Human pathogenic hantaviruses and prevention of infection

Detlev H. Krüger,^{1,*} Günther Schönrich¹ and Boris Klempa^{1,2}

¹Institute of Medical Virology; Helmut Ruska Haus; University Medicine Charité; Charitéplatz; Berlin, Germany;

²Institute of Virology; Slovak Academy of Sciences; Bratislava, Slovakia

Key words: hantavirus, vaccines, protective immunity, immunopathogenesis

Hantaviruses are emerging viruses which are hosted by small mammals. When transmitted to humans, they can cause two clinical syndromes, hemorrhagic fever with renal syndrome or hantavirus cardiopulmonary syndrome. The review compiles the current list of hantaviruses which are thought to be pathogenic in humans on the basis of molecular or at least serological evidence. Whereas induction of a neutralizing humoral immune response is considered to be protective against infection, the dual role of cellular immunity (protection versus immunopathogenicity) is discussed. For immunization, inactivated virus vaccines are licensed in certain Asian countries. Moreover, several classical and molecular vaccine approaches are in pre-clinical stages of development. The development of hantavirus vaccines is hampered by the lack of adequate animal models of hantavirus-associated disease. In addition to active immunization strategies, the review summarizes other ways of infection prevention, as passive immunization, chemoprophylaxis and exposition prophylaxis.

Introduction

Hantaviruses are emerging viruses which cause Hemorrhagic Fever with Renal Syndrome (HFRS) in Asia and Europe.^{1,2} Infection by New world hantaviruses leads to Hantavirus Cardiopulmonary Syndrome (HCPS) in the Americas with case fatality rates of up to 35%.^{3,4} In Asian HFRS, the kidney failure is frequently accompanied by superficial hemorrhages explaining why hantaviruses belong to the group of Hemorrhagic Fever viruses.² In Europe, increasing numbers of disease cases are reported. In Germany alone, two thousands of clinical cases were notified in 2010—more than in any of the previous 10 years since implementation of the reporting system.⁵ Very recently, the occurrence of hantaviruses and HFRS was shown also in Africa.^{6,7} This emergence of hantaviruses requires new approaches to efficiently prevent infection by these pathogens.

The Virus, Reservoirs and Transmission

Hantaviruses form a unique genus *Hantavirus* within the *Bunyaviridae* family. Hantavirus virions are enveloped, spherical particles of 80–110 nm in size. The virus genome consists of three segments of negative-stranded RNA; the large (L) segment encodes the viral RNA polymerase, the medium (M) segment the glycoprotein precursor which is co-translationally cleaved into the envelope glycoproteins Gc and Gn, and the small (S) segment the nucleocapsid protein (N).⁸

Hantaviruses produce a chronic infection with no apparent harm in their natural hosts, small mammals. They are very strictly associated with their reservoir hosts, mainly rodents but, as recently reported, also insectivores (shrews and moles). Usually, a particular hantavirus is transmitted only by one or few closely related rodent/insectivore species. Thereby, geographic range of every hantavirus is limited (although not necessarily determined) by the geographic range of its natural host. This association is reflected also in their phylogeny; rodent-associated hantaviruses form three major evolutionary clades which correspond to the three *Murinae* subfamilies of their natural hosts. Hantaan virus (HTNV), Seoul virus (SEOV) and Dobrava-Belgrade virus (DOBV) are most important examples of pathogenic *Murinae*-associated hantaviruses, Puumala virus (PUUV) belongs to the *Arvicolinae*-associated hantaviruses while Sin Nombre virus (SNV) and Andes virus (ANDV) causing HCPS in Americas are members of *Sigmodontinae*-associated hantaviruses. Several additional hantaviruses were reported to be pathogenic for humans with varying severity. Hantaviruses currently recognized as human pathogens together with their natural hosts, geographical range and clinical severity are summarized in Table 1. Not in all cases clear molecular evidence for the presence of these viruses in HFRS or HCPS patients exists but indirect (serological) evidence indicates the virus to be pathogenic. It should be mentioned that various genetic lineages of a virus, e.g., of DOBV, can exert quite different degrees of virulence towards humans.^{9,10} In addition, there are hantaviruses with unclear pathogenicity for humans or being regarded even as non-pathogenic. Prospect Hill virus (PHV) is the most prominent example of a non-pathogenic hantavirus, often used as a model virus in comparative pathogenesis studies. Pathogenicity and public health relevance of the recently discovered shrew- and mole-borne hantaviruses is currently unclear and remains to be investigated.

*Correspondence to: Detlev H. Krüger; Email: detlev.kruger@charite.de
Submitted: 12/22/10; Revised: 02/08/11; Accepted: 02/15/11
DOI: 10.4161/hv.7.6.15197

Table 1. Hantaviruses reported to be pathogenic in humans^a

Disease/ continent	Virus ^b	Abbreviation	Rodent host	Severity of disease	Case fatality	Patient-derived sequence available in GenBank
HFRS/Europe						
	Dobrava-Belgrade^c	DOBV				
	DOBV-Aa		<i>Apodemus agrarius</i>	mild/moderate	0.5–0.9%	yes
	DOBV-Af		<i>A. flavicollis</i>	severe	up to 10%	yes
	DOBV-AP		<i>A. ponticus</i>	moderate	up to 10%	yes
	Saaremaa ^c	SAAV	<i>A. agrarius</i>	? ^d		no
	Puumala^c	PUUV	<i>Myodes glareolus</i>	mild	0.1–0.4%	yes
	Tula^c	TULV	<i>Microtus arvalis</i>	? ^d		no
			<i>M. agrestis</i>			
			<i>M. rossiameridionalis</i>			
HFRS/Asia						
	Hantaan^c	HTNV	<i>Apodemus agrarius</i>	severe	up to 10%	yes
	Amur/Soochong		<i>A. peninsulae</i>	severe?		yes
	Seoul^{c,e}	SEOV	<i>Rattus spp.</i>	mild	<1%	yes
	Thailand^c	THAIV	<i>Bandicota indica</i>	? ^d		no
HFRS/Africa						
	Sangassou or related viruses^f	SANGV	<i>Hylomyscus simus</i>	? ^d		no
HCPS/Americas						
	Andes^c	ANDV	<i>Oligoryzomys longicaudatus</i>	severe	25–35%	yes
	Orán	ORNV	<i>O. longicaudatus</i>			yes
	Bermejo	BMJV	<i>O. chacoensis</i>			yes
	Lechiguanas	LECV	<i>O. flavescens</i>			yes
	Rio Mamore ^c	RIOMV	<i>O. microtis</i>			no ^g
	Choclo	CHOV	<i>O. fulvescens</i>			yes
	Maciel	MCLV	<i>Necomys benefactus</i>			no
	Laguna Negra ^c	LNV	<i>Calomys laucha</i>			yes
	Araraquara	ARAV	<i>Bolomys lasiurus</i>			yes
	Hu39694		unknown			yes
	Castelo dos Sonhos	CASV	unknown			yes
	Juquitiba	JUQV	<i>O. nigripes</i>			yes
	Bayou^c	BAYV	<i>Oryzomys palustris</i>			yes
	Black Creek Canal ^c	BCCV	<i>Sigmodon hispidus</i>			no
	Sin Nombre^c	SNV	<i>Peromyscus maniculatus</i>	severe	25–35%	yes
	New York ^c	NYV	<i>P. leucopus</i>			yes
	Monongahela	MGLV	<i>P. maniculatus nubiterrae</i>			no

^aCompiled from Hammerbeck, et al.¹² Jonsson, et al.²¹ Kariwa, et al.²⁰ Krüger & Klempa¹⁰ Muranyi, et al.¹⁹ ^bViruses are grouped according to rather strict species definition criteria recently proposed by Maes et al.²² ^cSpecies listed in the latest report of the International Committee for Taxonomy of Viruses, see Fauquet, et al.²³ and www.ictvonline.org/virusTaxonomy.asp ^dNot to be estimated since only few patients are described. ^eProbably world-wide distributed. ^fLow anti-SANGV neutralizing antibodies detected in patients which can be also caused by infection with a related African hantavirus. ^gDesignated as Anajatuba virus in GenBank but probably RIOMV.

