JOURNAL OF VIROLOGY, Aug. 1974, p. 307-314

Vol. 14, No. 2
Printed in U.S.A.

Inhibition of Herpes Virus-Induced Cell Fusion by Concanavalin A, Antisera, and 2-Deoxy-D-Glucose

H. LUDWIG, H. BECHT, AND R. ROTT
Institut für Virologie, Justus Liebig-Universität, Giessen, Germany

Received for publication 25 March 1974

Pseudorabies virus-induced cell fusion in rabbit kidney cells can be prevented by Concanavalin A added early after infection. The infected cells are not agglutinated and the infectivity of cell-free virus is not reduced. Sera from productively infected animals also inhibit polykaryocytosis, whereas a hyperimmune serum directed against virus structural components has no effect. 2-Deoxy-D-glucose reversibly disturbs virus-induced fusion and reduces significantly the virus infectivity.

The alteration of the cell surface after infection with certain herpes viruses leads to polykaryocytosis (6, 26), which seems to be an early event during virus replication (7, 19). The finding that Concanavalin A (Con A) could block fusion of BHK cells, which had been infected with the parainfluenza virus SV 5 (28), prompted investigations to determine whether the phytagglutinin would also interfere with the fusion process induced by a virus belonging to a totally different group and would therefore represent a more general phenomenon.

Since Con A is a multivalent agent which is capable of lattice formation and which binds to specific receptors at the surface of normal cells (20, 24), corollary experiments were set up with virus-specific antibodies. In contrast to Con A, antibodies complex with virus-induced determinants appearing at the surface of the altered cell membrane. Such an approach seemed to be promising, since herpes virus-specific antigens and structural glycoproteins (10, 13, 22) become exposed at the cell surface (4, 10, 11, 15, 21). A still different possibility to disturb cell fusion on the level of glycoprotein synthesis became available through the observation that 2-deoxy-D-glucose (DOG) interferes with the synthesis of glycoproteins in a number of cells infected with different enveloped viruses (5, 12, 14, 16).

We therefore describe in this communication the possibility to block cell fusion induced by pseudorabies (PsR) virus with Con A and virus-specific antibodies and to interfere with the fusion process by disturbing the synthesis of glycoproteins with the sugar analogue DOG.

MATERIALS AND METHODS

Cells. Rabbit kidney (RK) cell monolayers were prepared from 3- to 4-week-old rabbits. The kidneys were trypsinized, the cells were sedimented by centrifugation, and the cell pellet was resuspended in an 150-fold volume of Lavit-medium PM 13 (Serva, Heidelberg), containing 5% fetal calf serum. Usually the cells formed a complete monolayer within 3 days and were then used for the fusion experiments. Other primary and permanent cell cultures were propagated according to standard procedures. The permanent cell lines were grown in Eagle minimal essential medium (MEM) supplemented with 10% fetal calf serum.

Viruses and infectivity assays. Pseudorabies (PsR) virus, strain DEK, originally obtained by G. Plummer was used in all experiments. Herpes simplex virus type 1 (HSV-1) was strain KOS, and type 2 (HSV-2) was strain 196. The viruses had been plaque purified three times, and one stock preparation was used for the experiments. Their origin and characterization has been described (17, 18). PsR virus was propagated in RK cells and reached titers of 10^8 to 10^9 PFU per ml in cell-free stocks. Infectious virus was assayed by plaque titration (25) in RK cells or in chicken embryo fibroblasts (CEF) by using 0.2 ml of inoculum per dilution step and the sodium salt of carboxymethyl cellulose (0.75%) in Lavit medium with 2% serum as an overlay.

Fowl plaque virus was the Rostock strain grown in the allantoic cavity of embryonated chicken eggs.

Determination of DNA. DNA was extracted from infected cells and was centrifuged in a Beckman analytical ultracentrifuge as described in detail elsewhere (17, 18).

Antisera. Antiserum 1 was kindly supplied by H. R. Frey and B. Liess, Hannover. A pig had been infected with cell-free PsR virus; 5 days postinfection (p.i.) the animal had shown typical clinical symptoms of pseudorabies and had recovered completely. Four intramuscular injections with the same virus followed in intervals of 5 days. The animal was bled 10 days after the last injection.

Antiserum 2 was kindly provided by H. C. Borgen, Copenhagen. It had been produced in a sow by subcutaneous injection of photoinactivated PsR virus. The virus no longer produced plaques and did not

induce any clinical signs in rabbits or in the sow. The inactivation of the virus and immunization procedure has been outlined in detail elsewhere (3).

The field sera from pigs with antibodies to PsR virus were kindly furnished by F. Steck, Bern.

