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Pseudotypes of Vesicular Stomatitis Virus with the Envelope Properties of Mammalian and Primate Retroviruses

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By employing improved techniques it has been possible to produce and characterize a representative spectrum of mammalian and primate retrovirus pseudotypes of vesicular stomatitis virus (VSV). Selection of appropriate cell lines for both the production and subsequent detection of the VSV pseudotypes has been the most important factor in permitting their demonstration. The host range for penetration of these retrovirus pseudotypes of VSV has been defined and found to differ from that reported for the replication of the corresponding retroviruses. Additionally, retroviruses having an identical host range for replication were distinguishable by differences in their host range for penetration, implying that restriction of replication may be occurring by different mechanisms. Studies of the plaque-forming efficiency of retrovirus pseudotypes of VSV in cell lines nonpermissive for replication of the corresponding retroviruses permitted a distinction to be made between the restriction of replication occurring as a consequence of postpenetration events and that occurring as a consequence of a block to penetration itself. The demonstration of primate retrovirus pseudotypes of VSV permits the use of VSV as a probe for the detection of this group of viruses.

Phenotypic mixing between viruses of disparate classes has been well documented (19) and has been exploited for the study of both cellular receptors for viruses and viral penetration of cell membranes (4, 5, 12, 13). The ability of vesicular stomatitis virus (VSV) to replicate efficiently in a wide range of cell types and to acquire readily the envelope properties of other, coinfecting viruses has enabled investigators to use pseudotype formation by VSV as a probe to identify viral presence not only in productively but also in latently infected cell lines (7). Although VSV pseudotypes of avian and mammalian retroviruses have been described, studies of the interaction of VSV with primate retroviruses have failed to detect similar phenotypic mixing (15). By employing improved techniques successfully used in the detection of VSV pseudotypes of mouse mammary tumor virus (J. Zavada, C. Dickson, and R. Weiss, Virology, in press), it has been possible to produce and characterize VSV pseudotypes of a representative spectrum of mammalian and primate retroviruses. The study of these pseudotypes has confirmed and extended data regarding host range and interference specificity of these retroviruses and has allowed the definition of optimal conditions for the use of VSV in detecting the presence of latent primate retroviruses in both laboratory and clinical materials.

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MATERIALS AND METHODS

Viruses. Wild-type VSV (Indiana strain) and the thermolabile mutant *tlB*17 (18) were used for all experiments. Stock virus was recloned and grown up in Vero cells. The simian sarcoma-associated virus (SSAV)-like component (8) of the HL23V-1 virus complex (2), the M7 strain of baboon endogenous virus (BEV) (1), and RD-114 were kindly provided by N. Teich as infected cultures of Kirsten sarcoma virus-transformed normal rat cells (KNRK), canine thymus cells (A7573), and human rhabdomyosarcoma (RD) cells, respectively. SSAV (16) and another M7 isolate of BEV were kindly provided by G. Todaro as infected cultures of canine thymus and human rhabdomyosarcoma cells (A204), respectively. Supernatant virus of each was filtered through a 0.22-μm filter and employed to establish chronic infection in Tb-1-lu and 7605L cells that were then maintained separately. Murine xenotropic retrovirus (MuLV-X) isolated from BALB/c mice and grown in rabbit SIRC cells (Zavada et al., in press) was kindly provided by N. Teich.

Cells. Tb-1-lu bat lung cells (CCL/88), NRK cells, mink lung cells (CCL/64), Vero cells, and NIH/3T3 cells were obtained and used as previously described (Zavada et al., in press). 7605L cells, a strain of human diploid fibroblasts derived from fetal lung (8), were used in passages 12 to 17. Rat-1 cells (F2408) (7a) were kindly provided by J. Wyke. Nor-

mal baboon lung cells (BLG) (11) were kindly provided by G. Todaro.

Primary cultures of chicken embryo cells derived from Leghorn flocks (C/E) were used after one passage. All other avian cells (Muscovy duck, turkey, Japanese quail, Sonnerat jungle fowl, Japanese green pheasant) were derived from embryo cultures as previously described (15).

All cell lines were maintained in Dulbecco modified Eagle medium containing antibiotics. For mammalian cell lines this culture medium was supplemented with 10% heat-inactivated (56°C for 30 min) fetal calf serum (20% for BLG cultures), whereas all avian cells were maintained in medium supplemented with 10% tryptose phosphate broth, 1% heat-inactivated chick serum, and 1% heat-inactivated fetal calf serum. Conditions for cell maintenance during VSV plaque assays were as previously described (14).

