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INDEXING WORDS

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SYNOPSIS

Mechanisms of polykaryocyte formation induced by virus infection have been studied in various cells for their virological and cytopathological properties. Several plant lectins provide useful means for the investigation of alteration in the surface properties of cell membranes. In this report, effect of concanavalin A (Con A) on cytopathological changes was investigated in certain mammalian and mosquito cells infected with vesicular stomatitis virus (VSV). The cell fusion induced by VSV in BHK-21 cells was markedly inhibited by Con A treated in early post-infection period. Con A prevented also to varied extent the virus release from different cell lines. The inhibition of virus release was most effective when the infected cells were treated with Con A without replacement of the medium until each indicated hours harvesting the culture fluids. Con A reduced significantly the infectivity of cell-free VSV and the degree of neutralization varied considerably for the virions grown in the different host-cells. The results presented here suggest that a detailed analysis of the membrane-receptors responsible for the cell fusion by VSV may be possible by using different types of plant lectin.

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INTRODUCTION

Vesicular stomatitis virus (VSV), like many other enveloped viruses, is formed by budding at the outer membrane of infected cells. The final step in the maturation of this virus alters the surface structure and properties of cell membranes.[15] Many kinds of mammalian and mosquito cell lines are susceptible to VSV and, in general, it has a rapid growth cycle in the infected cells. Recently it has been reported that marked polykaryocyte formation was observed in monolayers of some cell lines infected with VSV.[2,8,12] In addition to detection of changes in the cell surface induced by virus infections, several plant lectins including concanavalin A (Con A) have been used to identify the process which is inhibited by them during virus replication.[1,6,9,10,11]

This communication describes the modification of VSV-induced cytopathogenicity in certain mammalian cells treated with Con A. In addition, experiments were undertaken to study the relationship between inhibition of cytopathic effect (CPE) and virus maturation by Con A using different cell lines. The neutralization of viral infectivity by Con A was also investigated.

MATERIALS AND METHODS

Cell Cultures.

The cell lines used in these experiments include a strain of baby hamster kidney cells designated BHK-21-KB,[12] rabbit kidney (RK 13), mouse L, and *Aedes albopictus* cells. BHK-21, RK 13 and L cells were grown in Eagle's minimum essential medium (MEM) containing 0.03% L-glutamine and 5% inactivated calf serum, and incubated at 37°C.[13] *A. albopictus* cell line had been originally maintained in Department of Microbiology, Rutgers Medical School, New Jersey, U.S.A. and thereafter was kindly supplied by Dr. Igarashi of Research Institute for Microbial Diseases, Osaka University, Japan. The cells were cultivated at 28°C in Eagle's MEM containing 0.03% L-glutamine, supplemented with non-essential amino acids and 10% fetal calf serum.[4]

Virus and Virus Infection.

New Jersey strain of vesicular stomatitis virus (VSV) was prepared in primary chick embryo (CE) cell cultures. The virus

titers were determined by plaque assay in BHK-21 cells. The virus was inoculated at an input of 1 to 10 plaque forming units (PFU) per cell. For the neutralization test on cell-free VSV by Con A, the viruses grown in the different host-cells were prepared by serial passages more than ten times in BHK-21, CE or *A. albopictus* cell cultures.

Morphological Observation and Quantitation of Fusion.

Cell cultures to be tested for cell fusion were grown on cover-slips in Leighton tubes or small bottles for plaque assay until confluent monolayers were formed. These cells were infected with 1 to 10 PFU/cell of VSV and incubated at 37°C for the indicated time intervals after infection. Observation and extent of cell fusion were estimated by the method described previously.[12,13)]

Chemicals.

Concanavalin A (Con A) was purchased from Pharmacia, Uppsala, Sweden; α-methylmannoside (α-MM), from Nakarai Chemicals, Japan. These reagents were dissolved in Ca- and Mg-free phosphate buffered saline (PBS) and diluted to indicated concentrations.

RESULTS

Cytopathological Changes and Virus Yields in Different Cell Lines Infected with VSV.

Table 1 shows the summary of results on the cytopathological changes and the virus yields in certain mammalian and mosquito cell cultures. The ability of VSV to induce cytopathogenicity was different in the cell culture systems used. Marked polykaryocyte formation was observed in both BHK-21 and mouse L cells at 18 to 24 hours post-infection (p.i.). There were considerable differences in the extent of susceptibility to polykaryocytosis among the cell lines tested. The BHK-21-KB cells were the most susceptible to the VSV-induced cell fusion. The strongest CPE was induced in BHK-21, L and RK 13 cells infected with VSV. However, no distinct morphological changes were shown in *A. albopictus* cells even after a long period of infection. The highest yields of VSV were obtained in BHK-21, L and RK 13 cell cultures at 18 to 24 hours p.i., whereas *A. albopictus* cells produced less infectious virus.