A portion of antiserum 1 was absorbed with PsR virus-infected cells. Approximately 5×10^7 cells were harvested 5.5 h after infection, when fusion had spread over the whole monolayer. The medium was decanted, the cells were washed three times with cold phosphate-buffered saline (PBS), and the monolayer was fixed with 1% glutaraldehyde for 30 min at 4 C. The cells were then scraped from the dishes, and fixation was continued for another 30 min in suspension. After two washings in cold PBS, one half of the fixed cells were suspended in 1.0 ml of antiserum, incubated for 45 min at 37 C, and centrifuged. After a second absorption cycle with the remaining fixed cells the serum was used.

Fusion experiments. Cells in plastic petri dishes (60 mm in diameter) were used. After removal of the medium, the cells were infected with a multiplicity of infection of 20 to 50. The monolayers were incubated at 37 C for 90 min in a humidified CO_2-incubator (5% CO_2) and were carefully rocked every 20 min. Then the plates were washed five times with prewarmed PBS and flooded with 2.0 ml of MEM, containing the indicated concentrations of Con A, DOG, or antiserum. Fusion events were counted with the aid of a graded plastic dish in an inverted microscope and expressed as a percentage of multinucleated cells.

Agglutination tests with Con A. The test procedure for agglutination of single cell suspensions in the presence of the phytagglutinin has been outlined before (1).

Electronmicroscopy. Cells had been fixed in glutaraldehyde (1.75%) and OsO_4 (1%) and were embedded in Epon. Thin sections were contrasted with uranyl acetate and lead citrate and examined in an Elmiskop 101.

Chemicals. Con A was purchased from Calbiochem. DOG and α-methyl-D-glucopyranoside (α-MG) were purchased from Sigma Chemical Co., St. Louis, Mo., and kept as a 1 M stock solution in Aq. dest. at −30 C.

RESULTS

Fusion of PsR virus-infected rabbit kidney cells. Primary, subcultured, as well as cells of a permanent line (RK 13) of RK, fuse after infection with PsR virus (Fig. 1A).

This kind of cytopathic effect cannot be observed in other cell systems like L-cells, BHK-cells, HeLa-cells, and primary cells of bovine fetuses which replicate the virus under the same conditions. These cell types round up after PsR virus infection. MDBK-cells inoculated with this virus show a tendency for syncytia formation. However, no complete fusion takes place as is the case in RK cells.

Since RK 13 cells exhibit spontaneous polykaryocytes in the noninfected monolayer, they are less suitable for experiments about herpes-induced fusion. Primary RK cells were therefore chosen. Syncytia formation by PsR virus is optimally displayed in 3- to 4-day-old cells forming a dense monolayer; older cells react with retarded cell fusion. The amount of newly synthesized infectious virus parallels the alteration of the cell morphology, which starts 3 to 4 h p.i.; 8 to 10 h p.i. the whole monolayer has formed one syncytium (Fig. 1A and 2). In contrast, infection of RK cells with HSV-1 (strain KOS) or HSV-2 (strain 196) leads to rounding of the cells with no tendency to fuse.

Inhibition of fusion by Con A. In the presence of Con A the PsR virus-infected cells were not fused. Virtually all of them had rounded up 10 h after infection and about one third had detached from the petri dish (Fig. 1B). The dose which did not affect control cells morphologically (Fig. 1Aa) and prevented syncytia formation completely was between 15 and 25 μg of Con A per ml (Fig. 3). Fusion could only be prevented when Con A was added between 0 and 4 h p.i. to the culture medium (Fig. 4). If the medium containing Con A was removed at any time during this period and incubation was continued with normal medium, fusion occurred as in the nontreated controls. Pretreatment of the cells with Con A for 24 h followed by infection and incubation with normal medium did not influence their fusion.

Cell fusion by Con A was no longer inhibited when 100 mM of α-MG had been added to the medium containing the phytagglutinin. Under these conditions the total amount of infectious virus was not reduced (Table 1).

Influence of Con A on the agglutinability of PsR virus-infected cells. It was of interest to know whether the concentration of Con A which inhibits fusion of RK cells by PsR virus and which agglutinates many cell types infected with other enveloped viruses (1) would also clump the PsR virus-infected cells. At hourly intervals from 0 to 8 h p.i. and at 22 h p.i. the single cells or the disrupted syncytia were incubated with increasing concentrations of Con A from 10 to 250 μg per mixture. No specific agglutination was detectable by macroscopic inspection. PsR virus-infected CEF or BHK-21 cells, which also replicate the virus to high titers, remained individually in suspension. However, control preparations of fowl plaque virus-infected CEF were readily agglutinated by 10 μg of Con A per mixture. Repeated experiments with changing CEF and RK cell batches and varying PsR virus passages gave the same negative results.

