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Growth of Mouse Hepatitis and Other Indigenous Mouse Viruses in Tracheal Organ Cultures

By

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With 1 Figure

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Summary

Organ cultures of mouse trachea were infected with some indigenous mouse viruses. Mengovirus and reovirus type 3 grew to high titer; inocula of, respectively, 10^2 and 10^3 $TCID_{50}$ were required to initiate infection. Organ cultures supported also the growth of mouse hepatitis viruses, MHV-3 and MHV-S, though to a lesser extent. Viral production was noted for periods of as long as two weeks. None of the viruses had a noticeable effect on the ciliary activity or acquired such capacity on serial passage in organ cultures.

1. Introduction

Organ cultures of ciliated epithelium have been successfully employed in virus research. By use of this technique many respiratory viruses have been shown to multiply in differentiated cells of respiratory epithelium (cf. review by Hoorn and Tyrrell, 1969). Only limited use of organ cultures of respiratory tissue has been made in studies of viral infections which occur *in vivo* primarily as enteric infections. The respiratory tract, though not being the principal host target organ for the infections, might yet play a role at the initiation or in the transmission of infection. It was therefore thought worthwhile investigating whether some murine viruses which are found primarily as agents of enteric infections might grow in organ cultures of mouse trachea.

2. Materials and Methods

2.1. Viruses

The strains of mouse hepatitis viruses (MHV-3 and MHV-S), mengovirus, and reovirus type 3 were described previously (4, 5). The viruses were titrated in, respectively, NCTC-1469 cells, L cells, and primary rhesus monkey kidney cells. The identity

of the viral strains was confirmed in neutralization tests by use of mouse antisera. Cell cultures and viral suspensions used were culturably free of mycoplasmas.

2.2. Organ Cultures

Organ cultures of trachea were prepared and maintained as described previously (9, 17). Unless otherwise stated, 4-week-old male mice of a specific pathogen-free colony were used. Fragments of trachea were planted on scratched areas on the bottom of a 60 mm plastic Petri dish, with the epithelial surface of each fragment uppermost. Cultures were maintained in medium 199 with 0.13 per cent sodium bicarbonate and 0.2 per cent bovine plasma albumin. The dish cultures were incubated at 35° C in 5 per cent CO_2 in air.

Cultures were infected on the second day of incubation by removing medium and adding 0.1 ml of virus and 0.9 ml of fresh medium. Mouse hepatitis viruses and mengovirus were allowed to adsorb to the cells for 2 hours at 35° C. Adsorption of reovirus type 3 continued for 4 hours (7). After the adsorption period, unadsorbed virus was removed by 3 washes with medium, and fresh medium was added. Each day, medium was changed, and cultures were examined carefully for ciliary activity. Medium removed was stored at —70° C until titrated. In some experiments virus was inoculated in parallel into control tubes or dishes without tissue; medium was collected every hour or every day. This control enabled the testing of thermal viral inactivation.

2.3. Viral Titration

Each day, medium was removed from 5 infected organ cultures. Medium from each culture was titrated separately. The mean value at each incubation time is given. Viral titrations were performed by the tube dilution method, 4 tubes being used for each 10-fold dilution. The 50 per cent end point ($TCID_{50}$) was calculated by the method of KÄRBER (11).

3. Results

The results of a representative experiment in which organ cultures of mouse trachea were infected with approximately 10^4 $TCID_{50}$ of MHV-3 and viral multiplication characterized throughout a period of 12 days are summarized in Figure 1 a. The titer of MHV-3 fell to a low level within 24 hour and rose 10- to 100-fold between days 5 and 9. Substantial quantities of virus were still released into the medium by day 12. In cultures inoculated with MHV-S no significant increase in viral titer was observed, but infectious virus was still found by day 9. Since it is assumed that very young animals are generally more susceptible to viral infections, attempts were made to grow MHV-S in organ cultures of trachea from 1-day-old mice (Fig. 1 b). Each culture received an inoculum of 10^5 $TCID_{50}$ of virus. There was a 10-fold increase in viral titer between days 5 and 9, and virus continued to appear in the medium throughout the observation period of 14 days. Figure 1 shows further that MHV-3 and MHV-S disappeared from tissue-free control dishes within 28 hours. Taking into consideration the rapid thermal inactivation of the viruses and the multiple dilution of complete medium changes, it was concluded that MHV-3 and MHV-S multiplied in the culture system.

Mengovirus grew rapidly and to very high titer in organ cultures of trachea (Fig. 1 c). By 3—6 days after inoculation the titer was approximately 1000-fold higher than that at the time of inoculation. Experiments with varying dosages of virus indicated that an inoculum of 10^2 $TCID_{50}$ was sufficient to initiate infection. The virus appeared to grow equally well after serial passages in organ cultures of trachea. Three passages were performed at dilutions of 1 in 1000. The maximum titer at the third passage was as high as that after primary inoculation.