Hantaviruses differ from other bunyaviruses in one important ecological aspect; they are not transmitted by arthropod vectors. Transmission occurs via inhalation of aerosolized rodent urine, saliva and feces. This way of transmission is assumed to be dominant between reservoir host animals as well as for

accidentally infected humans. In addition, direct transmission through bites seems to be important for the circulation of the virus in rodent populations in nature. Moreover, the capability to induce lethal ANDV infection of Syrian hamsters by intragastric administration¹¹ allows to speculate that transmission

of hantaviruses through ingestion of contaminated food might also occur.¹²

Humans are usually considered as dead-end host which does not transmit the virus further. So far, ANDV is the only hantavirus for which person-to-person transmission has been documented.^{13–17} This might represent a serious public health concern since ANDV is associated with high case fatality rates (25–35%). A recent prospective study showed that viral RNA could be detected in peripheral blood cells for up to 2 weeks before the onset of symptoms¹⁵ and the infectious virus was isolated from a seronegative, asymptomatic child 2 days before he developed HCPS.¹⁸ Thus, the virus could be spread by asymptomatic patients to other parts of the world. However, an influenza-like pandemic scenario seems to be unlikely because human-to-human transmission of ANDV appears to be very inefficient, occurs mainly within households, and requires relatively intimate interpersonal contact.¹⁵

The Diseases: Hemorrhagic Fever with Renal Syndrome and Hantavirus Cardiopulmonary Syndrome

The severity of hantavirus diseases can range from mild to severe with case fatality rates of up to 35%. Both HFRS and HCPS are associated with acute thrombocytopenia and changes in vascular permeability and both syndromes can include renal and pulmonary symptoms. However, the syndrome caused by Old World hantaviruses dominantly shows renal affections and is called HFRS, whereas New World hantavirus disease is mainly associated with cardiopulmonary failure and is therefore called HCPS.^{1–4}

The clinical incubation time before onset of symptoms is typically 2–3 weeks long, however, time periods ranging between 1 and 6 weeks have been reported. In the early (prodromal) phase of about 3–5 days, HFRS and HCPS patients exhibit fever, myalgia, malaise, headache, backache, abdominal pain and often nausea and diarrhea. For HFRS patients, also vision disorders have been reported.

In the next phases (about 2–7 days) following the prodrome, hypotension occurs and can result in cardiogenic shock and death. In HCPS patients lung edema and even lung failure develop and require, depending on severity, supplemental oxygen, intubation with mechanical ventilation or extracorporeal membrane oxygenation (ECMO). HFRS patients mainly show renal impairment or failure and require, in severe cases, hemodialysis during this oligouric/anuric phase. In addition to cardiogenic shock, lung or renal failure are also common reasons for fatal outcome.

The beginning of the diuretic phase is a positive prognostic sign for the patient. Clinical improvement is usually rapid. After a few days, the convalescence period starts which can last over several weeks. Hantavirus syndromes are regarded as acute diseases and it is a matter of discussion to what extent they can result in long-term sequela.

There are wide differences between the severities of clinical courses dependent on the hantavirus type causing infection (see Table 1) and probably additional properties of the host. In some cases the different phases of disease are difficult to distinguish.

Human Adaptive Immune Responses to Hantaviruses

Dendritic cells (DC) bridge the innate and adaptive immune system and play a key role in orchestrating the immune components during infection. HTNV has been demonstrated to productively infect DCs.²⁴ Unlike many other viruses HTNV does not cause any obvious loss in DC function but instead induces DC maturation. Thus, hantavirus-infected DC most likely serve as a vehicle for hantavirus dissemination as maturing DCs migrate to regional lymph nodes where they induce a powerful adaptive immune response consisting of virus-specific antibodies and T cells.

After hantavirus infection virus-reactive IgM and IgA responses are rapidly generated whereas IgG antibody titers rise more slowly.²⁵ In addition both total and specific IgE levels are increased in patients with NE and HFRS.^{26,27} However, the role of IgE antibodies for virus clearance and virus-induced pathogenesis is unclear so far. After onset of the acute phase IgM as well as IgG antibodies can be detected that react with hantaviral N protein, which represents the major target antigen.^{28–35} Antibodies against Gc and Gn appear later during the progress of disease.³⁶

It remains still enigmatic why hantaviral N protein is the dominant antigen. It forms the helical nucleocapsid inside the virion whereas Gn and Gc project from the virion surface and bear epitopes that are easily accessible for neutralizing antibodies.³⁴ Possibly, the N protein contributes to viral evasion by distracting the humoral response from the glycoproteins in the viral envelope. On the other hand infection experiments with suckling mice suggest that N protein-reactive antibodies may confer at least some protection and prolong survival.³⁷

The humoral immune response towards hantaviruses is long-lasting. Several studies have shown that in HCPS-convalescent individuals neutralizing IgG antibodies persist at high levels for years.^{38,39} Moreover, high titers of neutralizing IgG antibodies were detected in sera of convalescent patients decades after PUUV infection.^{40,41} Accordingly, repeated symptomatic infection with hantaviruses have not been observed so far indicating that previously infected individuals are protected life-long from re-infection. Surprisingly, titers of neutralizing antibodies even increased in the majority of individuals after acute ANDV infection.⁴² In some of these individuals repeated exposure to ANDV could be excluded as they were not residents of endemic areas suggesting that re-stimulation of antiviral B-cell responses might be due to viral persistence.

Several studies indicate that B-cell epitopes located on N, Gc and Gn proteins are conserved between different hantavirus types. Sera from patients with previous HFRS or HCPS cross-neutralized heterologous hantaviruses with varying efficiency.⁴³ In hamsters, cross-neutralizing antibodies were detected after hantavirus infection or vaccination with DNA vaccines expressing hantaviral glycoproteins.⁴⁴ Moreover, vaccination of rhesus macaques with DNA vaccines encoding Gc and Gn elicited cross-neutralizing antibodies.^{45,46} Boosting with the same DNA vaccine enhanced the cross-neutralizing capacity of sera.⁴⁵ In another report, bank voles immunized with recombinant N protein from Topografov virus, ANDV or DOBV developed cross-reactive

antibodies against PUUV N protein and were cross-protected to a certain degree from challenge with PUUV.⁴⁷ Collectively, these results demonstrate the feasibility to generate vaccines that protect against multiple hantavirus types.