Events in the herpesvirus-infected cells treated with Con A. Since Con A was able to

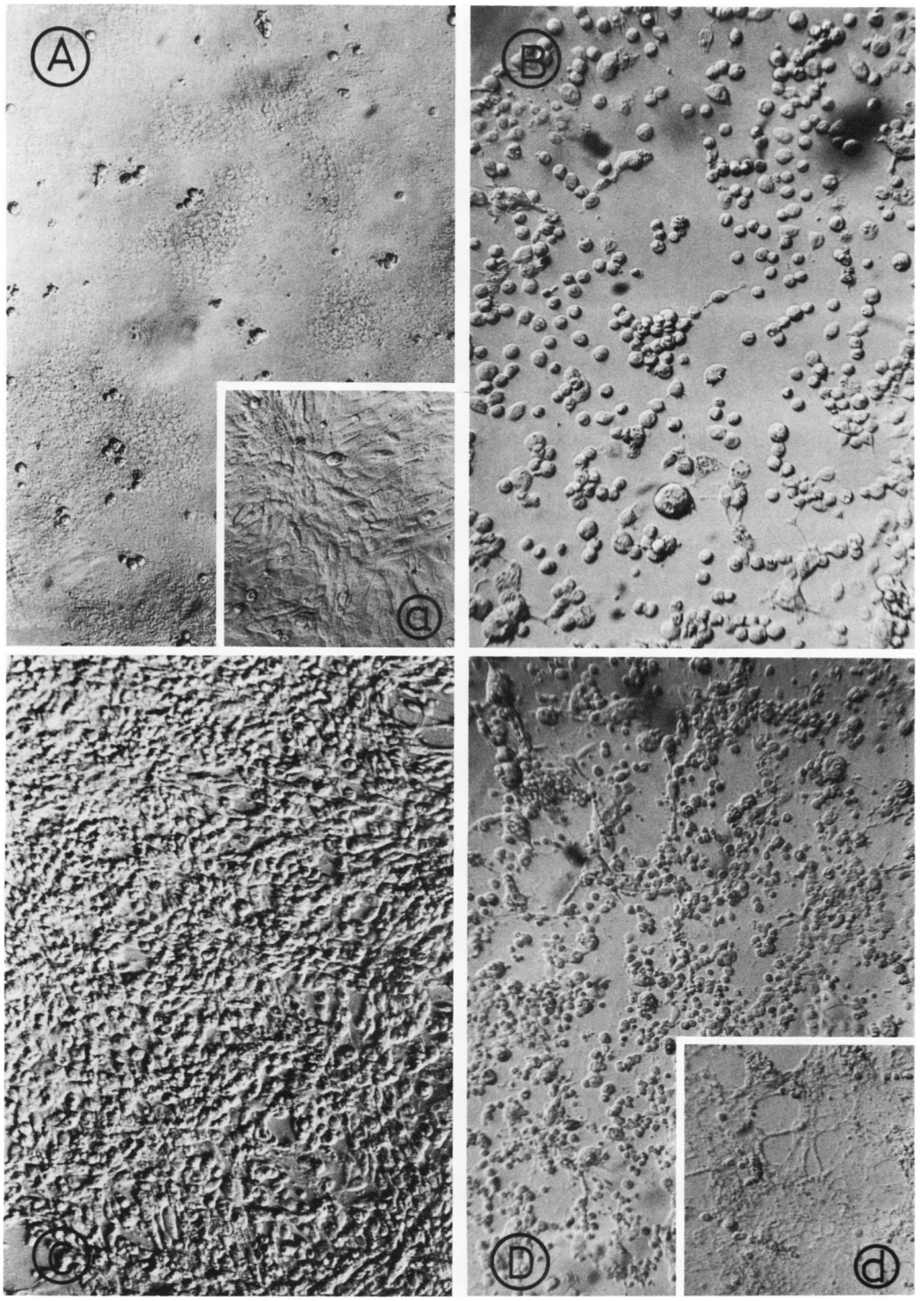

FIG. 1. *Inhibition of syncytia formation in RK cells infected with PsR virus. (A) An infected monolayer forming one total syncytium 10 h p.i.; (Aa) noninfected control cells treated with 20 μg of Con A per ml; (B) infected culture after incubation for 15 h with 20 μg of Con A per ml; (C) infected cells after incubation for 8 h with 10 mM DOG; (D) infected cells after incubation for 12 h with convalescent serum (antiserum 1) diluted 1:8, which completely inhibits cell fusion, and (Dd) with hyperimmune serum (antiserum 2), which does not influence syncytium formation.*

