Interference Induced in GL-V3 Monkey Kidney Cells by Rabies Virus Strains

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SUMMARY

Resistance to superinfection with vesicular stomatitis virus (VSV) occurred in GL-V3 monkey kidney cells infected with the CVS-11, Pitman Moore, LEP Flury, but not the ERA strain of rabies virus. Specific immunofluorescent staining of intracellular rabies antigen showed that the number and size of fluorescent foci increased after the onset of interference, and that this was paralleled by increasing yields of infectious virus. Although CVS-11 and ERA differed in their ability to induce interference, the virus yields from monolayers infected with either strain were similar. Interference apparently had no effect on the replication or dissemination of the inducing virus, and seems unrelated to the long incubation period or aberrant forms of infection in vivo.

The study of rabies virus-infected cells in tissue culture may provide valuable information on the mechanisms underlying both the prolonged incubation periods of the disease and aberrant forms of infection. Persistent infections with rabies virus can be established in a variety of mammalian cells in tissue culture; no cytopathic effect is observed and such cultures can be maintained by cell transfer for many passages. The mechanism(s) underlying the maintenance of a carrier type of infection remains unclear. Kawai et al. (1975) isolated a fraction containing defective interfering (DI) particles from the supernatants of cultures persistently infected with rabies virus; no interferon could be detected and the monolayers resisted challenge with homologous virus only. By contrast, Fernandes et al. (1964) and Wiktor & Clark (1972) established persistent infections in cultures which resisted challenge with heterologous virus and they were unable to consistently detect interferon. Many cell lines resist superinfection with homologous and/or heterologous viruses shortly after infection with rabies (see Nicholson et al., 1979a), but the significance of these events remains uncertain. Recently, we reported (Nicholson et al., 1979a) that GL-V3 monkey kidney cells (Christofinis, 1970) become resistant to superinfection with vesicular stomatitis virus (VSV) after infection with the CVS-11 strain of rabies virus. This interference was highly reproducible and could be used to titrate neutralizing antibody. In this paper, CVS-11 and related rabies virus strains have been used to determine the relation of heterologous interference to the replication and dissemination of the inducing virus together with their suitability for the interference inhibition test (Nicholson et al., 1979a) in GL-V3 cells.

Different samples of fixed strains of rabies virus were obtained from various sources and were passaged in BHK-21 cells prior to use. The Indiana strain of VSV was used as the challenge virus. Stocks of each virus were stored at −70 °C. Infectivity of rabies virus was titrated by the intracerebral inoculation of suckling mice, and endpoints were calculated by the Spearman-Kärber method (Lorenz & Bögel, 1973). Infectivity of VSV was titrated by plaque assay in GL-V3 cells. The plaque-inhibition technique used for the assay and identification of interferon has been described previously (Nicholson et al., 1979b). Immunofluorescent staining was carried out using hyperimmune fluorescein-conjugated anti-rabies horse globulin (Center for Disease Control, Atlanta, Ga., U.S.A.). After fixing and staining, the cell monolayers were then examined in a Zeiss microscope with dark-field illumination, an HBO-200 mercury vapour lamp and a UGI 1,41 filter combination.
Fig. 1. Percentage inhibition of VSV plaque count by monolayers inoculated 96 h earlier with increasing log_{10} m.o.i. (LD_{50}/cell) of different strains of rabies virus. (a) CVS-11 (●, 5th passage level in BHK-21 cells; O, 8th passage level); (b) CVS-11 (HK-112, BHK-7: 2nd passage level); (c) CVS-11 (HK-113: 2nd passage level); (d) Pitman Moore (●, 2nd passage level; O, 5th passage level); (e) LEP Flury (●, 2nd passage level; O, 5th passage level); (f) ERA and ERA-ATCC strains of rabies virus (3rd passage level).

