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Treatment of hantavirus pulmonary syndrome

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Abstract

Viruses in the genus *Hantavirus* can cause one of two serious illnesses when transmitted from rodents to humans: hemorrhagic fever with renal syndrome (HFRS) or hantavirus pulmonary syndrome (HPS). Of the two diseases, HPS is more severe with an approximate 40% mortality across the Americas. The high rate of mortality could be reduced if effective therapeutics could be discovered for treatment of this illness. Herein we review approaches being explored for the discovery of therapeutics for HPS and how they could be employed in treatment and prevention of disease.

Keywords: Hantavirus; HPS; HFRS; Hantavirus pulmonary syndrome; Bunyavirus; Antiviral therapy; Ribavirin

1. Introduction

Hantaviruses cause two types of serious illness when transmitted from their rodent reservoirs to humans: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) (Lee and Johnson, 1982; Wong et al., 1988; Peters and Khan, 2002). These viruses are harbored by both Old-World and New-World rodents, and hence, their epidemiology reflects the geographical restriction imposed by the host range of the rodent vector (Plyusnin and Morzunov, 2001). Hantaviruses first came to the attention of western medicine in the early 1950s when more than 3000 US troops fighting in the Korean war became ill with Korean hemorrhagic fever, which later came to be known as HFRS (Johnson, 2004; Maes et al., 2004). The wave of HFRS cases presumably resulted from a high contact rate with rodents chronically infected with Hantaan virus (HTNV) as soldiers lived and fought in the open fields. The second category of illness, HPS, was first recognized in 1993 when an outbreak of severe respiratory disease struck in the Four Corners region of the US (Nichol et al., 1993). The hantavirus responsible for this disease, Sin Nombre virus (SNV), is harbored by the deer mouse (*Peromyscus maniculatus*).

Since the Four Corners outbreak, more than 2000 cases of HPS have occurred in sporadic fashion throughout the Americas, leading to the discovery of many different strains of these viruses and their associated rodent reservoirs (Barclay and Rubinstein, 1997; Bayard et al., 2004; Bohlman et al., 2002; Chu et al., 2006; Figueiredo et al., 2003; Fulhorst et al., 1997; Hjelle et al., 1996; Johnson et al., 1997, 1999; Levis et al., 1997; Lopez et al., 1996; Vincent et al., 2000; Williams et al., 1997). In addition to the United State and Canada, HPS cases have been confirmed in Argentina, Bolivia, Brazil, Chile, Paraguay, Panama and Uruguay. The initial Four Corners outbreak, followed by the many others throughout the Americas over the past 15 years, has elevated attention to these viruses as a global health problem.

In addition to their recognition as a global health problem, hantaviruses have been on and off the Centers for Disease Control and Prevention Category A list of potential bioterror agents, reflecting ambiguity as to the threat posed by these viruses (Bronze et al., 2002). When hantaviruses are viewed individually, rather than as a genus, it becomes obvious why certain hantaviruses pose a greater bioterror threat than others. For example, the South American Andes virus (ANDV) has continued to have a high mortality (30–50% HPS case–fatality rate) and is the only hantavirus for which there is evidence of person-to-person transmission (Wells et al., 1997). All of the HPS-causing viruses show a rapid disease course with serious pulmonary symptoms. Cases usually appear in rural areas, mandating transport of the patient to the nearest hospital. Hence,

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the clinical course of the disease, whether it occurs through an intentional act or accidental exposure, requires rapid diagnostic tests and treatment.

2. Classification, structure and replication strategy

Members of the genus *Hantavirus*, family *Bunyaviridae*, have a tri-segmented, negative-sense, single-strand RNA genome enclosed within a membrane derived from the Golgi apparatus (Schmaljohn et al., 1983; Schmaljohn and Hooper, 2001). The three gene segments, L, S, and M encode the L protein, nucleocapsid protein (N), and envelope glycoproteins (Gn and Gc; previously G1 and G2), respectively. Hantaviruses enter host endothelial cells via interaction of the larger viral glycoprotein (Gn) with the host's cell surface receptor(s); β1 and β3 integrins (Gavrilovskaya et al., 1999, 1998). Following entry, the precise steps are unknown, however, it is presumed that the virus is uncoated to liberate the three nucleocapsids that contain genomic RNA complexed with N and L proteins. Transcription, replication and assembly occur within the cytoplasm with L and S transcripts translated on free ribosomes, while the Gn/Gc precursor is co-translated on the rough endoplasmic reticulum (Schmaljohn and Hooper, 2001). For the Old-World hantaviruses, experimental evidence clearly shows that the viral envelope derives from budding into the Golgi apparatus, while New-World viruses may mature at the plasma membrane (Ravkov and Compans, 2001). The molecular determinant(s) responsible for differences in the clinical course of the two diseases are unknown.