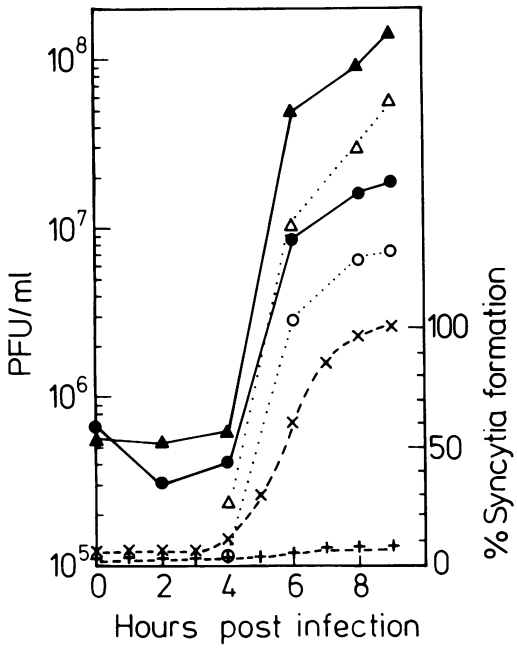

FIG. 2. *Effect of Con A on growth of PsR virus and syncytium formation in RK cells. Syncytium formation in the presence (+) and absence (×) of 15 μg of Con A per ml. Cell-free virus in the presence (○) and absence (△) of Con A. Cell-associated virus in the presence (●) and absence (▲) of Con A.*

alter the morphology of PsR virus-infected cells drastically, it was of interest to know whether virus DNA and infectious virus were formed in those cells. When the rounded Con A-treated cells were gently pelleted (10 min, 800 × *g*) and the total DNA was extracted and banded in the analytical ultracentrifuge (17), they carried about 50% more virus DNA than the untreated infected cells (Fig. 5). The total yield of infectious virus in the Con A-treated cells was between 10 and 50%, varying from cell to cell batch, as compared to the cultures without phytagglutinin (Fig. 3 and 4). However, this loss of infectivity was only evident when Con A was added between 0 and 2 h p.i. (Fig. 4). When Con A was added to the medium, the yield of extracellular virus was reduced by approximately 90% (Fig. 2). Further experiments were undertaken to determine the effect of Con A on the infectivity of cell-free virus. In one series of experiments the virus preparation was diluted to give 500 to 1,000 plaques per ml. An equal volume of medium containing 20, 40, or 100 μg of Con A per ml was added, the mixture kept at 37 C for 60 min and assayed for residual infectivity by plaque titration. In a second series the cell-free virus stock preparation containing approximately 10^7 PFU per ml was mixed with a solution containing 20 to 40 μg of Con A, incubated at 37 C for 10 min, 3 and 21 h, and diluted serially for plaque assays. Essentially the same number of plaques were formed if the stock virus or diluted virus was incubated with Con A or with control medium (Table 2).

Influence of specific antisera on virus-induced fusion. Antibodies, the other multivalent agent employed, also inhibited cell fusion. The convalescent serum 1 blocked the formation of any syncytium in a dilution of 1:8 (Fig. 1D). Virtually all cells were individually visible, and the monolayer as a whole had a similar appearance as after treatment with Con A. On the other hand, the hyperimmune serum 2 did not interfere with cell fusion at all (Fig. 1Dd) in spite of the fact that it had a neutralizing titer of 1:310 as compared to 1:220 for antiserum 1.

In an attempt to remove the specific antibodies against the component responsible for fusion, antiserum 1 was absorbed with glutaral-

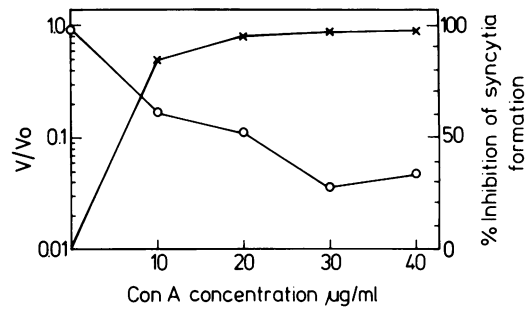

FIG. 3. *Effect of different concentrations of Con A on the production of syncytium formation (×) and infectious virus (○). Con A was added at hour 0 and the cells were harvested for titration of total virus yield 20 h p.i. (V_0) plaque counts in controls and (V) in Con A-treated cells.*

