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)

(Received for publication 22 January 1971)

Since the original description of the murine leukemia virus (MLV) 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 dom-
inant; 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

* Support in part under Contract 65–612 within the Special Virus-Cancer Program of the
National Cancer Institute.

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 \( \text{Fe-1} \) system in vivo. These findings suggested that the N-B gene system might be identical to \( \text{Fe-1} \).

The identity of the N-type and \( \text{Fe-1} \) sensitivity could be established in reciprocal experiments involving the viruses and mouse strains used to define the \( \text{Fe-1} \) 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 \( \text{Fe-1}^- \) than in \( \text{Fe-1}^+ \) 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 \( \text{Fe-1}^- \) and in \( \text{Fe-1}^+ \) 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 \((N \times B)\) 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\(^b\) strain, a partially congenic DBA/2 strain carrying the \( \text{Fe-1}^- \) allele from C57BL/6 (7) should be resistant to N-tropic viruses, and the partially congenic D2-R\(^b\) strain, which carries the \( \text{Fe-1}^- \) allele, should be sensitive to N-tropic viruses; (e) mice known to show N-type sensitivity in vitro but with unknown \( \text{Fe-1} \) sensitivity in vivo should carry the \( \text{Fe-1}^- \) 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\(^b\) and D2-R\(^b\) were developed for sensitivity or resistance at the \( \text{Fe-1} \) and \( \text{Fe-2} \) loci. The D2-R\(^b\) strain is homozygous for resistance at \( \text{Fe-1} \) and for sensitivity at \( \text{Fe-2} \); the D2-R\(^b\) strain is homozygous for sensitivity at \( \text{Fe-1} \) and resistance at \( \text{Fe-2} \) (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 \( \times \) D2)F\(_1\) X 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). Titered 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*, was reduced slightly or not at all from that seen on ME cells of the Fv-2* 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)F1 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 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 F1 hybrid cells due to the dominance of resistance, while NB-tropic types grow equally well on ME cells of F1 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.

| 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 |

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

The pattern of virus growth on ME cells of the D2-Rb strain indicates the
correlation of N-type and Fe-1 sensitivity. In addition, the finding of B-type sensitivity in the D2-R8 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)F1 × DBA/2 with further backcrossing to DBA/2 (7); thus the D2-R8 mouse derives most of its genetic material from DBA. If the N and B genes were on different linkage groups, D2-R8 should show the resistance pattern of (N × B)F1 hybrids. As B-type sensitivity was found in D2-R8, the possibility of allelism appears more likely.

Classification of the N-Type C57BR Strain as Fe-1.—Mice of the C57BR strain are homozygous for Fe-28 and therefore totally refractory to the induction of spleen foci by either F-S or F-B virus. Consequently, no direct method was available to test in vivo for their Fe-1 type. Since the results in vitro indicated that C57BR mice are N-type (8), and thus presumably homozygous for Fe-1*, it was of interest to verify this prediction by the indirect genetic method of progeny testing. As sensitivity is dominant at the Fe-2 allele, the effect of resistance at Fe-2 is not seen in heterozygotes. Thus, the cross (DBA/2 × C57BR)F1 should possess the genotype Fe-1*/Fe-1* and Fe-2*/Fe-2* and therefore be strongly susceptible to focus formation by both F-S virus and F-B virus; the cross (D2-R8 × C57BR)F1 should be Fe-1*/Fe-1* and Fe-2*/Fe-2* and be much more strongly susceptible to focus formation by F-B than by F-S virus; the cross (D2-R8 × C57BR)F1 should be Fe-1*/Fe-1* and Fe-2*/Fe-2* and be refractory to focus formation by both viruses. These predictions were completely borne out, as shown in Table IV.