Infectivity and neutralization assays. Antiserum against VSV was obtained, and immunoglobulin fractions were prepared, as previously described (Zavada et al., in press). Antiserum to HL23V-1, prepared in rabbits by the use of gradient-purified virus disrupted with Nonidet P-40, was kindly provided by N. Hogg. Goat anti-SSV(SSAV) was obtained from C. Bergholz and f. Deinhardt in lyophilized form and reconstituted directly before use. Antiserum to BEV (ID 4-S-0504) was supplied by The Virus Cancer Program, National Institutes of Health, Bethesda, Md.

Procedures used for antiserum neutralization of both parent VSV and the pseudotypes produced, for heat inactivation of *tlB*17 viral harvests, and for the determination of residual viral infectivity by plaque assay were identical to those previously reported (Zavada et al., in press).

RESULTS

Production and host range of mammalian and primate retroviruses. One cell line derived from bat lung, Tb-1-lu, was employed for the production of all VSV pseudotype stocks used in these studies except VSV(MuLV-X), as this cell line was capable of being productively infected by all mammalian retroviruses examined and was free of known endogenous viruses. However, VSV superinfection of other retrovirus-infected cell lines also yielded pseudotype virus.

The conditions of VSV superinfection of retrovirus-infected cells (e.g., cell density, multiplicity of infection) were not found to have a significant effect on the resultant titer of VSV pseudotype virus produced, although highest yields were obtained by VSV infection of rapidly growing subconfluent cultures at a multiplicity of infection between 0.5 and 5.0. The harvesting of supernatant fluids was always performed 10 to 14 h postadsorption as no further increase in pseudotype titer occurred after that time (data not shown). The use of wild-type VSV rather than the *tlB*17 mutant was due to the generally higher pseudotype fraction obtained, perhaps a consequence of the more rapid thermal inactivation of pseudotypes formed with *tlB*17.

Superinfection of retrovirus-infected cell cultures with VSV regularly resulted in the production of more than 10^8 PFU of VSV per ml when assayed on any of the indicator cells used. The detection of VSV pseudotypes from this lytic harvest was accomplished by the treatment of supernatant fluids with anti-VSV globulin in the manner previously described, followed by the inoculation of virus in the presence of polybrene and appropriate indicator cells. Figure 1 shows that such treatment of VSV grown through uninfected cells regularly reduced the infectivity titer of VSV to less than 100 PFU/ml. Subsequent to VSV passage through retrovirus-infected cells, however, a substantially greater titer of residual infectious virus could be detected on appropriate indicator cells after antiglobulin treatment. That this infectivity did not represent unneutralized VSV was demonstrated by the fact that further anti-VSV globulin treatment failed to reduce the titer, whereas specific antiserum against the retrovirus present in the superinfected cell line was capable of completely neutralizing this residual infectivity. On the basis of these results, it was assumed that the infectivity remaining after anti-VSV globulin treatment of such harvests represented the consequence of adsorption and subsequent penetration of pseudotypes of VSV bearing envelope components specified by the retrovirus present in the superinfected cells.

The relative efficiency of penetration and, thus, the apparent pseudotype titers were often found to vary widely, depending on the indicator cell type. By employing a spectrum of both mammalian and avian cells, a host range for penetration was established for each of the VSV pseudotypes produced (Fig. 1). The HL23V-1 pseudotype of VSV penetrated primate cells, in which it replicates well (8), with only moderate efficiency, but gave 20- to 40-fold-higher titers in cells of rat origin (NRK, Rat-1). An absolute block to penetration was demonstrated in mouse cells, including both the continuous line NIH/3T3 as well as BALB/c secondary cultures (data not shown). Cells of chicken origin (C/E) were found to be highly resistant to penetration, whereas several other avian cell types (duck, turkey, quail) were more susceptible. Results obtained with the VSV(SSAV) pseudotype were identical to those found for VSV(HL23V-1), whereas VSV-(MuLV-X), while demonstrating a qualita-