Tracheal epithelium also supported the growth of reovirus type 3 (Fig. 1d). The virus multiplied to high titer. It reached peak titer a little later than did mengovirus. An inoculum of 10^3 $TCID_{50}$ of reovirus type 3 was required to infect organ cultures. In addition four serial passages were performed at dilutions of 1 in 100. At each passage the level of virus increased 100-fold or more during the course of the experiment.

Infected and uninfected control cultures were examined every day. Ciliary activity in uninfected cultures was retained throughout the observation period. None of the viruses had a noticeable effect on ciliary activity, and infected cultures failed to reveal gross changes of the structure of the epithelium. In addition no obvious changes were observed after serial passages of the viruses in organ cultures.

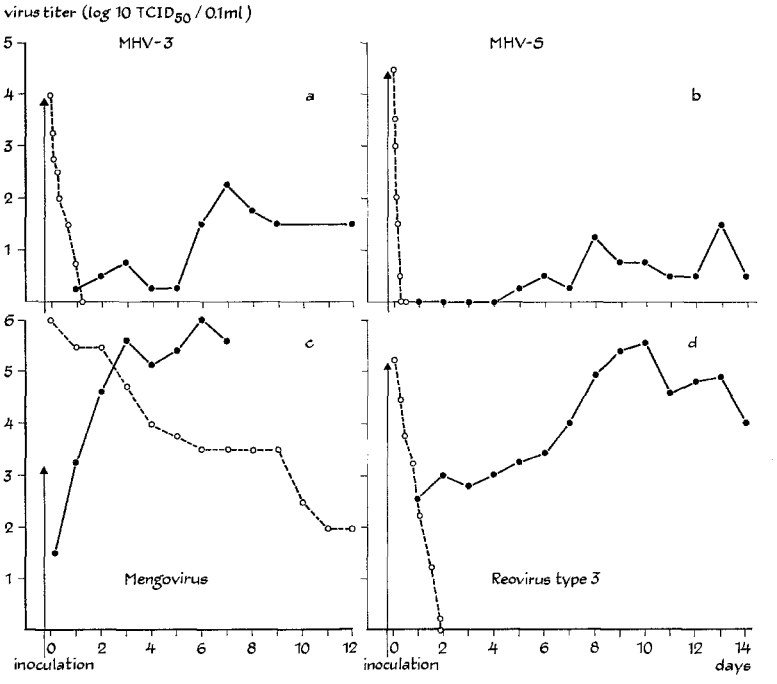

Fig. 1. Growth curves of murine viruses in mouse tracheal organ cultures
Medium was changed daily. Each point represents the mean value of titers of five cultures

Symbols: •———•, infected organ cultures; o— — —o, inactivation of virus without organ culture

4. Discussion

As far as we know, there have been no investigations on the infectiousness of MHV for the respiratory tract. The present study indicates that organ cultures of trachea support the growth of MHV-3 and MHV-S. It is of interest to note that other members of the coronavirus group, namely avian and human coronaviruses, have been found to cause respiratory illness in, respectively, chickens and man.

In addition some coronavirus strains could be propagated only in organ cultures of respiratory epithelium (12, 15, 16). Although natural reovirus infection as well as mengovirus and MHV infections of mice occur primarily as enteric infections (2), experimental studies have shown that reoviruses can induce a respiratory infection in mice *via* the intranasal route of administration (8). Similarly, studies of mengovirus infection in mice indicated that this virus can replicate in the lungs after aerosol exposure (1, 14) or intraperitoneal inoculation (3) and in the throat after administration of virus by the peroral route (6). Our finding that organ cultures of trachea are susceptible to reovirus type 3 and mengovirus is in agreement with the results of these *in vivo* experiments.

It has been shown that viruses which cause respiratory disease in man multiply in organ cultures of human respiratory epithelium. Many viruses stop the ciliary activity, but this is often observed only after serial passage (10). Similarly, Sendai virus, an indigenous respiratory virus of mice, was found to grow in organ cultures of mouse respiratory tissue with destruction of the ciliated epithelium (17). The murine viruses used in the present study induced no detectable cytological changes. This may reflect a low natural virulence of these viruses for respiratory epithelium. It is also possible that the viral strains were poorly adapted to respiratory tissue. On the other hand, we found no evidence of increased virulence on serial passage in organ cultures.

It is generally assumed that infections which are essentially restricted to the intestinal tract of mice are transmitted primarily, if not solely, through contact with virus-contaminated fecal material (2, 13). The present experiments indicate that substantial quantities of virus can be produced by respiratory epithelium. Although organ cultures of trachea differ from respiratory tissue in the intact host in several respects such as the influence of local immunity and the presence of an intact mucous layer, our observations seem to suggest that the respiratory tract might be involved in natural MHV, mengovirus, and reovirus infections. The possibility should be considered, therefore, that, in addition to fecal contact, the airborne route might play a role, though presumably a minor one, in the transmission of these infections.

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