Table 1 Relationships between cytopathogenicity and virus yields in the different cell lines infected with VSV.

Cell lines	Polykaryocyte* formation (%)	Cytopathic** effects	Virus yields*** (PFU/ml)
BHK-21-KB	80 - 100	3 +	3.6×10^{10}
Mouse L	50 - 70	3 +	2.3×10^{9}
RK 13	0	3 +	2.6×10^{8}
A. albopictus	0	-	5.5×10^{7}

* Polykaryocyte formation (mean per cent of total cells) at 24 hours p.i. (MOI = 1-10).

** Cytopathic effect at 24 hours p.i. 3+, Complete destruction of monolayer cells. -, No CPE.

*** The virus yields at 24 hours p.i. were assayed in BHK-21-KB cells.

Comparison of Inhibitory Effect on Cytopathological Changes and Virus Release by Con A in Different Cell Lines.

Treatment of VSV-infected BHK-21 cells with up to 200 μg/ml of Con A caused complete inhibition of polykaryocyte formation and significant reduction of virus yields (Table 2 and Fig. 1). However, there were considerable differences in the extent of inhibition of virus release from the infected cells, depending on the time interval of Con A addition to the medium. As shown in Table 2 (Exp. 1), when Con A was added 1 hour p.i. to the medium and then replaced with normal medium at 4 hours p.i., inhibitory effect on the virus release was significantly lower whereas cell fusion could be completely prevented. A long incubation without the medium change after addition of Con A profoundly prevented the release of infectious virus (Exp. 2 in Table 2). Similar results, as shown in Tables 3 and 4, were also obtained in RK 13 or *A. albopictus* cells infected with VSV. Table 3 and Figure 1 show inhibitory effect of Con A on CPE and virus release in the infected

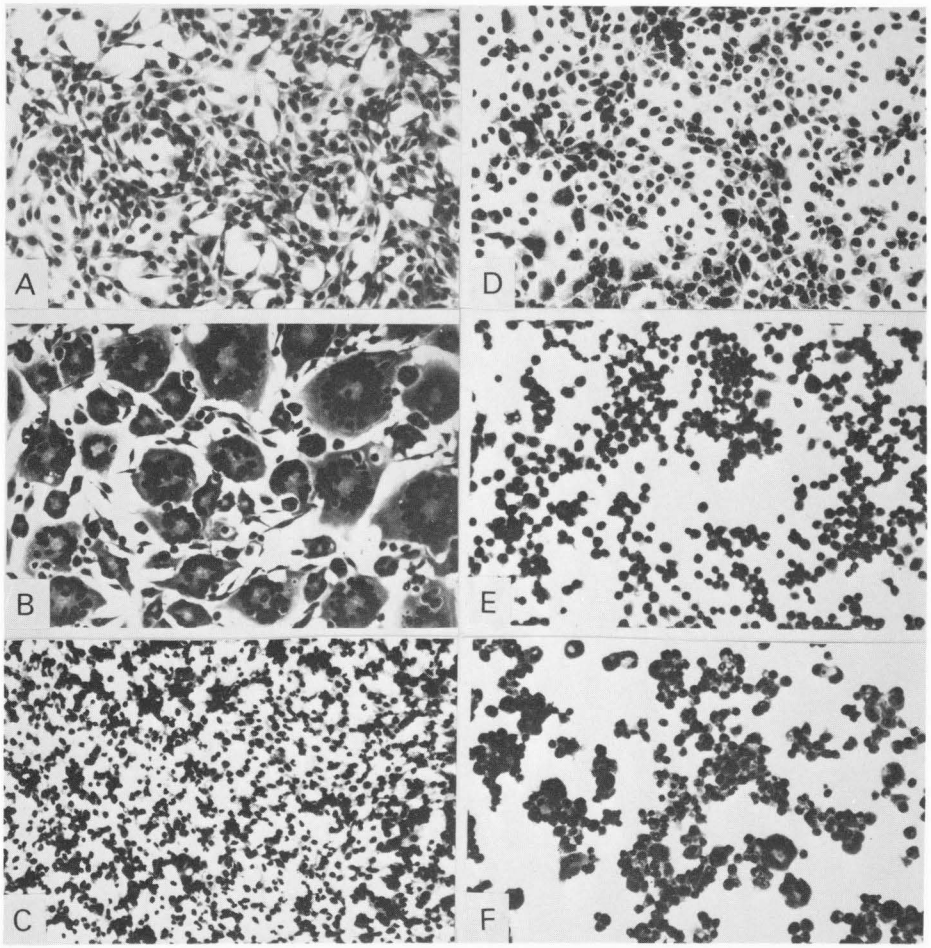

Fig. 1 Effect of Con A on cytopathological changes in VSV-infected BHK-21 and RK 13 cells. May-Grünwald-Giemsa stain. Magnification x 300. A. Uninfected BHK-21 cells; B. VSV-infected BHK cells (24 hours) untreated; C. Infected BHK cells treated with 100 µg/ml Con A; D. Uninfected RK 13 cells; E. Infected RK cells (24 hours) untreated; F. Infected RK cells treated with 100 µg/ml Con A.

