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QUANTITATIVE ANALYSIS OF RNA AND ACIDIC PROTEINS OF MONOBLASTS AND MONOCYTES INFECTED WITH AFRICAN SWINE FEVER VIRUS

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A single cell cytophotometry was used to analyze the amount of RNA and proteins in porcine cells that are susceptible to African Swine Fever Virus (ASFV). The obtained results show that ASFV infection results in depletion of total RNA in the nucleus of monoblasts and monocytes. The level of nuclear acidic proteins in monoblasts is also less, than in control during all time of infection, whereas in monocytes it is higher, than in uninfected cells at the final stage of infection. These data can be used in further researches to elucidate cytopathogenesis of ASFV infection.

Keywords: cytophotometry, infected cell, ASFV, cytopathogenesis, disease, nucleus, nucleolus.

Introduction. African Swine Fever Virus (ASFV) is an enveloped double-stranded DNA virus that is the sole member of the genus Asfivirus within the family of Asfarviridae. It is the causative agent of the significant disease of domestic swine (African swine fever) with high mortality rates, for which there is no effective vaccine. The primary cell types infected by ASFV belong to the mononuclear phagocytic system, including monoblasts and monocytes. Although the early stage of ASFV replication occurs in the host nucleus, little is known about nuclear alterations caused by this virus [1]. In our initial studies we have reported about changes in DNA content of ASFV-infected monoblasts and monocytes [2]. Here we describe the total amount of RNA and acidic proteins in the nucleus (and whole cell) of infected monoblasts and monocytes. These parameters may serve as a strong indicator of nuclear and subsequently cellular metabolic changes caused by ASFV infection.

Materials and Methods. The primary culture of porcine bone marrow was isolated and cultivated as described previously [2]. We used ASFV genotype II distributed in RA. The virus was added to culture vials simultaneously with cultivation of cells. The titer of ASFV in each experimental culture was 104 hemadsorbtion units/*ml*. For total RNA amount, preparations were stained by gallocyanin-chrome alum vital dye according to the method of Einarson adapted to quantitative and analytical requirements [3]. Measurements were performed by miscroscope-photometer SMP05 (Opton) at 620 *nm* wavelength with the scanning image analyzer BioScan. In order to quantify the total amount of acidic proteins, preparations were

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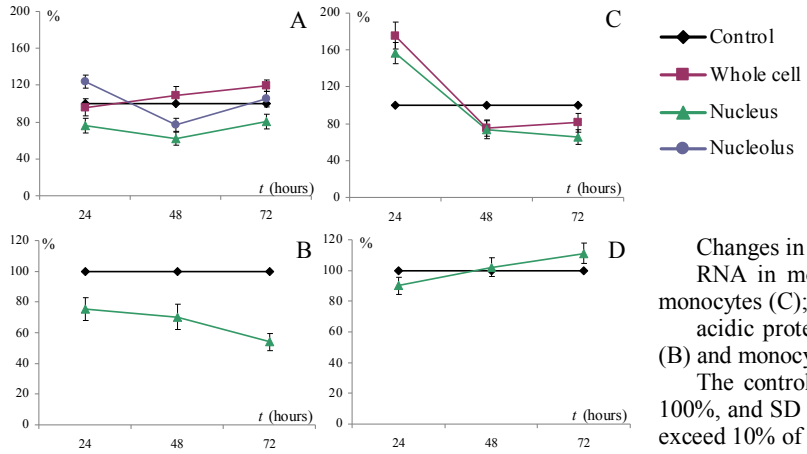
stained by Naphthol Yellow S vital dye as described previously [4]. Measurements were performed by the same microscope-photometer at 343 *nm* wavelength and analyzed by BioScan software. The experiments were repeated four times. At least 400 cells were observed for this study. Data were evaluated by a statistical Student's *t*-test, and differences between control and infection were considered significant at $p < 0.05$. Results are expressed as mean of 4 experiments ± standard deviation (SD).

