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Institutionen för Mikrobiologi, Tumör och Cellbiologi

Of Voles and Men: Novel Hantavirus *In Vitro* Models

AKADEMISK AVHANDLING

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ABSTRACT

Hantavirus-infection can cause severe disease in humans, with up to 40% case fatalities. Presently, no therapeutics or prophylaxis against hantaviral illness exists. The mechanisms underlying the onset of symptoms and the following pathogenesis are not fully known. *In vitro* research conducted on mono-layered human cell cultures with cell line adapted hantaviruses provides important knowledge for understanding the virus and the innate immune responses induced, but the results might not always correspond to infection in humans. Furthermore, neither the interactions between the virus and its natural animal host, nor why infections in them do not cause disease are well understood. To better understand hantavirus pathogenesis, new models and tools are needed.

In **paper I**, genetic properties of hantavirus were investigated by analyzing two substrains of Puumala hantavirus (PUUV) derived from virus propagated on cells lacking parts of the innate antiviral response. From this study we could conclude that the differences in phenotype and replication were caused without mutations in the viral glycoproteins. Mutations were however observed in the nucleocapsid protein and in the RNA dependent RNA polymerase. We also observed that the phenotypic differences between the substrains and the parental strain were cell line specific.

To be able to analyze hantavirus infection of cells from the natural host *in vitro*, vole embryonic fibroblasts derived from bank voles, the host for PUUV, were isolated in **paper II**. These cells were susceptible and permissive not only to PUUV, but to a range of other bank vole-borne viruses, indicating that these cells can be an important tool for studies of several zoonotic viruses. Regarding IFN- β and Mx, two important antiviral proteins, infection of the vole fibroblasts with PUUV induced a different response compared to that observed in human fibroblasts, indicating a possible species difference in innate immune response against hantavirus infection.

To better mirror the situation in human organs, complex *in vitro* models resembling human tissue might be valuable. In **paper III** we took the advantage of using a 3-dimensional organotypic model of human lung tissue to study early and long term infections of the highly pathogenic Andes virus (ANDV). With this model we could show that a peak in progeny virus production occurs more than a week after initial infection. We also observed increased extracellular levels of the pro-inflammatory cytokines IL-6 and IL-8, and of IP-10 and eotaxin-1 upon ANDV-infection, as well as suppression of RANTES-responses. These ANDV-induced effects were observed late after infection. VEGF-A are suggested to be involved in the pathogenesis of hantaviral illness, as it might be responsible for the increased vascular permeability observed in patients. We observed higher levels of VEGF-A after ANDV-infection, suggesting that infection of lung tissue *per se* might be responsible for the increased VEGF-A levels observed in patients.

The potential role of dendritic cells (DCs) during hantaviral illness is not known. In **paper IV**, we studied the effect DCs have on hantavirus-infection, by adding these cells to the organotypic human lung tissue model. We showed that DCs had an antiviral effect against hantavirus-infection, suggesting that DCs might be involved in limiting the infection.

In this thesis, establishment of novel *in vitro* models, and studies of different aspects of hantavirus-infection were performed. Genetic properties of PUUV were investigated and by using different *in vitro* models, the cellular responses of voles and humans during hantavirus-infection were analyzed.