JOURNAL OF VIROLOGY, June 1988, p. 2196–2199
0022-538X/88/062196-04$02.00/0

Vol. 62, No. 6

Reduced Yield of Infectious Pseudorabies Virus and Herpes Simplex Virus from Cell Lines Producing Viral Glycoprotein gp50

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Received 25 September 1987/Accepted 8 March 1988

Pseudorabies virus (PRV) glycoprotein gp50 is the homolog of herpes simplex virus (HSV) glycoprotein D. Several cell lines that constitutively synthesize gp50 were constructed. Vero cells, HeLa cells, and pig kidney (MVPK) cells that produce gp50 all gave reduced yields of PRV and HSV progeny viruses when compared with the parent cell line or the same cell line transfected to produce a different protein. The reduction in virus yield was greatest at low multiplicities of infection. The Vero and HeLa cells that produce gp50 showed an even greater reduction in HSV yield than in PRV yield. This phenomenon may be an example in a herpesvirus of the interference observed in retroviruses or cross-protection in plant virus systems.

Early work with avian retroviruses showed that infection of cells with a retrovirus blocked subsequent infection by another retrovirus of the same serogroup (26). This phenomenon, called interference, was shown to block infection at the level of penetration of cells by viruses (27), presumably by blocking of surface receptors. It was later demonstrated that production of an envelope glycoprotein by an endogenous retrovirus could make chicken cells less susceptible to infection by a virus of the same serogroup as the endogenous virus (22). A similar phenomenon was observed in various lines of mice. The resistance of DBA/2 mice to leukemogenesis by mink cell focus-inducing virus was shown to be due to expression of an envelope glycoprotein of an endogenous virus (2, 23). The *Rcfm* locus for the same resistance has also been shown to be due to expression of an interfering provirus envelope glycoprotein (3). Also, expression of endogenous virus envelope glycoprotein genes has been shown to be the basis for resistance to ecotropic murine leukemia viruses by $Fv\text{-}4^r$ (11, 12) and allelic $Akvr\text{-}1^r$ mice (5). Interference has also been demonstrated in cultured cells with the feline leukemia viruses (24).

An observation qualitatively similar to interference in retroviruses has long been known in plant virology. The term cross-protection has been applied to the resistance to viral disease obtained by preinfection of a plant with an avirulent strain of a similar virus (reviewed in reference 8). Although it has not been shown to be the basis of classical cross-protection, Abel et al. (1) recently showed that expression of the tobacco mosaic virus coat protein gene in transgenic tobacco plants makes the plants at least partially resistant to tobacco mosaic virus-induced disease.

This report describes an observation with a herpesvirus glycoprotein that may be an effect similar to interference in retroviruses or cross-protection in plants. Pseudorabies virus (PRV) is a herpesvirus of swine. In the course of work to develop a subunit vaccine to PRV, several viral glycoprotein genes of PRV have been cloned and sequenced (17–21). One of the viral glycoproteins, gp50 (28), was found to have homology with glycoprotein D (gD) of herpes simplex virus (HSV) (17). This protein has been shown to be an effective subunit vaccine against PRV (14). In the course of making cell lines that express gp50, it was noted that such cell lines have a reduced ability to propagate PRV.

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Expression of the cloned gp50 gene in an uninfected CHO cell line has been previously reported (17). CHO cells are naturally resistant to PRV. Therefore, for experiments involving virus infections, cell lines naturally permissive for PRV growth were used for expression of gp50. The expression plasmid used was the same as that previously described, except that pSV2neo (25) was used as a vector instead of pSV2dhfr. Briefly, the human cytomegalovirus immediate early promoter was cloned upstream from the gp50 gene, and the polyadenylation signal from the bovine growth hormone gene was cloned downstream. The entire expression construction was cloned into pSV2neo to produce pNIE50PA. This plasmid was transfected into Vero cells (ATCC CCL81) by calcium phosphate precipitation (9). Cells resistant to 1 mg of G418 (Geneticin; GIBCO Laboratories) per ml were selected. Clones of transfected cells were isolated by using cloning cylinders and screened for gp50 expression by an enzyme-linked immunosorbent assay to be described elsewhere (A. L. Meyer et al., manuscript in preparation). In the enzyme-linked immunosorbent assay, gp50 is detected by reaction with antiserum from a rabbit vaccinated with a vaccinia virus-gp50 recombinant (14). Two clones, A8 and B4, were selected from independent transfections and produced gp50 levels slightly lower than those of PRV-infected Vero cells. Expression of gp50 by these cell lines was confirmed by detection of a 60-kilodalton protein by Western blot (immunoblot) analysis with antiserum from a mouse infected with vaccinia virus-gp50 (data not shown). All transfected cell lines were maintained in Dulbecco modified Eagle medium with 1 mg of G418 per ml to select for stable maintenance of the gp50 construction.

