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Effect of Influenza A Virus and Bacterial Lipopolysaccharide on Proliferation and Expression of Cytokines and Other Cellular Factors in the Endothelial Cell Line ECV-304

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Abstract—Viral infection and bacterial lipopolysaccharide (LPS) cause endothelial-cell dysfunction. The aim of the current study was to investigate the effect of influenza A virus and LPS from *Escherichia coli* on the proliferative activity and gene expression of cytokines and cellular factors (*TNF*α, *TGF*β, *IFN*-γ, *MMP-9*, *NF*-κ*B*, *Rho A*, *eNOS*, and *iNOS*) in human endothelial cells ECV-304. It was found that ECV-304 cells infected with very low infectious doses of influenza virus acquired the capacity for the long-term active proliferation (over eight passages). Addition of LPS from *E. coli* reduced the virus-stimulated cell proliferation. It was shown that influenza virus and LPS affected the gene expression of cytokine and other cellular factors. When endothelial cells were infected with influenza A virus in the presence of LPS, there was a significant increase in the expression of several genes and the expression pattern of certain genes was modified. Expression of *MMP-9* gene inhibited by the virus and LPS separate exposure significantly increased during the first day after addition of the virus and LPS simultaneously. The same was true for the *IFN*-γ gene expression. *TNF*α gene was active only for 1–3 days whereas the expression of *TGF*β, *eNOS*, *iNOS*, *NF*-κ*B* and *Rho A* genes increased significantly on the fifth day, as it was observed with the cells treated with LPS only. Thus, the influenza A virus and LPS change the physiological state of endothelial cells. This occurred during various time periods (as well as at various degrees of viral infection) produced by different cellular factors and, possibly, involved different signaling pathways.

Keywords: endothelial cell line ECV-304, lipopolysaccharide (LPS), influenza A virus, cell proliferation, expression of cytokines

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INTRODUCTION

Influenza virus infects epithelial cells of the upper and lower respiratory tracts. It results in their damage, apoptosis, and desquamation. With a mild disease, the lesions are reversible. The cell layer is restored in several days (Armstrong et al., 2013). Influenza A viral infection enhanced the production of pro-inflammation cytokines: tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and their transcription regulator NF-κB. It causes cell dysfunction and apoptotic death (Julkunen et al., 2001; Bernasconi et al., 2005; Schmolke et al., 2009). The virus increased the level of cell proteases in endothelial cells, such as ectopic trypsin. The enzyme produces post-translation proteolytic cleavage of the viral hemagglutinin and converts the precursor of matrix metalloproteinase (pro-MMP-9) into active the MMP-9 form. Influenza-virus induction of these processes results in the degradation of basal membrane proteins and disruption of the cell's connection with the extracellular matrix. These lesions may alter the endothelial-cell permeability and cause organ failure (Wang et al., 2010; Kido et al., 2012). Increased production of TNFα, an apoptosis inducer, is a protective cell response to influenza-virus infection (Seo, Webster, 2002). TNFα enhances the production of nitrogen oxide (NO), followed by cell apoptosis. The process is modulated with interferon gamma (IFN-γ) (McKinney et al., 2003). NO is synthesized with the endothelial constitutive NO-syntase (e NOS) and inducible NO-syntase (i NOS). Their expression is triggered by cytokines, stress, and bacterial and viral agents. Apop-

Abbreviations: ID—infectious dose, MI—multiplicity of infection, eNOS—endothelial NO-synthase, iNOS—inducible NO-synthase, IFN-γ—interferon γ, IL—interleukin, LPS—lipopolysaccharide, MMP-9—matrix metalloproteinase 9, NO—nitrogen oxide, TGFβ—transforming growth factor β, TLR—Toll-like receptor, TNFα—tumor necrosis factor α.

tosis in cells infected with the influenza A virus is induced with the transforming growth-factor beta (TGFβ); viral neuraminidase is a TGFβ activator (Schultz-Cherry, Hinshaw, 1996).

Lipopolysaccharide (LPS), a Gram-negative bacteria endotoxin, circulating in blood may cause inflammation and endothelial-cell dysfunction known as "sepsis syndrome." Endothelial cells express two types of Toll-like (TLR) receptors: predominantly TLR-4 and, at a low level, TLR-2. They are regulated by LPS, TNFα, and IFN-γ, which, in turn, requires increased NF-κB expression (Peters et al., 2003). LPS stimulates the production of proinflammatory cytokines mainly via TLR-4, which triggers intracellular signaling cascades, resulting in activation and nuclear translocation of NF-κB (Meyer-Schwesinger et al., 2009). TNFα is a key LPS-induced cytokine. It causes endothelial-cell dysfunction both through apoptosis and via RhoA-produced activation of mitogen-activated protein kinases, which increases cell permeability (Mong et al., 2008; Meyer-Schwesinger et al., 2009).

