STUDIES ON THE EFFECT OF A NUCLEAR POLYHEDROSIS VIRUS OF THE SPRUCE BUDWORM ON THE REPLICATION OF VERTEBRATE VIRUSES IN MAMMALIAN, AVIAN AND FISH CELL LINES

by

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Abstract

Virus interference tests were carried out using cells of mammalian, avian or fish origin. The cells were inoculated with nuclear polyhedrosis virus (NPV) and then challenged with a virus that is known to replicate and to produce a cytopathogenic effect (e.g. chick embryo fibroblast cells with Sindbis virus, L cells with Encephalomyocarditis virus, RTG-2 and FHM fish cells with Infectious pancreatic necrosis virus). When cell destruction was evident, the progeny vertebrate virus was assayed and its titre was compared to that of virus produced in cells not inoculated with NPV. The results showed that NPV did not interfere with the replication of other viruses in vertebrate cell lines.

Résumé

L'auteur effectua des tests sur l'interférence virale en utilisant des cellules provenant de Mammifères, d'Oiseaux ou de Poissons. Les cellules furent inoculées avec le virus de la Polyhédrose nucléaire (VPN) et puis l'auteur inocula aussi un virus qui multiplie et produit l'effet cytopathogène (e.g. des cellules fibroblastiques d'embryons de poulets à virus Sindbis, des cellules L à virus Encephalomyocardite, des cellules de Poissons RTG-2 et FHM à virus de Nécrose pancréatique infectieuse). Lorsque la destruction des cellules se révélait, le virus des Vertébrés ci-dessus fut essayé et son titre comparé à celui de virus produit en des cellules pas inoculées avec du VPN. Il fut

observé que le VPN n'affecte pas la multiplication des autres virus dans les lignes cellulaires de Vertébrés.

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Introduction

Considerable effort has been expended to elucidate the effect of nuclear polyhedrosis viruses (NPV) on non-target organisms, particularly vertebrates, since these viruses present a potentially viable alternative to chemical insecticides in the control of forest pests. Following protocols and guidelines recommended by the World Health Organization an NPV of the spruce budworm was administered by various routes to mammals and birds and its effect was investigated thoroughly (Valli, 1974, 1975). No toxic effect was observed.

The effect of the virus on vertebrates could also be studied, very conveniently, by inoculating cells grown in vitro and then studying alterations in cellular growth and morphology. However, some viruses replicate in cells without the production of a cytopathic effect. One method of identifying such infection is by the ability of the infecting virus to interfere with the replication of another virus known to produce a cytopathic effect. This interference effect is typified by Rubella virus which interferes with the replication of ECHO virus 11 (Gresser and Enders, 1962).

In this report we describe the results of interference tests using NPV of the spruce budworm and four vertebrate cell lines.

Materials and Methods

Virus

NPV virions were prepared by solubilization of inclusion bodies with alkali followed by purification on sucrose density gradients (Arif and Brown, 1975). The infectivity of each virus preparation was determined by *per os* inoculation of fifth instar larvae of the spruce budworm and examining each larva microscopically for the production of virus inclusion bodies. All virion preparations were found to be infectious.

Virus Interference Tests

Replicate vertebrate cell monolayers were prepared in small (25 cm^2) Corning tissue culture flasks in 5 ml of minimum essential medium (MEM). One half of the cultures were inoculated with NPV and the other half were mock-infected. Virus adsorption was allowed to proceed at room temperature for 2 hrs in 0.5 ml MEM. The inoculum was removed and the cells were washed twice with a balanced salt solution (BSS) and overlaid with 5 ml MEM. At intervals of 2 hrs, 1, 2, 3, 4, 5 days post exposure to NPV the medium was removed and the cells were superinfected with a virus known to replicate and produce cytopathic effect (CPE). The cell and virus combination were: Chick embryo fibroblasts (CEF) and Sindbis virus, L cells and Encephalomyocarditis virus, RTG-2 trout cells and Infectious pancreatic necrosis virus (IPN), FHM minnow cells and IPN virus. The challenge virus inoculum was 0.5 ml at a multiplicity of infection of 10 plaque forming units (PFU) per cell. After an adsorption period of one hr at room temperature, the

inoculum was removed, the cells washed twice with BSS, overlaid with 5 ml of MEM and incubated at the appropriate temperature until cell disruption was evident.

The infected cultures were frozen and thawed once to release intracellular virus, the cell debris removed by centrifugation and the virus yield was assayed by plaque titration using duplicate cell monolayers for each dilution.

Plaque Titration

A 0.2 ml sample from 10-fold dilutions of the virus preparation was added to duplicate cell monolayers and the virus was allowed to adsorb for 1 hr. Growth medium containing 1% agarose was added and the cells were incubated at the appropriate temperature. When plaques developed, they were counted and the virus titre computed.

Results

Multiplication of Sindbis Virus in CEF Cells Inoculated with NPV

The cell monolayers were inoculated and mock-infected with NPV and at various periods later they were challenged with Sindbis virus. Approximately 1 day after superinfection the titre of Sindbis virus was determined. Since NPV is an insect virus and replicates best at temperatures slightly above ambient, the cultures were incubated at 27°C as well as 37°C following inoculation with NPV. The results of the experiment are presented in Table 1.

The data show that at 27°C there was no significant difference in the titre of Sindbis virus between NPV infected and mock-infected cultures. Even though the cultures were allowed to incubate with NPV for 3 days before superinfection, there was no interference with Sindbis virus multiplication. Similar data were obtained when the cells were incubated at 37°C after NPV addition. It is clear from these results that NPV does not interfere with the replication of Sindbis virus either at 27°C or at 37°C.