3. Clinical syndrome

Hantaviruses cause a spectrum of vascular-leak syndromes in humans ranging from proteinuria to pulmonary edema and frank hemorrhage (Khan and Khan, 2003; Peters and Khan, 2002; Peters et al., 1999; Plyusnin et al., 2001; Schmaljohn and Hjelle, 1997). Old-World hantaviruses have been associated with a mild-to-severe disease, HFRS, that is characterized by fever, vascular leakage resulting in hemorrhagic manifestations, and renal failure (Lee, 1982; Lee and van der Groen, 1989; Vapalahti et al., 2003).

HPS, caused by New-World hantaviruses, has been associated with a much higher rate of fatal illness. The illness is characterized by fever and vascular leakage resulting in noncardiogenic pulmonary edema followed in severe cases by shock with lactic acidosis, a low cardiac index and elevated systemic vascular resistance (Enria et al., 2001). Many authors prefer the term hantavirus cardiopulmonary syndrome (HCPS) to emphasize the important role of cardiogenic shock; among hospitalized patients, death almost invariably results from cardiogenic shock rather than respiratory failure (Hallin et al., 1996; Ferrés et al., 2007; Mertz et al., 2006, 2004; Saggioro et al., 2007; Vial et al., 2006). The illness caused by SNV and related New-World viruses bears some resemblance to HFRS, except that the lungs are targeted for capillary leakage instead of the kidneys (Moolenaar et al., 1997, 1995; Zaki et al., 1995). Case–fatality ratios for HPS caused by the most prevalent North American and South American hantaviruses, SNV and ANDV, respectively, range from 30 to 50% (Doyle et al., 1998), while other strains such as the Leguna Negra (Paraguay) and Juquitaba viruses (Brazil) have a much lower mortality (∼15%).

In striking contrast to all other HPS- and HFRS-causing viruses, ANDV, found in Chile and Argentina, is associated with person-to-person transmission (Chaparro et al., 1998; Enria et al., 1996; Ferrés et al., 2007; Martinez et al., 2005; Padula et al., 1998; Vitek et al., 1996; Wells et al., 1997). In Chile, the risk of person-to-person transmission is greatest among close household contacts (sex partners and persons who sleep in the same bed or room) of index cases. In a recent prospective study of household contacts of index patients with HPS in Chile, the overall risk of secondary infection within the household was 3.4%. However, the risk was 17.6% among sex partners of index cases, versus 1.2% among other household contacts (Ferrés et al., 2007).

Among HPS cases in which a short, defined exposure in a high-risk area could be determined, the median incubation period from exposure to the onset of symptoms was 18–19 days with a range of 11–32 days for ANDV (Mertz et al., 2006; Vial et al., 2006) and 9–33 days for SNV (Young et al., 2000). The true range is certainly greater (one reported case had an incubation period of 46–51 days), but well-documented short incubation periods have not been reported. Also, in a prospective study of household contacts of index cases with HPS in Chile, ANDV RNA could be detected in peripheral blood cells by RT-PCR up to 2 weeks before the onset of symptoms or the appearance of anti-hantavirus antibodies in persons who subsequently developed HPS (Ferrés et al., 2007).

The diagnosis, clinical course and supportive care for patients with New-World hantaviral infections have recently been reviewed (Mertz et al., 2006). The clinical course can be broken into five distinct phases, with some variation in incidence and severity of symptoms among patients (Fig. 1). Following the incubation period, the patient develops a prodrome of fever, myalgia, and progressively worsening thrombocytopenia, often accompanied by headache, back pain, abdominal pain, and diar-

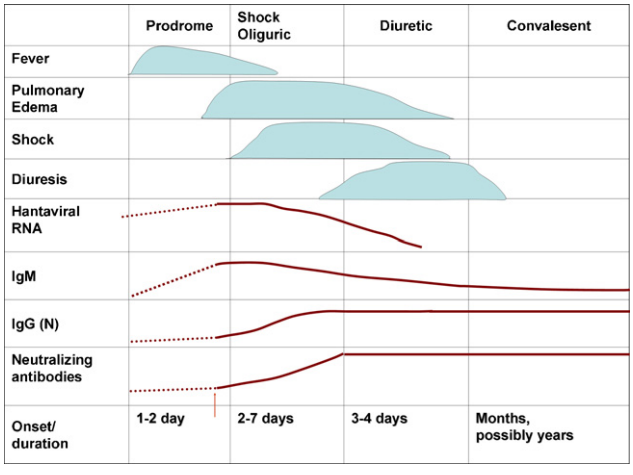

Fig. 1. Clinical course of hantavirus pulmonary syndrome.

