Serum-Borne Reovirus Type 1 Infection of Cultured Haemic Cell Lines

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SUMMARY

Accidental reovirus type 1 infection of cultured human haemic cells by calf serum is described for the first time. Most cultures died but one with unusual properties became a carrier line.

Reoviruses have been isolated from many species of vertebrates, including man. This group of cytopathic RNA viruses is associated with respiratory infections of intensively reared cattle (Andrewes & Pereira, 1967), and all three serotypes have been identified in cattle faeces (Rosen, Abinanti & Hovis, 1963). In this communication we wish to report the accidental calf serum-borne infection of cultured human haemic cells by reovirus type 1.

Newly established haemic cell lines derived from leukaemic patients were frozen for storage in liquid nitrogen after different periods of in vitro growth. The cell freezing medium was made up of RPMI-1640 (Flow Laboratories) containing 25% bovine serum and 7% dimethyl sulphoxide (DMSO). Usually, foetal bovine serum was used, but on one occasion the freezing medium was supplemented with calf serum. Five different cell lines were frozen with the medium containing calf serum. The same cell lines had been frozen at an earlier date with medium containing the usual foetal bovine serum.

After freezing with foetal bovine serum, each of the five lines could be recovered for continuous in vitro propagation. However, after freezing with the medium containing calf serum, only one of the five cell lines could be recovered; the other four cultures did not contain any viable cells after one week in culture.

The cell culture able to survive differed from the others in the possession of a unique cell-killing property derived from a soluble substance released into the medium. It appears distinct from other types of in vitro cell-mediated killing as described for T-cell and K-cell reactions (Granger et al. 1975) and a detailed description is provided by Karpas (1977). Even this culture only survived with considerable cell mortality.

Ultrastructural examination of this proliferating culture revealed the presence of large numbers of virus particles. Previous examinations, both at the time of setting up the culture and after nine months culture before exposure to calf serum showed no virus particles. Approximately one third of the cells contained virus and many of these appeared to be morphologically disrupted and were therefore presumably dead. The individual virus particles had a diam. of approx. 80 nm, and although significant numbers were seen extracellularly, the majority were located intracellularly as large aggregates forming cytoplasmic inclusions (Fig. 1) with microtubules (Fig. 2) resembling those formed by reovirus (Dales, 1973). The cell line continued to proliferate over three months as a virus carrier culture, with a drop in cell density from a maximum of $2.5 \times 10^4$ cells/ml to $5 \times 10^4$ viable cells/ml. Under optimal conditions over 95% of the cells in the uninfected culture were viable while virus-infected cultures contained between 20 to 40% dead or dying cells.

In order to determine which type of reovirus was responsible for the infection, neutralizing
Fig. 1. Infected cells from the carrier culture showing an intracytoplasmic reovirus 1 inclusion body. The cell structure appears to be relatively intact. Magnification × 50,000.
Fig. 2. Part of the intracytoplasmic virus inclusion showing microtubules typical of reovirus. Magnification ×160000.
Antisera against the three serotypes of reovirus type 1, 2 and 3 were each incubated (1 h at 37 °C) separately with a cell-free virus suspension prepared from the killer line. Each of the three virus preparations, together with a control virus preparation, was then used to infect, separately, a different and susceptible human haemic line. Incubation was continued for a week, and samples from each culture were then stained for viability.

Only the cells that were inoculated with virus incubated with the antiserum to reovirus type 1 contained viable cells, while the cultures which were infected with virus preparation alone, or with virus suspensions incubated with antiserum to reovirus type 2 and 3, did not contain any viable cells.

In another experiment, cells from the virus-carrier cell line were divided into four culture flasks. To a control flask, only culture medium was added, while neutralizing antiserum to each of the three reovirus serotypes was immediately added separately to the other three flasks. Incubation was continued at 37 °C and at weekly intervals cell samples were obtained to assess viability. Staining of the cells revealed that the control culture and the cultures grown in the presence of antisera to reovirus 2 and 3 all contained 20 to 40% of dead cells. On the other hand, the cells grown in the presence of anti-reovirus 1 serum continued to proliferate with a high viability rate and repeated ultrastructural examination over a period of four months has failed to reveal any reovirus particles. None of the original batch of calf serum remained for examination but five other batches of calf serum from the same manufacturer were examined for reovirus contamination and proved negative. We must therefore assume that the batch of serum which gave rise to the reovirus type 1 infection was probably contaminated by serum obtained from a viraemic calf. Since all three types of reovirus have been isolated from cattle, it is quite likely that calf serum could produce accidental contamination. Reoviruses will pass readily through all the filters used commercially in the preparation of sera for tissue culture, and their relative stability to various chemical and physical agents (Andrewes & Pereira, 1967) would explain the persistence of infectious particles during the prolonged period of commercial preparation. Consequently, it seems unlikely that manufacturers can effectively exclude reovirus contamination when they failed to detect viruses in 25% of bovine sera they examined (Kniazeff et al. 1975).

The production of a persistent latent infection with reovirus type 3 has been reported in human embryonic cells (Bell & Ross, 1966), Burkitt lymphoma cells (Levy et al. 1968) and Chinese hamster ovary cells (Taber, Alexander & Whitford, 1976). Therefore, it is not inconsistent that one of our haemic cell lines could continue to proliferate after being infected by a reovirus. However, this is the first report of a serum-borne infection by a reovirus, and also the first to describe a latent infection with reovirus type 1, a point which should be borne in mind by workers with cultured haemic cells.

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REFERENCES


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