Figure 1 shows the growth of PRV (Rice) in parental Vero cells; Vero cell lines A8 and B4, which produce gp50; and Vero-tPA, a Vero cell line transfected with a plasmid identical to pNIE50PA, except with the cDNA for human tissue plasminogen activator in place of the gp50 gene. At three different multiplicities of infection and throughout the infection, the virus yield was consistently lower from the Vero-gp50 cell lines. At least 10-fold less virus was consistently produced by the Vero-gp50 cell lines. The difference in virus yield was greatest early in infection and at lower multiplicities. This showed that PRV can replicate in Vero-gp50 cell lines and that high titers of progeny virus can eventually be achieved. However, the rate of progeny virus accumulation was reduced. The Vero-tPA cells were an important control,

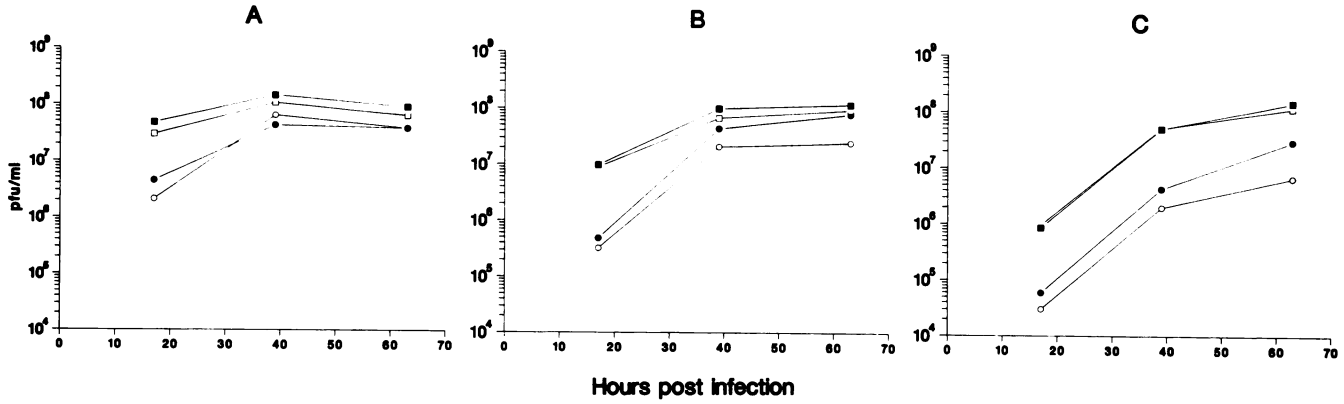

FIG. 1. Virus yields from PRV infection of Vero cell lines. Fresh confluent Vero cell monolayers (symbols: ■, Vero; □, Vero tPA; ●, Vero gp50-A8; ○, Vero gp50-B4) were infected with PRV (Rice) at different multiplicities (panels: A, 0.1; B, 0.01; C, 0.001) as previously described (19). At various times after infection, the virus was harvested by freezing, thawing, and sonication of a flask of infected cells. Virus yields were determined by titration on Vero cells.

eliminating the possibility that G418 could confer herpesvirus resistance to cells, as has been reported for another aminoglycoside, neomycin (13). Furthermore, Vero-gp50 cells passed in the absence of G418 showed similar reductions in virus yield (data not shown).