Intriguingly, HCPS patients developed a more benign course if high titers of neutralizing IgG antibodies were present in the acute phase of the disease.^{48,49} This suggests that an efficient antibody response in the early phase of disease is important to interfere with viral dissemination and prevent extensive damage of vascular endothelium. Furthermore, the induction of neutralizing antibodies is associated with protection against hantaviruses in infection models using small animals.⁵⁰⁻⁵³ Finally, passive immunization of Syrian hamsters with neutralizing antibodies against ANDV-encoded Gn and Gc that were produced in other species (macaques, rabbits) confers protective immunity against lethal challenge with ANDV.^{11,45,51,54} Collectively, these data support the concept that strong induction of neutralizing antibodies against envelope glycoproteins represents a prime goal in hantavirus vaccine development.

During acute infection vigorous T-cell responses involving CD8⁺ T cells are observed in patients with HFRS and HCPS.^{55,56} Moreover, a robust CD8⁺ T-cell memory develops in individuals after infection with PUUV and HTNV.^{57,58} Similarly, in patients recovering from ANDV infection long-lived and highly differentiated virus-specific effector memory T cells exist.⁴² Intriguingly, in the latter study virus-specific effector memory T cells were negative for CD127 (IL-7R α) implying that they are continuously activated. They resemble in this regard herpesvirus-specific memory T cells raising the possibility that hantaviral virions or antigen persists in humans. In line with this theory, Björkstöm et al. recently observed in PUUV-infected patients a rapid expansion of NK cells that remained functionally competent at elevated numbers for a long time.⁵⁹ However, viral RNA has not been detected so far in specimens from individuals previously infected with hantavirus.

Similar to the humoral response virus-reactive CD8⁺ T cells are mostly directed against immunodominant epitopes of the N protein although all hantaviral structural proteins (Gn, Gc, N) serve as a source for epitopes.⁶⁰ This may be due to the fact that the N protein represents the most conserved and abundant hantaviral protein produced during infection.⁶¹ In striking contrast, in humans previously infected with ANDV Gn-specific responses are predominant as compared to N- and Gc-specific responses.⁴² The reasons for these discordant results are unclear at the moment but differences in the time-point of T-cell analyses or experimental design could play a role. In addition, this phenomenon can be explained by the finding that the C-terminal region of Gn from ANDV contains sequences that direct Gn to proteasomal degradation.⁶² As a consequence presentation of Gn-derived epitopes through HLA class I molecules could be enhanced.

In terms of vaccine development the actual source of immunodominant T cells epitopes appears to be less relevant. It has been shown in the HCPS animal model that vaccination with adenoviral vectors expressing any of the individual hantaviral structural proteins (N protein, Gn or Gc) protects Syrian hamsters from lethal challenge.⁶³ Strikingly, in this study protective immunity was established in the absence of detectable neutralizing

antibodies. Supporting this notion a recent case report described that the appearance of CD8⁺ T cells but not neutralizing antibodies was associated with the elimination of ANDV from peripheral blood.⁶⁴ Moreover, in bank voles vaccination with recombinant N proteins from PUUV and other hantaviruses induces protection and even cross-protection against PUUV that is based on T-cell responses rather than production of neutralizing antibodies.^{47,65,66} Collectively, these findings underline the importance of cellular immunity for protection from hantavirus infection.

Role of Antiviral Immune Responses in Hantavirus-Induced Pathogenesis

T cells represent a double-edged sword during hantavirus infection. At one hand CD8⁺ T cells play a pivotal role in protective immunity as outlined above. On the other hand, antiviral CD8⁺ T cells are implicated in hantavirus-associated pathogenesis in humans.^{36,67,68} Supporting this notion, in patients with severe acute HCPS higher frequencies of SNV-specific CD8⁺ T cells were detected by tetramer-staining compared with SNV-infected individuals showing less severe symptoms.⁵⁶ In stark contrast, higher frequencies of IFN γ producing antiviral T cells during acute HTNV infection are linked to mild or moderate HFRS emphasizing the protective role of T cells.⁶⁹ The discrepancy between these results may be explained by the different methods used to visualize T cells. In general, tetramer staining reveals much higher virus-specific T cell frequencies than IFN γ ELISPOT and identifies virus-specific T cells irrespective of their function. However, there are different types of CD8⁺ T cells with different effector mechanisms, including cytolytic and non-cytolytic, contributing to resistance to virus infection.⁷⁰ T cells secreting IFN γ are known to control hepatitis B and C virus, which are regarded as non-cytopathic viruses, in a non-cytolytic fashion thereby avoiding cellular damage.⁷¹ For this reason, it is likely that IFN γ producing T cells are also crucial for non-cytolytic clearance of hantaviruses and other non-cytopathic pathogens like hepatitis B and C virus. In contrast, T cells that are predominantly cytotoxic may eliminate the hantaviruses at the cost of tissue damage resulting in pathogenesis. In conclusion, the quality rather than the quantity of antiviral T cells during acute infection with hantaviruses may be predictive of the disease outcome.

Intriguingly, a correlation between certain HLA class I haplotypes and disease outcome was observed in HFRS patients.^{72,73} Moreover, patients with a HLA-B35 haplotype were found to be more prone to severe HCPS than other patients.⁵⁶ In contrast, analysis of individuals previously infected with ANDV revealed that a strong HLA-B35-restricted response of memory CD8⁺ T cells is related to mild rather than to severe HCPS.⁴² Possibly, the quality and quantity of antiviral CD8⁺ T-cell responses during acute hantavirus infection is not reflected by the memory CD8⁺ T-cell pool years after hantavirus infection. Alternatively, different approaches to detect hantavirus-specific T-cells could be responsible for these seemingly discordant results. In any case, it is likely that besides HLA-class I-restricted T cells other factors also influence the outcome of hantavirus-associated disease.

Table 2. Summary of hantavirus vaccines used in humans and non-human primates

Hantavirus	Vaccine format	Immunogen(s)	State of development	Country
HTNV	Inactivated virus	Whole particle	Human mass vaccination	South Korea
HTNV, PUUV	Inactivated virus	Whole particle	Clinical studies	South Korea
HTNV, SEOV	Inactivated virus	Whole particle	Human mass vaccination	China
HTNV	Recombinant vaccinia virus	G _N , G _C , N	Clinical studies (not pursued)	US
HTNV, PUUV	DNA vaccine	G _N , G _C	Clinical studies	US
ANDV, HTNV, PUUV, SEOV	DNA vaccine	G _N , G _C	Pre-clinical studies (Rhesus, Cynomolgus)	US

Data compiled from Boudreau, et al.⁷⁸ Hammerbeck, et al.¹² Schmaljohn,⁷⁶ Zhang et al.⁷⁷ G_N, G_C, glycoproteins; N, nucleocapsid protein; for virus abbreviations see Table 1.

Active Immune Prophylaxis

Since excellent and very recent overviews on hantavirus vaccines exist,^{12,74-76} we like to summarize older studies by referring to these review articles and add more details only about some latest developments or aspects not particularly discussed in those reviews. Target groups for current and future vaccination approaches are inhabitants of hantavirus-endemic geographic areas and people with professional risks of infection, e.g., forest workers, workers in stables and animal houses/laboratories or soldiers living in military camps. Table 2 gives an overview about the most developed hantavirus vaccine approaches.