LPS is able to induce the conversion of endothelial cells into activated fibroblasts, also known as "myofibroblasts" (Echeverria et al., 2013), via the endothelial–mesenchymal transition. The inducers are TGFβ1 and TGFβ2 (van Meeteren, Dijke, 2012). TGFβ is a member of the multifunctional cytokine family, which regulates various biological processes, such as cell differentiation, proliferation, and apoptosis. The effect of TGFβ on the cell state and cell cycle is specific and is realized through different signaling pathways (Undevia et al., 2004). It is known as a factor that produces endothelial-barrier dysfunction involving p38 signaling and RhoA activation (Clements et al., 2005; Lu et al., 2006). TGFβ induces lung fibrosis and apoptosis in lung epithelial cells (Hagimoto et al., 2002).

Thus, the effects of viral infection and LPS on endothelial-cell dysfunction have much in common at the molecular level. It is worthwhile to consider their combined action on endothelial cells. The goal of this research was to study the impact of bacterial LPS and influenza virus on proliferation of human endothelial cells (ECV-304) and gene expression of cytokines, as well as *TNFα*, *TGFβ*, *IFN-γ*, *MMP-9*, *NF-κB*, *Rho A*, *eNOS*, and *iNOS* factors.

MATERIALS AND METHODS

Cell lines. Spontaneously transformed human endothelial cells ECV-304 and canine kidney cells MDCK have been obtained from the Cell Culture Collection at the Research Institute of Influenza of the Ministry of Healthcare of Russia, St. Petersburg. The cells were maintained in alpha-MEM medium with 2% bovine embryonic serum without antibiotics. The cells were subcultures every 6–7 days at a ratio of 1 : 3–1 : 5 using a trypsin/EDTA mixture. ECV-304 cells were plated with a density of 12×10^5 cells/mL and infected with influenza virus. The multiplicity of infection (MI) (a value equal to the ratio of the infectious dose (ID) to the number of infected cells) was 0.004 with ID 1000 and 0.000004 with ID 1.

LPS obtained from *Escherichia coli* (Sigma-Aldrich, United States) was used in a concentration of 100 ng/mL.

Infection of a 1-day monolayer of ECV-304 cells. Cells grown in 50-mL flasks (Nunc, Denmark) were infected with 0.5 mL of titrated liquid containing the virus according to the method described in (Danilenko et al., 2016). Infection of the cells was carried out in three variants: (1) only virus (A/Pert/16/11(H3N2), (2) only LPS, and (3) virus together with LPS. Cells not treated with virus or LPS served as control. At days 1, 3, and 5, the monolayer was removed with trypsin/EDTA and cells were pipetted in serum-free alpha MEM medium. An amount of 0.1 mL of cell suspension was taken for cell counting; other cells were centrifuged at 1000 rpm for 10 min. The pellet was frozen at –20°C to assay gene expression with real-time PCR.

Cell proliferation. Cells were grown in the medium with 1% serum in 50-mL flasks and infected with low doses (ID 1) of influenza virus A/Pert/16/11 (H3N2) or A/St. Petersburg/06/14 (H1N1pdm) as described above. The cells were subcultured in a ratio of 1 : 3. The number of cells not stained with trypan blue was counted in a Fuchs–Rosenthal chamber according to the method described in (Danilenko et al., 2016). Calculation for each time point was done in two replicates.

RT-PCR assay of gene expression. Expression of *TNFα, TGFβ, IFN-γ, MMP-9, NF-κB*, and *Rho A* genes was studied. RNA was isolated with AmpliPrime RIBO-prep kit (CNI epidemiology, Russia) according to the manufacturer's instructions. cDNA was synthesized on an RNA template with reverse-transcription technology using a Reverta-L reagent kit (CNI epidemiology, Russia). Amplification was performed in a Rotor-Gene 6000 thermocycler (Corbett Research, Australia) using several programs. For *MMP-9* and *Rho A*: 95°C 10 min, 40 cycles at 95°C 15 s, then 60°C 50 s. For *TNFα* and *TGFβ*: 95°C 5 min, 40 cycles at 95°C 20 s, then 60°C 40 s. For *IFN-γ* and *NF-κB*: 95°C 5 min, 37 cycles 95°C 10 s, then 55°C 30 s and 72°C 30 s. 25 μL reaction mixture was composed of 5 μL cDNA, 10 μL 2.5× PCR buffer (Syntol, Russia), 0.5 μL SynTaq DNA-polymerase (5 units/μL) (Syntol, Russia), 0.5 μL of each primer (10 pmol/μL).