For the comparative studies of interference, monolayers of rabies-infected cells in 96-well disposable plastic plates were tested for resistance to approx. 50 p.f.u. VSV as described previously (Nicholson et al., 1979a). The number of plaques formed in infected monolayers were compared with the number in uninfected control cultures. Interference was considered significant when the VSV plaque count in rabies-infected monolayers was below the 95% confidence limit of the mean count in the controls (Lorenz, 1962). Strain-dependent differences in the ability to induce interference were regularly observed. Neither the ERA strain provided by the Wistar Institute nor the ERA-ATCC strain obtained from the
Table 1. Percentage inhibition of VSV at different times after infection with CVS-11 rabies virus*

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<tr>
<th>CVS-11 m.o.i. (LD₅₀/cell)</th>
<th>Percentage inhibition at time (h)</th>
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<td>130</td>
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* Cultures of 1·5 x 10⁵ cells were challenged with about 50 p.f.u. VSV.

American Type Culture Collection induced interference (Fig. 1 f); both strains produced some cytopathic effects, and immunofluorescent staining showed they had infected the monolayers extensively. By contrast, partial interference was induced by the CVS-11 (HK-113) and CVS-11 (HK-112, BHK-7) strains [which were both derived from a strain adapted to primary hamster kidney (HK) at the Center for Disease Control (Kissling, 1958)] and by the LEP Flury strain provided by Professor Kuwert, Essen, F.R.G. (Fig. 1 c, b, e). This partial interference was dose-related and also occurred at low multiplicities of infection with CVS-11 (5th passage level, Fig. 1 a) and the Pitman Moore (PM) strain (2nd passage level, Fig. 1 d). Increasing the m.o.i. of these strains to 0·1 to 4 LD₅₀/cell gave a peak of complete interference followed by partial interference at m.o.i.s ≥ 4 LD₅₀/cell.

Autointerference by DI particles may have been responsible for the partial interference observed in monolayers inoculated at a high m.o.i. (≥ 4 LD₅₀/cell). The CVS-11, PM and LEP Flury strains were therefore passaged under conditions favourable for the accumulation of DI particles. After three serial passages in BHK-21 cells, using undiluted virus inocula for each passage, the harvests were tested for their ability to induce interference. As shown in Fig. 1 (a, d, e) interference was not inhibited, but was enhanced by further passage.

No interference was observed when cell monolayers were inoculated with a mixture containing 100 p.f.u. VSV and up to 40 LD₅₀/cell of the CVS-11 strain. Although not entirely excluding the possibility, the results of this experiment strongly suggest that this type of interference does not occur as a result of competition for common virus receptor sites. Table 1 shows that significant interference first became apparent when the monolayers were challenged with VSV 15 h after infection with the CVS-11 strain at an m.o.i. of 13 LD₅₀/cell. With the exception of monolayers inoculated with the CVS-11 strain at an m.o.i. of ≥ 13 LD₅₀/cell, which never became completely resistant to VSV, the degree of interference was related to both the incubation period and the m.o.i. of CVS-11. In a similar experiment, challenge of monolayers infected with CVS-11 with an increasing m.o.i. of VSV revealed that the interference could be partially overcome, and when challenged with ≥ 3 x 10⁴ p.f.u. VSV/monolayer (about 2 p.f.u./cell) all of the cells showed a cytopathic effect and the monolayers were completely destroyed.

Thirty h after infection of cell monolayers with 0·2 LD₅₀/cell of the CVS-11 strain, specific immunofluorescent staining showed intracellular viral antigen in 62% of 100 high-power fields (× 100); this was associated with a 48% inhibition of a VSV challenge of about 100 p.f.u./culture (0·5 x 10⁶ cells), and the release of 10⁻² LD₅₀/ml of CVS-11 into the cell
supernatants. By 48 h, 100% inhibition of VSV had occurred, the virus yield had increased 100-fold, and although the number of high-power fields positive for rabies antigen had not increased, both the size of individual foci and the intracellular content of antigen were observed to be greater. By 96 h, the yield of infectious virus had increased a further 40-fold to $10^5.8\,	ext{LD}_{50}/\text{ml}$, and 84% of high-power fields had become positive. These observations suggest that the heterologous interference was not associated with any inhibitory effect upon the replication of the inducing virus. To study this further, a comparison was made of the 96-h virus yields from monolayers containing about $2 \times 10^6$ GL-V3 cells that were inoculated with 0.5 log$_{10}$ dilutions of the CVS-11 strain, or the non-interfering ERA strain of rabies virus. Apart from autointerference which occurred in monolayers inoculated with CVS-11 at an m.o.i. of $\geq 0.5\,\text{LD}_{50}/\text{cell}$, there was no significant difference in the yields of infectious virus from monolayers infected with the CVS-11 or ERA strains.