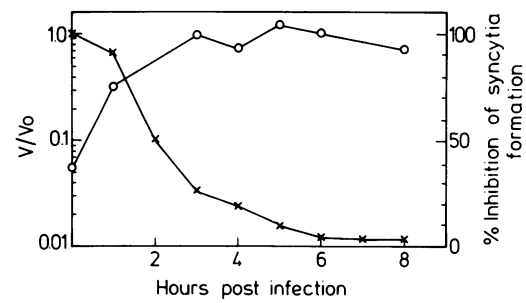

FIG. 4. *Influence of Con A (15 μg/ml), added at different times p.i., on syncytium formation (×) and production of infectious virus (○). The cells were harvested 18 h p.i. and the total virus yield was determined by plaque titration. (V_0) plaque counts in controls and (V) in the Con A-treated cells.*

TABLE 1. *Effect of Con A on PsR virus infection in RK cells*

Determination	Control	Con A	Con A + α-MG		
			10 mM	50 mM	100 mM
% Syncytia formation[a]	100	0	10	80	100
PFU/ml[a]	5.0×10^6	1.0×10^6	6.8×10^6	8.2×10^6	2.1×10^7
Cell agglutination[b]	None	None			

[a] Con A (15 μg/ml) alone or in a mixture with different amounts of α-MG was added to infected cells; 15 h p.i. the cytopathic effect was read, the cell culture was frozen and thawed three times, centrifuged for 10 min at 2,000 × *g*, and the supernatant (total virus yield) was titrated.

[b] PsR virus-infected RK-, CEF-, or BHK-cells were brought in single cell suspension and 0.3 ml was mixed with an equal volume of increasing concentrations of Con A solution up to 500 μg/ml.

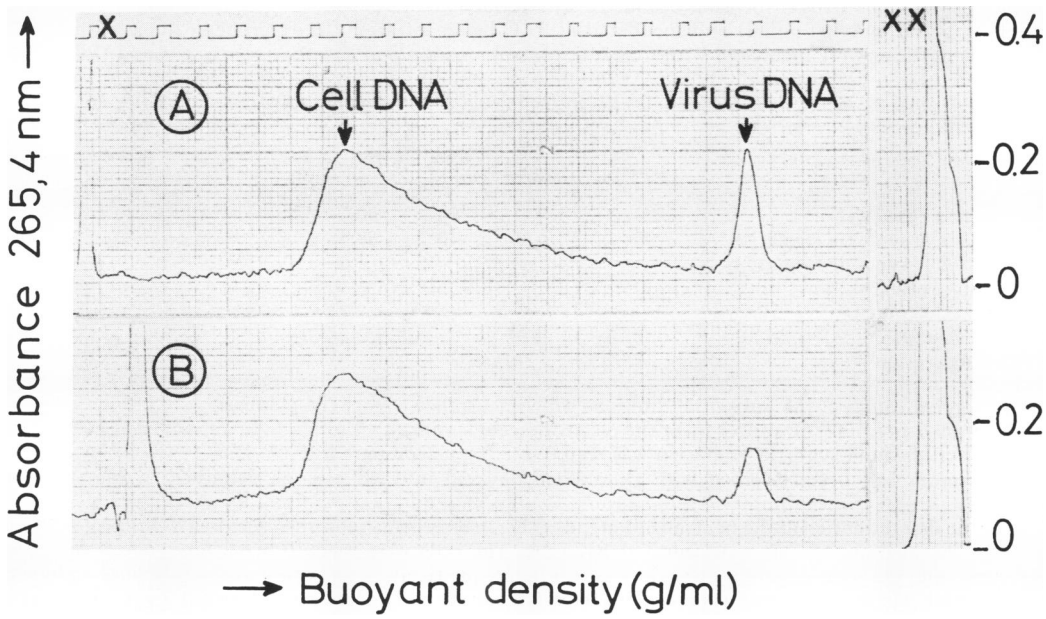

FIG. 5. *Photoelectric scannings of DNA preparations centrifuged to equilibrium in CsCl in a Beckman model E analytic ultracentrifuge (44,000 rpm, 20 h, 25 C). PsR virus-infected RK cells incubated with (A) and without (B) Con A (15 μg/ml) were harvested 18 h p.i.; the DNA was extracted and equal amounts were subjected to centrifugation. Virus DNA banded with a buoyant density of 1.731 g/ml (17, 18). The penmarker line (×) gives the reference line for a scan speed of 0.63 cm/min and a chart speed of 1.0 mm/s. The symbol ×× is for calibration steps.*

dehyde-fixed infected cells, which had been harvested 5.5 h after infection. The absorbed serum had lost considerable capacity to inhibit virus-induced cell fusion (Table 3), whereas the neutralizing potency was not affected. Field sera from pigs with relatively high neutralizing antibody titers inhibited cell fusion at least partially when dilutions of 1:10 were employed (Table 3). All these convalescent sera repressed cell fusion to various extents. No definite correlation between inhibition of cell fusion and their neutralizing potency could be established.