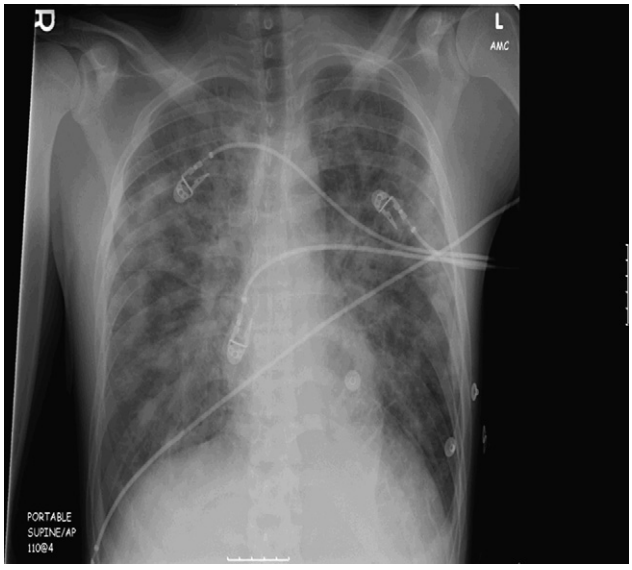

Fig. 2. Chest radiograph of an HPS patient. Portable, supine AP chest radiograph of a patient in the cardiopulmonary phase at the time of presentation to the critical care unit, showing diffuse alveolar filling and small, bilateral pleural effusions. The size of the cardiac silhouette appears normal. Within hours the patient required mechanical ventilation and extracorporeal membrane oxygenation (ECMO). Courtesy G. Mertz, University of New Mexico.

rhea. Both IgM and IgG antibodies can typically be detected at or shortly after the onset of the prodrome.

The cardiopulmonary phase typically begins with cough, shortness of breath and the development of bilateral pulmonary infiltrates (Fig. 2) as well as the development of typical abnormalities in peripheral blood smear findings that may allow clinicians to establish a presumptive diagnosis with a high degree of confidence (Ketai et al., 1994; Mertz et al., 2004, 2006). Patients with mild HPS may only require supplemental oxygen, but those with severe disease will progress quickly to respiratory failure, with or without shock. Deaths, almost invariably due to shock, may occur within hours of the onset of the cardiopulmonary phase. In the absence of extracorporeal membrane oxygenation (ECMO, Fig. 3), almost all deaths occur within 24–48 h of the onset of this phase. Cardiac output and respiratory function should be monitored carefully, and cardiac function should be followed particularly closely after intubation or initiation of positive end expiratory pressure (PEEP). Cardiac output should be maintained with agents such as dobutamine, and volume replacement should be used very cautiously. For hospitals in which it is feasible, the indications for venous-arterial ECMO have recently been reviewed (Mertz et al., 2006). After several days, surviving patients enter a diuretic phase. Improvement is usually rapid, and most ventilator-dependent patients can be extubated within a day or two. The convalescent phase, which is characterized by weakness, fatigue, and abnormal gaseous diffusion capacity of the lungs, may persist for months or years.

Because the timeframe in which patients become ill and seek hospitalization coincides with a decrease in the presence of the virus, therapeutic interventions that target viral replication may not be effective unless given very early. Unfortunately, while virus may be detectable by RT-PCR in peripheral blood cells up to 2 weeks before the onset of symptoms, and IgM and IgG antibodies are typically detectable for several days during the febrile prodrome, HPS is rarely recognized until after the onset of the cardiopulmonary phase, at a point when progression to respiratory failure, shock and death may occur within hours. The evaluation of the efficacy of antiviral and antibody therapies in animal models should take this into consideration.

4. Animal models of HPS

Animal models exist for both chronic hantavirus infection and acute disease. They include traditional laboratory animals, such as mice, hamsters, macaques, and natural rodent hosts of hantavirus infection, such as bank voles, cotton rats and deer mice. Models involving natural rodent hosts show persistent, life-long infections (Botten et al., 2003; Compton et al., 2004; Tanishita et al., 1986). In contrast, models utilizing mice, rats, gerbils, and nonhuman primates involve acute infection, which may be asymptomatic (Kurata et al., 1983; Xu et al., 1992; Yanagihara et al., 1988; Schmaljohn et al., 1990) or, in the recently described hamster model, involve lethal disease (Hooper et al., 2001b). Given that patients with HFRS and HPS are viremic during the acute phase of disease, then clear the virus (Antoniades et al., 1987; Yao et al., 1989), an acute model of viral infection is preferable for efficacy studies.