Amplification for *eNOS* and *iNOS* assay was done in one step using reagent kit OneStep RT-PCR Kit (Qiagen, Germany). An amount of 25 μL of the reaction mixture contained 3 μL RNA, 12.5 μL OneStep RT-PCR buffer (2×), 1 μL reverse transcriptase and HotStarTaq DNA-polymerase, 0.5 μL primers and

Sequences of primers and TaqMan-probes to assay gene expression for cytokines and other cellular factors

Gene	F-primer 5'–3'	Probe 5'–3'	R-primer 5'–3'
eNOS	TGGCTGGTACATGAGCACT-GAGAT	R6G–ACAACATCCTGGAG-GATGTGGCTGT–BHQ1	CACGTTGATTTCCACTGCTG-CCTT
iNOS	GCGTTACTCCACCAACAATG-GCAA	FAM–AGCGGAGTGATGG-CAAGCACGACTT–BHQ1	ATAGCGGATGAGCTGAGCAT-TCCA
RhoA	CGGGAGCTAGCCAAGAT-GAAG	FAM–AGAGATATGGCAAA-CAGGATTGGCG–BHQ1	GCTTGCAGAGCAGCTCTCGTA
MMP-9	CCTGGAGACCTGAGAAC-CAATC	FAM–TACCGCTATGGTTA-CACTCGGGTGGC–BHQ1	GATTTCGACTCTCCACGCATC
NF-κB	GCTCAGTGAGCCCATGGAAT	FAM–TCACCGGATTGAG-GAGAAAC–BHQ1	TGATGCTCTTGAAGGTCT-CATATGTC
IFN-γ	GCTTTTCAGCTCTGCATCGT	CY5–TGGCTGTTACTGCCAG-GACCCA–BHQ2	CCGCTACATCTGAATGACCTG
ACTB	TCTACAATGAGCTGCGTGTG-GCTCCC	CY5–CAAGGCCAACCGC-GAGAAGATGACCCAGATCAT–BHQ2	AGCAACGTACATGGCTGGG-GTGTTGAA

probes. Amplification was performed in the thermocycler: reverse transcription at 50°C for 30 min, denaturation at 95°C for 10 min, 37 cycles at 95°C for 15 s, then at 58°C for 30 s.

Sequences of oligonucleotide primers for *iNOS, MMP-9, NF-κB, Rho A* (Sintol, Russia), and *IFN-γ* (DNA-synthesis, Russia) are listed in the table. *TNFα* and *TGFβ* primers are components of commercial kits (Sintol, Russia). Primers to actin (*ACTB*) (DNA-synthesis, Russia), the expression of which is stable in endothelial cells, were used as control.

Experiments were repeated two or three times. The results were presented in relative units calculated according to the formula $2^{-\Delta\Delta Ct}$, where C_t is the value of the threshold cycle for the sample (Wong, Medrano, 2005).

The data were statistically treated with MS Office Excel 2007 and Statistica 6.0 software. The Mann–Whitney test was used to compare nonparametric samples. The difference was considered significant at $p < 0.05$.

RESULTS

ECV-304 cell proliferation. In this study, we used a higher cell-seeding dose (12×10^4 cells/mL) then previously (Smirnova et al., 2011, 2016; Danilenko et al., 2016). It resulted in maximum virus-stimulating proliferation 24 h after infection with a low virus dose (ID 1). Virus-stimulated cell proliferation gradually declined for 5 days (Fig. 1). An amount of 100 ng/mL of LPS enhanced cell proliferation for 1–5 days. LPS added to the cells infected with 1 ID virus reduced the virus-stimulated cell proliferation at all time periods (Fig. 1). ECV-340 cells once infected with A/Pert/16/11 (H3N2) or A/St. Petersburg /06/14 (H1N1pdm) viruses from the third passage displayed increased proliferative activity compared to control cells. Low decrease in cell proliferation at the seventh passage was caused by the higher ratio of the infected cell plating (1 : 5 rather than 1 : 3). Both viruses of different suptypes exhibit a similar stimulating effect on the endothelial cells (Fig. 2).

Gene expression. Here and below, the gene expression is compared to control at the same cultivation period. LPS in the early period (1–3 days) increased (by 2.1–2.4 times) only *TNFα* expression but the

