	Foci
DBA/2	N	*ss*	*ss*	1.12	≫100	0.93	>100	0.67	74
C57BR	N	*ss**	*rr**	0.24	0	NT	NT	0.19	0
D2-R^S	B	*rr*	*ss*	0.26	26	0.16	0	0.28	53
(DBA/2 × C57BR)F_1	(N × N)F_1	*ss**	*sr**	1.03	≫100	0.77	>100	0.64	68
(D2-R^S × C57BR)F_1	(B × N)F_1	*sr**	*sr**	0.19	43	0.12	0	0.44	89
(D2-R^B × C57BR)F_1	(N × N)F_1	*ss**	*rr**	0.31	0	0.18	0	0.14	0

NT = not tested.

* Predicted genotype, demonstrated to be the actual genotype by these results.

from the F-MLV system were tested on cells used to study naturally occurring MLV types (Tables I and II), and naturally occurring viruses were inoculated on cells of known host-range patterns in the F-MLV system (Table III). The results of these reciprocal studies are entirely consistent with one another, indicating the likelihood that the genetic locus controlling N- and B-type sensitivity and *Fv-1* are closely linked or identical. The complete correlation between *Fv-1^s* and N-type sensitivity in the various mouse strains would be highly unlikely

if the genes were only linked, and it is our working hypothesis that *Fv-1*^{s} and the gene for N-type sensitivity are identical.

A second conclusion derived from these studies is that the genetic determinants of N- and B-type sensitivity are allelic, i.e., the allele for B-type sensitivity is *Fv-1*^{r}. If the loci determining N- and B-type sensitivity were not closely linked, it would be likely that the D2-R^S strain would be resistant to both N-tropic viruses (through resistance at the presumably identical *Fv-1* and N-type loci) and B-tropic viruses (through the predominance of DBA/2 genetic material). However, as B-type sensitivity is seen in the D2-R^S strain (as in C57BL/6), it can be inferred that the determinants of N and B sensitivity are linked. Allelism, rather than linkage, is indicated by the finding that all inbred strains show one or the other of the two reciprocal patterns (8).

F-MLV preparations contain at least two distinct neoplastic viruses, spleen focus-forming virus and lymphatic leukemia virus (11–13), and there is evidence that both may play a role in focus formation. The host-range properties appear to reside in the lymphatic leukemia virus component; we do not know if the spleen focus-forming virus is detected in the tissue culture plaque assay.

As discussed in relation to the naturally occurring MLV isolates (8), genetic factors other than the *Fv-1* gene appear to influence susceptibility of cell cultures from mouse strains. For example, the D2-R^B mice showed 2–4-fold lower sensitivity to N-tropic viruses than the C57L strain, while D2-R^S showed comparably higher sensitivity to B-tropic viruses than the BALB/c strain. The factors producing relative resistance of the 129 and NZB strains to naturally occurring N-type viruses (8) do not appear significant in regard to the F-S virus, though a slight reduction in titer was seen with NZB ME cells.

The second gene determining sensitivity to F-MLV, *Fv-2*, segregates independently of *Fv-1* (7). Although mice which are homozygous for resistance at *Fv-2* are completely resistant to spleen-focus induction by either F-MLV variant (7), it is apparent that *Fv-2* does not exert a significant effect in vitro. This finding, together with the evidence for the absolute block exerted by *Fv-2* in vivo and the dominance of susceptibility, suggests that *Fv-2* may be expressed in the hematopoietic target cells for the virus in the mouse, but not in tissue culture fibroblasts. The prediction that the *Fv-2*–resistant C57BR strain should be *Fv-1* sensitive, on the basis of N-B data, was confirmed through matings with *Fv-2*–sensitive DBA/2 mice (Table IV).

