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ORAL ABSTRACT PRESENTATIONS
SESSION TITLE: NEW METHODS OF SAMPLE PREPARATION AND DATA PROCESSING FOR CRYO-ELECTRON MICROSCOPY

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Abstract OR-18: Entry of Hantavirus into the Innate Immune Cell

Natalia G. Plekhova[1], Evgeniy V. Pustovalov[2]

[1]Pacific State Medical University, Vladivostok, Russia; [2]Far Eastern Federal University, Vladivostok, Russia

Background: Hantaviruses cause hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome, which infects more than 200,000 people worldwide with case fatality ratios of 30%–40%. There are no specific therapies or vaccines for these diseases. Hantaviruses (HV) are enveloped, negative-strand RNA viruses of the family Bunyaviridae. Monocytes/macrophages have an important role in the spread of virus from the primary site of infection. However, the site and detailed mechanism of entry of HV in cells have not yet been defined. Therefore, this study focused on the entry of the pathogenic hantaviruses Hantaan into African green monkey kidney epithelial cells (Vero E6) and macrophages.

Methods: The cellular culture Vero E6, the human monocytic cell line THP-1, obtained from the Korean Cell Line Bank (KCLB) (Seoul, Korea) and cells of mice peritoneal exudate were used. The extracellular liquid of infected culture Vero E6 by strain pathogenic virus Hantaan PM-T79-95 included not less 100 infectious units on the macrophage was used. Mouse monoclonal [5E11] to HV nucleocapsid protein and caveolin-1 (Abcam, USA) at 1:200 dilution were used for immunohistochemical detection of localization in macrophages. Goat polyclonal Anti-Mouse IgG H&L (10 nm Gold) and Alexa Fluor® 488 (Abcam, USA) were used as secondary antibodies. Thin sections were examined in Jeol 100 S electron microscope. Series of optical sections were taken with a confocal scanning laser microscope LSM510META (Carl Zeiss, Germany). JEOL 100S, transmission electron microscopy, was used to obtain the images, which were acquired at 50,000X magnification.

Results: Using electron microscopy, we found that during the first 5 min of contact, the HV penetrates into the cytoplasm of macrophages by fusing with the plasma membrane. During this process, loosening and thickening of HV supercapsid were noted. The viruses with morphological signs deproteinization of the genome were predominantly detected in the perinuclear space of macrophage cytoplasm, as well as near the cisterns of the granular endoplasmic reticulum (2-3 hours of incubation). The space between the shell and the dense osmiophilic nucleoid, the thinning of the shell and an increase of viral size due to the nucleoid loosening and was determined. At the same time, the number of HV antigen-positive cells was noted minimum. The multichannel scanning of infected cells determined the

co-localization HV-antigen and caveolin 1 positive sites. A significant difference of the indicators intensity fluorescence at the sites of co-localization was definitude.

Conclusion: So, during the penetration into macrophages HV use the plasma membrane fusion mechanism associated with caveolin 1. In addition to this caveolin mediated endocytosis the HV can also penetrate in another way, which is the aim of further studies.

Key Words: Hantavirus • macrophages • ultrastructure • electron microscope

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