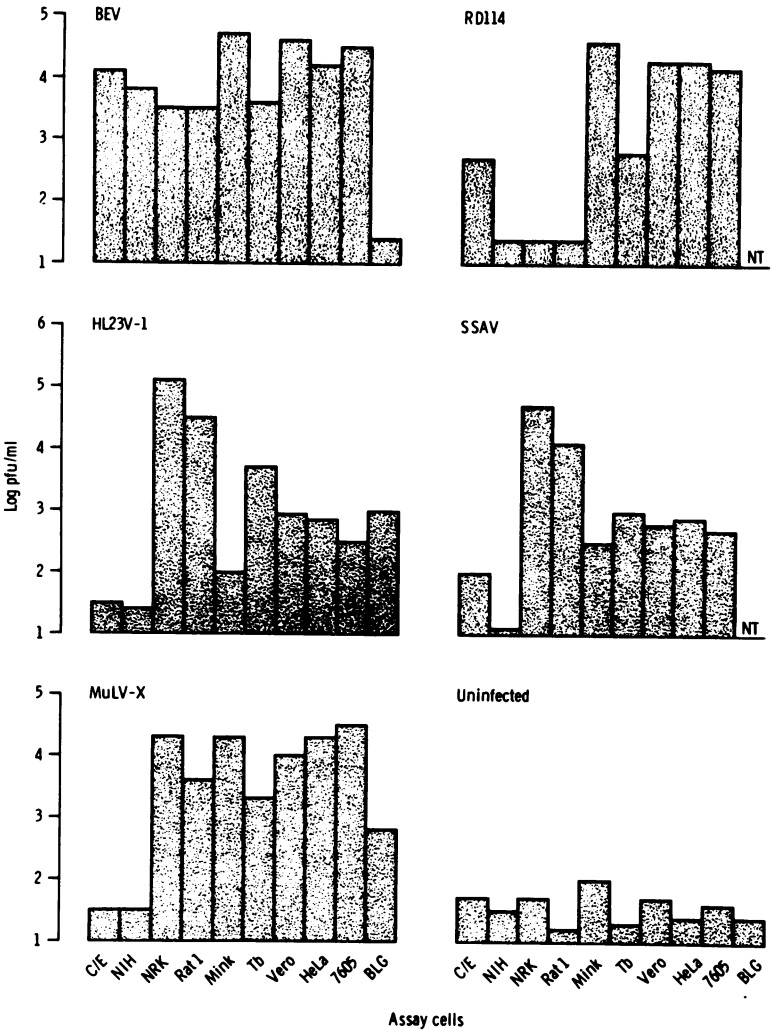

FIG. 1. *Host range for penetration of retrovirus pseudotypes of VSV. Pseudotype titers represent VSV infectivity remaining after anti-VSV globulin treatment of VSV harvests from the indicated retrovirus-infected and uninfected cells when assayed on the indicator cells shown. The titer of VSV prior to neutralization was greater than 10^8 PFU/ml for all harvests assayed simultaneously on the same cell lines. NT, Not tested.*

tively similar host range for penetration, differed quantitatively, having a relatively greater efficiency of penetration of mink and primate cells than that seen with either HL23V-1 or SSAV (Fig. 1).

VSV pseudotypes formed with the other endogenous xenotropic viruses, BEV and RD-114, showed both the broad host range as well as the specific restrictions expected. Although these two viruses have been reported to have similar host ranges for replication in cells of nonhost origin (1, 9, 10), the inability of VSV(RD-114) to penetrate NIH/3T3, NRK, or Rat-1 cells permitted it to be differentiated from the BEV pseudotype of VSV, which formed plaques in all uninfected cell types examined except baboon. When a second stock of BEV obtained from outside this laboratory was employed to produce VSV(BEV) pseudotypes, identical results were obtained, thus confirming these findings. The fact that the host range for penetration was determined by the retrovirus rather than by the cell type used for virus propagation was evident from the identical host ranges obtained when different cell lines infected with the same retroviruses were used to generate VSV pseudotypes (data not shown).

Interference with mammalian retrovirus pseudotypes of VSV in retrovirus-infected cells. Specific interference to the penetration of

sarcoma virus or VSV pseudotypes by appropriate retrovirus-infected cell lines has constituted one of the essential criteria for the definition of pseudotype production. As shown in Table 1, the various VSV pseudotypes produced with the mammalian retroviruses showed a greater than 100-fold reduction in titer when the indicator cell line was infected by the homologous virus. That this interference was a consequence of preexisting infection by the specific retrovirus rather than a consequence of the cell line employed was demonstrated by similar interference seen when either Tb-1-lu or 7605L retrovirus-infected cells were used for assay.