Newborn and adult rodents have been used extensively to evaluate vaccines and antivirals for the treatment of HFRS (Huggins et al., 1986). Suckling mice infected with HTNV develop a systemic lethal disease with pronounced signs of neuropathology, and die within 3 weeks (Kurata et al., 1983; McKee et al., 1985). The broad spectrum antiviral drug ribavirin was the only promising therapeutic brought forward from efficacy testing in suckling mice for clinical trials for HFRS (Huggins et al., 1991, 1986). Recently, ribavirin was tested for prophylactic efficacy in a deer mouse model of persistent infection (Medina et al., 2007). Ribavirin at 100 mg/kg administered concurrently with the challenge virus protected all mice against seroconversion; however, 4 of 6 animals demonstrated evidence of infection by immunohistochemistry and/or quantitative RT-PCR of heart tissue. In the same study, concurrent administration of the anti-$\alpha_v\beta_3$-integrin antibody, ReoPro, reduced viral load, as measured by quantitative RT-PCR. The potential of $\alpha_v\beta_3$-integrin as a drug target has also been demonstrated with in vitro studies of peptide inhibitors that blocked hantavirus entry (Larson et al., 2005).

Several years ago, ANDV was shown to cause a lethal disease resembling HPS in adult Syrian hamsters (Hooper et al., 2001b). The LD_{50} was only 8 PFU. Similarities between ANDV-associated disease in hamsters and HPS include an incubation period of 10–24 days in hamsters versus 12–27 days in humans; rapid progression from first symptoms to death; severe dyspnea, a fall in blood pressure and increased heart rate in the hours preceding death; pulmonary edema and fluid in the pleural cavity; similar histopathology in the lungs and spleen; hematologic abnormalities (i.e. lymphopenia, neutrophilia, mild thrombocytopenia, lymphocyte apoptosis) (Campen et al., 2006; Padula et al., 2000; Wahl-Jensen et al., 2007). However, hamsters show a

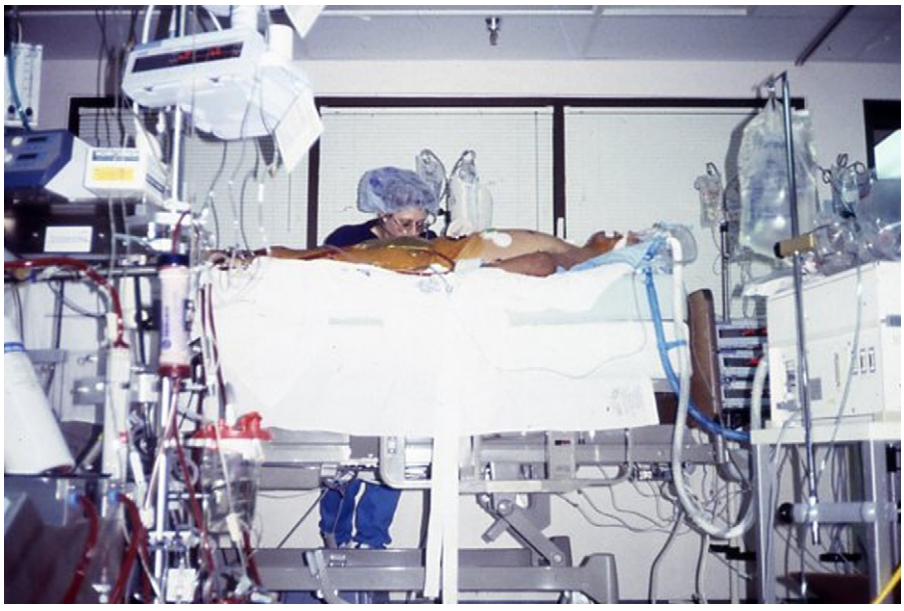

Fig. 3. Extracorporeal membrane oxygenation care of an HPS patient. An adult patient with HPS has been cannulated in the femoral artery and vein. The bed is elevated to improve the efficiency of the ECMO pump. Courtesy Mark Crowley, University of New Mexico.

high viral load in the liver, which is not generally present in HPS patients. The hamster model has been used to study the shock phase of HPS with ANDV (Campen et al., 2006). Although ANDV was infectious in mice and cynomolgus macaques (see below), it did not cause disease (Hooper et al., 2001a; McElroy et al., 2002). The mechanisms underlying the species-specific virulence of ANDV remain unknown.