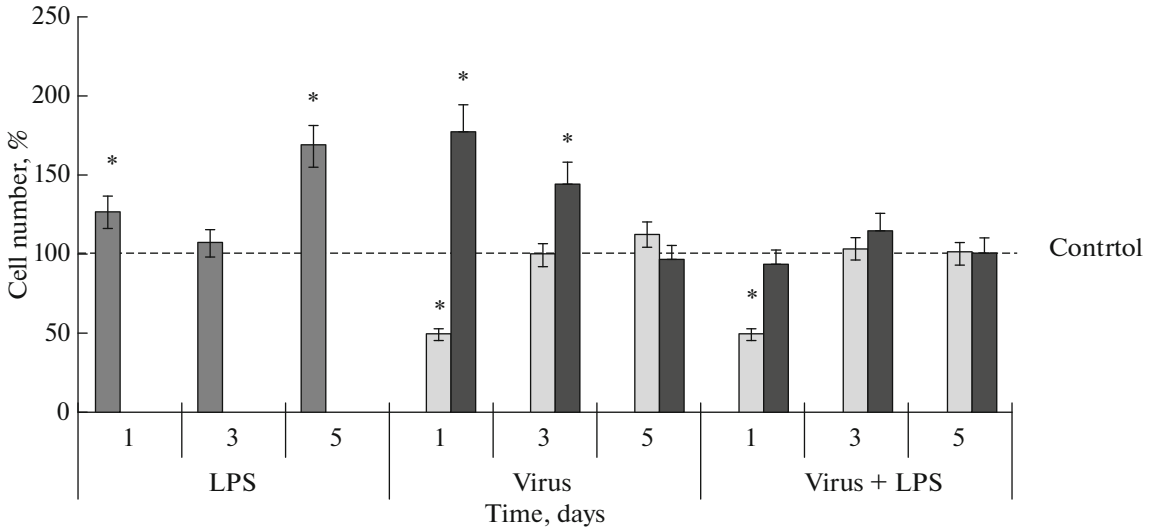

Fig. 1. ECV-304 cell proliferation for 5 days after treatment with 100 ng/mL lipopolysaccharide (LPS) from *Escherichia coli*, influenza virus A/Pert/16/11 (H3/N2), and their combination (virus+LPS). Infectious doses (ID) of the virus were 1 (black columns) and 1000 (light columns); control—cells cultivated without treatment. Here and in Figs. 2 and 3, the asterisk shows the significant difference, $p < 0.05$.

expression of its mRNA was not registered after 5 days. The expression of all other genes under the study during the first 3 days was at the same level or lower as in control cells, but was increased on the fifth day. The highest level was observed for TGFb mRNA (21.3 times higher than control). On the fifth day, mRNA for *MMP-9* was increased by 2.1 times, while for *IFN*-γ and *eNOS* genes it was increased by 2.4 times and for *iNOS* by 1.9 times. The increase was slightly higher for mRNA of *NF*-κ*B* (5.2 times) and *Rho A* (3.2 times) (Fig. 3).

eNOS, *iNOS*, *TGF*β, *IFN*-γ, and *NF*-κ*B* gene expression in cells infected with A/Pert/16/11 (N3H2) virus during the first 3 days was at the control level or lower. During the first day, enhanced expression was common only for *MMP-9* gene (2.4 times) with ID 1 (Fig. 3). At the third day, increased expression was observed for *TNF*α gene (by 3.6 times with ID 1 and 2.6 times with ID 1000) and *Rho A* gene (2.2 times with ID 1000). After 5 days, the expression of *TNF*α, *TGF*β, *eNOS*, and *iNOS* genes increased with high ID 1000 by 2.2, 5.9, 4.0, and 2.5 times, respectively, and *NF*-κ*B* gene with low ID 1 by 3.0 times. On the first day, the influenza virus inhibited expression of *Rho A* gene by 3.8 and 3.5 times (ID 1 and 1000, respectively), *TGF*β by 2.1 times (ID 1000), and *NF*-κ*B* by 2.0 times (ID 1). *TNF*α expression on the fifth day with ID 1 was not revealed (Fig. 3). Drastic inhibition of *MMP-9* expression was observed after treatment with only LPS (626 times) or only a high dose of virus in a (ID 1000) (592 times) on the first and third days (7.2 and 11.9 times, respectively).

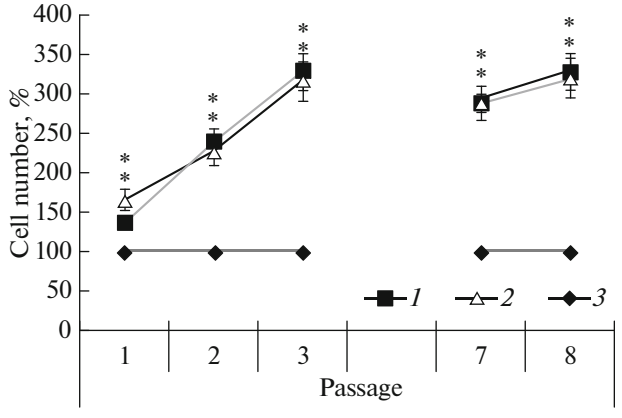

Fig. 2. Proliferation of ECV-304 cells infected with influenza virus A/Pert/16/11 (H3N2) (curve *1*) or A/St. Petersburg/06/14 (H1N1pdm09) (curve *2*) during eight passages. Curve *3*—control.

