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NOTES

Growth of Pseudotypes of Vesicular Stomatitis Virus with N-Tropic Murine Leukemia Virus Coats in Cells Resistant to N-Tropic Viruses

ALICE S. HUANG, PETER BESMER, LOUISE CHU, AND DAVID BALTIMORE

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115 and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Formation of pseudotypes between murine RNA tumor viruses and vesicular stomatitis virus (VSV) has been confirmed. Pseudotypes of VSV genomes coated by the surface envelope from an N-tropic tumor virus grew equally well in cells homozygous for either the *Fv-1*^{n} or *Fv-1*^{b} alleles. Therefore, the product of the *Fv-1* locus, which restricts growth of murine RNA tumor viruses, must act on an intracellular aspect of tumor virus replication, a step after attachment and penetration.

Formation of mixed viral pseudotypes among the enveloped RNA viruses was first observed between a rhabdovirus, vesicular stomatitis virus (VSV), and a paramyxovirus, simian virus 5 (1). This exchange involves only the viral glycoproteins and appears to occur during the process of budding out from the plasma membrane (11). Recently, Zavada reported the occurence of similar pseudotype formation between VSV and avian or murine RNA tumor viruses (22, 23). These mixed particles contain the VSV genome completely coated by the surface glycoprotein antigens of RNA tumor viruses. The pseudotypes were identified by three criteria: (i) their resistance to neutralization by anti-VSV antiserum; (ii) their inability to initiate VSV plaques in cells with surface restrictions for the RNA tumor virus which donated the glycoprotein; and (iii) their susceptibility to antiserum against the specific RNA tumor viruses.

Our objective was to confirm Zavada's observations and then to use VSV pseudotypes as a probe to study the major genetic locus of resistance in mice to RNA tumor viruses, *Fv-1* (5, 9). *Fv-1* maps on chromosome 4 (linkage group VIII) (14). Cells of mice homozygous for one of the two known alleles at this locus, designated *Fv-1*^{n} and *Fv-1*^{b}, are permissive for N-tropic or B-tropic RNA tumor viruses, respectively (12, 13). B-tropic virus grows poorly in cells from *Fv-1*^{n}/*Fv-1*^{n} mice, such as NIH Swiss, and N-tropic virus grows poorly in cells from *Fv-1*^{b}/*Fv-1*^{b} mice, such as BALB/c. Heterozygotes (*FV-1*^{n}/*Fv-1*^{b}) are resistant to infection by both N- and B-tropic viruses. Because VSV grows in a variety of cells (10), pseudotypes of VSV with coats from N-tropic RNA tumor viruses can be used to determine whether the product of the *Fv-1* locus acts as a barrier at the surface of the cell or if it mediates restriction directed at an aspect of the virus which is inside the envelope. If restriction is a surface phenomenon, then the VSV pseudotype of an N-tropic tumor virus should be restricted as if it were an N-tropic virus. If restriction is directed at the core of the virus, and is therefore presumably an intracellular event, then the VSV pseudotype should be insensitive to the restriction system.

Initially, to form VSV pseudotypes we used a line of BALB/c murine cells, called JLS-V11, which is a producer of Moloney murine leukemia virus (M-MuLV) (21). Medium from these cells contained M-MuLV at approxi-

mately 5 × 10^5 PFU/ml as determined by the XC assay (8, 15). JLS-V11 cells were superinfected with purified cloned VSV (17) at an input multiplicity of 0.1 for 18 h at 37 C. The medium was harvested and assayed for VSV plaques on Chinese hamster ovary (CHO) cells or JLS-V9 cells. The latter cells are identical to JLS-V11 cells except that they have never been infected with M-MuLV and are not producing detectable murine leukemia virus (MuLV) (21). VSV from JLS-V11 cells had a titer of 1.12 × 10^8 PFU/ml on JLS-V9 cells. Neutralization of this preparation by rabbit antiserum made against VSV revealed that 1/1,000 of the plaques on JLS-V9 cells were initiated by VSV particles which were resistant to neutralization by antiserum (Fig. 1a). A control VSV preparation grown in JLS-V9 cells in the absence of any M-MuLV was neutralized by antiserum to a residual fraction of 1/20,000 (Fig. 1a). The