Inactivated virus vaccines. Virus suspensions inactivated by formalin or β-propiolactone are in practical use for human vaccination in Korea and China. A commercial suckling mouse-brain derived HTNV vaccine formulated with alum adjuvant, Hantavax, is distributed in Korea. Also bivalent HTNV/PUUV inactivated vaccines produced in hamster brains as well as a HNTV-based vaccine produced in Vero cells were tested at least in mice. In China, inactivated vaccines were developed as monovalent or bivalent vaccines based on HTNV and/or SEOV propagated in Mongolian gerbil kidney cells, golden hamster kidney cells, suckling mouse brain or Vero cells. The first vaccine was approved for human use in 1993. Since 1995 the vaccines have been used in highly endemic regions of the country, and in 2007/8, a national Expanded Program on Immunization has been started. About 2 million people are reported to be vaccinated per year with only minor side effects. In general the vaccination is described as safe and efficacious.^{77,79} Whereas some authors describe a highly protective effect and significant case reduction by immunization with these vaccines, this efficacy is questioned in other studies (as discussed by Schmaljohn^{12,76}).

In Russia, an inactivated bivalent PUUV/DOBV vaccine has been developed very recently. PUUV Ufa-97 and DOBV-Aa Lipetzk-06 strains were grown on Vero E6 cells, concentrated and inactivated by formalin treatment. Aluminium hydroxide was used as adjuvant. Immunization of BALB/c mice showed neutralizing antibody activities against both PUUV and DOBV. This bivalent HFRS vaccine has passed pre-clinical trials conducted by Russian Control Authority Institution (E.A. Tkachenko, pers. communication).

One handicap for the development of hantavirus vaccines (based on live or killed virus) is the low replication efficiency of

hantaviruses in cell culture which makes the production of larger amounts of virus difficult. Adaptation of virus to cell culture (which should be deficient in the production of different interferons) should lead to higher virus yield as recently shown for a PUUV strain.⁸⁰

Live hantavirus vaccines. To our knowledge, no studies exist to use attenuated hantaviruses for immunization. Very recently the generation of genetic reassortants between pathogenic and non-pathogenic viruses has been proposed as putative vaccine approach, as exemplified by construction of a virus carrying the S and L segments of non-pathogenic Prospect Hill Virus and the M segment of PUUV origin.⁸¹ In vitro, this virus was shown to interact with elements of the innate immune defence in the same way as Prospect Hill Virus but—according to the origin of its M segment—should induce a PUUV-specific neutralizing immunity.

Molecular vaccines. Chimeric viruses. After promising protection studies in hamster or gerbil infection models, live recombinant vaccinia virus expressing N and glycoproteins of HTNV has been exploited in human phase 1 and 2 studies. Mainly because of the limited induction of neutralizing antibodies in vaccinia virus-immune persons and the potential side effects of live vaccinia virus these investigations have been stopped.^{12,76} Hantavirus antibody induction in animals has been shown after administration of recombinant *Peromyscus* cytomegalovirus expressing SNV Gn or vesicular stomatitis virus pseudotypes carrying HNTV glycoprotein. Very recent progress has been made by use of non-replicating adenovirus vectors. In a hamster model, recombinant adenovirus expressing ANDV N, Gn or Gc protein protects the animals against lethal challenge with this hantavirus.⁶³ Induction of neutralizing antibodies and protection against SEOV challenge were also observed after immunization with replication-competent recombinant canine adenovirus expressing SEOV Gn or Gc.^{82,83}

Virus-like particles. Virus-like particles (VLPs), e.g., hepatitis B virus or polyomavirus core particles, are useful carriers of foreign epitopes.⁸⁴ It is interesting to note that the insertion of segments of hantavirus N protein into those VLPs induces long-lasting (cross)immunity and protection against hantavirus challenge in rodent models without induction of neutralizing antibodies. One can speculate that this protection is caused by cellular immunity. Moreover, it was demonstrated that vesicular stomatitis virus pseudotypes with HTNV glycoproteins in their surface induced protective neutralizing antibodies in mice. Without heterologous carrier, Li et al. have generated “autochthonous” HTNV particles

consisting of N protein and glycoproteins after expression in Chinese hamster ovary cells and self-aggregation.⁸⁵ In mice, these VLPs induced specific humoral and cellular immune responses.

Recombinant proteins. Recombinant hantavirus proteins have been expressed in *Escherichia coli*, yeast, transgenic plants or the baculovirus system. N protein as well as glycoprotein preparations were shown to induce immunogenicity and protectivity in hamster, mice or bank voles. Whereas this effect is explained by induction of neutralizing antibodies by the glycoproteins, the protective immune response against N, which is an internal viral protein, can be best explained by triggering cellular immunity. The immunogenicity of these proteins can be further enhanced by use of adjuvants. Since the N protein is more conserved within related hantaviruses than the glycoproteins, an advantage of N protein use seems to be the induction of broader cross-reactive immunity against various virus types.

To our knowledge, no immunogenicity/protection studies on the basis of these recombinant proteins have been performed in a lethal disease model or nonhuman primates. Furthermore, it is still unclear whether the viral RNA-dependent RNA polymerase can be used for immunization approaches.

DNA vaccines. It is generally accepted that DNA vaccines are able to induce long-lasting humoral and cellular immunity. Moreover, they offer an easy way to construct multivalent vaccines. In numerous approaches plasmid DNA, linear DNA and packaged alphavirus replicons expressing N protein and/or glycoproteins of SEOV, PUUV, SNV, HTNV, ANDV were delivered to hamsters, mice, deer mice, rabbits or nonhuman primates. As shown in the compilation of Hammerbeck et al. the extent of immune response (detected by ELISA for anti-N antibodies and by neutralization assays for anti-Gc and Gn antibodies) and protection against challenge by homologous virus were generally more efficient for glycoprotein constructs than for N protein constructs.¹² In certain cases, there were differences in the immunogenicity of the DNA vaccines in different animal models, e.g., the ANDV M segment was found to be immunogenic in nonhuman primates and rabbits but not in hamsters.¹²

Using M segment constructs of HTNV and PUUV, a phase I clinical study was undertaken to determine the safety, tolerability and immunogenicity of those DNA vaccine administered by a particle-mediated epidermal delivery device in a group of 28 volunteers.^{76,78} The data show significant levels of neutralizing antibodies against these viruses. Because of the proposed group-specific cross reactivities, the PUUV component of the vaccine should be also reactive towards the related TULV but the HTNV component towards the *Murinae*-associated viruses DOBV and SEOV.