RK 13 cells. Con A (100 and 200 μg/ml) appeared to be less effective against CPE in RK cells infected with VSV, but all the infected cells had rounded or agglutinated. The virus production in RK cells was also inhibited by Con A at 24 hours p.i., but the rate of inhibition was lower than in BHK-21 or *A. albopictus* (Table 4).

Table 2 Effect of Con A on cell fusion and virus release from BHK-21 cells infected with VSV.

Exp.	Con A (μg/ml)	Polykaryocyte formation (%)	Infectivity (PFU/ml) of released viruses 7 hours	24 hours
1	200	0	3.3×10^5	7.3×10^8
	100	0	4.8×10^5	5.4×10^9
	0	90 - 100	8.5×10^7	6.3×10^{10}
2	200	0	8.6×10^2	4.7×10^3
	100	0	9.5×10^3	2.2×10^5
	0	90 - 100	9.2×10^6	3.5×10^{10}

Con A was added at 1 hour p.i. to the serum-free medium. Polykaryocyte formation and infectious virus yields in the treated and untreated cells were determined at 24 hours p.i. In Exp. 1, the medium was replaced with normal medium without Con A at 4 hours p.i. In Exp. 2, Con A-treated cell cultures were incubated without medium change until each indicated hours for virus assay. The virus infectivity was assayed in BHK-21 cells.

Table 3 Effect of Con A on cell lysis and virus release from RK 13 cells infected with VSV.

Exp.	Con A (μg/ml)	Cell lysis	Infectivity (PFU/ml) of released viruses	
			7 hours	24 hours
1	200	2 +	1.8×10^2	1.8×10^5
	100	3 +	5.0×10^2	3.5×10^5
	0	3 +	1.0×10^4	4.3×10^6
2	200	2 +	2.5×10^3	2.4×10^3
	100	3 +	9.1×10^3	3.8×10^3
	0	3 +	1.0×10^4	3.1×10^6

Con A was added at 1 hour p.i. In Exp. 1, the medium was replaced with the normal medium without Con A at 4 hours p.i. In Exp. 2, Con A-treated cell cultures were incubated without medium change until virus assay. Cell lysis was observed at 24 hours p.i. 3+, Complete destruction of monolayer cells. 2+, Extensive cell lysis.

Table 4 Effect of Con A on virus release from *A. albopictus* cells infected with VSV.

Exp.	Con A (μg/ml)	Cell lysis	Infectivity (PFU/ml) of released viruses	
			24 hours	48 hours
1	200	-	7.8×10^4	6.7×10^6
	100	-	6.3×10^5	2.2×10^7
	0	-	5.0×10^7	1.3×10^8
2	200	-	4.8×10^4	1.8×10^4
	100	-	8.1×10^4	1.0×10^5
	0	-	7.8×10^7	4.5×10^8

Con A was added at 1 hour p.i. In Exp. 1, the medium was replaced with the normal medium without Con A at 4 hours p.i. In Exp. 2, Con A-treated cell cultures were incubated without medium change until virus assay. -, No CPE.

Effect of Con A on Cell-Free VSV.

Table 5 shows the results in which different concentrations of Con A were incubated for 1 hour at 37°C with VSV diluted with PBS to 100 PFU/ml. Each sample was titrated for residual infectivity by plaque assay using BHK-21 cells. Con A directly neutralized the infectivity of VSV to a large extent in the plaque reduction rates. The neutralization rate was different considerably for each VSV grown in BHK-21 or *A. albopictus* cells. Virions grown in *A. albopictus* cells were neutralized strongly by Con A more than those grown in BHK-21 cells. The neutralization by Con A could be reversed by addition of 250 mM α-MM (Data not shown). Similar results were also obtained from the kinetic experiments in which effects of Con A on the infectivity of each VSV grown in the different host-cells were tested (Fig. 2). Con A (100 μg/ml) was incubated for 2 hours at 37°C with each stock virus solution. The samples were then diluted with PBS and titrated at the indicated hours. Under these experimental conditions, VSV grown in *A. albopictus* cells was neutralized rapidly more than those grown in BHK-21 or CE cells.

