The aim of the project is to investigate the influence of ectromelia (mousepox) virus (ECTV) on the noncanonical nuclear factor (NF)-κB signaling pathway in epithelial cells, fibroblasts, and macrophages *in vitro*. Mousepox virus, which is harmless to humans, has become a valuable tool for research on closely related viruses belonging to the same *Orthopoxvirus* genus and *Poxviridae* family, which can cause serious zoonoses. A virus that enters the body's cell, uses a variety of strategies in order to secure favorable conditions for effective replication. Previous studies on the mechanisms of response modification to orthopoxvirus infection focused on the impact of the members of this genus on the canonical NF-κB activation, which is essential for innate antiviral response mechanisms. These viruses effectively block NF-κB stimulation, thus avoiding the activation of the innate antiviral response. Surprisingly, recently it has been shown that the noncanonical NF-κB pathway is also involved in the innate mechanisms of antiviral immunity. Considering the emerging role of noncanonical pathway activation in antiviral immunity and the fact that there is a crosstalk between the canonical and noncanonical NF-κB signaling pathways, we assume that ECTV modulates the activation of the components of the noncanonical signaling of NF-κB.

In our experiments, human endothelial HeLa cells, mouse embryonic fibroblasts MEFs, and murine macrophage RAW 264.7, which are susceptible to ECTV, will be infected with highly virulent strain Moscow ECTV (ECTV-MOS), UV-inactivated ECTV-MOS or will be left uninfected. Additionally, infected and uninfected cells will be treated with noncanonical NF-κB activation pathway inducers, such as interferon (IFN)-γ, lipopolysaccharide (LPS), lymphotoxin (LT)-β, tumor necrosis factor (TNF)-α, or 12-O-tetradecanoylphorbol-13-acetate (TPA). At 4, 8, 12, 18 and 24 hours post infection (h.p.i.) the cells will be fixed or harvested for further analysis. To visualize cellular localization of ECTV and cellular inhibitors of apoptosis (cIAP) 1/2, TNF receptor-associated factors (TRAF) 2/3, NF-κB-inducing kinase (NIK), inhibitor κB kinase complex (IKK) α subunit and RelB proteins, immunofluorescence assay will be used. In order to evaluate cIAP1/2, TRAF2/3, NIK, IKKα, p100/p52, phospho-p100, and RelB levels in whole cell extracts and nuclear and cytoplasmic fractions, Western Blot technique and chemiluminescence will be performed. DNA binding by NF-κB RelB and p52 subunits will be evaluated using DNA-binding ELISA and colorimetric analysis. In order to evaluate the expression of genes encoding proteins of the noncanonical NF-κB signaling pathway and responsive to noncanonical NF-κB signal transduction, real-time PCR analysis will be performed.

Despite the spectacular success of vaccination campaign of the World Health Organization (WHO), smallpox caused by variola virus (VARV), which is closely related to ECTV, is still regarded as a threat for humans because it may be used as a biological weapon. Moreover, the use of vaccinia virus (VACV) as a vaccine against smallpox, which also provided protection against other orthopoxviral diseases, is no longer used. Therefore, VACV, along with cowpox virus (CPXV) and monkeypox virus (MPXV) are the sources of emerging zoonoses, which may be fatal. Therefore, studies on orthopoxviral pathogenesis are still necessary.

The studies on the influence of ECTV infection on the activation of the components of the noncanonical NF-κB signaling pathway will be novel and will enable indication of the potential targets of orthopoxviral manipulation. Studying influence of viruses on cell signaling may facilitate therapeutic target identification and vaccine development. It is also important that immunomodulatory abilities of poxviruses may be utilized to inhibit unwanted immune response, which is a hallmark of autoimmune diseases and graft rejection process.