Passive Immunization

There are numerous results demonstrating the ability of polyclonal or monoclonal antibodies to prevent (or delay) hantavirus infection of mice, hamsters or even nonhuman primates (for a summary see Hammerbeck et al.¹²). In their lethal ANDV/hamster model, Hooper et al. found a protective effect of anti-ANDV sera even as late as 5 days after virus infection.¹¹ On the basis of these findings, one can speculate about the future establishment

of post-exposure prophylaxis measures which could protect persons after virus exposition. Even in the case that virus replication is not completely inhibited, the people could benefit from this treatment since high antibody titers are generally accepted to be correlated with a rather benign clinical course of infection.^{48,49}

Chemoprophylaxis

Ribavirin is the only established antiviral drug with some in vitro- and in vivo-efficacy against hantavirus replication.⁸⁶ However, its action is rather virus-unspecific, its therapeutic use leads to side effects (as anaemia), and there are controversial results about benefit on the basis of clinical studies. The efficacy of ribavirin therapy given to HTNV-infected suckling mice showed higher survival rate in the ribavirin-treated mice than the placebo control group.⁸⁷ A double-blind placebo-controlled trial with HFRS patients in China showed a sevenfold reduction in morbidity in the ribavirin-treated group as well as reduction in fatalities.⁸⁸ A clinical study using intravenous ribavirin for treating Department of Defense personnel with HFRS acquired in Korea from 1987 to 2005 demonstrated a low rate of oliguria (3%) and the absence of dialysis requirement in the treatment cohort (33 individuals); these findings support previous reports that ribavirin given early results in decreased severity of renal insufficiency.⁸⁹ On the other hand, the results of trials in patients suffering from HCPS yielded disappointing results.^{90,91}

Recently, 1-beta-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR) was identified to be promising antiviral candidate with activity against HTNV and ANDV. However, it protected suckling mice from infection with HTNV only to a degree similar to that seen with ribavirin.⁹² In addition, N¹-aryl purines as a novel structural class and potential scaffold for drug discovery were analysed for activity against hantaviruses by Chung et al.⁹³ N¹-3-fluorophenyl-inosine (FPI) showed significant activity against HTNV but (rather surprisingly) not against ANDV. Interestingly, FPI neither decreased viral RNA levels nor increased the mutation frequency of the viral RNA. Therefore, the antiviral activity of FPI might be due to the interaction of FPI or its metabolites with viral or host proteins involved in post-replication events that would affect the levels of infectious virus released.

Other antiviral strategies have also been evaluated, such as the use of cyclic peptides which bind $\alpha\beta_3$ integrin as a virus receptor and thereby block SNV and HTNV infection of Vero E6 cells,^{94,95} or multivalent cyclic peptides presented on nanoparticles which specifically prevented SNV infection in vitro.⁹⁶ As the latest development, new peptidomimetic compounds were selected based on similarity to a cyclic peptide known to bind the $\alpha\beta_3$ receptor. Three compounds showed potency in the nanomolar range in dose-response studies and were at least 2,000 times more efficient than the original cyclopeptide. Selectivity assays with a panel of hantaviruses supported the mechanism of inhibition by targeting the $\alpha\beta_3$ receptor, through the β_3 integrin subunit. However, the therapeutic potential of these compounds needs to be further refined by the optimization of solubility along with the pharmacokinetics, as well as in vivo studies using animal models.⁹⁷ In the case that efficient and well-tolerated drugs can

be introduced, Jonsson et al.⁹⁸ have proposed prophylaxis studies among recent household contacts of index cases with ANDV infection in Chile and Argentina where person-to-person transmission of ANDV and case clusters are described.

Exposition Prophylaxis

It is obvious that current immune- and chemoprophylactic measures are not sufficient to reach a sustained success in the fight against hantavirus infections.

For prevention of hantavirus infections and disease, exposition prophylaxis is still the most important task. To reduce the risk of virus “spill over” from the reservoir hosts to men, one should avoid exposition to rodents and their excreta. This includes control of mice inside and outside of human dwellings. During activities in rooms with potential mice infestation (for example stables, sheds, summer houses after winter) disposable gloves and, if possible, face masks should be worn. The swirling of dust particles containing mice excreta or nesting material should be avoided. During outdoor activities (for example camping, farm or forest work) contact with mice nests and mice excreta should also be avoided. Further precautions are the safe storage of food inside and outside of human dwellings as well as the disinfection and disposal of trapped or dead mice. Because the infection can also be transmitted by laboratory rodents, they should be examined for a possible persisting hantavirus infection.

Conclusions

(A) Up to now hantavirus research is hampered by the lack of adequate animal models of hantavirus-associated disease. The Old World hantaviruses do not cause overt disease in any animal species apart from nonhuman primates, which have been shown

to develop mild symptoms similar to HFRS in humans after infection with PUUV.^{54,99,100} In general, New World hantaviruses also do not induce symptoms that mirror human disease in animals. So far, only ANDV has been reported to cause HPCS-like disease in syrian hamster.¹⁰¹ Therefore, it is difficult to dissect the role of host defense components in protective immunity against hantaviruses after infection/vaccination or to proof the concept of hantavirus-induced immunopathogenesis.

(B) Regarding the diversity of hantavirus species it appears paramount to generate vaccines that induce a high degree of cross-reactivity. This might become an even more urgent problem in the case that shrew- and mole-borne hantaviruses turn out to be significant human pathogens, too.

(C) There is already strong evidence that hantavirus-reactive CD8⁺ T cells in the absence of neutralizing antibodies are sufficient for protection from hantavirus-associated disease and hantavirus clearance. Future transfer studies using animal models have to verify this idea.

(D) It is conceivable that not every human individual infected with pathogenic hantavirus develops symptoms. Thus, it should be investigated whether different qualities of virus-induced CD8⁺ T cell responses are responsible for the different outcomes of hantavirus infection, i.e., hantavirus clearance without or with symptoms (immunopathogenesis).

Acknowledgements

The authors are grateful for the continuous support of their work by Deutsche Forschungsgemeinschaft (currently grants KR1293/9-1 and Graduiertenkolleg 1121). In addition, B.K. acknowledges financial support by the European Commission (European Virus Archive, FP7 CAPACITIES project—GA n° 228292) and the Slovak Scientific Grant Agency VEGA (grant 2/0189/09). We thank Brian Hjelle and Jiro Arikawa for critical comments.