### Table II

| 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)F1| (C57BR/cdJ × C57L/J)F1| 5.8 | 5.7 |
| (B × B)F1| (A/J × BALB/cN)F1| 3.0 | 5.4 |
| (N × B)F1| (C57BR/cdJ × BALB/cN)F1| 3.0 | 5.6 |
|          | (C57L/J × A/J)F1| 3.0 | 5.7 |
|          | (C57L/J × BALB/cN)F1| 3.0 | 5.6 |
| (B × N)F1| (BALB/cN × DBA/2N)F1| 2.5 | 5.2 |
|          | (BALB/cN × C57BR/cdJ)F1| 2.5 | 5.7 |
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

| N-B type | Cells | N-tropic viruses | B-tropic viruses |
|----------|-------|------------------|------------------|
| N        | C57L/J | 4.9 4.7 3.5 0.5 0.9 0.8 |
|          | D2-Rs  | 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-Rs  | <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-Rs, and D2-Rb Strains and Their F1 Hybrids

| Mouse strain | N-B type | Genotype | F-S virus | F-B virus |
|--------------|----------|----------|-----------|-----------|
|              |          | Fv-1 type | 0.2 ml i.v. | 0.2 ml i.v. | 0.2 ml i.v. | 0.2 ml i.v. |
|              |          | Fv-2 type | 1:10 dilution | 1:100 dilution | 1:1000 dilution | 1:1000 dilution |
| Foci | Foci | Foci | Foci |
| Spleen wt. | Spleen wt. | Spleen wt. | Spleen wt. |
| DBA/2        | N        | ss        | 1.12      | 0.93      | >100         | 0.67         |
| C57BR        | N        | ss*       | 0.24      | NT        | NT          | 0.19         |
| D2-Rs        | B        | rr        | 0.26      | 0.16      | 0           | 0.28         |
| (DBA/2 × C57BR)F1 | ss*       | 1.03      | >100      | 0.77      | >100         | 0.64         |
| (DBA/2 × C57BR)F1 | rs*       | 0.10      | 0.12      | 0          | 0.44         | 0.89         |
| (D2-Rs × C57BR)F1 | ss*       | 0.31      | 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 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 $Fe-I^*$ 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 $Fe-I^*$. If the loci determining N- and B-type sensitivity were not closely linked, it would be likely that the D2-R strain would be resistant to both N-tropic viruses (through resistance at the presumably identical $Fe-I^*$ 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 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 $Fe-I^*$ gene appear to influence susceptibility of cell cultures from mouse strains. For example, the D2-R mice showed 2-4-fold lower sensitivity to N-tropic viruses than the C57L strain, while D2-R 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, $Fe-2$, segregates independently of $Fe-I^*$ (7). Although mice which are homozygous for resistance at $Fe-2$ are completely resistant to spleen-focus induction by either F-MLV variant (7), it is apparent that $Fe-2$ does not exert a significant effect in vitro. This finding, together with the evidence for the absolute block exerted by $Fe-2$ in vivo and the dominance of susceptibility, suggests that $Fe-2$ may be expressed in the hematopoietic target cells for the virus in the mouse, but not in tissue culture fibroblasts. The prediction that the $Fe-2$-resistant C57BR strain should be $Fe-I^*$ sensitive, on the basis of N-B data, was confirmed through matings with $Fe-2$-sensitive DBA/2 mice (Table IV).

The allele symbols of the $Fe-I^*$ system were originally assigned superscripts denoting susceptibility ($Fe-I^*$) and resistance ($Fe-I^*$) 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 $Fe-I^*$ will be called $Fe-I^*$, and the allele $Fe-I^*$ will be called $Fe-I^*$. Thus $Fe-I^*$ homozygotes are highly susceptible
to infection with N-tropic viruses, and $F_{v-1}^p$ 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 F1 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 $F_{v-1}$ 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 $F_{v-1}$ show N-type sensitivity; strains resistant at $F_{v-1}$ show B-type sensitivity. The $F_{v-2}$ locus does not appear to exert significant effect in tissue culture. Knowledge of N-B type has been useful in predicting $F_{v-1}$ sensitivity.

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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.
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