Recently, a second hantavirus, Maporal, which was originally isolated from an arboreal rice rat captured in central Venezuela, was reported to cause disease in Syrian golden hamsters (Milazzo et al., 2002). The illness is clinically and pathologically similar to HPS with regard to its time course, the presence of virus-specific IgG at the onset of clinical disease, subacute pneumonitis, diffuse alveolar edema, mononuclear cell infiltrates in the lungs and liver, and the widespread distribution of hantaviral antigen in endothelial cells of the microvasculature of lung.

Old-World hantaviruses, including Prospect Hill virus and Puumala virus injected intravenously have been shown to infect nonhuman primates with some evidence of mild disease (i.e. proteinuria) (Yanagihara et al., 1988). Similarly, nonhuman primates infected intratracheally with single-passage Puumala virus developed mild disease similar to that observed in humans (Groen et al., 1995). The first demonstration of a New-World hantavirus infection of nonhuman primates by an HPS-causing virus was reported for ANDV in 2002 (McElroy et al., 2002). The natural history of ANDV was examined in cynomolgus macaques following intravenous or aerosol challenge. In contrast to PUUV infection, the monkeys did not manifest clinical disease, however, they did show a significant decrease in circulating lymphocytes between days 8 and 11 post-challenge. All animals developed IgM and IgG antibodies against the viral N protein and a neutralizing antibody response.

5. Antiviral therapy

Ribavirin was tested for efficacy in HFRS patients in China and shown to have a statistically significant beneficial effect if initiated early in the disease course (Huggins et al., 1991). Two double-blind, placebo-controlled efficacy trials have been performed in persons with HPS in the cardiopulmonary phase (Chapman et al., 1999; Mertz et al., 2004). The first was a trial of ribavirin conducted in the US and Canada by the NIAID-sponsored Collaborative Antiviral Study Group, and the second is an ongoing NIH-NIAID-sponsored controlled trial of intravenous methylprednisolone in Chile (Mertz et al., 2004; G. Mertz, personal communication). In the first trial, the majority of the patients were in the cardiopulmonary stage when they enrolled, and treatment with ribavirin had no clinical benefit, suggesting that its efficacy may depend on the phase of infection and the severity of disease when treatment is initiated and calling attention to the need for early intervention.

Using clinical, radiological and peripheral blood smear criteria, over 90% of the subjects enrolled in these trials based on a presumptive diagnosis of HPS in the cardiopulmonary phase have subsequently had the diagnosis confirmed. As such, few study subjects who are not infected with hantavirus are needlessly exposed to study drug. Another advantage of this approach is that any effective treatment could presumably be adopted wherever severe HPS is present, including Argentina, Bolivia, Brazil, Canada, Chile, Paraguay, the US and Uruguay. The major impediment of this design is that progression to respiratory failure, shock and death typically occurs within hours of presentation in the cardiopulmonary phase, leaving little time for the study intervention to have an effect.

Although intervention has not been attempted during the incubation period of HPS, and attempts to identify and treat patients during the febrile prodrome have not been successful, it

is conceivable that prophylaxis or early treatment studies could be feasible among recent household contacts of index cases with ANDV infection in Chile and Argentina. An established clinical trials network is in place in Chile, and person-to-person transmission of ANDV and case clusters are well described in both countries (Chaparro et al., 1998; Enria et al., 1996; Martinez et al., 2005; Padula et al., 1998). Subjects could be enrolled in prophylaxis studies based on risk factors that were significantly associated with development of HPS among recent household contacts and/or based on detection of ANDV RNA in peripheral blood cells by RT-PCR. Alternatively, recent contacts counseled about the risk of HPS could be enrolled in treatment studies at the onset of the febrile prodrome.

6. Immunotherapy

Administration of human neutralizing antibodies during the acute phase of HPS might prove effective for the treatment and/or prophylaxis of hantaviral infections. Bharadwaj et al. measured antibodies at hospital admission and found that patients with lower titers of neutralizing antibodies often had severe disease, while those with higher titers had mild disease (Bharadwaj et al., 2000). The authors speculated that a strong neutralizing antibody response, or passive immunotherapy, might effectively reduce viremia and promote recovery. Reduced levels of viremia at hospital admission, as measured by numbers of viral genomes in plasma, were associated with reduced severity of HPS caused by SNV (Xiao et al., 2006).