ECV-304 cell treatment with the influenza virus A and LPS on the first day markedly increased the expression of only *MMP-9* gene (3.3 and 3.5 times with ID 1 and 1000, respectively) and *IFN*-γ gene (4.6 times, ID 1000). In this period, the expression of other genes was inhibited: *TNF*α (3.4 times, ID 1000), *TGF*β (2.4 and 3.0 times), NF-κ*B* (4.9 and 1.9 times), and *Rho A* (24.0 and 4.7 times) with ID 1 and 1000, respectively (Fig. 3). After 3 days, the gene expression in cells infected with virus and LPS was predominantly at the control level and with a high dose of viral infection did *TNF*α expression increase (by two times) and *MMP-9* expression decrease (2.4 times). However, by the fifth day, the expression of many genes had increased: *TGF*β (18.4 and 17.8 times), *NF*-κ*B* (8.2 and 13.2 times), and *eNOS* (7.2 and 6.0 times), as well as, to a lesser extent, *Rho A* (3.2 and 4.6 times), *iNOS*

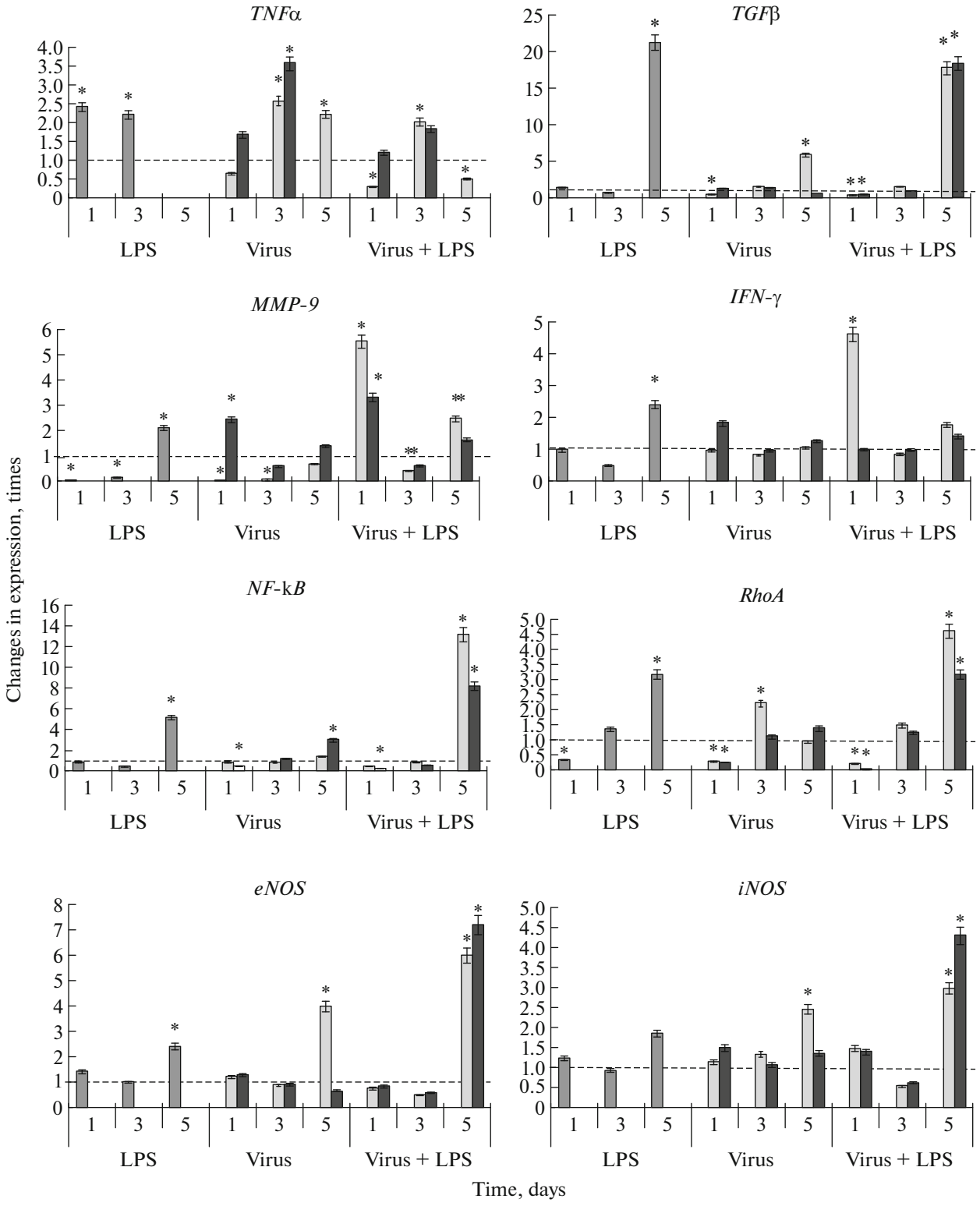

Fig. 3. Expression of *TNFα, TGFβ, MMP-9, IFN-γ, NF-κB, RhoA, eNOS*, and *iNOS* genes for 5 days in ECV-304 cells treated with 100 ng/mL lipopolysaccharide (LPS) from *Escherichia coli*, influenza virus A/Pert/16/11 (H3/N2), and their combination. Black columns—virus ID 1; light columns—virus ID 1000. Dotted line—untreated cells; control value was taken as 1 (see MATERIALS AND METHODS).