Effect of 2-deoxy-D-glucose on cell fusion. In view of the finding that herpes virus-specific glycoproteins are incorporated into the plasma membrane (10), it appeared feasible that the formation of these structures which was probably responsible for cell fusion might be inhibited by DOG, since this compound interferes with the glycoprotein synthesis of a number of enveloped viruses (12, 16). In fact, DOG in a concentration of 5 to 10 mM or more was capable of stopping cell fusion in RK cells (Fig. 6A and B). Control cells were morphologically unchanged even in the presence of 100 mM DOG. Infected cells treated with DOG presented the same morphological picture as the ones kept in the presence of Con A or antiserum

TABLE 2. *Effect of Con A on cell-free PsR virus*

Incubation time[a]	Control	Con A treated (μg/ml)		
		10	20	50
Expt 1				
1. 60 min	52, 57, 50, 49	86, 96, 98, 97	91, 91, 97, 103	68, 74, 144, 107
2. 60 min	67, 81, 66, 74		38, 27, 34, 20	43, 42, 31, 37
Expt 2				
10 min	7.5×10^6	6.4×10^6	7.0×10^6	
3 h	8.0×10^6	6.7×10^6	7.3×10^6	
21 h	2.2×10^6	1.1×10^6	1.6×10^6	

[a] In experiment 1 plaques of two different experiments are given: the virus was diluted to 500 to 1,000 plaques per ml. This virus suspension was mixed with equal amounts of control medium or Con A solutions (20 μg/ml, 40 μg/ml and 100 μg/ml) and incubated at 37 C before titration. In experiment 2 approximately 10^7 infectious particles were incubated with Con A or control medium and the mixture was titrated (PFU/ml).

TABLE 3. *Effect of PsR virus-specific antisera and reconvalescent (R) sera on cell fusion*

Determination	R anti-serum 1	Anti-serum 2	R 607	R 569	R 572	R 570	R 606	Control
Neutralizing capacity[a]	220	310	90	50	30	70	30	<4
% Inhibition of syncytia formation[b]	95	0	40	60	50	30	50	0
% Inhibition of syncytia formation after absorption[c]	10							
Neutralization index								
Before absorption	2.75	3.70						
After absorption[d]	2.62							

[a] Reciprocal values of the antiserum dilutions which neutralize 80% of the plaques.

[b] Medium containing the antiserum or the reconvalescent serum at a dilution 1:10 was added at hour 0 to the infected cultures and syncytia formation was read 6 to 8 h p.i. (compare Fig. 1A, D,d).

[c] The serum had been absorbed with glutaraldehyde-fixed infected cells as described in Materials and Methods.

[d] For the determination of the neutralization index the unabsorbed or absorbed sera in a dilution of 1:10 were given to the medium of PsR virus-infected RK cells. The cells were read 10 h p.i. for syncytia formation, harvested, and the total infectivity was determined by plaque titration and compared with that of an infected culture which had received control serum. This reference culture had an infectivity titer of 1.7×10^7 PFU/ml.

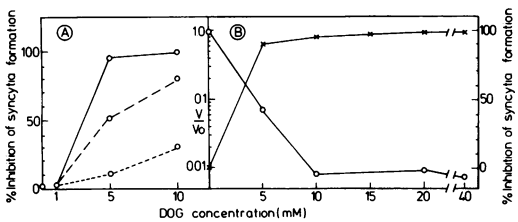

FIG. 6. *Effect of DOG on PsR virus-induced cell fusion. (A) Different concentrations of DOG were added at 0 (O——O), 1 (O- - -O), and 2.5 h (O···O) p.i. to the culture medium and syncytium formation was evaluated 12 h p.i. (B) DOG was added at 0 h and syncytium formation (×) and total virus yield (O) were determined 20 h p.i. (V_0) plaque counts in controls and (V) in DOG-treated cells.*

at least for the first 6 to 8 h p.i. (Fig. 1C). Virus-induced fusion was not affected when RK cells were pretreated up to 1 day with 10 mM DOG before infection. Similarly, fusion reappeared when infected cells were incubated with DOG for 6 to 8 h after infection and the drug then was washed off and incubation continued with fresh medium.

The morphological alterations of the infected cells in the presence of DOG was accompanied by a significant loss of infectivity (Fig. 6B). Electron micrographs of thin sections showed viral nucleocapsids in the nucleus and enveloped virus in the cytoplasm. A similar loss of infectivity with an unchanged number of physical particles had been reported for BSC 1-cells infected with herpes simplex virus and treated with DOG (5).