Effect of NPV on the Growth of EMC Virus in L Cells

An experiment similar to the one above was carried out using mouse-derived L cells and EMC virus (a picornavirus). The results are summarized in Table 2 and clearly show that EMC virus multiplied in L cells both at 27 C and 37 C without interference from NPV. If NPV did multiply in L cells without producing CPE, then these cells would have become refractory to EMC virus or at best permitted very

TABLE 1

Growth of Sindbis virus in NPV infected chick embryo fibroblasts at 27°C and 37°C

Length of exposure of cells to NPV	Yield of Sindbis virus (pfu/ml)			
	Infection with NPV at 27°C		Infection with NPV at 37°C	
	NPV-infected cultures	Mock-infected cultures	NPV-infected cultures	Mock-infected cultures
2 hr	8.3×10^8	5.1×10^8	6.5×10^8	4.5×10^8
1 day	5.0×10^8	3.6×10^8	4.3×10^8	3.6×10^8
2 days	4.6×10^8	7.0×10^8	1.6×10^8	7.9×10^8
3 days	2.8×10^8	2.4×10^8	2.2×10^8	1.3×10^8

TABLE 2

Growth of EMC virus in NPV infected L cells at 27°C and 37°C

Length of exposure of cells to NPV	Yield of Sindbis virus (pfu/ml)			
	Infection with NPV at 27°C		Infection with NPV at 37°C	
	NPV infected cultures	Mock-infected cultures	NPV infected cultures	Mock-infected cultures
2 hr	0.8×10^9	1.7×10^9	5.3×10^9	2.4×10^9
1 day	1.0×10^9	2.0×10^9	2.2×10^9	3.6×10^9
2 days	3.5×10^9	1.0×10^9	1.0×10^9	5.9×10^9
3 days	2.2×10^9	0.9×10^9	0.9×10^9	1.9×10^9

limited replication of the virus. The data demonstrate that there was no significant difference in the titre of EMC produced in cells that were inoculated or mock-infected with NPV.

Replication of IPN Virus in Fish Cells and Treated with NPV

The effect of NPV on fish cells grown in vitro was examined using two cell lines derived from minnows (FHM) and trout (RTG-2). After exposure to NPV the cells were incubated at 22°C. The monolayers could not be incubated above 22°C since higher temperatures are lethal to the cells. Exposure of the cells to NPV was extended up to 5 days since replication of the virus, if it does occur, is expected to be much slower at 22°C than at 27°C. Tables 3 and 4 show that NPV did not interfere with the multiplication of IPN virus. Even a 5 day exposure to NPV did not render the cells refractory to superinfection.

TABLE 3

Growth of IPN virus in NPV infected RTG-2 cells

Infection with NPV at 22°C

Length of exposure of cells to NPV	Yield of IPN virus (pfu/ml)	
	NPV infected cultures	Mock-infected cultures
2 hr	1.3×10^7	7.1×10^7
1 day	5.4×10^7	4.0×10^7
2 days	7.3×10^7	5.8×10^7
3 days	4.0×10^7	2.5×10^7
4 days	2.8×10^7	1.0×10^7
5 days	3.1×10^7	6.4×10^7

TABLE 4

Growth of IPN virus in NPV infected FHM cells

Infection with NPV at 22°C

Length of exposure of cells to NPV	Yield of IPN virus (pfu/ml)	
	NPV infected cultures	Mock-infected cultures
2 hr	3.0×10^7	4.1×10^7
1 day	2.2×10^7	0.8×10^7
2 days	5.1×10^7	6.3×10^7
3 days	0.9×10^7	3.0×10^7
4 days	4.4×10^7	3.6×10^7
5 days	2.8×10^7	3.1×10^7

Discussion

The most common result of mixed and multiple infections of single cells is interference, a phenomenon recognized by drastic depression in yield of challenging viruses. The experiments described in this report clearly reveal that vertebrate cells are non-susceptible to NPV at 22°, 27° or 37°C. This is shown by the fact that a challenging virus known to multiply in these vertebrate cells did so to titres comparable to those produced in mock-infected cells.

There are several mechanisms by which viruses interfere with each other in multiple infection experiments.

1. One type of interference involves intracellar changes, namely modification or destruction of the membrane receptor sites. Rubin (1960) showed that chicken cells infected with an avian leukosis virus (ALV), a non-cytocidal infection, are refractory to infection by Rouse sarcoma virus (RSV). In this case, ALV infection results in alteration of the receptor sites for RSV and the virus becomes incapable of attaching to the cells.

2. Another type of interference is mediated by interferon. It is a protein produced by many vertebrate cells in response to virus infection and inhibits the multiplication of a second virus (Isaacs, 1963).

3. A phenomenon has been described in bacterial viruses in which 'phage induces the synthesis of a deoxyribonuclease which hydrolyzes cellular DNA as well as the DNA of a superinfecting virus (Knight, 1974). Thus the challenging virus may enter the cell but its DNA is

destroyed immediately upon decoating.

These examples give an insight into the process of viral replication and the different steps at which interference may occur. The experiments described in this report show that the challenging virus replicated normally, proving that NPV neither multiplies in vertebrate cells nor modifies the cellular structures, such as surface membranes; the cells do not become refractory to a superinfecting virus. This information provides further evidence that NPV is a "safe" virus for vertebrates.

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