Results and Discussion. Below we present data regarding the total amount of RNA and acidic proteins not only for the nucleus of monoblasts /monocytes, but also for the whole cell. It is important to note that due to the fact that monocytes are more specialized than monoblasts, most of them do not have nucleoli. Thus, the nucleolar data of monocytes is absent. Moreover, there was no significant difference in the nucleolar level of acidic proteins between infected and control monoblasts, therefore, this datum is also not presented below.



Changes in amount of –
RNA in monoblasts (A) and monocytes (C);
acidic proteins in monoblasts (B) and monocytes (D).
The control is represented as 100%, and SD of control doesn't exceed 10% of value.

Figure A clearly shows that the amount of nuclear RNA in infected monoblasts was significantly ($p < 0.02$) less, than in control at 24 hours post-infection (*hpi*). The highest decrease in the level of nuclear RNA was detected at 48 *hpi*, when they fell down up to 39% of control level ($p < 0.001$). The total amount of nucleolar RNA in monoblasts was higher, than in control ($p < 0.02$) only at 24 *hpi*, after which it significantly decreased ($p < 0.02$) at 48 *hpi*. The level of RNA in the whole cell became significantly higher ($p < 0.05$), than in control only at 72 *hpi*.

Figure B shows that the quantity of nuclear acidic proteins was constantly less, than in the cells of control group during the entire course of infection ($p < 0.01 - p < 0.001$). These data are in a good accordance with morphometric measures, particularly with nuclear and nucleolar areas that reduced in size simultaneously with changes in the amount of nuclear RNA and proteins at 24 and 48 *hpi* (data are not presented).

In contrast to monoblasts, the concentration of nuclear RNA in monocytes was higher (about 56% of control, $p < 0.001$), than in control at the beginning of infection (Figure C). However, the level of nuclear RNA significantly decreased and became 27% less, than in control at 48 *hpi* ($p < 0.02$). The same decrease in the

level of RNA was observed for the whole cell. At the final stage of infection (72 *hpi*) the amount of nuclear RNA remained lower than in uninfected monocytes. Like in monoblasts, the quantity of nuclear acidic proteins was significantly less ($p < 0.05$), than the amount in control at 24 *hpi* (Figure D). At 48 *hpi* their concentration increased and reached levels similar to those in control group and became significantly higher ($p < 0.05$) at the final stage of infection. Morphometric measures indicate the simultaneous changes in nuclear area with the changes in the concentration of nuclear RNA particularly at 72 *hpi* (data are not presented).

Here, for the first time, we reported the suppression of the main biological processes, such as the host gene transcription and translation by ASFV. It has been shown before, that this virus is able to impact on the host gene expression [5]. Particularly, the most significant changes are observed in redox-related proteins and nucleoside diphosphate kinases. The depletion of nucleoside diphosphate kinases, which are required for the synthesis of nucleoside triphosphates, may lead to the suppression of the level of RNA and consequently a decrease in the level of acidic proteins in the host cell nucleus. Interestingly, we observed high concentration of nuclear RNA in monocytes at 24 *hpi*. This phenomenon can be explained by the facts that ASFV induces the synthesis of some polypeptides early after infection, which account for most of the information content of the virus DNA, as well as that virus triggers antiviral response at the beginning of infection [6]. Another interesting observation is the difference between monocyte and monoblast response to ASFV infection. This requires further studies, but our working hypothesis is that less-differentiated cells like monoblasts are more responsive to environmental factors, including ASFV-induced cytokines such as TNF-α.

Conclusion. For the first time, we used single cell cytophotometry to analyze the total amount of RNA and acidic proteins in the nucleus of infected monoblasts and monocytes. The above data show that ASFV infection results in the significant changes in the level of nuclear RNA and acidic proteins in infected cells. Further studies may shed light on all aspects of viral cytopathogenesis and help to develop effective vaccines against ASFV.

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