Comparative study of extracellular enveloped virus formation by different variola virus strains.

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The Orthopoxvirus genus comprises the viruses with huge differences in pathogenicity for humans. Thus, variola virus (VARV) is highly pathogenic for a man whereas vaccinia and cowpox viruses were actually used as a vaccine to protect humanity from smallpox. The mechanisms responsible for these differences in the virulence of poxviruses are still unclear. Virus dissemination inside an organism is one of the principal pathogenetic events of any viral disease. The essential link of orthopoxviral disease is the formation of extracellular enveloped virus (EEV), which provides dissemination of the infection in the organism. The necessity of the EEV for dissemination of vaccinia virus infection *in vitro* and *in vivo* has been shown. Data about the EEV formation in other poxvirus infections are poor, and nothing is known about EEV formation in cells infected by variola virus. The goal of the present study was to examine the ability of VARV strains differing in pathogenicity for a human to produce an enveloped virus in different cells.

Variola virus strains Ind-3a, Butler, and Congo-9 from the Russian National Collection of SRC VB "Vector" were used in the study. Cell cultures Vero, CV-1, L-68, and BHK-21 were infected with 0.1 – 1 PFU of all strains, and propagated at 37^{o} C. Chick embryos received VARV strains to produce 5-10 single pocks on the chorioallantoic membrane. Infected cell cultures were fixed in 4% paraformaldehyde at 7, 9, 18, 24 and 48 h postinfection. Single pocks were dissected and fixed at 48 and 72 h postinfection. All VARV infected samples were prepared in 2002. The samples were routinely processed for electron microscopy. Morphometric analysis of VARV strains infection was performed on 20 randomly selected cells. The degree of accuracy between the data was determined by χ^2 – criteria ($p<0.05$) using 4-field tables.

Electron microscopy examination of infected cell cultures and cells of chick chorioallantoic membrane revealed that morphologic parameters of assembly were identical for all examined strains of VARV. Different strains showed different production of immature and mature virions in different cell cultures. However, there was no correlation between cell origination and production of VARV progeny. Electron microscopy revealed the wrapping of virus particles by Golgi membranes and formation of "enveloped" virus in all cell cultures and chick embryos infected by all three VARV strains. A morphometric study showed that different strains of VARV produced different amounts of EEV, even in the same type of cell culture. No correlation between the amount of EEV produced by different VARV strains and the cell culture origin was found. Thus, the Ind-3a strain, which was the most pathogenic for humans, produced a low amount of EEV in human fibroblasts (L-68 culture), while the less pathogenic Butler strain produced a higher percentage of EEVs, in the same type of cell culture. So, no evidence of relation between EEV amount and pathogenecity of VARV strains in cell culture was found. However, all VARV strains showed the ability to produce considerable amounts of EEV, suggesting the potential efficacy of spreading in the organism. In pock cells of the chick embryos, all the three VARV strains showed high production of EEV.

Role of the EEV for orthopoxvirus infection pathogenesis and virulence is not clear yet. Our study showed that VARV possesses an obvious ability to produce EEV in various cell cultures, including cells of human origination. Large amounts of EEV were observed in cells of chick embryos, against the background of an immature immune system. VARV clearly demonstrates the ability of the virus to use the cellular machinery for spreading the infection. We

propose that ability of VARV to produce EEV is one of the critical features necessary for virulence.