At present, there have been no published reports of controlled clinical trials of immunotherapy for HFRS or HPS. However, studies in mice, hamsters and rats have indicated that passive transfer of neutralizing mAbs or polyclonal sera to HTNV can passively protect animals from challenge with the same virus (Arikawa et al., 1992; Custer et al., 2003; Schmaljohn et al., 1990; Xu et al., 2002; Zhang et al., 1989). HTNV Gc-specific neutralizing mAbs, administered up to 4 days after challenge protected hamsters from infection, and up to 2 days after challenge protected suckling mice from lethal disease (Liang et al., 1996; Xu et al., 2002). Similarly, hamsters treated with immune plasma from ANDV patients and deer mice treated with plasma from SNV patients were protected against homologous virus challenge (Custer et al., 2003; Medina et al., 2007). Post-exposure administration of antibodies has been shown to confer protection. Passive transfer of sera from rhesus macaques, vaccinated with a DNA vector expressing the ANDV M segment, protected hamsters against lethal challenge with ANDV (250 LD_{50}) even when administered 5 days after challenge (Custer et al., 2003). Hamsters treated with immune serum 1 day before challenge with ANDV were either sterilely protected or developed HPS after several weeks; the late deaths were probably caused by the emergence of virus that was not eliminated by the passive antibody treatment (Custer et al., 2003). These data suggest that a post-exposure prophylaxis regimen consisting of passive immunoprophyaxis and active vaccination would be effective for HPS, as has been shown for other viral diseases such as rabies, hepatitis A and B, and varicella (Sawyer, 2000).

7. Vaccines

No FDA-approved vaccine for HPS is available in the United States. A killed-virus vaccine, similar to approaches used in China and Korea, is not being pursued for several reasons, including the dangers associated with the mass production of virus under high-containment (BSL-4 for large preparations of virus) and unresolved questions of the efficacy of killed-virus vaccines. However, a number of laboratories have been working to develop vaccines that employ viral antigens delivered by DNA vectors or as recombinant proteins. A vaccinia virus-vectored vaccine containing the M and S genes of HTNV was found to elicit neutralizing antibodies in humans; however, pre-existing immunity to vaccinia (smallpox vaccination) lessened its efficacy (McClain et al., 2000). In another approach, HTNV glycoprotein genes were used to pseudotype vesicular stomatitis virus (Lee et al., 2006). This vaccine elicited neutralizing antibodies in mice and protected against HTNV infection.

For plasmid DNA approaches, the basic strategy of most vaccine efforts has involved the M segment products which elicit a protective neutralizing antibody response. The first M segment-based DNA vaccine to elicit neutralizing antibodies contained the full-length SEOV M gene (Hooper et al., 1999). Mice or hamsters vaccinated with the SEOV M gene-based DNA vaccine using a gene gun developed neutralizing antibodies, and the hamsters were protected from infection with SEOV. A similar vaccine containing the full-length HTNV M gene elicited neutralizing antibodies in hamsters that cross-neutralized SEOV and DOBV, and protected against infection with those viruses (Hooper et al., 2001b). Both the SEOV and HTNV M gene-based DNA vaccines administered by gene gun elicited high-titer neutralizing antibodies in macaques (Hooper et al., 2001b).

The first DNA vaccine to elicit high-titer neutralizing antibodies against HPS hantaviruses contained the full-length ANDV M gene (Custer et al., 2003). In that study, rhesus macaques vaccinated with a gene gun developed neutralizing antibody titers as high as 1:20480. Sera from these animals cross-neutralized SNV and BCCV and protected hamsters against lethal challenge with ANDV. A DNA vaccine containing fragments of the SNV M gene protected deer mice from infection with SNV (Bharadwaj et al., 2002). A plasmid containing both the HTNV and ANDV full-length M genes will elicit antibodies that neutralize both HFRS and HPS-associated hantavirus, albeit with lower titers than the single gene constructs (Hooper et al., 2006). In that study, a long-range boost promoted a rapid memory response (high-titer neutralizing antibodies) in nonhuman primates vaccinated with HTNV, ANDV or dual HTNV/ANDV DNA vaccines. More recently a PUUV M gene-based DNA vaccine was produced (Hooper, unpublished data), and this construct, combined with the HTNV M gene-based DNA vaccine forms an HFRS DNA vaccine that the United States Army Medical Research Institute of Infectious Diseases is moving towards clinical trials.

The hantavirus N protein delivered as a recombinant protein (e.g. made in baculovirus, yeast, or *E. coli* systems; or on hepatitis B virus core particles) has been shown to provide protection against hantavirus challenge in small animal models (e.g. mice,

voles) for both Old-World and New-World viruses (Dargeviciute et al., 2002; de Carvalho Nicacio et al., 2002; Klingstrom et al., 2004; Maes et al., 2006; Lindkvist et al., 2007; Geldmacher et al., 2005). In addition, a SNV S gene-based DNA vaccine and a Puumala truncated S gene-based DNA vaccine demonstrated some protective efficacy in the deer mouse or bank vole models (2 of 5 animals), respectively (Bharadwaj et al., 2002; Bucht et al., 2001). The mechanism of protection afforded by nucleocapsid vaccines is unknown but presumably N promotes a protective cellular immune response. The fact that previous infection of hamsters with essentially any hantavirus protects them against a lethal ANDV infection, despite the absence of ANDV neutralizing antibodies, suggests that cell mediated immunity is sufficient to protect against hantaviruses (Hooper et al., 2001a,b).

