JOURNAL OF VIROLOGY, Apr. 1976, p. 370-373

Vol. 18, No. 1
Printed in U.S.A.

Host Restriction of Friend Leukemia Virus Coat Protein Synthesis

USHARANJAN RAY, RUY SOEIRO,* AND BERNARD N. FIELDS[1]

Departments of Medicine and Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461*

Received for publication 27 October 1975

Fv-1 gene-mediated host restriction of Friend leukemia virus replication was investigated in terms of coat protein synthesis. By using the assay of pseudotype formation with vesicular stomatitis virus, it was shown that under restricting growth conditions the availability of leukemia virus coat protein for pseudotype formation was decreased. These studies appear to eliminate a pure assembly defect as the mechanism of *Fv-1* host restriction.

The host response to murine RNA tumor viruses is multigenic. One gene in particular, the *Fv-1* locus in mice, determines susceptibility or resistance to infection by exogenous Friend (LLV-F) and other murine leukemia viruses (6, 8, 10). Most naturally occurring murine oncornaviruses have been classified as N-tropic or B-tropic depending upon their ability to replicate in N-type (prototype NIH Swiss; $Fv\text{-}1^n/Fv\text{-}1^n$) or B-type cells (prototype BALB/c; $Fv\text{-}1^b/Fv\text{-}1^b$) (3). Some murine leukemia viruses, however, are restricted in neither cell type and are denoted NB-tropic. This gene influences the sensitivity of cells to exogenous infection (8, 10), but the mechanism of action of the *Fv-1* gene is not understood. Our earlier studies have focused on the early events of exogenous infection by LLV-F and have shown that virus adsorption and penetration are not affected by the *Fv-1* gene (5). Whereas it appears that the effect of the *Fv-1* gene acts at a stage in replication after penetration, the exact stage at which LLV-F replication is blocked by the nonpermissive *Fv-1* allele has not been finally established.

One approach to determining the site at which viral replication is blocked under nonpermissive growth conditions is to test for the presence of viral-specific proteins in the cell. We have attempted to show whether viral envelope glycoproteins are formed under nonpermissive conditions, that is, N-tropic LLV-F grown in BALB/c cells or B-tropic LLV-F grown in NIH cells. We have tested for the ability of cells infected with LLV-F to allow the formation of pseudotype virions with vesicular stomatitis virus (VSV). Such pseudotypes (denoted VSV LLV-F) would contain VSV cores and envelope glycoproteins characteristic of the murine leukemia virus. This approach focuses on the question of whether LLV-F envelope glycoproteins are synthesized during a nonpermissive infection and also whether such envelope proteins are accessible to the superinfecting VSV to form phenotypically mixed virions. Were we able to show that equal numbers of VSV pseudotypes could be produced by superinfecting the permissive versus the restrictive LLV-F infection, this would suggest that equal amounts of envelope glycoproteins were being produced in each case. Furthermore, the finding that equal amounts of VSV pseudotype were formed in each case might suggest that *Fv-1* gene restriction occurred at the level of virion assembly. On the other hand, if significantly decreased titers of VSV pseudotypes were formed in the restrictive infection for LLV-F, this would suggest a block earlier in virus replication, that is, for example, at the level of transcription or translation. A more complex possibility, that equal amounts of glycoprotein could be formed in the permissive versus the restrictive infection, but that the envelope glycoprotein was unavailable for pseudotype formation either due to geographic or mechanical reasons, or as a result of the *Fv-1* mechanism itself, could not be ruled out by this approach.

The experimental plan for the formation of VSV pseudotypes with envelope glycoprotein from LLV-F and the definition of these pseudotypes by differential antibody neutralization are essentially as described initially by Zavada (15) and, subsequently, in earlier experiments by this and other laboratories (4, 5, 7).