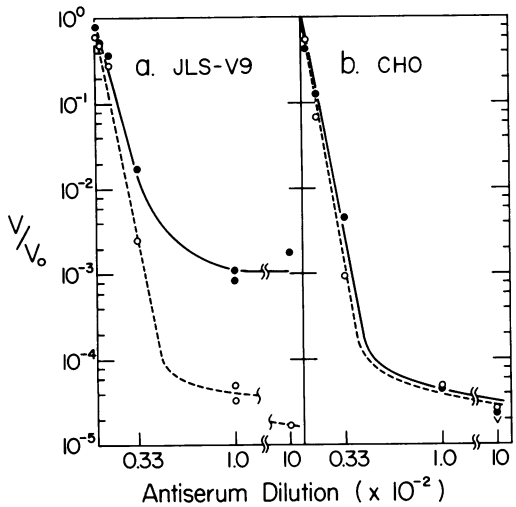

FIG. 1. *Detection of pseudotypes of VSV(M-MuLV). Rabbit anti-VSV antiserum was prepared against purified cloned VSV (17), as previously described (16), except the virus was not inactivated. JLS-V9 and JLS-V11 cells were grown as monolayers in the same medium as CHO cells in suspension (16). VSV grown in either JLS-V11 or JLS-V9 cells was tested against antiserum essentially by the plaque-reduction technique of multiplicity analysis (3). In summary, VSV was adjusted to a titer of 2 × 10^7 PFU/ml and mixed with an equal volume of medium containing rabbit anti-VSV antiserum at the indicated dilutions. Control mixtures were made containing normal rabbit serum. The mixtures were incubated for 1 h at 37 C and then diluted and plated on either JLS-V9 cell monolayers or CHO cell monolayers. The plaque assay on both cell types was performed as described previously for CHO cells (16). The surviving fraction of plaques, V/V_0, was plotted versus antiserum concentration. Symbols: ●, VSV made in JLS-V11 cells; ○, VSV made in JLS-V9 cells.*

difference between the two preparations was indicative of the presence of VSV pseudotypes (VSV[M-MuLV]). Assay of the same VSV preparations on CHO cells showed that all of the plaques on these cells were initiated by VSV which was susceptible to anti-VSV antiserum (Fig. 1b). These results indicate that VSV(M-MuLV) was capable of forming plaques on JLS-V9 cells and not on CHO cells, presumably due to their inability to enter into the CHO cells.

VSV(M-MuLV) cannot be used for studying the *Fv-1* locus because Moloney virus is NB-tropic, that is, it is not restricted by the *Fv-1* locus (5). Therefore, to obtain pseudotypes of VSV in coats of N-tropic MuLV (VSV[MuLV-N]), NIH/3T3 cells chronically infected with N-tropic MuLV were infected with VSV at a multiplicity of 0.5 to 1. The co-infected NIH/3T3 cells were incubated at 37 C for 15 h. (N-tropic MuLV was kindly supplied by Janet Hartley and Wallace P. Rowe. When tested in the XC assay in our laboratory, this virus showed a 100-fold higher titer in NIH/3T3 cells than in BALB/3T3 cells.)

VSV obtained from the co-infected cells was assayed on CHO, NIH/3T3 (derived from NIH Swiss mice; reference 7), 3T6 (derived from random-bred Swiss mice; reference 19), and JLS-V9 cells. 3T6 cells contain the *Fv-1*^{b} locus (18) as do JLS-V9 cells which derive from BALB/c mice (21). Tests in our laboratory confirmed the selective sensitivity of those two cell lines to B-tropic MuLV. The VSV preparation grown on co-infected NIH/3T3 cells had a titer of 200 × 10^6 PFU/ml of CHO cells, 6.75 × 10^6 PFU/ml on NIH/3T3 cells, 1.78 × 10^6 PFU/ml on 3T6 cells, and 7.10 × 10^6 PFU/ml on JLS-V9 cells.

