



Alemka Markotic, MD, PhD



Dr. Alemka Markotic is a Researcher at the Institute of Immunology, Zagreb, Croatia and the Associate Professor at the Medical Faculty University of Rijeka, Croatia. Her group is focused on research of innate immunity to intracellular pathogens especially to hantaviruses and is mostly supported by the Croatian Ministry of Science, Education and Sport.

We are grateful to the ISID for supporting this study.

Ljiljana Cebalo, MD, Institute Immunology of Zagreb, Croatia performed most of the experiments, and

Connie Schmaljohn, PhD, US Army Medical Research Institute of Infectious Diseases (USAMRIID), Frederick, MD, USA served as a consultant.

## ISID Small Grants Program Final Report

by Alemka Markotic, MD, PhD, Associate Professor • Cellular Immunology Unit  
Department for Research and Development, Institute of Immunology • Zagreb • Croatia

### Mechanisms of Apoptosis in 293HEK cells infected with Hantaviruses

The genus *Hantavirus* (family *Bunyaviridae*) is responsible for two different syndromes: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS), both important problems in global public health. Following the incubation period, HFRS is manifested mainly by fever, variable degrees of circulatory failure, hemorrhage, and renal failure, but symptoms demonstrating the involvement of other organs and systems are also observed. In HPS there is initially a febrile phase associated with myalgia, headache, and malaise followed by progressive pulmonary edema with hypotension and hypoxemia.

Hantaviruses are maintained in nature in persistently infected rodents and can also persistently infect cultured mammalian cells, causing little or no cytopathology. During our studies of persistence, we unexpectedly observed cytopathic effects (CPE) and apoptosis in hantavirus-infected human embryonic kidney cells (HEK293). Our initial results (A. Markotic *et al.*, J Gen Virol 2003) indicated that members of the TNF receptor superfamily did not contribute to the apoptosis that we saw in the infected HEK293 cells.

To better understand kidney immunopathology in HFRS/HPS and the mechanisms of viral persistence, it would be of great importance to identify the apoptosis pathways and factors that lead to cytopathology. We looked for the expression of some TNF ligands and TNF receptors that were not tested in our previous study, bcl-2, caspases, IAP, TRAF, CARD, death domain family members, death effector domain family members, CIDE domain family members, as well as genes involved in the p53 and ATM pathways. To investigate the possible role of adhesion molecules and extracellular matrix proteins, which may be involved in cytopathogenicity during the interaction of HFRS- and HPS-causing hantaviruses and 293HEK cells, we looked for the expression of various types of cell adhesion molecules (such as the integrins, IgG superfamily members, cadherins and catenins, and selectins) as well as extracellular matrix proteins, proteases (such as the matrix metalloproteinases and the serine and cysteine proteinases) and their inhibitors.

We used Focused Gene Expression cDNA Array Analysis (GEArrays, SuperArray Bioscience, Frederick, MD, USA) to compare expression profiles of genes in RNA samples from HEK293 cells infected with Hantaan virus, Andes virus, and Sin Nombre virus. Two different GEArray™ were used: GEArray Q series Human Apoptosis Gene Array and GEArray Q series Human Extracellular Matrix & Adhesion Molecules Gene Array. Selected genes were identified by RT-PCR to verify transcriptional responses.

Increases in gene expression of p53, BCL2, BCL2-interacting killer (BIK), and caspase 7 in infected cells indicated that one of the mechanisms of apoptosis might involve the release of apoptotic factors from the mitochondria. Changes were also detected in cysteine cathepsins, which are implicated in a multitude of physiological and pathophysiological processes: degradation of extracellular matrix, apoptosis, and events of inflammatory and immune responses etc. A lysosomal pathway, characterized by partial rupture of lysosomal membranes, may be engaged simultaneously with mitochondrial permeabilization and caspase activation. The changes in gene expression for alpha (v) beta3 integrin (vitronectin receptor, receptor for pathogenic hantaviruses) and “bridging” thrombospondin (TSP), together with several metalloproteinases (MMP1, MMP2, MMP3, MMP13, and MMP24) and their inhibitors, which are critical modulators of extracellular matrix (ECM), may be involved in apoptosis. Metalloproteinases are known specifically to degrade ECM components such as fibronectin, vitronectin, and laminin.

Our findings at the gene expression level indicated that the mechanisms of apoptosis in HEK293 cells induced by hantaviruses are the result of the complex interplay among various molecules.

We have presented evidence that one of the mechanisms of apoptosis may involve the release of factors from the mitochondria. It is also possible that lysosomal rupture precedes and is necessary for the activation of the mitochondrial pathway of cell death.

Additionally, several important adhesion and extracellular matrix molecules may play roles in hantavirus-induced apoptosis in HEK293 cells. We also would like to highlight the importance of matrix metalloproteinases and their inhibitors, whose role in the immunopathogenesis of hantaviruses is shown here for the first time.

Further in vitro experiments should confirm the mechanisms of apoptosis at the gene expression level. Additionally, some of our findings direct us towards further clinical studies among HFRS and HPS patients, especially focused on kidney disorders.

An understanding of the mechanisms leading to apoptosis in HEK293 cells infected with HFRS- and HPS-causing viruses may yield to improved approaches to control the immunopathogenesis of kidney disorders, especially in HFRS. Additionally, it may also improve our understanding of the mechanisms of the persistence and pathogenesis of hantaviral infections in general. ❖