References

- Kruger DH, Ulrich R, Lundkvist AH. Hantavirus infections and their prevention. *Microbes Infect* 2001; 3:1129-44.
- Lee HW, van der GG. Hemorrhagic fever with renal syndrome. *Prog Med Virol* 1989; 36:62-102.
- Mertz GJ, Hjelle B, Crowley M, Iwamoto G, Tomicic V, Vial PA. Diagnosis and treatment of new world hantavirus infections. *Curr Opin Infect Dis* 2006; 19:437-42.
- Peters CJ, Simpson GL, Levy H. Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. *Annu Rev Med* 1999; 50:531-45.
- Faber MS, Ulrich RG, Frank C, Brockmann SO, Pfaff GM, Jacob J, et al. Steep rise in notified hantavirus infections in Germany, April 2010. *Euro Surveill* 2010; 20:15.
- Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, et al. Hantavirus in African wood mouse, Guinea. *Emerg Infect Dis* 2006; 12:838-40.
- Klempa B, Koivogui L, Sylla O, Koulemou K, Auste B, Kruger DH, ter Meulen J. Serological evidence of human hantavirus infections in Guinea, West Africa. *J Infect Dis* 2010; 201:1031-4.
- Schmaljohn CS, Nichol ST. *Bunyaviridae*. In: Knipe DM and Howley PM, Eds. *Fields Virology*. Philadelphia: Lippencott Williams & Wilkins 2007; 5:1741-89.
- Klempa B, Tkachenko EA, Dzagurova TK, Yunicheva YV, Morozov VG, Okulova NM, et al. Hemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. *Emerg Infect Dis* 2008; 14:617-25.
- Kruger DH, Klempa B. Dobrava-Belgrade virus. In: Liu D, Ed. *Molecular detection of human viral pathogens*. Boca Raton: CRC Press 2011; 631-8.
- Hooper JW, Ferro AM, Wahl-Jensen V. Immune serum produced by DNA vaccination protects hamsters against lethal respiratory challenge with Andes virus. *J Virol* 2008; 82:1332-8.
- Hammerbeck CD, Wahl-Jensen V, Hooper JW. Hantavirus. In: Barrett ADT, Stanberry LR, Eds. *Vaccines for biodefense and emerging and neglected diseases*. London: Academic Press/Elsevier 2009; 379-412.
- Chaparro J, Vega J, Terry W, Vera JL, Barra B, Meyer R, et al. Assessment of person-to-person transmission of hantavirus pulmonary syndrome in a Chilean hospital setting. *J Hosp Infect* 1998; 40:281-5.
- Enria D, Padula P, Segura EL, Pini N, Edelstein A, Posse CR, Weissenbacher MC. Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission. *Medicina (B Aires)* 1996; 56:709-11.
- Ferres M, Vial P, Marco C, Yanez L, Godoy P, Castillo C, et al. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile. *J Infect Dis* 2007; 195:1563-71.
- Martinez VP, Bellomo C, San Juan J, Pinna D, Forlenza R, Elder M, Padula PJ. Person-to-person transmission of Andes virus. *Emerg Infect Dis* 2005; 11:1848-53.
- Padula PJ, Edelstein A, Miguel SD, Lopez NM, Rossi CM, Rabinovich RD. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology* 1998; 241:323-30.
- Galeño H, Mora J, Villagra E, Fernandez J, Hernandez J, Mertz GJ, Ramirez E. First human isolate of Hantavirus (Andes virus) in the Americas. *Emerg Infect Dis* 2002; 8:657-61.
- Muranyi W, Bahr U, Zeier M, van der Woude FJ. Hantavirus infection. *J Am Soc Nephrol* 2005; 16:3669-79.
- Kariwa H, Yoshimatsu K, Arikawa J. Hantavirus infection in East Asia. *Comp Immunol Microbiol Infect Dis* 2007; 30:341-56.
- Jonsson CB, Figueiredo LT, Vapalahti O. A global perspective on hantavirus ecology, epidemiology and disease. *Clin Microbiol Rev* 2010; 23:412-41.
- Maes P, Klempa B, Clement J, Matthijssens J, Gajdusek DC, Kruger DH, Van Ranst M. A proposal for new criteria for the classification of hantaviruses, based on S and M segment protein sequences. *Infect Genet Evol* 2009; 9:813-20.
- Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA. *Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses*. London: Elsevier Academic Press 2005.
- Raftery MJ, Kraus AA, Ulrich R, Kruger DH, Schonrich G. Hantavirus infection of dendritic cells. *J Virol* 2002; 76:10724-33.

