Rapid Cytopathic Effect with Rabies Virus in Fused Hamster Embryo Cells

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With 4 Figures

Received June 13, 1973

Summary

A line of hamster embryo cells fused with inactivated Sendai virus was infected with a strain of fixed rabies virus. The growth of rabies virus in such a system produced a characteristic cytopathic effect.

1. Introduction

NEFF and ENDERS (1) fused hamster embryo (Iota) cells with inactivated Sendai virus and thus made those naturally resistant cells susceptible to poliovirus. They suggested that poliovirus is incorporated into the cells during the fusion process, when the natural barrier to infection is reduced. Under those conditions poliovirus produced a typical and reproducible cytopathic effect (CPE).

Even though rabies virus can be propagated in a variety of primary cells and cell lines, CPE has been found only sporadically (2—12), and usually 10 days or more after infection. MATSUMOTO et al. (12), however, recently observed CPE 3 days after infection of organ cultures of mammalian neural tissues.

This report describes the rapid CPE obtained when Iota cells were inoculated with rabies virus after fusion with inactivated Sendai virus.

2. Materials and Methods

2.1. Cells

Iota cells (13) were supplied by Elizabeth Grogan of the Children’s Hospital Medical Center, Boston, Massachusetts. The cells were grown as monolayers in Leighton tubes, with Eagle’s basal medium supplemented with 10 per cent fetal calf serum (FCS) used as growth medium. The same medium supplemented with 2 per cent FCS, pH 7.0—7.2, was used as maintenance medium after virus infection.

¹ Work done while the senior author was on leave from the Panamerican Zoonosis Center, Casilla de Correos 23, Ramos Mejía, Buenos Aires, Argentina.
2.2. Viruses

ERA (8) and CVS (5) strains of rabies virus adapted to growth in Iota cells were used. The ERA virus had infectivity titers of $10^{4.5}$ weanling mouse intracerebral LD$_{50}$ (MICLD$_{50}$)/0.03 ml.

Sendai virus for fusion was obtained from Dr. Walter Dowdle, Laboratory Division, Center for Disease Control, Atlanta, Georgia. The virus was inoculated into the allantoic cavities of 10-day-old chicken embryos. The allantoic fluid was harvested 3 days later, concentrated, and inactivated with 0.13—0.15 per cent β-propiolactone as described by NEFF and ENDERS (1). The hemagglutination titer of the Sendai virus concentrate was 1:20,480. Bovine albumin was added at a final concentration of 0.5 per cent, the concentrate was stored at $-70\,^\circ\mathrm{C}$ to prevent decay of the fusion factor activity, and used within 2 weeks of preparation.

2.3. Fluorescent Antibody Staining (FA)

The staining procedure was the one described by DEAN (14). Fluorescence was observed with a Zeiss microscope with UG 1 exciter filter and #65 barrier filter; the light source was an Osram HBO 200 mercury vapor lamp. The conjugate was prepared from equine serum at this laboratory, by classic methods.

2.4. Hematoxylin and Eosin (H & E) Staining

The staining procedure was the one described by MERCHANT (15).

2.5. General Procedures

Monolayer cultures were prepared by seeding Leighton tubes with $1-2 \times 10^5$ Iota cells suspended in 1.5 ml of Eagle’s medium. The growth medium was removed after incubation for 3 days at $37\,^\circ\mathrm{C}$, and the tubes were then infected with 0.15 ml of undiluted rabies virus for 60 minutes at $37\,^\circ\mathrm{C}$; the inoculum was then removed and each tube was inoculated with 0.8 ml of Eagle’s medium with 2 per cent FCS and 0.1 ml inactivated Sendai virus concentrate. Incubation was continued for 4 hours at $37\,^\circ\mathrm{C}$. This fluid was then removed and replaced with 1.5 ml of Eagle’s medium containing 2 per cent FCS; the cultures were incubated at $35\,^\circ\mathrm{C}$. Controls not infected with rabies virus were prepared in each experiment. At 24, 48, and 72 hours after infection, the tissue culture supernates were removed, centrifuged at 1000 r.p.m. for 10 minutes, and titrated intracerebrally in weanling mice for virus infectivity (0.03 ml/mouse).

Two coverslips from each time period were removed from the Leighton tubes and fixed, one in acetone for FA staining, the other in Bouin’s fixative for H & E staining.

3. Results

Fluorescent antibody staining indicated that Iota cells were more sensitive to rabies virus after cell fusion than were normal cells; 12 hours after being infected with rabies virus 30 per cent of the fused cells fluoresced, compared with less than 10 per cent of the normal cells (Table 1). By 48 hours all of the fused cells fluoresced, but infection of all of the infected unfused Iota cells was not noted until 72 hours.

The titer of rabies virus in the supernate of the fused cell cultures increased to a maximum 48 hours after being infected, and were slightly higher than those in the unfused cultures at 48 and 72 hours.

The addition of inactivated Sendai virus has been shown (1) to cause fusion of about 90 per cent of Iota cell monolayers. Fusion is apparent several hours after the addition of Sendai virus, with syncytial formation complete by 24 hours. Normal Iota cells are fibroblastic with an oval central nucleus, a clear nuclear

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The results referred to in the Figures and Tables were found using the ERA strain; similar results in all cases were also found with CVS.