Figure 2 shows that when the VSV stock from co-infected NIH/3T3 cells was neutralized by anti-VSV antiserum and then assayed on NIH/3T3, 3T6, and JLS-V9 cells, pseudotypes of VSV(VSV[MuLV-N]) were detected. No pseudotypes were detected on CHO cells, which gave results similar to that shown in Fig. 1b. VSV genomes coated by the surface proteins of an N-tropic MuLV were therefore apparently equally capable of penetrating and replicating in murine cells with either the *Fv-1*^{n} or *Fv-1*^{b} locus. Lack of infection of CHO cells indicates that the VSV(MuLV-N) contained absorption sites specific for murine cells. The slight increase in titer of VSV(MuLV-N) on NIH/3T3 and JLS-V9 cells compared to the titer on 3T6 cells was probably not significant and correlated with the differences in plating efficiency of VSV on the three types of cells.

This report confirms Zavada's findings of the

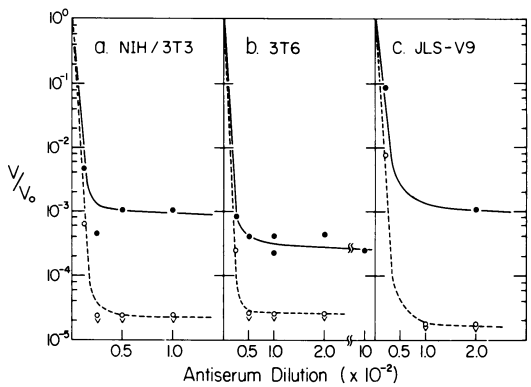

FIG. 2. *Growth of VSV(MuLV-N) on NIH/3T3, 3T6 cells, and JLS-V9 cells. NIH/3T3 and 3T6 cells were grown as monolayers in the presence of Dulbecco medium (2) plus 10% calf serum. JLS-V9 cells were grown as described in Fig. 1. VSV from NIH/3T3 cells or from NIH/3T3 cells co-infected with N-tropic MuLV were neutralized exactly as described for Fig. 1 except that the initial virus titer was approximately 2 × 10⁶ to 4 × 10⁶ PFU/ml. Plaque assays were done on NIH/3T3, 3T6, and JLS-V9 monolayers by the procedure for CHO cells, except that the appropriate culture medium was used for each type of cell. Symbols: ●, VSV made in NIH/3T3 cells co-infected with N-tropic MuLV; ○, VSV made in uninfected NIH/3T3 cells.*

formation of pseudotypes of VSV genomes in envelopes of RNA tumor viruses (22, 23). The studies also indicate that VSV pseudotypes made in cells co-infected with N-tropic MuLV will grow in cells homozygous for either the *Fv-1*^{n} or *Fv-1*^{b} alleles. Thus *Fv-1* restriction against murine tumor viruses apparently is not due to lack of receptor sites on the cell surface but rather is due to intracellular restriction. Such an interpretation is consistent with the finding that the *Fv-1* alleles show genetic dominance for resistance (12). Whether the restriction acts directly on the nucleic acid, in analogy with bacterial restriction systems, or on another target remains to be determined. Fenyö et al. (4) and Yoshikura (21a) also recently suggested that the *Fv-1* product can have an intracellular site of action.

The inability of VSV(MuLV) to infect Chinese hamster cells, and Zavada's earlier demonstration of their inability to infect human cells and their inefficient infection of Syrian hamster cells (23), demonstrates that across species barriers a true surface restriction does exist. Also, with avian tumor viruses surface resistance is evident (20). The VSV pseudotypes thus allow one to readily distinguish two types of cellular resistance to RNA tumor viruses.

Further experiments using a variety of cell genotypes and using antiserum directed against specific MuLV strains are in progress. Of particular interest is the possibility of forming not only pseudotypes containing VSV genomes but also of forming particles containing MuLV genomes coated by VSV antigens. We are presently testing for the existence of these pseudotypes in our viral preparations.

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ADDENDUM IN PROOF

Similar experiments and results using VSV-N-tropic and B-tropic Friend leukemia virus pseudotypes were performed by T. G. Krontiris, R. Soeiró, and B. N. Fields (personal communication).

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