Failure of the RA 27/3 Strain of Rubella Virus to Induce Intrinsic Interference

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SUMMARY

Unlike other strains tested, the RA 27/3 vaccine strain of rubella virus, attenuated in human fibroblasts, failed to inhibit superinfection with Newcastle disease virus (i.e. induce intrinsic interference). Since proteins of the input virion are known to lead to intrinsic interference, these may differ in the RA 27/3 strain from those in other rubella strains.

The haemadsorption-negative plaque test for the assay of rubella virus, described by Marcus & Carver (1965), revealed a special type of virus interference which they termed intrinsic interference. In this assay, cells infected with rubella virus (the agent inducing interference) become resistant to superinfection with Newcastle disease virus (NDV), but remain susceptible to infection with many other viruses. It was determined that the genome of the inducing virus codes for the protein(s) responsible for the intrinsic interference effect, so that intrinsic interference is distinct from interferon-mediated resistance to viruses (Marcus & Carver, 1967). Subsequently, it has been shown that infection with many other non-cytopathic virus agents results in cells becoming refractory to infection with NDV (Beard, 1967; Marcus & Carver, 1967; Wainwright & Mims, 1967; Seto & Carver, 1969; Rott et al. 1972). In extensive studies of the mechanism of intrinsic interference, Marcus & Zuckerbraun (1970a, b) used temperature-sensitive (ts) mutants of Sindbis virus to show that only RNA + mutants were capable of inducing intrinsic interference; RNA − mutants failed to do so when grown at non-permissive temperatures. Their studies showed that an early protein, coded for by the Sindbis virus and thought to be a polymerase protein, subsequently interfered with an early step in synthesis of NDV RNA, although the exact mechanism was not described. In a later study, Mifune & Matsuo (1975) showed that a particular ts mutant of rubella virus, which was defective in RNA production, also failed to induce intrinsic interference at non-permissive temperatures. However, to date, there has been no description of a strain or RNA + ts mutant of rubella virus which fails to induce interference to NDV superinfection. Indeed, the ability or inability of a virus to induce intrinsic interference appears to be a consistent property of all strains of that virus.

The RA 27/3 strain of rubella virus was initially grown from a kidney explant of a rubella virus infected foetus, and was attenuated and characterized by Plotkin, Cornfield, & Ingalls (1965) and Plotkin et al. (1967). After four subcultures of fibroblasts from the explant, virus-containing supernatant fluid was used to infect human diploid fibroblasts of the WI-38 strain, and the virus was subsequently propagated through 25 passages in WI-38 cells. Early passages were incubated at temperatures of 35 °C and later at 33 °C, and subsequent passages were performed at 30 °C. Late passage virus grew better at 30 °C than at 37 °C. Twenty-fifth passage virus, as tested in vaccine trials, was used in the present study (lot number RU/4/1, obtained from Dr Stanley A. Plotkin).
A stock of RA 27/3 virus was made by inoculating a flask of WI-38 cells in the 25th cell passage (Microbiological Associates, Bethesda, Maryland) with a m.o.i. of 1 TCD₅₀/cell. After 10 days the supernatant was harvested and clarified, and samples were stored at -70 °C. This stock virus contained 10⁶ TCD₅₀/ml when assayed on RK₁₃ cells.

The California strain of NDV was grown in the chorioallantoic sac of 10-day-old chick embryos. Allantoic fluid was collected after a 48 h incubation period, clarified, filtered and stored at -70 °C. NDV plaque assays were performed in primary chick embryo fibroblasts.

RA 27/3 virus was assayed in the RK₁₃ line of rabbit kidney cells (Flow Laboratories, Rockville, Maryland) using a TCD₅₀ method. Briefly, confluent cell monolayers in screw top tubes were aspirated and then inoculated with 0.3 ml each of serial tenfold dilutions of virus-containing fluids. Absorption was carried out on a roller wheel at 25 °C for 1 h, and 1.5 ml of Eagle’s minimal essential medium (MEM) supplemented with 3% calf serum was added to each tube. The tubes were incubated at 33 °C and the monolayers were examined daily for cytopathic effect and compared with uninoculated controls. Fresh media was added twice weekly and the tubes were observed for two weeks.

In intrinsic interference studies, RA 27/3 virus was added to confluent WI-38 monolayers which contained approx. 10⁶ cells/tube. The passage level of the cells ranged from 19 to 25 but was uniform within individual experiments. Experiments were done in screw top tubes with a 0.3 ml inoculum and with m.o.i. from 0.01 to 10 TCD₅₀/cell. Absorption was carried out on a roller wheel for 1 h at 35 °C, after which the infected cells were covered with 1.5 ml of MEM containing 3% calf serum. Incubation temperatures of 30 and 35 °C were used in separate experiments. Under these conditions, RA 27/3 virus added at 10 TCD₅₀/tube increased to a maximum amount of approx. 1 × 10⁶ TCD₅₀/tube after 10 days.