Restriction of N- or B-tropic LLV-F in mouse cells of the appropriate allele is not absolute but rather is multiplicity dependent (9, 14). To demonstrate this multiplicity effect and to test its effect on pseudotype formation, cells of either the permissive or the restrictive genotype were

[1] Present address: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. 02115.

infected with an LLV-F of each tropism and at two different multiplicities of infection (MOI), 0.1 and 1.0. At an input MOI of 0.2 or less, N-tropic virus replicates from 10^{-2}- to 10^{-3}-fold less on BALB/c cells than on NIH cells. B-tropic LLV-F infections gives the reciprocal result on NIH cells. However, at multiplicities of >1, the restrictive effect of the *Fv-1* gene may be overcome. Flasks containing subconfluent monolayers of cells (2×10^5 to 3×10^5) were infected with either N- or B-tropic LLV-F at the indicated MOI according to published methods (5). After adsorption for 1 h at 37 C, unadsorbed virus was removed and the cells were incubated in fresh medium. On day 4 or 5 postinfection, when the monolayer had become confluent, supernatant medium was collected to assay for LLV-F yield using the X-C plaque assay on the permissive cell type. The results (Table 1) confirm previous observations that, at a low MOI, both N- and B-tropic LLV-F yield a low titer when grown on the nonpermissive cell in comparison with that obtained on the permissive cell type. Furthermore, these results demonstrate that at high multiplicity (1.0) this effect is largely overcome.

On day 5, each infected flask, as well as uninfected cells of each type, was infected with VSV-ts45 (a temperature-sensitive [ts] mutant with thermolabile coat protein) (2) at an MOI of 5 and incubated at 31 C with medium containing 5% serum. After 24 to 36 h, at which time the cells demonstrated extensive cytopathic effects, the cells were harvested by mechanical scraping into the medium. The combined cells and medium were frozen and thawed and subjected to a 15-s pulse of ultrasonic disruption, and the resultant suspension was clarified by centrifugation at $2,000 \times g$ for 10 min. Supernatant aliquots were frozen at −80 C. Plaque assay for VSV and VSV pseudotypes was carried out as previously described (5).

The ability of permissively versus nonpermissively infected cells to support the formation of VSV (LLV-F) pseudotypes is shown in Table 2. The titers of VSV obtained from cells uninfected by LLV-F are shown in lines 9 and 10. Since VSV-ts45 is thermolabile, exposure of this virus to heat results in a drop in titer of 3 to 4 logs. Furthermore, the remaining virus can be neutralized by exposure to anti-VSV antiserum. These heat-resistant VSV produced in the cells not infected with LLV-F represent either revertants from the ts phenotype or a small "leaky" fraction of heat-stable virus. At any rate, these are completely neutralized by anti-VSV antiserum and therefore do not represent pseudotype virions. Infectious VSV plaques that survive heat and anti-VSV antiserum

TABLE 1. *FLV yield at different MOIs of N- and B3T3 cells*[a]

Cell	Virus	MOI	Log_{10} titer of FLV yield
N3T3	N-FLV	0.1	5.57
		1.0	5.50
	B-FLV	0.1	2.78
		1.0	4.00
B3T3	N-FLV	0.1	2.90
		1.0	4.60
	B-FLV	0.1	4.90
		1.0	5.15

[a] T75 flasks containing N- or B3T3 cells (2×10^5 to 3.0×10^5) were treated with DEAE-dextran (20 μg/ml) for 1 h at 37 C and washed once, and virus (2 ml) was added to adsorb for 1 h at 37 C. Excess virus was removed, the cell monolayer was washed, and infected cells were incubated with 15 ml of fresh medium. When cell confluency was reached, supernatants were collected for progeny Friend leukemia virus (FLV) titer by the X-C method (11). The cells were then superinfected with VSV-ts45 and incubated at 31 C for 24 to 36 h.

treatment must have their envelope glycoproteins replaced by those of LLV-F. The degree of glycoprotein replacement necessary for this effect is not known. In lines 3 and 5 of Table 2 are shown the results of VSV pseudotype formation in the cells restrictedly infected with LLV-F. Low-multiplicity infection of NIH cells by B-tropic LLV-F, or BALB/c cells by N-tropic LLV-F, results in no VSV plaques that resist treatment by heat or anti-VSV antiserum. Therefore, in both restricted cases, as in the cells uninfected by Friend leukemia virus, no pseudotypes of VSV have been formed. However, in the permissive infections (lines 1, 2, 7, and 8, Table 2), although the titer of VSV does not demonstrate significant protection from heat treatment, virtually all of the heat-resistant VSV plaques are resistant to neutralization by anti-VSV antiserum. To prove that these doubly resistant VSV virions are pseudotypes, an aliquot was treated not only with heat, but also with both anti-VSV and an anti-LLV-F antiserum, which alone demonstrates no neutralization of VSV. There was a further reduction in titer of between 1 and 2 logs of VSV by this combined antiserum treatment, indicating that LLV-F envelope glycoproteins were present in these pseudotypes.