The yield of HSV from both A8 and B4 cells was reduced to an even greater extent than the yield of PRV (Table 1). It is known that PRV and HSV have many molecular similarities, with both homologous genes and at least partially colinear genome organization (e.g., see reference 18). One of the homologous genes is gp50, which is homologous to HSV gD. As a control for the possibility that Vero-gp50 cells have some general virus resistance trait, the series of Vero cell lines was infected with vaccinia virus. All four Vero cell lines supported growth of vaccinia virus equally well.

Table 2 shows a similar set of experiments for two HeLa cell lines transformed with pNIE50PA. HeLa cells were obtained from F. Rottman (Case Western Reserve University, Cleveland, Ohio) and transfected with pNIE50PA, and G418-resistant clones were screened for gp50 expression by indirect immunofluorescence with monoclonal antibody 3A-4 (17). Two clones that produce gp50, 1-7 and 9-2, were tested for ability to support herpesvirus growth. PRV does not grow well in HeLa cells, but virus yield was reduced 10-fold in cell lines that produce gp50 compared with control HeLa lines. HeLa cells are good host cells for HSV. Both gp50-producing cell lines were very resistant to HSV infection, with no virus detected at a dilution that would detect the viral inoculum. As a control cell line grown in G418, a HeLa line that produces bovine growth hormone (transfected with a plasmid containing the genomic bovine growth hormone gene under control of the cytomegalovirus immediate early promoter cloned into pSV2neo) supported normal growth of both PRV and HSV. As with Vero-gp50 cells, HeLa cells that produce gp50 supported growth of vaccinia virus as well as did control HeLa cells.

Although PRV has a very broad host range (reviewed in reference 10), its natural host is the pig. Therefore, a line of pig cells, MVPK (obtained from M. Wathen, The Upjohn Co., Kalamazoo, Mich.), was transfected with pNIE50PA. The G418-resistant transfectants were cloned and screened for gp50 expression by enzyme-linked immunosorbent assay. One of these gp50-producing lines, MVPK-2, supported replication of PRV poorly compared with an MVPK line transfected with the tPA vector (Fig. 2). An MVPK line that was transfected with pNIE50PA but does not produce gp50 (line MVPK-4) and one that produces low levels of gp50 (line MVPK-7) supported replication of PRV normally. Because MVPK is not a good host line for HSV or vaccinia virus, no informative data about other viruses were obtained with these cell lines. It can be concluded from Fig. 2 that pig cells, like Vero and HeLa cells, are less permissive for PRV when they constitutively express gp50.

The mechanism of the reduced replication of PRV in gp50-producing cell lines is unknown. It may be a general phenomenon that inappropriate levels or timing of viral protein synthesis can interfere with virus replication. The

TABLE 1. Growth of viruses on Vero cell lines that express gp50[a]

Cell line	PFU/ml of:		
	PRV	HSV-1	Vaccinia virus
Vero	1.4×10^8	1.3×10^8	5.3×10^6
Vero-tPA	1.0×10^8	1.4×10^8	7.4×10^6
A-8	1.0×10^7	1.5×10^6	5.8×10^6
B-4	1.5×10^7	1.6×10^7	6.6×10^6

[a] Lines A-8 and B-4 express PRV gp50. Fresh confluent Vero cell monolayers were infected with PRV (Rice), HSV-1 (F^+; obtained from B. Roizman), and vaccinia virus (WR) at a multiplicity of infection of 0.01 as previously described (19). The cells were collected 29 h postinfection by freezing, thawing, and sonication. Virus yields were determined by titration on Vero cells.

TABLE 2. Growth of viruses on HeLa cell lines that express gp50[a]

Cell line	PFU/ml of:		
	PRV	HSV-1	Vaccinia virus
HeLa	2.9×10^7	7.5×10^8	1.5×10^8
bGH	1.0×10^7	1.3×10^8	1.6×10^8
1-7	1.0×10^6	$<10^5$	1.9×10^8
9-2	1.3×10^6	$<10^5$	2.0×10^8

[a] Lines 1-7 and 9-2 express PRV gp50. Fresh confluent HeLa cell monolayers were infected with PRV (Rice), HSV-1 (F^+), and vaccinia virus (WR) at a multiplicity of infection of 0.01. The cells were collected 48 h postinfection by freezing, thawing, and sonication. Virus yields were determined by titration on Vero cells.

