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EXAMINATION OF THE ACTIVITY OF GLYCOPROTEIN HN AND F ANTIGENS OF THE OUTER ENVELOPE OF THE PARAINFLUENZA VIRUS TYPE 3 BY USING FUSIONAL, HEMOLYTIC AND HEMAGGLUTINATION TESTS, *IN VITRO*

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The objective of our study was to examine fusional, hemolytic and hemagglutinating activities of the surface glycoprotein HN and F antigens of parainfluenza virus type 3, in vitro. The samples of activated PI3 virions, induced Vero cell fusion after 24 h and 48 h. After treatment of inoculated Vero cells was the dilutions of specific immune sera against PI3 virus, cell fusion were not registered at dilutions of 1:2, 1:4 and 1:8 of the immune sera. The samples of PI3 virions, activated by 0.025 g/dL trypsin-versen, induced hemolysis at antigen dilution of 1:8. The samples of activated PI3 virions, expressed an intensive hemagglutinating activity of 128 HAU/0.1 mL. After treatment of the abovementioned samples with specific immune sera against PI3 virus, hemolytic activities were not detected at the dilution of 1:16 of specific immune sera. These results showed that the fusional and hemolytic tests can be used for fast detection of immunologically important glycoprotein antigens of PI3 virus and their identification with specific immune sera.

Key words: virus PI3, fusional test, hemagglutination, hemolysis

INTRODUCTION

The most important investigation of the complex antigenic structure and fusional, hemagglutinating and hemolytic activities of the outer glycoprotein HN and F antigens of parainfluenza virus type 3 were made by Scheid and Choppin (1974), Heminway *et al*. (1994), Breker-Klassen *et al*. (1996), Tanabayashi and Compans (1996), Yao, Hu and Compans (1997), Milic *et al*. (2001), Milic *et al*. (2003) and Ferreira *et al*. (2004). Important studies related to conformational structure of the glycoprotein HN and F antigens of the outer envelope of parainfluenza virus type 3 were published by Morrison (2003) and Lawrence (2004).

Hemagglutinin-neuraminidase antigen of the outer envelope of parainfluenza virus type 3 belong to the glycoprotein type 2 and represent a tetramer which consists of a two disulfide linked dimer. Hemagglutinin-

neuraminidase antigen is a multifunctional molecule with three different activities: receptor binding activity, neuraminidase activity and fusion promotion activity.

Fusion (F) protein of the outer envelope of parainfluenza virus type 3 is a transmembrane glycoprotein type 1 which consists of three regions: head, neck and stalk. This protein is syntetized as a inactive precursor Fo. Fusion protein become responsible for cell fusion and hemolysis after proteolytic cleavage, by cell proteases, of the inactive precursor Fo when from the abovementioned subunit became two disulfide linked subunits as F1 and F2.

MATERIAL AND METHODS

Virus and Tissue culture

The bovine PI3 virus, strain SD2, with a titre of $LD_{50}=10^{-3,1}$ (log $10^{-3,1}$ TCID/0.1 mL) and hemagglutination titre of 64HAU/0.1 mL, was used. The fusional test and inhibition of cell fusion with specific immune sera against PI3 virus were performed on Vero cell lines.

Examination of fusional activities of parainfluenza virus type 3, in vitro (fusional test on Vero cells)

The fusional activities of the glycoprotein antigens of PI3 virus were examined on Vero cells. The samples of PI3 virus (500 μL) with a hemagglutination titre of 64HAU/0.1 mL were individually activated with 5 μl of 0.025 g/dL trypsin-versen in 0.2 mol/L PBS (pH 7.2) according to Scheid and Choppin (1974) and Milić (1993). After 10 min of incubation at 37°C, the samples of virus suspension (500 μl) were treated with 75 μL Eagle MEM containing 10 g/dL fetal sera in order to inactivate trypsin – versen. Samples of the so activated PI3 virus were individually inoculated in microplate wells with Vero cells and incubated for 6 h, 12 h, 24 h i 48 h at 36°C. Inoculated Vero cell lines were observed under polarized light on Karl Zaissler microscope, at magnification of 4x8x3.

Inhibition of cell fusion - neutralization of PI3 virus with specific hiperimmune sera

The samples of PI3 virus with a titre of $100LD_{50}$, were activated with 5 μL of 0.025 g/dL trypsin-versen in 0.2 mol/L PBS (pH 7.2) and treated with an equal volume of a dilution of specific immune sera from 1:2 to 1:512. These mixtures of activated PI3 virus and hiperimmune sera were individually inoculated in microplate wells and incubated for 1 h at 37°C. After that, these mixtures of activated PI3 virus and specific immune sera were inoculated in microplate wells with Vero cell lines and incubated for 48 h at 36°C.