When the restricted virus infections were carried out at high multiplicity (lines 4 and 6, Table 2), clear indication of pseudotype formation was found. These results correlate directly with the titer of LLV-F formed at high MOIs in the restricted situation shown in Table 1.

The control of exogenous virus infection ex-

TABLE 2. *VSV (Friend leukemia virus) pseudotype yield after coinfection of N- or B3T3 cells with VSV-ts45 and N- or B-FLV*[a]

FLV-genotype	MOI	VSV-ts	Host cell	Log_{10} titer of VSV			
				No heat (no serum)	Heat		
					No serum	Anti-VSV	Anti-FLV + anti-VSV
1. N-FLV	0.1	+	N3T3	6.1	2.72	2.65	1.0
2. N-FLV	1.0	+	N3T3	6.0	3.0	2.9	1.0
3. B-FLV	0.1	+	N3T3	6.57	2.1	<1	<1
4. B-FLV	1.0	+	N3T3	6.30	3.14	3.0	2.0
5. N-FLV	0.1	+	B3T3	6.41	2.75	<1	<1
6. N-FLV	1.0	+	B3T3	6.39	3.06	1.74	<1
7. B-FLV	0.1	+	B3T3	6.39	3.70	2.62	<1
8. B-FLV	1.0	+	B3T3	6.54	4.0	3.04	1.60
9.		+	N3T3	6.1	3.1	<1	<1
10.		+	B3T3	6.42	2.32	<1	<1

[a] N- or B3T3 mouse cells were infected, respectively, with N-Friend leukemia virus (FLV) or B-FLV (see Table 1). The cells were superinfected with ts45 VSV, and the progeny of the double infection were characterized as to thermal stability and neutralization by antiserum. The results are expressed as logarithm of infectivity titer remaining after heat and/or antiserum treatment.

erted by the *Fv-1* gene has been investigated at several points in the replicative cycle. Krontiris et al. (5) as well as Huang et al. (4) and Yoshikura (14) have independently studied the effect of virus penetration in permissive versus restrictive cells by the use of pseudotypes of VSV. Krontiris et al. (5) also showed that virus adsorbed equally well to each cell type. These experiments ruled out the possibility that the *Fv-1* gene effected a restriction at these early steps. Sveda et al. (12) furthermore carried out studies using ^{32}P-labeled input virus and were able to conclude not only that equal amounts of radiolabeled virus were adsorbed by each cell type, but that equal amounts of viral specific genome RNA appeared to enter both permissive and restricted cell nuclei and form RNA-DNA covalently linked hybrid molecules. These studies suggested that provirus formation occurs equally in both permissive and restrictive infections.

These studies of pseudotype formation represent the first clear-cut difference we have found in the replication of LLV-F in *Fv-1*^{n}- versus *Fv-1*^{b}-type cells. They indicate that viral envelope glycoproteins are not available for the formation of VSV pseudovirions in cells restrictedly infected by LLV-F. The conclusion suggested by these studies is that viral glycoproteins, as measured by VSV pseudotype formation, are formed at very reduced amounts in the restrictedly infected cell. These results have been confirmed by direct immunoprecipitation of viral specific proteins under similar conditions of infection (T. G. Krontiris, B. N. Fields, and R. Soeiro, unpublished data).