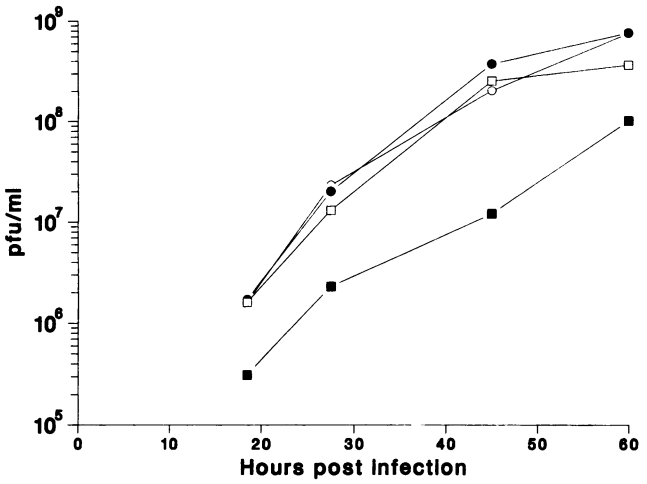

FIG. 2. Virus yields from PRV-infected MVPK cell lines. Fresh confluent MVPK cell monolayers (symbols: ■, MVPK-2; □, MVPK-4; ●, MVPK-7; ○, MVPK-tPA) were infected with PRV (Rice) at a multiplicity of infection of 0.01. At various times after infection, the virus was harvested by freezing, thawing, and sonication of a flask of cells. Virus yields were determined by titration on Vero cells.

extensive work cited earlier on retroviruses and plant viruses might suggest this. A similar observation has been made for herpesviruses by Orberg and Schaffer (16), who found that cell lines which overproduced the HSV DNA-binding protein ICP8 on infection showed inhibited HSV growth. Interestingly, as observed with gp50-producing cells, ICP8-overproducing cells were more resistant to low-multiplicity infection than high-multiplicity infection.

Although there may be many examples of inhibition of virus infection by inappropriate synthesis of viral gene products, it is likely that different gene products will be found to inhibit infection by different mechanisms, depending on the function of the protein. No direct evidence is available for the function of gp50. However, gp50 is homologous to HSV gD (17) and is therefore likely to have a similar function. HSV gD has been shown to be involved in binding of virus to cells (6) and in the cell fusion function of HSV (15). Recent studies by Fuller and Spear (7) have shown that some monoclonal antibodies that neutralize viruses by reaction with gD do not prevent virus binding to cells but prevent entry into the cells. This work implied that gD function is important for some fusion event that allowed the virion to cross the cell membrane. It seems possible that in cell lines that constitutively produce gp50 the site where the gp50 or gD of an incoming virion would react is already blocked with endogenous gp50. Although the known function of gD suggests that gp50-producing cells produce lower virus yields because of interference with virus entry, it is also possible that gp50 interferes with some other step in virus replication, such as virion assembly or release.

A consistent observation throughout this work was that the Vero and HeLa cells that produce gp50 were even more resistant to HSV than to PRV. This may suggest that HSV is more dependent on whatever step is blocked in gp50-producing cells than is PRV. For example, if the block is at the stage of virus entry, perhaps PRV can use an alternative pathway for entry more efficiently than can HSV. Alternatively, if competitive inhibition is involved it may be that gp50 competes more effectively than gD for whatever receptor is involved.

Recently, an observation similar to that reported here was made for HSV (4). Cells that constitutively express gD were found to be refractory to HSV infection, and the block was located at the point of virus entry. The similarity of these results with the data in this report indicates that gD and gp50 probably have functional similarities, as well as sequence homology.

We thank Darrell Thomsen and Roger Brideau for the HeLa cells that express bovine growth hormone and Michael Wathen for the parent MVPK cells. We thank Kathy Hiestand for typing the manuscript.

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