25. Leduc JW, Ksiazek TG, Rossi CA, Dalrymple JM. A retrospective analysis of sera collected by the Hemorrhagic Fever Commission during the Korean Conflict. *J Infect Dis* 1990; 162:1182-4.
26. Alexeyev OA, Ahlm C, Billheden J, Settergren B, Wadell G, Juto P. Elevated levels of total and Puumala virus-specific immunoglobulin E in the Scandinavian type of hemorrhagic fever with renal syndrome. *Clin Diagn Lab Immunol* 1994; 1:269-72.
27. Markotic A, Dasic G, Gagro A, Sabioncello A, Rabatic S, Kuzman I, et al. Role of peripheral blood mononuclear cell (PBMC) phenotype changes in the pathogenesis of haemorrhagic fever with renal syndrome (HFRS). *Clin Exp Immunol* 1999; 115:329-34.
28. Gott P, Zoller L, Darai G, Bautz EK. A major antigenic domain of hantaviruses is located on the aminoproximal site of the viral nucleocapsid protein. *Virus Genes* 1997; 14:31-40.
29. Kallio-Kokko H, Lundkvist A, Plyusnin A, Avsic-Zupanc T, Vaheri A, Vapalahti O. Antigenic properties and diagnostic potential of recombinant Dobrava virus nucleocapsid protein. *J Med Virol* 2000; 61:266-74.
30. Kallio-Kokko H, Leveelahti R, Brummer-Korvenkontio M, Lundkvist A, Vaheri A, Vapalahti O. Human immune response to Puumala virus glycoproteins and nucleocapsid protein expressed in mammalian cells. *J Med Virol* 2001; 65:605-13.
31. Lundkvist A, Björsten S, Niklasson B, Ahlberg N. Mapping of B-cell determinants in the nucleocapsid protein of Puumala virus: definition of epitopes specific for acute immunoglobulin G recognition in humans. *Clin Diagn Lab Immunol* 1995; 2:82-6.
32. Lundkvist A, Kallio-Kokko H, Sjolander KB, Lankinen H, Niklasson B, Vaheri A, Vapalahti O. Characterization of Puumala virus nucleocapsid protein: identification of B-cell epitopes and domains involved in protective immunity. *Virology* 1996; 216:397-406.
33. Lundkvist A, Vapalahti O, Plyusnin A, Sjolander KB, Niklasson B, Vaheri A. Characterization of Tula virus antigenic determinants defined by monoclonal antibodies raised against baculovirus-expressed nucleocapsid protein. *Virus Res* 1996; 45:29-44.
34. Tischler ND, Galeno H, Roseblatt M, Valenzuela PD. Human and rodent humoral immune responses to Andes virus structural proteins. *Virology* 2005; 334:319-26.
35. Zoller L, Scholz J, Stohwasser R, Giebel LB, Sethi KK, Bautz EK, Darai G. Immunoblot analysis of the serological response in Hantavirus infections. *J Med Virol* 1989; 27:231-7.
36. Maes P, Clement J, Gavrilovskaya I, Van Ranst M. Hantaviruses: immunology, treatment and prevention. *Viral Immunol* 2004; 17:481-97.
37. Yoshimatsu K, Yoo YC, Yoshida R, Ishihara C, Azuma I, Arikawa J. Protective immunity of Hantaan virus nucleocapsid and envelope protein studied using baculovirus-expressed proteins. *Arch Virol* 1993; 130:365-76.
38. Valdivieso F, Vial P, Ferres M, Ye C, Goade D, Cuiza A, Hjelle B. Neutralizing antibodies in survivors of Sin Nombre and Andes hantavirus infection. *Emerg Infect Dis* 2006; 12:166-8.
39. Ye C, Prescott J, Nofchissey R, Goade D, Hjelle B. Neutralizing antibodies and Sin Nombre virus RNA after recovery from hantavirus cardiopulmonary syndrome. *Emerg Infect Dis* 2004; 10:478-82.
40. Horling J, Lundkvist A, Huggins JW, Niklasson B. Antibodies to Puumala virus in humans determined by neutralization test. *J Virol Methods* 1992; 39:139-47.
41. Lundkvist A, Horling J, Niklasson B. The humoral response to Puumala virus infection (nephropathia epidemica) investigated by viral protein specific immunoassays. *Arch Virol* 1993; 130:121-30.
42. Manigold T, Mori A, Graumann R, Llop E, Simon V, Ferres M, et al. Highly differentiated, resting gn-specific memory CD8⁺ T cells persist years after infection by andes hantavirus. *PLoS Pathog* 2010; 6:1000779.
43. Chu YK, Jennings G, Schmaljohn A, Elgh F, Hjelle B, Lee HW, et al. Cross-neutralization of hantaviruses with immune sera from experimentally infected animals and from hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome patients. *J Infect Dis* 1995; 172:1581-4.
44. Hooper JW, Custer DM, Thompson E, Schmaljohn CS. DNA vaccination with the Hantaan virus M gene protects hamsters against three of four HFRS hantaviruses and elicits a high-titer neutralizing antibody response in Rhesus monkeys. *J Virol* 2001; 75:8469-77.
45. Custer DM, Thompson E, Schmaljohn CS, Ksiazek TG, Hooper JW. Active and passive vaccination against hantavirus pulmonary syndrome with Andes virus M genome segment-based DNA vaccine. *J Virol* 2003; 77:9894-905.
46. Hooper JW, Custer DM, Smith J, Wahl-Jensen V. Hantaan/Andes virus DNA vaccine elicits a broadly cross-reactive neutralizing antibody response in nonhuman primates. *Virology* 2006; 347:208-16.
47. de Carvalho NC, Gonzalez D, Padula PV, Björling E, Plyusnin A, Lundkvist A. Cross-protection against challenge with Puumala virus after immunization with nucleocapsid proteins from different hantaviruses. *J Virol* 2002; 76:6669-77.
48. Bharadwaj M, Nofchissey R, Goade D, Koster F, Hjelle B. Humoral immune responses in the hantavirus cardiopulmonary syndrome. *J Infect Dis* 2000; 182:43-8.
49. MacNeil A, Comer JA, Ksiazek TG, Rollin PE. Sin Nombre virus-specific immunoglobulin M and G kinetics in hantavirus pulmonary syndrome and the role played by serologic responses in predicting disease outcome. *J Infect Dis* 2010; 202:242-6.
50. Schmaljohn CS, Chu YK, Schmaljohn AL, Dalrymple JM. Antigenic subunits of Hantaan virus expressed by baculovirus and vaccinia virus recombinants. *J Virol* 1990; 64:3162-70.
51. Hooper JW, Kamrud KI, Elgh F, Custer D, Schmaljohn CS. DNA vaccination with hantavirus M segment elicits neutralizing antibodies and protects against seoul virus infection. *Virology* 1999; 255:269-78.
52. Pensiero MN, Jennings GB, Schmaljohn CS, Hay J. Expression of the Hantaan virus M genome segment by using a vaccinia virus recombinant. *J Virol* 1988; 62:696-702.
53. Xu X, Ruo SL, McCormick JB, Fisher-Hoch SP. Immunity to Hantavirus challenge in Meriones unguiculatus induced by vaccinia-vectored viral proteins. *Am J Trop Med Hyg* 1992; 47:397-404.
54. Klingstrom J, Stoltz M, Hardestam J, Ahlm C, Lundkvist A. Passive immunization protects cynomolgus macaques against Puumala hantavirus challenge. *Antivir Ther* 2008; 13:125-33.
55. Huang C, Jin B, Wang M, Li E, Sun C. Hemorrhagic fever with renal syndrome: relationship between pathogenesis and cellular immunity. *J Infect Dis* 1994; 169:868-70.
56. Kilpatrick ED, Terajima M, Koster FT, Catalina MD, Cruz J, Ennis FA. Role of specific CD8⁺ T cells in the severity of a fulminant zoonotic viral hemorrhagic fever, hantavirus pulmonary syndrome. *J Immunol* 2004; 172:3297-304.
57. Van Epps HL, Terajima M, Mustonen J, Arstila TP, Corey EA, Vaheri A, Ennis FA. Long-lived memory T lymphocyte responses after hantavirus infection. *J Exp Med* 2002; 196:579-88.
58. Van Epps HL, Schmaljohn CS, Ennis FA. Human memory cytotoxic T-lymphocyte (CTL) responses to Hantaan virus infection: identification of virus-specific and cross-reactive CD8(+) CTL epitopes on nucleocapsid protein. *J Virol* 1999; 73:5301-8.
59. Björkström NKL T, Stoltz M, Fauriat C, Braun M, Michaëlsson J, Klingström J, et al. Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. *J Exp Med* 2011; 208: 13-21.
60. Wang PZ, Huang CX, Zhang Y, Li ZD, Ye HT, Zhang Y, et al. Analysis of the immune response to Hantaan virus nucleocapsid protein C-terminal-specific CD8(+) T cells in patients with hemorrhagic fever with renal syndrome. *Viral Immunol* 2009; 22:253-60.
61. Kaukinen P, Vaheri A, Plyusnin A. Hantavirus nucleocapsid protein: a multifunctional molecule with both housekeeping and ambassadorial duties. *Arch Virol* 2005; 150:1693-713.
62. Sen N, Sen A, Mackow ER. Degradation at the C terminus of the pathogenic but not the nonpathogenic hantavirus G₁ tail direct proteasomal degradation. *J Virol* 2007; 81:4323-30.
63. Safronetz D, Hegde NR, Ebihara H, Denton M, Kobinger GP, St JS, et al. Adenovirus vectors expressing hantavirus proteins protect hamsters against lethal challenge with Andes virus. *J Virol* 2009; 83:7285-95.
64. Manigold T, Martinez J, Lazzano X, Ye C, Schwartz S, Cuiza A, et al. Case report: T-cell responses during clearance of Andes virus from blood cells 2 months after severe hantavirus cardiopulmonary syndrome. *J Med Virol* 2008; 80:1947-51.
65. Dargeviciute A, Brus SK, Sasnauskas K, Kruger DH, Meisel H, Ulrich R, Lundkvist A. Yeast-expressed Puumala hantavirus nucleocapsid protein induces protection in a bank vole model. *Vaccine* 2002; 20:3523-31.
66. Ulrich R, Lundkvist A, Meisel H, Koletzki D, Sjolander KB, Gelderblom HR, et al. Chimeric HBV core particles carrying a defined segment of Puumala hantavirus nucleocapsid protein evoke protective immunity in an animal model. *Vaccine* 1998; 16:272-80.
67. Schonrich G, Rang A, Luttkes N, Raftery MJ, Charbonnel N, Ulrich RG. Hantavirus-induced immunity in rodent reservoirs and humans. *Immunol Rev* 2008; 225:163-89.
68. Terajima M, Hayasaka D, Maeda K, Ennis FA. Immunopathogenesis of hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome: Do CD8⁺ T cells trigger capillary leakage in viral hemorrhagic fevers? *Immunol Lett* 2007; 113:117-20.
69. Wang M, Wang J, Zhu Y, Xu Z, Yang K, Yang A, Jin B. Cellular immune response to Hantaan virus nucleocapsid protein in the acute phase of hemorrhagic fever with renal syndrome: correlation with disease severity. *J Infect Dis* 2009; 199:188-95.
70. Harty JT, Twinnereim AR, White DW. CD8⁺ T cell effector mechanisms in resistance to infection. *Annu Rev Immunol* 2000; 18:275-308.
71. Guidotti LG, Chisari FV. Nontoxic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; 19:65-91.
72. Makela S, Mustonen J, Ala-Houhala I, Hurme M, Partanen J, Vapalahti O, et al. Human leukocyte antigen-B8-DR3 is a more important risk factor for severe Puumala hantavirus infection than the tumor necrosis factor-alpha(-308) G/A polymorphism. *J Infect Dis* 2002; 186:843-6.
73. Mustonen J, Partanen J, Kanerva M, Pietila K, Vapalahti O, Pasternack A, Vaheri A. Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. *Kidney Int* 1996; 49:217-21.
74. Hjelle B. Vaccines against hantaviruses. *Expert Rev Vaccines* 2002; 1:373-84.
75. Maes P, Clement J, Van RM. Recent approaches in hantavirus vaccine development. *Expert Rev Vaccines* 2009; 8:67-76.
76. Schmaljohn C. Vaccines for hantaviruses. *Vaccine* 2009; 27:61-4.
77. Zhang YZ, Zou Y, Fu ZF, Plyusnin A. Hantavirus infections in humans and animals, China. *Emerg Infect Dis* 2010; 16:1195-203.
78. Boudreau E, Sellers K, Rusnak J, Rivard R, Bovni D, Joselyn M, et al. Phase I clinical study on the safety, tolerability and immunogenicity of Hantaan and Puumala DNA vaccines. Abstracts of the VIII International Conference on HFRS HPS and Hantavirus, Athens, Greece 2010; 83.
79. Li D. Trends of HFRS epidemiology and the expanded program on immunization with hantavirus vaccines in China. Abstracts of the VIII International Conference on HFRS HPS and Hantavirus, Athens, Greece 2010; 82.