DISCUSSION

The results presented here demonstrate that PsR virus-induced cell fusion can be prevented early after infection (0 to 4 h p.i.) by different types of treatment. The sugar analogue DOG completely stops the fusion event, whereas virus

products are continuously made in the infected cell (manuscript in preparation). Since evidence has been presented that DOG affects glycoprotein synthesis (16) one can suggest that the fusion factor is a glycoprotein. For paramyxoviruses this has recently been shown (30). Our findings underline preliminary results (27), and they are in agreement with a report by Gallaher et al. (8), who found that DOG inhibited herpes simplex virus-induced fusion of BHK cells. They substantiate the suggestion that glycoproteins play a role in the alteration of the herpes virus-infected cell monolayer (10).

A totally different manipulation of the PsR virus-infected cultures gave the same results. Cell fusion could also be inhibited by treatment with Con A, provided that the phytagglutinin was added early in the infectious cycle before the fusion process had started. The same conditions had to be applied and the same dose range of Con A was effective to prevent fusion of BHK cells by the paramyxovirus SV 5 (28). Inhibition of virus release, which is one of the prominent effects on myxovirus-infected cells (28) may also be the reason for the reduced yield of PsR virus when Con A is added to culture medium of RK cells. This interpretation is compatible with data presented by other authors (23).

Steric hindrance of a complete membrane to membrane contact is probably the decisive effect for the prevention of cell fusion, because Con A has to be present before the fusion process starts. A mere adsorption of Con A to the cell surface does not seem to be the dominant factor, because pretreatment of the cells before infection could not prevent the fusion process. The nuclear membrane as the principle site of budding (2, 21) and consequently inadequate exposure of receptors at the cell surface is probably the reason for the lack of agglutinability of PsR virus-infected BHK cells and chicken embryo fibroblasts. The very high doses which other authors had to use to agglutinate HSV-infected chicken embryo fibroblasts (31) were never employed.

It is not clear whether cell fusion is inhibited by binding of Con A to specific glycoproteins which are still part of the host cell membrane or which have been translocated to the virus-specific protein, perhaps the putative fusion factor. Antibodies, however, the third agent found to interfere with cell fusion, are directed against the virus-specific component appearing at the cell surface. There is evidence that this fusion factor is a nonstructural glycoprotein, because a hyperimmune serum produced against inactivated whole virus did not prevent cell fusion in spite of its high neutralizing capacity. Convalescent sera, however, which presumably contain antibodies against the whole spectrum of viral structural and nonstructural antigens, had varying degrees of an inhibitory effect on cell fusion. Since after absorption of convalescent serum with infected cells the antifusion activity decreased considerably while the neutralizing power of the serum was not significantly changed, the assumption seems to gain support that the viral structure involved in the early event of cell fusion is located at the surface of the cell membrane and that it is different from the antigen reacting with neutralizing antibodies. It remains to be determined whether it is related to any of the early antigens described in herpes virus-infected cells (9, 29).

The mechanism by which Con A or antibodies block fusion might be identical. Either agent could act by sterical interference of the cell to cell contact and to some extent by formation of a lattice at the cell membrane. Thereby the membrane could lose its normal flexibility and the possibility to merge with a neighboring cell.

ACKNOWLEDGMENTS

For excellent technical assistance we are grateful to T. Bremer, M. Orlich, and E. Heller. The work was supported by the Sonderforschungsbereich 47 (Virologie).

LITERATURE CITED

1. Becht, H., R. Rott, and H.-D. Klenk. 1972. Effect of Concanavalin A on cells infected with enveloped RNA viruses. J. Gen. Virol. **14**:1-8.
2. Ben-Porat, T., and A. S. Kaplan. 1972. Studies on the biogenesis of herpesvirus envelope. Nature (London) **235**:165-166.
3. Borgen, H. C., and L. Espensen. 1970. Immunizing effect of photoinactivated pseudorabies virus. Zentralbl. Bakteriol. Abt. Parisitenk. Infektionskr. 1 Orig. **214**: 13-16.
4. Chen, J. H., and H. G. Purchase. 1970. Surface antigen on chick kidney cells infected with the herpesvirus of Marek's disease. Virology **40**:410-412.
5. Courtney, R. J., S. M. Steiner, and M. Benyesh-Melnick. 1973. Effects of 2-deoxy-D-glucose on herpes simplex virus replication. Virology **52**:447-455.
6. Ejercito, P. M., E. D. Kieff, and B. Roizman. 1968. Characterization of herpes simplex virus strains differing in their effects on social behaviour of infected cells. J. Gen. Virol. **2**:357-364.
7. Falke, D., and G. F. Kahl. 1971. The inhibitory effect of compound 48/80 on the formation of giant cells induced by herpesvirus hominis. J. Gen. Virol. **10**:273-277.
8. Gallaher, W. R., D. B. Levitan, and H. A. Blough. 1973. Effect of 2-deoxy-D-glucose on cell fusion induced by Newcastle disease and herpes simplex viruses. Virology **55**:193-201.
9. Hamada, C., and A. S. Kaplan. 1965. Kinetics of synthesis of various types of antigenic proteins in cells infected with pseudorabies virus. J. Bacteriol. **89**:1328-1334.
10. Heine, J. W., P. G. Spear, and B. Roizman. 1972. Proteins specified by herpes simplex virus. VI. Viral proteins in the plasma membrane. J. Virol. **9**:431-439.
11. Ito, M., and A. L. Barron. 1973. Surface antigens produced by herpesviruses: Varicella-Zoster virus. Infect. Immunity **8**:48-52.