None of the supernatants from cells infected with the ERA strain possessed antiviral activity. By contrast, low titres of interferon (1 to 2 units/ml) were detected in the supernatants from partially resistant and fully resistant monolayers inoculated with the CVS-11 strain at an m.o.i. of $\geq 0.5\,\text{LD}_{50}/\text{cell}$; however, no interferon was demonstrable from other fully resistant monolayers inoculated with a lower m.o.i. Thus, like Kaplan et al. (1960), we were unable to explain the extent of the antiviral activity by the low titres of interferon that were found.

The ubiquitous nature of the interferon system makes it necessary to differentiate its contribution from that of other types of interference. Pretreatment of cell monolayers with actinomycin D inhibits cellular RNA synthesis and may be used to distinguish between intrinsic and interferon-mediated heterologous interference (Marcus, 1977). Accordingly, GL-V3 cell monolayers were pretreated with 0.5 μg actinomycin D in 1 ml maintenance medium for 1 h; they were then washed and inoculated with log$_{10}$ dilutions of the CVS-11 strain. Forty-eight h later, the monolayers were challenged with about 40 p.f.u. VSV and the plaque counts compared with those in control monolayers. The results showed actinomycin D to have no inhibitory effect on interference. On the contrary, it enhanced the reduced levels of interference that were observed with monolayers inoculated with CVS-11 at an m.o.i. of $\geq 4\,\text{LD}_{50}/\text{cell}$. Thus, the inhibition of interferon synthesis may have enhanced both the replication of rabies virus and the resistance to superinfection with VSV.

Rabies-infected cells of human (Wiktor et al., 1964), rabbit (Fernandes et al., 1964), viper and lizard (Wiktor & Clark, 1972) origin have all been shown to resist heterologous virus challenge in the absence of detectable interferon. The results of this communication are consistent with intrinsic interference as the mechanism underlying heterologous interference in GL-V3 cells. As regards the differing abilities of CVS-11 and ERA strains to induce interference, this may be analogous to the failure of the RA 27/3 strain of rubella virus to induce intrinsic interference in WI-38 cells (Kleiman & Carver, 1977). Moreover, VSV is among the various unrelated viruses that may be inhibited by this means (Rott et al., 1972; Hunt & Marcus, 1974). Although intrinsic interference blocks VSV replication, it does not inhibit cell killing (Marcus, 1977); this would explain why the interference induced by the CVS-11 strain could be completely overcome by increasing the m.o.i. of VSV to about 2 p.f.u./cell. Whatever the mechanism underlying this type of heterologous interference, we found no evidence that it was associated with any subsequent inhibition of rabies virus replication or dissemination in vitro. Also, as the incubation periods are longer and the development of rabies is much less certain in laboratory animals inoculated with the ERA strain, compared to those inoculated with the CVS-11 strain, the development of interference by these strains in vitro cannot be related to the evolution of the disease in vivo.

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REFERENCES

CHRISTOFINIS, G. J. (1970). Biological characteristics of cell line GL-V3 derived from the kidney of a vervet
monkey (Cercopithecus aethiops). Journal of Medical Microbiology 3, 251–258.

FERNANDES, M. V., WIKTOR, T. J. & KOPROWSKI, H. (1964). Endosymbiotic relationship between animal viruses and


infected BHK cells. Virology 67, 520–533.


NICHOLSON, K. G., HARRISON, P. & TURNER, G. S. (1979a). Rabies neutralising antibody determination by the
interference inhibition test (IIT) and the mouse neutralisation test (MNT). Journal of Biological
Standardization 7, 253–261.


ROTT, R. C., SCHOLTISSEK, C., KLENK, H.-D. & KALUZA, G. (1972). Intrinsic interference between different enveloped

WIKTOR, T. J. & CLARK, H. F. (1972). Chronic rabies virus infection of cell cultures. Infection and Immunity 6,
988–995.


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