(4.3 and 3.0 times) with ID 1 and 1000, and *MMP-9* only with ID 1000 (2.5 times) (Fig. 3). In this period *TNF*α expression was either suppressed (2.0 times, ID 1000) or was not identified at all (ID 1) as it was found in cells treated with only virus or LPS (Fig. 3). After 5 days, *TNF*α expression was switched to *TG*Fβ expression. LPS stimulated the virus-induced gene expression for most cellular factors on the fifth day.

Correlation analysis showed a direct relationship in the expression of *TGF*β with *eNOS, NF-κB*, and *RhoA* genes (Spierman correlation coefficient r_s was 0.51, 0.66, and 0.67, respectively). *NF-κB* expression was also directly correlated with *iNOS* ($r_s = 0.51$) and *RhoA* ($r_s = 0.73$) and *eNOS* with *iNOS* ($r_s = 0.57$). *MMP-9* expression was directly correlated with *IFN*-γ ($r_s = 0.69$) and *iNOS* ($r_s = 0.56$) and was in the inverse correlation with *TNF*α ($r_s = -0.51$). Thus, the results we obtained demonstrated that there is a modified pattern of gene expression and, therefore, cell state at the early and late stages after pathogen treatment (influenza virus and LPS).

DISCUSSION

We have found that endothelial-cell proliferation stimulated by influenza virus depends on ID and the cell-plating density (Smirnova et al., 2011, 2016; Danilenko et al., 2016). Increased cell proliferation was accompanied by apoptosis induction. As a result, some cells were eliminated whereas survived cells acquired more intense proliferative activity. In the present work, we also showed that ECV-304 cells infected with a low dose of influenza A virus gained the capacity for long-term active proliferation (eight passages). Similar results have been obtained with immortalized T cells (Jurkat) infected with a low dose of influenza virus A (Smirnova et al., 2015).

ECV-304 cells infected with various doses of influenza A with or without the presence of LPS exhibited a modified pattern of gene expression for cytokines and other factors of the intracellular signaling pathways. The activity of *TNF*α, *MMP-9*, and *IFN*-γ genes (involved in cell permeability and apoptosis) was most apparent during the first 3 days after infection. Further cultivation was accompanied by enhanced activity of *TGF*β, *RhoA, NF-κB, eNOS*, and *iNOS* genes engaged in enhanced cell proliferation and modified cell permeability.

The most drastic changes in the gene expression profile were observed in endothelial cells infected with the influenza A virus in LPS presence. MMP-9 expression (strongly inhibited in cells treated with the virus and LPS independently) was robustly enhanced in the first day. *IFN*-γ and *TNF*α were active only for 1–3 days. The activity of other genes under study was considerably increased only to the fifth day, as it was observed with cells treated with LPS only. *TGF*β and *eNOS* genes involved in the cell proliferation and cell permeability (Lu et al., 2006; Di Lorenzo et al., 2013) showed the highest activity.

Influenza virus A is able to induce G_0/G_1 cell-cycle arrest favorable for its replication (He et al., 2010). The event requires viral protein NS1, which inhibits the expression and activity of RhoA protein. It inhibits the phosphorylation of retinoblastoma protein Rb and delays the cell transition from G_1 into S phase (Jiang et al., 2013). Viral unstructured protein NS1 is multifunctional and crucial for the virus replication and virulence. The protein binds with various cell proteins and inhibits the expression of key antiviral and other genes in host cells (Hale et al., 2008). Supposedly, the disturbed reproduction of influenza virus A maintained on nonpermissive cells is shown as reduced or totally suppressed NS1 synthesis. It attenuates the blockage of expression of *RhoA* and other cell-cycle genes, which stimulates cell proliferation. Our findings showed that *RhoA* expression was suppressed during the first day after the viral and/or LPS infection. It increased by the fifth day after treatment with LPS or LPS together with virus.

The expressions of the genes that we studied are differently connected with each other and many other cellular factors. We investigated the impact of the influenza A virus and LPS on components of the signaling pathways at the transcription level. However, it is still early to draw an unambiguous conclusion about their connection with observed functional changes in the endothelial cells, because the activity of cellular factors regulated at the level of translation and post-translational modifications has not been considered in this study.

It can be suggested that the modified state of endothelial cells cultivated with the influenza A virus and LPS at various points of time (as well as various viral ID) is caused by different cellular factors that activate various signaling pathways. The influenza virus and LPS have a similar effect on cells. Their combined application enhanced the cell response on these pathogenic agents. Further cultivation probably will be accompanied by increased cell proliferation and permeability, as well as endothelial-cell transition into fibroblast- and myofibroblast-like cells , which may provoke fibrosis development in various organs.