12. Kaluza, G., C. Scholtissek, and R. Rott. 1972. Inhibition of the multiplication of enveloped RNA-viruses by glucosamine and 2-deoxy-D-glucose. J. Gen. Virol. **14**:251-259.
13. Kaplan, A. S., and T. Ben-Porat. 1970. Synthesis of proteins in cells infected with herpesvirus. VI. Characterization of the proteins of the viral membrane. Proc. Nat. Acad. Sci. U.S.A. **66**:799-806.
14. Kilbourne, E. D. 1959. Inhibition of influenza virus multiplication with a glucose antimetabolite (2-deoxy-D-glucose). Nature (London) **183**:271-272.
15. Klein, G., G. Pearson, J. S. Nadkarni, J. J. Nadkarni, E. Klein, G. Henle, W. Henle, and P. Clifford. 1968. Relation between Epstein-Barr viral and cell membrane immunofluorescence of Burkitt tumor cells. I. Dependence of cell membrane immunofluorescence on presence of EB virus. J. Exp. Med. **128**:1011-1020.
16. Klenk, H.-D., C. Scholtissek, and R. Rott. 1972. Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. Virology **49**:723-734.
17. Ludwig, H. 1972. Untersuchungen am genetischen Material von Herpesviren. Med. Microbiol. Immunol. **157**:186-238.
18. Ludwig, H., N. Biswal, and M. Benyesh-Melnick. 1972. Studies on the relatedness of herpesviruses through DNA-DNA hybridization. Virology **49**:95-101.
19. Munk, K., and G. Sauer. 1964. Relationship between cell DNA metabolism and nucleocytoplasmic alterations in herpes virus-infected cells. Virology **22**:153-154.
20. Nicolson, G. L. 1971. Difference in topology of normal and tumor cell membranes shown by different surface distributions of ferritin-conjugated Concanavalin A. Nature (London) **233**:244-246.
21. Nii, S., C. Morgan, H. M. Rose, and K. C. Hsu. 1968. Electron microscopy of herpes simplex virus. IV. Studies with ferritin-conjugated antibodies. J. Virol. **2**:1172-1184.
22. Olshevsky, U., and Y. Becker. 1972. Surface glycopeptides in the envelope of herpes simplex virions. Virology **50**:277-279.
23. Okada, Y., and J. Kim. 1972. Interaction of Concanavalin A with enveloped viruses and host cells. Virology **50**:507-515.
24. Ozanne, B., and J. Sambrook. 1971. Binding of radioactively labelled Concanavalin A and wheat germ agglutinin to normal and virus transformed cells. Nature (London) **232**:156-160.
25. Rapp, F. 1963. Variants of herpes simplex virus: isolation. characterization and factors influencing plaque formation. J. Bacteriol. **86**:985-991.
26. Roizman, B. 1962. Polykaryocytosis. Cold Spring Harbor Symp. Quant. Biol. **27**:327-342.
27. Rott, R., H. Becht, G. Hammer, H.-D. Klenk, and C. Scholtissek. 1974. Changes of the surface of the host cell after infection with enveloped viruses. *In* R.D. Barry and B. W. J. Mahy (ed.), Negative strand viruses. Academic Press Inc., New York.
28. Rott, R., H. Becht, H.-D. Klenk, and C. Scholtissek. 1972. Interactions of Concanavalin A with the membrane of influenza virus infected cells and with envelope components of the virus particle. Z. Naturforsch. **27b**:227-233.
29. Russel, W. C., E. Gold, H. M. Keir, H. Omura, D. H. Watson, and P. Wildy. 1964. The growth of herpes simplex virus and its nucleic acid. Virology **22**:103-110.
30. Scheid, A., and P. W. Choppin. 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**:475-490.
31. Tevethia, S. S., S. P. Lowry, W. E. Rawls, J. L. Melnick, and V. McMillan. 1972. Detection of early cell surface changes in herpes simplex virus infected cells by agglutination with Concanavalin A. J. Gen. Virol. **15**:93-97.