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Monitoring of coxsackievirus replication in clones of NIH 3T3 cell line

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Introduction & Background: Coxsackieviruses (CV) are small positive-stranded RNA viruses. They are important human pathogens that cause both acute and chronic diseases. Coxsackieviral infections show diverse clinical manifestations which are closely linked to their genetics, complex tissue tropism and host genetics and immune response. These viruses induce lytic infections, their cytopathic effect (CPE) includes morphological changes and destruction of the host cell monolayer in vitro, their viral RNA can remain persistent for prolonged times in cells and organs post infection. Literature reports show moderate replication of enteroviruses in NIH 3T3 cells.

Aim: Our aim was to study the replication of coxsackieviruses in NIH 3T3 clones with resistance to puromycin alone or along with blastomycin, in combination with presence of a truncated variant of the dicer ribonuclease.

Materials & Methods: Four genetically modified clones of NIH 3T3 cells were infected with CVB3 (Nancy strain). This virus was previously passaged in Vero cells (monkey kidney epithelial cells), with a titer of $10^{6.75}TCID_{50}$. Cells were infected with a multiplicity of infection of 0.1. The cells were cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum, further trypsinized and passaged every third/fourth day. 2% FBS was used for infection. We checked the cells for presence of viral replication (CPE) and viral RNA by the reverse transcriptase polymerase chain reaction (PCR) and Nested-PCR. We also made an attempt to adapt the virus to the cells by blind passages in the NIH 3T3 cells.

Results: Morphological changes were absent in the infected cells as compared to the mock-infected control of the NIH 3T3 cell lines. After third and fourth passages of the virus in the same cell line, rounding of cells was observed, but this effect did not increase on further passages of the virus. When checked for presence of viral RNA, the cultures were found to be positive irrelevant of the rounding effect. Considering the viral kinetics, we hypothesize a slow viral replication in the first phase of infection in these cells which might allow a time frame for the induction of cytokines, resulting in further slowing down the virus replication and spread of infection between cells and ensure cell survival and leading to persistence of viral RNA.

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Biography

Benkoova B, MSc, is a Virologist, currently working at the Enterovirus Laboratory and the National Reference Centre for Identification of Enteroviruses at the Medical Faculty of the Slovak Medical University in Bratislava, Slovakia. She received her BSc and MSc Virology degrees at Comenius University in Bratislava, Faculty of Natural Sciences. Her bachelor's thesis contains a literature review of neonatal enteroviral infections, their pathogenesis and clinical manifestation. Her MSc research thesis was focused on adaptive and innate immune response caused by coxsackievirus infection.

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