Table 5 Effect of Con A on the infectivity of VSV_{CE} grown in CE and VSV_{Aalb} in *A. albopictus* cells.

Con A (μg/ml)	VSV_{CE}		VSV_{Aalb}	
	Average plaque number	Plaque reduction (%)	Average plaque number	Plaque reduction (%)
100	5	92	0	100
50	13	71	0	100
25	27	56	3	97
12.5	14	77	5	96
0	62	—	120	—

Con A solution of the indicated concentrations was mixed at an equal volume with the virus suspensions of about 100 PFU/ml. Average plaque number was counted in BHK-21 cells after incubation at 37°C for 1 hour.

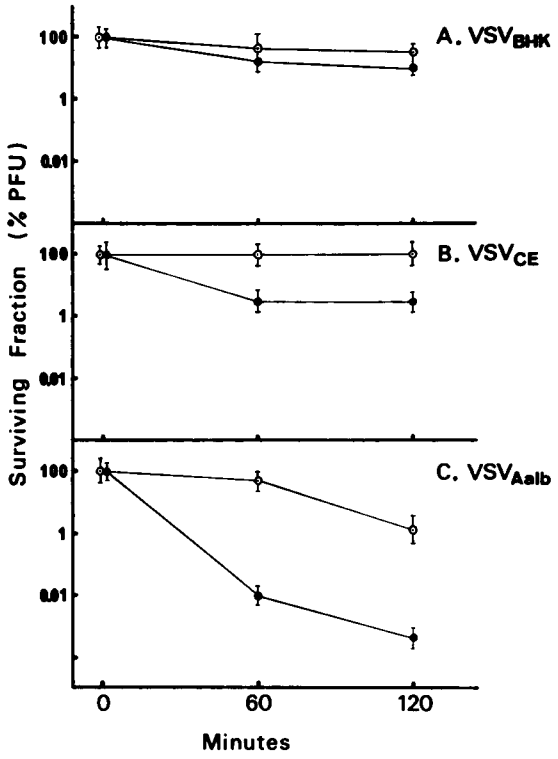

Fig. 2 Effect of Con A on the infectivity of each VSV grown in BHK-21 (A), CE (B) and *A. albopictus* (C) cells. ●, Con A (100 μg/ml); ⊙, Untreated control.

DISCUSSION

When the cytopathic effect by VSV infection was studied in several cell lines, a marked polykaryocyte formation has been observed in monolayers of certain strains of BHK-21 cells infected with VSV.[12] Recently, mechanisms of cell fusion induced by VSV have been investigated for their biological and cytopathological properties using the different cell lines.[2,13] The results presented here show that VSV-induced cell fusion can be inhibited by adding Con A to the BHK-21 cell culture early after infection. Con A also reduced VSV yields in infected cells. In addition, mature virions of VSV were directly neutralized by Con A after incubation at 37°C for 1 hour. As shown in Figure 2, it is of interest that the neutralization rates of VSV by Con A binding on the envelope glycoproteins varied for the virions which were produced on the different host-cell membranes. Although the

mechanism for these differences on the neutralization by Con A is not yet clear, it seems likely that the host effect on the virions is due to differences in carbohydrate content of each viral envelope.[7] In another experiment, mature virions of VSV were also neutralized by wheat germ agglutinin after incubation at 37°C for 1 hour, while both soybean agglutinin and phytohemagglutinin had less neutralizing activity.[14]

Con A affected considerably the cell fusion and virus yields in VSV-infected cells, even if it was added at about 6 hours p.i. The virus yields and rates of polykaryocyte formation were also markedly inhibited by adding anti-VSV sera early after infection, although the addition of anti-VSV sera had varying effect on the anti-fusing activity (Unpublished results).

From the results presented in Tables 2, 3 and 4, it was shown that VSV release from three kinds of cell culture was inhibited by treatment of the virus-infected cells with different concentrations of Con A early after infection. The inhibition of virus release was most effective when the infected cells were treated with Con A without replacement of the medium until each indicated hours harvesting the culture fluids. The irreversible binding of Con A or modification may occur on the infected-cell surface after long period of treatment.[3,5,6] There are differences in the inhibitory rates of virus release from three different kinds of cell line. Treatment of the cells with Con A before infection could not markedly prevent the cell fusion while the virus production was reduced to the same extent as addition of Con A in the early period of infection (Unpublished results). In RK 13 cells, the inhibition of virus production was lower than in BHK-21 or *A. albopictus* cells, and the cell lysis was protected to a less extent by Con A. The site or specificities of different lectin receptors on the cytopathogenicity of VSV in various types of cell require further investigation.

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