8. Conclusion

HPS is a significant health threat in endemic areas because of the sporadic and unpredictable occurrence of disease in formerly healthy adults, its high case–fatality rate and absence of vaccines or drugs. Vaccination of individuals in endemic areas or those who could be exposed to the virus in military, clinical or research settings would be an important strategy toward reducing the incidence of disease. If vaccines are successfully developed and licensed, it is likely they would be used in populations at high risk of exposure, including persons in endemic regions performing tasks that put them in contact with rodent excreta (e.g. farm and forest workers, military personnel). In Chile and Argentina, medical personnel treating HPS patients would be considered an at-risk population because of the possibility of person-to-person transmission of ANDV.

Antiviral therapeutics offer an alternative strategy for dealing with outbreaks in areas previously unrecognized to harbor disease, accidental exposure in laboratory workers or deliberate introduction through bioterrorism. Unfortunately, except for ribavirin, no potential antivirals have been reported to show efficacy in animal models of HFRS or HPS. Thus, it is imperative to develop countermeasures to treat unprotected persons who have been exposed to these virulent pathogens.

References

Antoniades, A., Grekas, D., Rossi, C.A., LeDuc, J.W., 1987. Isolation of a hantavirus from a severely ill patient with hemorrhagic fever with renal syndrome in Greece. J. Infect. Dis. 156, 1010–1013.

Arikawa, J., Yao, J.S., Yoshimatsu, K., Takashima, I., Hashimoto, N., 1992. Protective role of antigenic sites on the envelope protein of Hantaan virus defined by monoclonal antibodies. Arch. Virol. 126, 271–281.

Barclay, C.M., Rubinstein, G., 1997. Hantavirus in Argentina. Nature 386, 320.

Bayard, V., Kitsutani, P.T., Barria, E.O., Ruedas, L.A., Tinnin, D.S., Munoz, C., de Mosca, I.B., Guerrero, G., Kant, R., Garcia, A., Caceres, L., Gracio, F.G., Quiroz, E., de Castillo, Z., Armien, B., Libel, M., Mills, J.N., Khan, A.S., Nichol, S.T., Rollin, P.E., Ksiazek, T.G., Peters, C.J., 2004. Outbreak of hantavirus pulmonary syndrome, Los Santos, Panama, 1999–2000. Emerg. Infect. Dis. 10, 1635–1642.

Bharadwaj, M., Nofchissey, R., Goade, D., Koster, F., Hjelle, B., 2000. Humoral immune responses in the hantavirus cardiopulmonary syndrome. J. Infect Dis. 182, 43–48.

Bharadwaj, M., Mirowsky, K., Ye, C., Botten, J., Masten, B., Yee, J., Lyons, C.R., Hjelle, B., 2002. Genetic vaccines protect against Sin Nombre hantavirus challenge in the deer mouse (Peromyscus maniculatus). J. Gen. Virol. 83, 1745–1751.

Bohlman, M.C., Morzunov, S.P., Meissner, J., Taylor, M.B., Ishibashi, K., Rowe, J., Levis, S., Enria, D., St Jeor, S.C., 2002. Analysis of hantavirus genetic diversity in Argentina: S segment-derived phylogeny. J. Virol. 76, 3765–3773.

Botten, J., Mirowsky, K., Kusewitt, D., Ye, C., Gottlieb, K., Prescott, J., Hjelle, B., 2003. Persistent Sin Nombre virus infection in the deer mouse (Peromyscus maniculatus) model: sites of replication and strand-specific expression. J. Virol. 77 (2), 1540–1550.

Bronze, M.S., Huycke, M.M., Machado, L.J., Voskuhl, G.W., Greenfield, R.A., 2002. Viral agents as biological weapons and agents of bioterrorism. Am. J. Med. Sci. 323, 316–325.

Bucht, G., Sjölander, K.B., Eriksson, S., Lindgren, L., Lundkvist, A., Elgh, F., 2001. Modifying the cellular transport of DNA-based vaccines alters the immune response to hantavirus nucleocapsid protein. Vaccine 19, 3820–3829.

Campen, M.J., Milazzo, M.L., Fulhorst, C.F., Obot Akata, C.J., Koster, F., 2006. Characterization of shock in a hamster model of hantavirus infection. Virology 356, 45–49.