NDV challenge was performed as previously described (Carver & Seto, 1971). Briefly, the supernatant fluid was aspirated from RA 27/3 infected and control tubes, and the cell monolayers were inoculated with 0.3 ml of MEM containing NDV at a m.o.i. of 10 p.f.u./cell. After absorption on a roller wheel at 37 °C for 1 h, the NDV-containing residual inoculum was removed by aspiration and the cells were washed with 0.3 ml of phosphate buffered saline (PBS). Next, the rinsed cell monolayers were exposed to 0.3 ml of NDV antiserum for 30 min at 37 °C in order to prevent binding of red cells to residual input NDV. The antiserum was removed by aspiration and the monolayers were washed twice with 3 ml of PBS and then covered with 1.5 ml of MEM supplemented with 3% calf serum. After 15 h incubation at 37 °C, the monolayers were aspirated and covered with 3 ml of a suspension of guinea pig erythrocytes (6 × 10⁷/ml) in cold PBS. Red cell adsorption was carried out for 1 h at 4 °C. The cells were gently rinsed with cold PBS to remove unadsorbed RBCs and the tubes were covered with 1.5 ml of cold PBS and examined microscopically. As described previously (Marcus & Carver, 1965), interpretation of the results was based upon the presence or absence of haemadsorption. Erythrocytes adsorb to cells in which NDV is replicating (Marcus, 1962); however, rubella virus infected cells, which are refractory to NDV superinfection, fail to adsorb the erythrocytes and thus appear as haemadsorption negative areas.

In this series of experiments, the RA 27/3 strain of rubella virus consistently failed to induce intrinsic interference to NDV infection as judged by the development of haemadsorption. (Fig. 1). The F-8L (Marcus & Carver, 1965) and the M₃₃ strains of rubella virus (American Type Culture) were both used as positive controls, and each of these strains induced intrinsic interference under conditions identical to those in which the RA 27/3 strain failed (Fig. 2). Failure to induce intrinsic interference with RA 27/3 virus occurred in
Fig. 1. Haemadsorption following NDV challenge of WI-38 fibroblasts: (a) infected 10 days previously with 10 TCD<sub>50</sub>/cell of RA 27/3 rubella virus and (b) cell control (×150).
Fig. 2. Haemadsorption following NDV challenge of WI-38 fibroblasts infected 10 days previously with 0.1 TCD₅₀/cell of M33 strain of rubella virus (×150).

Experiments carried out at temperatures of 30°C as well as 35°C; with RA 27/3 virus at m.o.i. ranging from 10⁻⁴ TCD₅₀/cell to 10 TCD₅₀/cell; and after incubation periods ranging from 5 to 15 days. Cytopathic effect was never observed in rubella virus infected WI-38 cells. In all experiments, virus titrations were performed on supernatant fluids from RA 27/3 infected cell monolayers prior to NDV challenge, and it was established that RA 27/3 virus had replicated.

In order to test whether failure to induce intrinsic interference might be a marker of rubella virus attenuation, experiments were performed with two other currently available vaccine strains of rubella virus. Both the HPV-77 (Merck, Sharp and Dohme, West Point, Pennsylvania) and Cendehill (Smith, Kline and French, Philadelphia, Pennsylvania) strains induced intrinsic interference under the described experimental conditions. It appears then that inability to induce intrinsic interference is a specific characteristic of the RA 27/3 strain.

As discussed above, Marcus & Zuckerbraun (1970a, b) presented evidence that proteins of the inducing virus, probably the virus polymerase(s), were responsible for inducing intrinsic interference. In later investigations, using vesicular stomatitis virus (VSV) as a challenge virus, Hunt & Marcus (1974) showed that the interfering proteins produced their
effect after transcription of the input virion RNA, but before there was synthesis of new virus RNA.

Our results at 30 and 35°C indicate that the RA 27/3 virus is not ts for the expression of intrinsic interference. This failure shows that there are strain to strain differences with respect to the ability of a rubella virus to induce intrinsic interference. Possible explanations for this include: (1) The presence in RA 27/3 virus of minimally functional (Marcus & Sekellick, 1976) virus polymerase proteins which are not recognized at the intrinsic interference target site, but which function normally in RNA replication. (2) The presence of an inhibitor substance which is either coded for by the RA 27/3 virus genome itself, or results from a virus-cell interaction. This substance may then interfere with polymerase activity at the intrinsic interference target site.

We plan studies with early passage levels of RA 27/3 virus, to see if the prototype isolate did not induce intrinsic interference or whether this property developed in the process of attenuation. In addition, virus agents known to induce intrinsic interference will be propagated in WI-38 cells to see what effect this may have on the ability of a virus to induce intrinsic interference. Ultimately, direct comparisons of the polymerase proteins of closely related inducing and non-inducing rubella virus strains may allow the exact mechanism of intrinsic interference to be elucidated.

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