In addition to the homologous interference demonstrated, significant interference between different pairs of retroviruses, e.g., RD-114 with BEV-infected cells and SSAV with HL23V-1-infected cells, was also shown. In each of these cases interference was reciprocal and complete. No interference was seen between HL23V-1 and MuLV-X, despite the similar host range for penetration observed.

Neutralization of mammalian retrovirus pseudotypes of VSV. The reduction of VSV pseudotype infectivity by antiserum known to have neutralizing activity directed specifically against the retrovirus used in the production of that pseudotype is a third criterion for the definition of the VSV pseudotypes produced. One antiserum raised against HL23V-1 and another raised against SSAV were available for testing. In both cases the neutralization of VSV(HL23V) could be demonstrated to be specific and of high titer. When 100 PFU of VSV(HL23V-1) were used in a neutralization assay, the 50% plaque reduction titer of the anti-HL23V-1 serum was 1:1,200, and the titer of the anti-SSAV serum was 1:16,000. No neutralizing activity could be demonstrated against VSV(MuLV-X) or VSV(BEV) at dilutions of 1:10 or greater with either of the antisera. Similar assays with antiserum against BEV demonstrated neutralization of the homologous retrovirus pseudotype of VSV with a 50% plaque reduction titer of 1:320.

Heat stabilization of *tlB*17 infectivity. The *tlB*17 mutant of VSV incorporates a defect that permits free virions to be rapidly and extensively inactivated when maintained at 45°C for more than several minutes (18). It had been observed previously that the growth of this mutant at permissive temperatures through retrovirus-infected cell lines resulted in the production of a new heat-stable population of virus particles, composed largely of virus not neutralized by antiserum directed against VSV, but capable of being neutralized by specific anti-retrovirus serum and having the host range for penetration identical to that of pseudotypes of that retrovirus produced with wild-type VSV (17, 18). Similar findings (Table 2) were demonstrated upon examination of the harvest from retrovirus-infected Tb-1-lu cells superinfected with *tlB*17. Whereas the infectivity of *tlB*17 grown through uninfected Tb-1-lu cells could be reduced by almost 10^6, the surviving fraction after the growth of *tlB*17 through either HL23V-1- or BEV-infected cell lines was at least 100 times greater. The titer of this heat-resistant fraction was generally slightly higher than the pseudotype titer obtained after anti-VSV globulin treatment of the same non-heat-inactivated harvest. Treatment of the heat-resistant fraction with anti-VSV globulin caused a 10- to 50-fold reduction in infectivity, indicating that within the heat-resistant fraction there still remained a significant number of virus particles with exposed VSV antigens. The

TABLE 2. *Effect of heat inactivation and neutralization on VSV tlB17 infectivity*

*tlB*17-infected cell line	Titer (log_{10} PFU/ml)				
	No treatment	HI[a]		Neut.[b]	
		−Neut.	+Neut.	−HI	+HI
Tb-control	9.2	3.5	<1.3	<1.3	<1.3
Tb-BEV	9.3	5.7	4.0	4.6	3.7
Tb-HL23V	9.4	>6.0	5.6	5.6	5.5

[a] HI, Heat inactivation at 45°C for 60 min.
[b] Neut., Neutralization with $10^{-1.5}$ dilution of anti-VSV globulin for 24 h at 4°C.

TABLE 1. *Interference assays of VSV pseudotypes*

Pseudotype	Log_{10} reduction in pseudotype titer[a]									
	7605L assay cells preinfected with:					Bat assay cells preinfected with:				
	BEV	HL23V	SSAV	MuLV-X	RD-114	BEV	HL23V-1	SSAV	MuLV-X	RD-114
VSV(BEV)	3.0	0.0	0.0	0.1	3.2	>2.8	−0.8	ND[b]	0.2	>1.8
VSV(RD-114)	3.2	−0.1	ND	0.1	2.9	>1.1	−0.5	ND	0.1	>2.1
VSV(HL23V-1)	−0.4	>1.0	ND	0.0	0.0	−0.1	1.8	ND	0.2	−0.1
VSV(SSAV)	ND	>1.7	>1.7	ND	ND	−0.1	2.1	>2.4	−0.2	ND
VSV(MuLV-X)	−0.1	−0.1	ND	3.5	−0.1	−0.2	−0.6	ND	2.6	−0.1

[a] Compared with uninfected human 7605L cells and uninfected bat Tb-1-lu cells.
[b] ND, Not determined.

titer of pseudotype particles remaining after the combination of heat inactivation and anti-globulin treatment of the original *tlB*17 harvest appeared to be independent of the procedure used initially. Heat inactivation subsequent to neutralization of these harvests demonstrated that VSV(BEV) pseudotypes defined by anti-globulin treatment maintained a residual heat-labile fraction, as shown by the 10-fold reduction in titer, whereas VSV(HL23V) pseudotypes so formed appeared to be largely heat stable.