Determination of hemolytic activities of activated PI3 virus

The samples of 25 μL of PI3 virus, activated with 0.025 g/dL trypsin-versen in 0.2 mol/L PBS, were diluted in PBS as dilutions from 1:4 to 1:512 in microplate wells. The aliquotes of 50 μL PBS containing 0.5% quinea-pig erythrocyte

suspension were added to each well. Microtitre plates were incubated for 45 min at 37°C, followed by gently shaking the microplates.

Inhibition of hemolytic activities of activated PI3 virus

The samples of 25 μL of PI3 virus, activated with 0.025 g/dL trypsin-versen in 0.2 mol/L PBS, and with hemagglutination titre of 4 HAU/0.1 mL, were used. Samples of specific immune sera against PI3 virus of 25 μL were diluted in PBS from 1:2 to 1:1024 in microplate wells. After that, 25 μL samples of PI3 virus with hemagglutination titre of 4HAU/0.1 mL were added to each well. These mixtures of the activated virus and specific immune sera were incubated for 30 min at 37°C. The aliquotes of 50 μL PBS containing 0.5% quinea-pig erythrocyte suspension were added to each well. The samples were incubated for 45 min at 37°C.

Examination of hemagglutinating activity of PI3 virus

Hemagglutinating activity of PI3 virus was examined by the method of direct hemagglutination in microplates according to Clarke-u and Cassals-u (1958).

Hemagglutination – inhibition test – HI test

Determination of the titre of specific antibodies against PI3 virus in immune sera and aditional identification of this virus was performed by the method of hemagglutination-inhibition test according to Clark-u and Cassals-u (1958).

RESULTS

The samples of PI3 virus with a hemagglutination titre of 128 HAU/0.1 mL, activated with 0.025 g/dL trypsin-versen, induced cell fusion in inoculated Vero cells followed by formation of smaller groups of merged cells. The intensity of cell fusion of the inoculated cells got reinforced from low intensive to intensive cell fusion and resulted in the formation of agglomerations of Vero cells into groups with multinuclear cell forms and syncitium. The intensity of cell fusion depended from the antigen dilution, as well. After 24 h of incubation of inoculated Vero cells, cells fusion was intensive at antigen dilution from 1:2 to 1:4 (Figure 1), low at antigen dilution of 1:8 and least intensive at antigen dilution of 1:16. After 48 h of incubation of Vero cells inoculated with PI3 virus, intensive cell fusion was detected at antigen dilution of 1:32 (Figure 2), low intensive at antigen dilution of 1:64 and least intensive at the antigen dilution of 1:128. At other antigen dilutions, the intensity of cell fusion was not significant. In all the control Vero cells, inoculated with non-activated PI3 virus with hemagglutination titre of 64 HAU/ 0.1 mL, cell fusion was not detected.

After 48 h of inoculation of Vero cells with the samples of activated PI3 virions and specific immune sera against PI3 virus at dilutions of 1:2, 1:4 and 1:8, inhibition of cell fusion was detected. Cell fusion was registered in inoculated Vero cells treated with the dilution of specific immune sera of 1:16, as in inoculated Vero cells tretaed with higer dilutions of specific immune sera and constant concentration of PI 3 virus of 100 LD_{50}. The samples of PI3 virus, activated with

0.025 g/dL trypsin-versen, induced hemolysis of quinea – pig erythrocyte at antigen dilution of 1:8. This was followed with a significant increase of hemagglutinating activities of activated PI3 virus which was 128 HAU/0.1 mL. After treatment of the samples of activated PI3 virus with 0.025 g/dL trypsin-versen with the dilutions of specific immune sera of 1:2, 1:4 and 1:8, hemolysis of quinea – pig erythrocytes was not registered. Hemolysis of quinea-pig erythrocyte was detected at the dilution of the 1:16 of the specific immune serum. In the control group of samples which contained non-activated PI3 virions, hemolysis of quinea – pig erythrocytes was not registered. After treating the samples of PI3 virions with the dilutions of specific immune sera against PI3 virus, aglutination of quinea – pig erythrocyte was not detected at dilutions of 1:2, 1:4:, 1:8, 1:16 and 1:32.