The search for novel antiviral preparations and approaches to influenza A virus and the complications it causes has intensified in recent years. The application of antiviral remedies to block the replication of virus particles during acute infection of influenza virus is often ineffective and can lead to the emergence of viral particles resistant to this medicine. Antibacterial drugs destroy bacteria, which results in the accumulation of bacterial components, LPS in particular, in the bloodstream. In this regard, the search for remedies with antioxidant and anti-inflammatory activity appropriate for simultaneous application with antiviral drugs intensified in recent years (Eropkin et al.,

2007; Sgarbanti et al., 2014). Of particular interest is a new generation of medications capable of recovering normal functioning of signaling pathways (Zarubaev, Smirnov, 2014). The ECV-304 cellular model was used for preliminary testing of medical preparations with antioxidant, anti-inflammatory, and angioprotective activity on the physiological state of cells infected with various doses of the influenza A virus and LPS (Smirnova et al., 2016).

REFERENCES

Armstrong, S., Mubareka, S., and Lee, W., The lung microvascular endothelium as a therapeutic in severe influenza, *Antiviral Res.*, 2013, vol. 9, pp. 113–118.

Bernasconi, D., Amici, C., La, Frazia, S., Ianaro, A., and Santoro, G., The IκB kinase is a key factor in triggering influenza A virus-induced inflammatory cytokine production in airway epithelial cells, *J. Biol. Chem.*, 2005, vol. 280, pp. 24127–24134.

Clements, R., Minnear, F., Singer, H., Keller, R., and Vincent, P., Rho and Rho-kinase dependent and independent signals mediate TGF-β-induced pulmonary endothelial cytoskeletal reorganization and permeability, *Am. J. Physiol. Lung Mol. Physiol.*, 2005, vol. 288, pp. L294–L306.

Danilenko, D.M., Smirnova, S.S., Smirnova, T.D., Pisareva, M.M., Plotnikova, M.A., Drobintseva (Durnova), A.O., and Eropkin, M.Yu., Human lung carcinoma (A-549) continuing cell line and human endothelial (ECV-304) continuing cell line responses to the influenza virus at different multiplicities of infection, *Cell Tissue Biol.*, 2016, vol. 10, no. 4, pp. 305–313.

Di Lorenzo, A., Lin, M.I., Murata, T., Landskroner-Eiger, S., Schleicher, M., Kothiya, M., Iwakiri, Y., Yu, J., Huang, P.L., and Sessa, W.C., ENOS-derived nitric oxide regulates endothelial barrier function through VE-cadherin and Rho GTPases, eNOS-derived nitric oxide regulates endothelial barrier function through VE-cadherin and Rho GTPases, *J. Cell Sci.*, 2013, vol. 126, pp. 5541–5552.

Echeverria, C., Montorfano, I., Sarmiento, D., Becerra, A., Nunez-Villena, F., Figueroa, X., Cabello-Verrugio, C., Elorza, A., Riedel, C., and Simon, F., Lipopolysaccharide induces a fibrotic-like phenotype in endothelial cells, *J. Cell. Mol. Med.*, 2013, vol. 17, pp. 800–814.

Eropkin, M.Yu., Gudkova, T.M., Konovalova, H.I., Shchelkanova, S.M., Iaglovskaia, I.B., Eropkina, E.M., and Kiselev, O.I., Antiviral action of some antioxidants/antihypoxants and their combinations with rimantadine against human influenza A(H3N2) virus studied on in vitro models, *Eksp. Klin. Farmakol.*, 2007, vol. 70, no. 1, pp. 33–37.

Hagimoto, N., Kuwano, K., Inoshima, I., Yoshimi, M., Nakamura, N., Fujita, M., Maeyama, T., and Hara, N., TGF-beta 1 as an enhancer of Fas-mediated apoptosis of lung epithelial cells, *J. Immunol.*, 2002, vol. 168, pp. 6470–6478.

Hale, B., Randall, R., Ortin, J., and Jackson, D., The multifunctional NS1 protein of influenza A viruses, *J. Gen. Virol.*, 2008, vol. 89, pp. 2359–2376.

He, Y., Xu, K., Keiner, B., Zhou, J., Czudai, V., Li, T., Chen, Z., Liu, J., Klenk, H-D., Shu, Y., and Sun, B., Influenza A virus replication induces cell cycle arrest in G_0/G_1 phase, *J. Virol.*, 2010, vol. 84, pp. 12832–12840.

Jiang, W., Wang, Q., Chen, Sh., Gao, Sh., Song, L., Lui, P., and Huang, W., Influenza A virus NS1 induces G_0/G_1 cell cycle arrest by inhibiting the expression and activity of RhoA protein, *J. Virol.*, 2013, vol. 87, pp. 3039–3052.

Julkunen, I., Saraneva, T., Pirhonen, J., Ronni, T., Melen, K., and Matikainen, S., Molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression, *Cytokine Growth Factor Rev.*, 2001, vol. 12, pp. 171–180.

Kido, H., Okumura, Y., Takahashi, E., Pan, H-Y., Wang, S., Yao, D., Yao, M., Chida, J., and Yano, M., Role of host cellular proteases in the pathogenesis of influenza and influenza-induced multiple organ failure, *Biochim. Biophys. Acta*, 2012, vol. 1824, pp. 186–194.