DISCUSSION

Although previous attempts to demonstrate pseudotype formation between VSV and primate retroviruses had been unsuccessful, in this report such phenotypic mixing has been shown to occur with moderate efficiency under appropriate conditions. The most important factor for both the production and also subsequent detection of these pseudotype particles was the use of appropriate cell lines. Although a number of different cell lines could be and were employed to produce VSV pseudotypes of the primate retroviruses, Tb-1-lu consistently gave the highest yields of pseudotype virus and the greatest percentage of VSV pseudotype with respect to total VSV produced, approximately 0.01% of the total VSV infectivity remaining after the treatment of viral harvests with anti-VSV globulin. There was no apparent correlation noted between retrovirus production by the various cell lines, measured by XC assay, sarcoma rescue, or reverse transcriptase activity, and the level of production of pseudotype particles upon VSV superinfection (data not shown). These observations are in accord with results found in the avian system, in which only the expression of viral glycoprotein rather than the release of retrovirus particles is required for pseudotype formation with VSV (7, 13). No attempt was made in these studies to quantitate the amount of retrovirus antigen present on the membranes of the infected cells, although such investigations could explain the quantitative differences in pseudotype production noted with the different cell lines.

Appropriate cell lines, in addition to allowing an enhanced production of pseudotype virus, were also essential for the subsequent detection of pseudotype virus. The use of cells of rat origin as indicator assay cells permitted the initial detection of VSV(HL23V-1) and VSV(SSAV) pseudotype viruses as these cells were 30-fold more sensitive to penetration by such particles when compared with the next most sensitive cell line, Tb-1-lu, and over 1,000-fold more sensitive than mink cells, a cell type in which HL23V-1 and SSAV have been reported to replicate efficiently (3, 8).

The relative plaque-forming efficiency of these mammalian retrovirus pseudotypes of VSV on different cell lines coincides with much of the previously published data on host range replication of the retroviruses themselves. The finding that some VSV pseudotypes produce plaques in cell types in which replication of the corresponding retrovirus has been reported to be restricted, e.g., BEV in rat and mouse cells (1, 10), suggests that the block to replication in these cases may be occurring primarily post-penetration of the retrovirus particle. In those cell lines in which the restriction of retrovirus replication is known to exist, the inability of the VSV pseudotypes to produce plaques, a process dependent solely on the functions of adsorption and penetration, suggests that the restriction of replication could be occurring at the stage of penetration rather than of replication. Whether there exists, as well, a postpenetration block to replication in these cases can only be answered by methods such as fusion or appropriate pseudotype formation that bypass the penetration block and permit the introduction of the retrovirus genome into the cell.

In addition to providing a better definition of penetration and replication restrictions, the examination of the VSV pseudotypes has permitted distinctions to be made between related viruses, e.g., RD-114 and BEV. Although these studies confirmed the strong reciprocal interference previously noted between these two viruses (3), the examination of the host range of penetration of their respective VSV pseudotypes has allowed the two viruses to be clearly distinguished. Thus, although both retroviruses have been reported to be unable to replicate on mouse and rat cell lines (6, 9, 10), restriction for BEV probably occurs only after penetration, whereas for RD-114 restriction of penetration alone is sufficient to explain the restriction of replication reported. Whether an additional postpenetration block exists can only be answered by studies as suggested above.

Further investigations made feasible by the production of mammalian retrovirus pseudotypes of VSV include studies of the genetics of cell receptors for these viruses, using either primary cell lines derived from appropriately bred animals, as has been done in the avian system (12), or interspecies cell hybrids derived by fusion. The measurement of neutralizing titers in human serum to the whole range of mammalian retroviruses should be facilitated by the use of the retrovirus pseudotypes of VSV for these permit the development of a quick, specific plaque assay. In addition, as VSV has

now been demonstrated to be capable of forming pseudotypes with the primate and putative human retroviruses and conditions for the detection of such pseudotypes have been defined, studies using VSV as a probe for the detection of latent or defective retroviruses in human clinical material have become practicable.

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