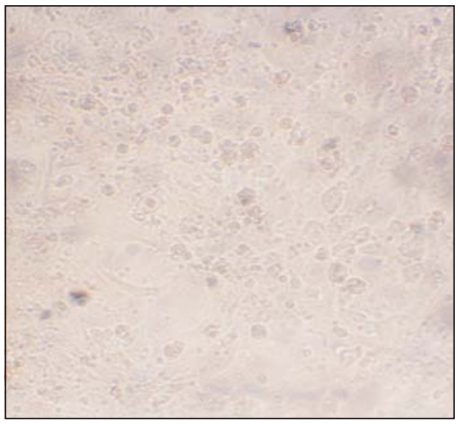

Figure 1. Fusion of Vero cells 24 h after inoculation with activated PI3 virus

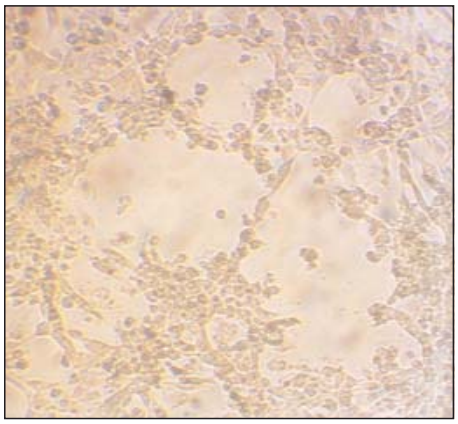

Figure 2. Fusion of Vero cells 48 h after inoculation with activated PI3 virus

DISCUSSION

Ferreira *et al*. (2004) examined the hemagglutination activities of the HN antigen of the Newcastle disease virus. The results suggest that the Newcastle virus requires different sialic acid – containing compounds, gangliosides and glycoproteins for entry into the target cell. They proposed that gangliosides act as primary receptors while N-linked glycoproteins function as the second receptor critical for viral entry. Removing sialic acid – containing receptors from the cell surface, Ah Tye *et al*. (1999) blocked cell fusion. Zhuhui Huang *et al.* (2004) thus confirming that the hemagglutinin-neuraminidase (HN) protein of Newcastle disease virus plays a crucial role in the process of infection and that the same protein determines tropism and virulence of the virus. Milić *et al*. (2003, 2001) examined the antigenic structure, fusional and hemolytic activities of the surface glycoprotein HN and F antigens of purified PI3 virus activated with 0.025 g/dL trypsin-versen. The obtained results confirmed that the fusional and hemolytic tests can be used for the fast detection of immunologically important glycoprotein

antigens of PI3 viruses and their identification with specific immune sera. Scheid and Choppin (1974) examined some biological activities of the glycoprotein antigens of the PI3 virus. The results showed that the fusion (F) protein of the outer envelope of Sendai virus has an important role in the cell fusion process and in hemolysis. Tanabayashi and Compans (1996), Yao *et al*. (1997) and Heminway *et al*. (1994) confirmed that a specific interaction between both paramyxoviral glycoproteins is required for the cell fusion process and hemolysis. Breker *et al*. (1996) examined hemolytic and fusional activities of glycoprotein antigens of the outer envelope of PI3 virus and showed that there are significant differences between the different viral strains that were examined. Their results suggested that PI3 viruses which exhibit greater syncytium – inducing activity, *in vitro*, have greater pathogenicity, *in vivo*.

On the basis of these results, it can be concluded that the described methods, among the other standard methods of virological diagnostics, can be succesfully used for the detection and identification of the crucial glycoprotein antigens of the outer envelope of parainfluenza virus type 3.

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ISPITIVANJE AKTIVNOSTI GLIKOPROTEINSKIH HN I F ANTIGENA SPOLJAŠNJEG OMOTAČA VIRUSA PARAINFLUENCE 3 PRIMENOM TESTOVA FUZIJE, HEMOLIZE I HEMAGLUTINACIJE *IN VITRO*

NIŠAVIĆ J i MILIĆ N

SADRŽAJ

Cilj ovih istraživanja je bio ispitivanje fuzionih, hemolitičkih i hemaglutinacionih aktivnosti glikoproteinskih antigena virusa parainfluence 3, *in vitro*. Rezultati testova ćelijske fuzije pokazali su da je virus PI3 posle aktivacije sa 0,025 g/dl tripsin-versena indukovao fuziju Vero ćelija posle 24h koja se intenzivirala posle 48h od inokulacije virusa. Tretiranjem inokulisanih Vero ćelija sa razređenjima imunih seruma protiv virusa PI3, ustanovljena je inhibicija ćelijske fuzije u razređenjima seruma od 1:2, 1:4 i 1:8. Uzorci virusa PI3, indukovali su posle aktivisanja sa 0,025 g/dl tripsin-versena, hemolizu eritrocita zamorca u razređenjima antigena od 1:8. Oni su ispoljili i intenzivnu hemaglutinacionu aktivnost posle aktivacije tripsin-versenom koja je iznosila 128 HJ/0,1 ml za virus PI3. Posle tretiranja uzoraka aktivisanih virusa PI3 sa razređenjima specifičnih imunih seruma, nije ustanovljena njihova hemolitička aktivnost do razređenja seruma od 1:16. Rezultati rada su ukazali na mogućnost korišćenja fuzionog i hemolitičnog testa za brzu detekciju imunološki značajnih glikoproteinskih antigena virusa PI3 i njihovu identifikaciju primenom specifičnih imunih seruma.