INFECTIOUS RHINOVIRUS RNA IN SUSCEPTIBLE AND NON-SUSCEPTIBLE TISSUE CULTURE SYSTEMS

R. Fletcher, A. Davis

Dept. of Microbiology, School of Dental Medicine, University of Pittsburgh, Pennsylvania 15213, U.S.A.

Received October 2, 1968

An attempt was made to infect normally non-susceptible tissue culture systems with an infectious rhinovirus RNA. Earlier, DeSomer *et al.* (*1*) successfully infected normally non-susceptible chicken embryo cells with infectious polio RNA. Because this infection produced one-cycle of new virions, it was expected to duplicate the findings with another member of the Picornavirus group, rhinovirus 2060. The rhinovirus was grown in monolayers of embryonic human lung (WI-38) maintained in Earle's medium plus 2% calf serum at 33° C. Infectious viral RN Awas phenol extracted in the presence of bentonin te using the procedure of Pons (*2*). The intact virions titered about 10^7 $TCID_{50}$/ml in sensitive WI-38 cells and the ribonuclease-sensitive extract titered about 4 $\log_{10}$ less. The susceptible tissue (WI-38 and HeLa cells) and the non-susceptible tissue (L, BHK and MDCK cells) were propagated stationary at 37° C in 16 × 125 mm screw cap tubes containing 2 ml of Earle's medium plus 10% calf serum. Before inoculation with virions or viral RNA extract, the tissue monolayers were washed three times with 2 ml volumes of Earle's medium with no serum. Then inocula were added to each tissue-containing tubes in the presence and absence of 10% dimethyl sulfoxide (DMSO) and the tubes incubated at 33° C on a 3 rev/min roller drum for 15 min; then each tube was drained, and supplied with 2 ml fresh Earle's medium plus 2% calf serum. Incubation was continued and the cultures were observed twice daily for any cytopathic effect (CPE). Infectious rhinovirus RNA produced a CPE in selected tissue culture systems as follows (0 = no CPE observed; — = not tested):

Tissue treatment prior to challenge	$TCID_{50}$/ml at 72 hr in tissue culture system				
	BHK	HeLa	L	MDCK	WI-38
10% MDSO	0	10^3	0	0	10^3
Ribonuclease	—	0	—	—	0
Antiserum	—	10^1	—	—	10^1
None	0	10^1	0	0	10^1

As the table indicates, extracted rhinovirus 2060 RNA produced infection within 72 hours in human lung and HeLa cells, but failed to infect mouse fibroblast (L), baby hamster kidney (BHK) and canine kidney (MDCK) cells. These latter tissue systems remained non-susceptible to infectious rhinovirus RNA even when observed for CPE at 7 days subsequent to inoculation. Similar findings were obtained using intact rhinovirus 2060. Other inoculated non-susceptible tissues, incubated for 24 hours at 37° C, were subjected to 2 repetitions of freezing and thawing and assayed in WI-38 cells. This procedure also failed to detect rhinovirus activity. Rhinovirus-specific serum neutralized intact virions, but did not neutralize the extract; ribonuclease inactivated the extracted RNA, but did not affect the intact virion.

In an effort to increase sensitivity in detecting infectious viral RNA, the tissue monolayer systems were treated by different methods. The most sensitive technique was to add an equal volume of viral RNA to tissue systems containing 20% DMSO (final concentration 10%). As indicated above, DMSO was removed after 15 min to reduce the concentration below the toxic level. Concentrations >1.25% were toxic for WI-38 cells. Similar to the findings (*3*) with Mengo virus RNA, higher and lower percentages of DMSO did not enhance RNA infection. A lower grade of infection was obtained with tissue treated with 0.85% NaCl or with tissue washed three times with medium containing no serum. Viral extracted RNA did not produce an apparent infection in susceptible cells when inoculated into tissue systems containing 0.25% $CaCO_3$, 1.7% NaCl in M/15 phosphate buffer, or 100 μg/ml DEAE-dextran sulfate or in tissue systems heated (42° C, 15 min) before inoculation.

References

1. DeSomer, P., Prinzie, A., and Schonne, E., *Nature (Lond.)* **184** : 652, 1959.
2. Pons, M., *Virology* **24** : 467, 1964.
3. Tovell, D. R., and Colter, J. S., *Virology* **32** : 84, 1967.