

SUMMARY

Hantaviruses are rodent-borne RNA viruses within the family Bunyaviridae. They are found worldwide and are associated with two severe disease syndromes: hemorrhagic fever with renal syndrome in Asia and Europe, and hantavirus pulmonary syndrome in the Americas. Human infection is usually initiated by inhalation of aerosols of excreta from infected rodents and can result in high mortality rates. These viruses are currently divided into 22 distinct species based on four rules defined by *The International Committee on Taxonomy of Viruses* or ICTV. A correct classification of hantavirus is an essential tool for antiviral therapy and vaccine development since researchers and medical doctors need to be able to identify for example the correct hantavirus species circulating in a specific geographic region. Unfortunately, the current classification as defined by the ICTV is rather difficult to comply and therefore often ignored by the descriptors of novel hantavirus strains. In order to obtain a scientifically based genetic classification in addition to the current ICTV classification, we analyzed in a first part of this thesis, complete S, M and L segment sequences found in Genbank by using similarity comparison, together with maximum likelihood and neighbor-joining analysis. S segment amino acid sequence comparison allowed a clear distinction between the different hantavirus species and lead us to propose an adjustment of the second rule of the ICTV classification guidelines (“*a hantavirus species exhibits an at least 7% difference in amino acid identity when comparing the complete S segment and M segment sequences*”) to a more appropriate rule, “*a 10% difference in S segment similarity or a 12% difference in M segment similarity based on complete amino acid sequences*“ in accordance with the current situation in the hantavirus field. Furthermore, the proposed guideline can replace the neutralization tests, to genetically identify them. For hantaviruses discovered without a virus isolate, these guidelines can form an alternative, scientifically based approach in identifying the correct hantavirus species. In order to draw clear and distinct dividing lines between species of hantaviruses, we developed an automated web-based tool which uses amino acid sequences from the S segment. This online GeTHa (Genotyping Tool for Hantaviruses) application was developed using Java and PERL scripts and combines phylogenetic analyses with similarity comparison for the genetic classification of hantaviruses. The analyses of previously well-characterized hantavirus sequences found in Genbank showed that all tested sequences could be correctly classified when using the GeTHa web application.

In Europe, the Dobrava-Belgrade virus and Puumala virus are the major hantaviruses that cause hemorrhagic fever with renal syndrome in humans. As hantaviruses can cause diseases with high morbidity and mortality, and as to date there is no specific treatment, research efforts are concentrated on the development of vaccines and antiviral therapies. In a second part of this doctoral thesis, we characterized the immunogenicity of recombinant nucleocapsid proteins of the Dobrava-Belgrade virus and Puumala virus, some of them linked to a carrier protein corresponding to the outer membrane protein A from *Klebsiella pneumoniae* (rP40). This rP40 molecule is a novel carrier protein which facilitates exogenous antigen uptake by dendritic cells. We cloned and expressed several recombinant Dobrava-Belgrade virus and Puumala virus proteins in the *E. coli* mutant ICONE 200 using the tryptophan promoter controlled pTEXmp18 expression vector. To test these recombinant proteins for their immunogenicity in NMRI mice, we developed an ELISA based on the Dob118 and Puu118 recombinant proteins, two proteins consisting of the first 118 amino-terminal amino acids of respectively the Dobrava-Belgrade virus and Puumala virus S segment. Using this ELISA, all recombinant proteins were found to be highly immunogenic after 3 immunizations with 10 µg of the different recombinant proteins. The immunizations resulted in the induction of a strong nucleocapsid-specific IgG response with a predominance of IgG1 over IgG2b and IgG2a. A specific IgG3 response could not be detected. NMRI mice immunized with recombinant proteins without rP40 showed lower nucleocapsid-specific antibody responses in comparison with the rP40 conjugated constructs.

Although several species of rodents, rabbits and non-human primates can be infected with the Dobrava-Belgrade virus or Puumala virus, only humans have been shown to present symptoms of hemorrhagic fever with renal syndrome. We have used an NMRI mouse model to test the capacity of our recombinant proteins to induce protection against virus challenge. Infection in this NMRI model is scored by the presence of neutralizing antibodies. In our experiments, neutralizing antibodies were detected by using a new technique called the replication reduction neutralization test (RRNT). With this RRNT, the degree of reduction in virus replication after incubation with sera containing specific neutralizing antibodies is determined by means of quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). The detection of hantavirus genomes with qRT-PCR eliminates the need for animal anti-hantavirus antibodies to detect the residual hantavirus genomes as needed for other neutralization protocols. In the challenge experiments with NMRI mice, only the truncated constructs containing the first 118 N-terminal amino acids conjugated to rP40 (P40-Dob118, P40p-Dob118 and P40-Puu118) gave 100% protection after 3 immunizations with 10 µg

protein. These results suggest that these recombinant proteins are good candidates for a recombinant subunit vaccine against the Dobrava-Belgrade virus and the Puumala virus.

In a last part of this dissertation, we examined chloroquine, a 4-aminoquinoline, as an effective inhibitor of the replication of hantavirus *in vitro* and *in vivo*. Chloroquine is a clinically approved drug which is effective against malaria, and was tested *in vitro* with several New World and Old World hantavirus strains in Vero E6 cell culture. Results indicate that the IC₅₀ of chloroquine for antiviral activity ($10.2 \pm 1.43 \mu\text{M}$) was significantly lower than its cytostatic activity, CC₅₀ ($260 \pm 2.52 \mu\text{M}$), yielding a selectivity index of 25.5. In the *in vivo* experiments with newborn C57Bl/6 pups, The highest survival rate (72.7%) was seen with a concentration of 10 mg/kg. Survival rates declined in a dose dependent manner, with 47.6% survival when treated with 5 mg/kg chloroquine and 4.2% survival when treated with 1 mg/kg chloroquine. Our results show that chloroquine can be highly effective against hantavirus infection in newborn mice and may be considered as a future drug against hantaviruses.