Lu, Q., Harrington, E., Jackson, H., Morin, N., Shannon, Ch., and Rounds, Sh., Transforming growth factor-β1–induced endothelial barrier dysfunction involves Smad2-dependent p38 activation and subsequent RhoA activation, *J. Appl. Physiol.*, 2006, vol. 101, pp. 375–384.

McKinney, L., Galliger, S., and Lowy, R., Active and inactive influenza virus induction of tumor necrosis factor-α and nitric oxide in J774.1 murine macrophages: modulation by interferon-γ and failure to induce apoptosis, *Virus Res.*, 2003, vol. 97, pp. 117–126.

Meyer-Schwesinger, C., Dehde, S., von Ruffer, C., Gatzemeier, S., Klug, Ph., Wenzel, U., Stahl, R., Thaiss, F., and Meyer, T., Rho kinase inhibition attenuates LPS-induced renal failure in mice in part by attenuation of NF-kB p65 signaling, *Am. J. Physiol. Renal Physiol.*, 2009, vol. 296, pp. F1088–F1099.

Mong, Ph., Petrulio, Ch., Kaufman, H., and Wang, Q., Activation of Rho kinase by TNF-α is required for JNK activation in human pulmonary microvascular endothelial cells, *J. Immunol.*, 2008, vol. 180, pp. 550–558.

Peters, K., Unger, R., Brunner, J., and Kirkpatrick, J., Molecular basis of endothelial dysfunction in sepsis, *Cardiovasc. Res.*, 2003, vol. 60, pp. 49–57.

Schmolke, M., Viemann, D., Roth, J., and Ludwig, S., Essential impact of NF-κB signaling on the H5N1 influenza A virus-induced transcriptome, *J. Immunol.*, 2009, vol. 183, pp. 5180–5189.

Schultz-Cherry, S. and Hinshaw, V., Influenza virus neuraminidase activates latent transforming growth factor β, *J. Virol.*, 1996, vol. 70, pp. 8624–8629.

Seo, S. and Webster, R., Tumor necrosis factor alpha exerts powerful anti-influenza virus effects in lung epithelial cells, *J. Virol.*, 2002, vol. 76, pp. 1071–1076.

Sgarbanti, R., Amatore, D., Celestino, I., Marcocci, M-E., Fraternale, A., Ciriolo, M-R., Magnani, M., Saladino, R., Garaci, E., Palamara, A.-T., and Nencioni, L., Intracellular redox state as target for anti-influenza therapy: are antioxidants always effective?, *Curr. Topics Med. Chem.*, 2014, vol. 14, pp. 2529–2541.

Smirnova, T.D., Danilenko, D.M., Ilyinskaya, E.V., Smirnova, S.S., and Eropkin, M.Yu., Impact of various multiplicity of infection of influenza A virus on proliferation and apoptosis induction in cultured cell lines of lymphocytic and monocytic origin (Jurkat, NC-37, THP-1 and U-937), *Tsitologiia*, 2015, vol. 57, no. 7, pp. 526–532.

Smirnova, T.D., Danilenko, D.M., Gudkova, T.M., Pisareva, M.M., Kadyirova, R.A., and Slita, A.V., Influence of different doses of infectious influenza A viruses on proliferation of human monolayer cell cultures, *Cell Cultures. Inform. Bull.*, 2011, vol. 27, pp. 3–12.

Smirnova, T.D., Smirnova, S.S., Sivak, K.V., Eropkina, E.M., and Eropkin, M.Yu., Human cell line ECV-304 as a model for the study of the effect of antioxidant preparations on the cellular proliferation and apoptosis under infection with influenza A virus and in the presence of bacterial lipopolysaccharide, *Cell Cultures. Inform. Bull.*, 2016, vol. 32, pp. 14–28.

Undevia, N., Dorscheid, D., Marroquin, B., Gugliotta, W., Tse, R., and White, S., Smad and P38-MAPK signaling mediates apoptotic effects of transforming growth factor-β1 in human airway epithelial cells, *Am. J. Physiol. Lung Cell Mol. Physiol.*, 2004, vol. 287, pp. L515–L524.

Van Meeteren, L. and Dijke, P., Regulation of endothelial cell plasticity by TGF-β, *Cell Tissue Res.*, 2012, vol. 347, pp. 177–186.

Wang, S., Le, T., Kurihara, N., Chida, J., Cisse, Y., Yano, M., and Kido, H., Influenza virus-cytokine-protease cycle in the pathogenesis of vascular hyperpermeability in severe influenza, *J. Infect. Dis.*, 2010, vol. 202, pp. 991–1001.

Wong, M.L. and Medrano, J.F., Real-time PCR for mRNA quantitation, *BioTechniques*, 2005, vol. 39, pp. 75–85.

Zarubaev, V.V. and Smirnov, V.S., Influence on cellular targets for treating influenza infection, *Infect. Immun.*, 2014, vol. 4, no. 1, pp. 15–26.

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