80. Abu Daude NH, Kariwa H, Tkachenko E, Dzagurova T, Medvedkina O, Tkachenko P, et al. Genetic and antigenic analyses of a Puumala virus isolate as a potential vaccine strain. *Jpn J Vet Res* 2008; 56:151-65.
81. Handke W, Oelschlegel R, Franke R, Wiedemann L, Kruger DH, Rang A. Generation and characterization of genetic reassortants between Puumala and Prospect Hill hantavirus in vitro. *J Gen Virol* 2010; 91:2351-9.
82. Yuan ZG, Li XM, Mahmmod YS, Wang XH, Xu HJ, Zhang XX. A single immunization with a recombinant canine adenovirus type 2 expressing the seoul virus Gn glycoprotein confers protective immunity against seoul virus in mice. *Vaccine* 2009; 27:5247-51.
83. Yuan ZG, Luo SJ, Xu HJ, Wang XH, Li J, Yuan LG, He LT, Zhang XX. Generation of E3-deleted canine adenovirus type 2 expressing the Gc glycoprotein of Seoul virus by gene insertion or deletion of related terminal region sequences. *J Gen Virol* 2010; 91:1764-71.
84. Ulrich R, Nassal M, Meisel H, Kruger DH. Core particles of hepatitis B virus as carrier for foreign epitopes. *Adv Virus Res* 1998; 50:141-82.
85. Li C, Liu F, Liang M, Zhang Q, Wang X, Wang T, et al. Hantavirus-like particles generated in CHO cells induce specific immune responses in C57BL/6 mice. *Vaccine* 2010; 28:4294-300.
86. Severson WE, Schmaljohn CS, Javadian A, Jonsson CB. Ribavirin causes error catastrophe during Hantaan virus replication. *J Virol* 2003; 77:481-8.
87. Huggins JW, Kim GR, Brand OM, McKee KT Jr. Ribavirin therapy for Hantaan virus infection in suckling mice. *J Infect Dis* 1986; 153:489-97.
88. Huggins JW, Hsiang CM, Cosgriff TM, Guang MY, Smith JJ, Wu ZO, et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis* 1991; 164:1119-27.
89. Rusnak JM, Byrne WR, Chung KN, Gibbs PH, Kim TT, Boudreau EF, et al. Experience with intravenous ribavirin in the treatment of hemorrhagic fever with renal syndrome in Korea. *Antiviral Res* 2009; 81:68-76.
90. Chapman LE, Mertz GJ, Peters CJ, Jolson HM, Khan AS, Ksiazek TG, et al. Intravenous ribavirin for hantavirus pulmonary syndrome: safety and tolerance during 1 year of open-label experience. Ribavirin Study Group. *Antivir Ther* 1999; 4:211-9.
91. Mertz GJ, Miedzinski L, Goade D, Pavia AT, Hjelle B, Hansbarger CO, et al. Placebo-controlled, double-blind trial of intravenous ribavirin for the treatment of hantavirus cardiopulmonary syndrome in North America. *Clin Infect Dis* 2004; 39:1307-13.
92. Chung DH, Kumarapperuma SC, Sun Y, Li Q, Chu YK, Arterburn JB, et al. Synthesis of 1-beta-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole and its in vitro and in vivo efficacy against Hantavirus. *Antiviral Res* 2008; 79:19-27.
93. Chung DH, Strouse JJ, Sun Y, Arterburn JB, Parker WB, Jonsson CB. Synthesis and anti-Hantaan virus activity of N(1)-3-fluorophenyl-inosine. *Antiviral Res* 2009; 83:80-5.
94. Hall PR, Malone L, Sillerud LO, Ye C, Hjelle BL, Larson RS. Characterization and NMR solution structure of a novel cyclic pentapeptide inhibitor of pathogenic hantaviruses. *Chem Biol Drug Des* 2007; 69:180-90.
95. Larson RS, Brown DC, Ye C, Hjelle B. Peptide antagonists that inhibit Sin Nombre virus and hantaan virus entry through the beta3-integrin receptor. *J Virol* 2005; 79:7319-26.
96. Hall PR, Hjelle B, Brown DC, Ye C, Bondu-Hawkins V, Kilpatrick KA, Larson RS. Multivalent presentation of antihantavirus peptides on nanoparticles enhances infection blockade. *Antimicrob Agents Chemother* 2008; 52:2079-88.
97. Hall PR, Leitao A, Ye C, Kilpatrick K, Hjelle B, Oprea TI, Larson RS. Small molecule inhibitors of hantavirus infection. *Bioorg Med Chem Lett* 2010; 20:7085-91.
98. Jonsson CB, Hooper J, Mertz G. Treatment of hantavirus pulmonary syndrome. *Antiviral Res* 2008; 78:162-9.
99. Groen J, Gerding M, Koeman JP, Roholl PJ, van Amerongen G, Jordans HG, et al. A macaque model for hantavirus infection. *J Infect Dis* 1995; 172:38-44.
100. Sironen T, Klingstrom J, Vaheri A, Andersson LC, Lundkvist A, Plyusnin A. Pathology of Puumala hantavirus infection in macaques. *PLoS One* 2008; 3:3035.
101. Hooper JW, Larsen T, Custer DM, Schmaljohn CS. A lethal disease model for hantavirus pulmonary syndrome. *Virology* 2001; 289:6-14.