Work by Tennant et al. (13) and Declève et al. (1) has shown that, under conditions of *Fv-1*-mediated restriction of virus replication, infected cells fail to demonstrate positive fluorescence when stained with antiserum directed against viral-specific proteins and, therefore, that under conditions of *Fv-1* gene-mediated host restriction of murine leukemia virus replication viral proteins appear to be absent in the infected cell. Our studies confirm and extend these studies and emphasize particularly the envelope protein gp 69/71. Furthermore, the difficulties of quantitation of the immunofluorescence assay, and the lack of specificity for Friend leukemia virus envelope protein, are obviated by the pseudotype assay.

Studies of viral-specific proteins by either method, therefore, when taken in conjunction with our earlier studies, suggest that the *Fv-1* gene effect is mediated before viral assembly, perhaps at the level of transcription of mRNA or at the level of translation of viral-specific proteins.

This work was supported by Public Health Service contract n O1 CP43380 from the National Cancer Institute, Virus Cancer Program. R.S. was supported by Public Health Service Career Development Award K04 CA70580 from the National Cancer Institute, and B.N.F. was supported by Health Research Council of the City of New York Career Scientist Award U-2522 and an Irma T. Hirschl Trust Career Scientist Award.

LITERATURE CITED

1. **Declève, A., O. Niwa, E. Gelmann, and H. S. Kaplan.** 1975. Replication kinetics of N- and B-tropic murine leukemia virus on permissive and nonpermissive cells *in vitro*. Virology 65:320–332.
2. **Flamand, A., and C. R. Pringle.** 1971. The homologies

of spontaneous and induced temperature-sensitive mutants of vesicular stomatitis virus isolated in chick embryo and BHK 21 cells. J. Gen. Virol. 11:81–85.

3. **Hartley, J. W., W. P. Rowe, and R. J. Huebner.** 1970. Host-range restrictions of murine leukemia viruses in mouse embryo cells. J. Virol. 5:221–225.
4. **Huang, A. S., P. Besmer, L. Chu, and D. Baltimore.** 1973. Growth of pseudotypes of vesicular stomatitis virus with N-tropic murine leukemia virus coats in cells resistant to N-tropic viruses. J. Virol. 12:659–662.
5. **Krontiris, T. G., R. Soeiro, and B. N. Fields.** 1973. Host restriction of Friend Leukemia Virus. Role of the viral outer coat. Proc. Natl. Acad. Sci. U.S.A. 70:2549–2553.
6. **Lilly, F.** 1970. Fv-2. Identification and location of a second gene governing the spleen focus response to Friend Leukemia Virus in mice. J. Natl. Cancer Inst. 45:163–169.
7. **Lowe, D. N., and R. A. Weiss.** 1974. Pseudotypes of vesicular stomatitis virus determined by exogenous and endogenous avian RNA tumor virus. Virology 57:271–278.
8. **Pincus, T., J. W. Hartley, and W. P. Rowe.** 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. J. Exp. Med. 133:1219–1233.
9. **Pincus, T., J. W. Hartley, and W. P. Rowe.** 1975. A major genetic locus affecting resistance to infection with murine leukemia viruses. III. Dose response relationships in Fv-1 sensitive and resistant cell cultures. Virology 65:333–342.
10. **Pincus, T., W. P. Rowe, and F. Lilly.** 1971. A major locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to Friend Leukemia Virus. J. Exp. Med. 133:1234–1241.
11. **Rowe, W. P., W. E. Pugh, and J. W. Hartley.** 1970. Plaque assay techniques for murine leukemia viruses. Virology 42:1136–1139.
12. **Sveda, M. M., B. N. Fields, and R. Soeiro.** 1974. Host restriction of Friend Leukemia Virus: fate of input virion RNA. Cell 2:271–277.
13. **Tennant, R. W., B. Schluter, W.-K. Yank, and A. Brown.** 1974. Reciprocal inhibition of mouse leukemia virus infection by Fv-1 allele cell extracts. Proc. Natl. Acad. Sci. U.S.A. 71:4241–4245.
14. **Yoshikura, H.** 1973. Host range conversion of the murine sarcoma-leukemia complex. J. Gen. Virol. 19:321–327.
15. **Zavada, J.** 1972. Pseudotypes of vesicular stomatitis virus with the coat of murine leukaemia and of avian myeloblastosis viruses. J. Gen. Vriol. 15:183–191.