The allele symbols of the *Fv-1* system were originally assigned superscripts denoting susceptibility (*Fv-1*^{s}) and resistance (*Fv-1*^{r}) with respect to an N-tropic MLV (F-S virus). Since these symbols are clearly inconsistent with the effect of the alleles with respect to B-tropic viruses, we propose to alter these symbols for future use. The allele heretofore called *Fv-1*^{s} will be called *Fv-1*^{n}, and the allele *Fv-1*^{r} will be called *Fv-1*^{b}. Thus *Fv-1*^{n} homozygotes are highly susceptible

to infection with N-tropic viruses, and *Fv-1*^{b} homozygotes highly susceptible to B-tropic viruses; both these susceptibilities are recessive.[2]

Tissue culture titrations of mouse-grown F-MLV closely paralleled the patterns obtained with the spleen-focus assay, for both the F-S and F-B variants in inbred strains and F_1 hybrids (7, 8). The finding that cellular susceptibility to naturally occurring viruses appears to be governed by a gene identical to one well characterized in the study of F-MLV indicates that this locus might be of widespread importance in MLV biology.

SUMMARY

The N-B locus affecting tissue culture infectivity with naturally occurring murine leukemia viruses appears to be identical to the *Fv-1* locus described for sensitivity to Friend leukemia virus. Results of tissue culture studies were parallel to results of studies in vivo and indicate that the F-S virus is N-tropic and the F-B virus is NB-tropic. Inbred and partially congenic mouse strains sensitive at *Fv-1* show N-type sensitivity; strains resistant at *Fv-1* show B-type sensitivity. The *Fv-2* locus does not appear to exert significant effect in tissue culture. Knowledge of N-B type has been useful in predicting *Fv-1* sensitivity.

REFERENCES

1. Friend, C. 1957. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J. Exp. Med.* **105:**307.
2. Fieldsteel, A. H., P. J. Dawson, and W. L. Bostick. 1961. Quantitative aspects of Friend leukemia virus in various murine hosts. *Proc. Soc. Exp. Biol. Med.* **108:**826.
3. Odaka, T., and T. Yamamoto. 1962. Inheritance of susceptibility to Friend mouse leukemia virus. *Jap. J. Exp. Med.* **32:**405.
4. Axelrad, A. 1966. Genetic control of susceptibility to Friend leukemia virus in mice: studies with the spleen focus assay method. *Nat. Cancer Inst. Monogr.* **22:**619.
5. Lilly, F. 1967. Susceptibility to two strains of Friend leukemia virus in mice. *Science (Washington).* **155:**461.
6. Odaka, T. 1969. Inheritance of susceptibility to Friend mouse leukemia virus. V. Introduction of a gene responsible for susceptibility in the genetic complement of resistant mice. *J. Virol.* **3:**543.
7. Lilly, F. 1970. Fv-2: identification and location of a second gene governing the spleen focus response to Friend leukemia virus in mice. *J. Nat. Cancer Inst.* **45:**163.
8. Pincus, T., J. W. Hartley, and W. P. Rowe. 1971. A major genetic locus affecting

[2] This alteration in nomenclature was agreed upon at an informal conference on "Genetic Factors in the Friend Disease Syndrome" in January 1971 at Bethesda, Maryland, attended by Drs. A. A. Axelrad, L. B. Crittenden, G. Cudkowicz, E. De Harven, C. Friend, J. W. Hartley, F. Lilly, H. Meier, T. Odaka, T. Pincus, W. P. Rowe, R. A. Steeves, and O. Stutman.

resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. *J. Exp. Med.* **133:**1219.

9. Axelrad, A. A., and R. A. Steeves. 1964. Assay for Friend leukemia virus: rapid quantitative method based on enumeration of macroscopic spleen foci in mice. *Virology.* **24:**513.
10. Hartley, J. W., W. P. Rowe, and R. J. Huebner. 1970. Host range restrictions of murine leukemia viruses in mouse embryo cell cultures. *J. Virol.* **5:**221.
11. Dawson, P. J., R. B. Tacke, and A. H. Fieldsteel. 1968. Relationship between Friend virus and an associated lymphatic leukaemia virus. *Brit. J. Cancer.* **22:**569.
12. Steeves, R. A., and E. A. Mirand. 1969. Separation of members of the Friend virus complex by sucrose gradient centrifugation. *Proc. Amer. Ass. Cancer Res.* **10:**86.
13. Steeves, R. A., and R. J. Eckner. 1970. Host-induced changes in infectivity of Friend spleen focus-forming virus. *J. Nat. Cancer Inst.* **44:**587.