Chaparro, J., Vega, J., Terry, W., Vera, J.L., Barra, B., Meyer, R., Peters, C.J., Khan, A.S., Ksiazek, T.G., 1998. Assessment of person-to-person transmission of hantavirus pulmonary syndrome in a Chilean hospital setting. J. Hosp. Infect. 40, 281–285.

Chapman, L.E., Mertz, G.J., Peters, C.J., Jolson, H.M., Khan, A.S., Ksiazek, T.G., Koster, F.T., Baum, K.F., Rollin, P.E., Pavia, A.T., Holman, R.C., Christenson, J.C., Rubin, P.J., Behrman, R.E., Bell, L.J., Simpson, G.L., Sadek, R.F., 1999. Intravenous ribavirin for hantavirus pulmonary syndrome: safety and tolerance during 1 year of open-label experience. Ribavirin Study Group. Antivir. Ther. 4, 211–219.

Chu, Y.K., Milligan, B., Owen, R.D., Goodin, D.G., Jonsson, C.B., 2006. Phylogenetic and geographical relationships of hantavirus strains in eastern and western Paraguay. Am. J. Trop. Med. Hyg. 75, 1127–1134.

Compton, S.R., Jacoby, R.O., Paturzo, F.X., Smith, A.L., 2004. Persistent Seoul virus infection in Lewis rats. Arch. Virol. 149, 1325–1339.

Custer, D.M., Thompson, E., Schmaljohn, C.S., Ksiazek, T.G., Hooper, J.W., 2003. Active and passive vaccination against hantavirus pulmonary syndrome with Andes virus M genome segment-based DNA vaccine. J. Virol. 77, 9894–9905.

Dargeviciute, A., Brus Sjolander, K., Sasnauskas, K., Kruger, D.H., Meisel, H., Ulrich, R., Lundkvist, A., 2002. Yeast-expressed Puumala hantavirus nucleocapsid protein induces protection in a bank vole model. Vaccine 20, 3523–3531.

de Carvalho Nicacio, C., Gonzalez Della Valle, M., Padula, P., Bjorling, E., Plyusnin, A., Lundkvist, A., 2002. Cross-protection against challenge with Puumala virus after immunization with nucleocapsid proteins from different hantaviruses. J. Virol. 76, 6669–6677.

Doyle, T.J., Bryan, R.T., Peters, C.J., 1998. Viral hemorrhagic fevers and hantavirus infections in the Americas. Infect. Dis. Clin. North Am. 12, 95–110.

Enria, D., Padula, P., Segura, E.L., Pini, N., Edelstein, A., Posse, C.R., Weissenbacher, M.C., 1996. Hantavirus pulmonary syndrome in Argentina. Possibility of person to person transmission. Medicina (B. Aires) 56, 709–711.

Enria, D.A., Briggiler, A.M., Pini, N., Levis, S., 2001. Clinical manifestations of New World hantaviruses. Curr. Top. Microbiol. Immunol. 256, 117–134.

Ferres, M., Vial, P., Marco, C., Yanez, L., Godoy, P., Castillo, C., Hjelle, B., Delgado, I., Lee, S.J., Mertz, G.J., 2007. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile. J. Infect. Dis. 195, 1563–1571.

Figueiredo, L.T., Moreli, M.L., Campos, G.M., Sousa, R.L., 2003. Hantaviruses in Sao Paulo State, Brazil. Emerg. Infect. Dis. 9, 891–892.

Fulhorst, C.F., Monroe, M.C., Salas, R.A., Duno, G., Utrera, A., Ksiazek, T.G., Nichol, S.T., de Manzione, N.M., Tovar, D., Tesh, R.B., 1997. Isolation, characterization and geographic distribution of Cano Delgadito virus, a newly discovered South American hantavirus (family Bunyaviridae). Virus Res. 51, 159–171.

Gavrilovskaya, I.N., Brown, E.J., Ginsberg, M.H., Mackow, E.R., 1999. Cellular entry of hantaviruses which cause hemorrhagic fever with renal syndrome is mediated by beta3 integrins. J. Virol. 73, 3951–3959.

Gavrilovskaya, I.N., Shepley, M., Shaw, R., Ginsberg, M.H., Mackow, E.R., 1998. beta3 Integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc. Natl. Acad. Sci. U.S.A. 95, 7074–7079.

Geldmacher, A., Skrastina, D., Borisova, G., Petrovskis, I., Kruger, D.H., Pumpens, P., Ulrich, R., 2005. A hantavirus nucleocapsid protein segment exposed on hepatitis B virus core particles is highly immunogenic in mice when applied without adjuvants or in the presence of pre-existing anti-core antibodies. Vaccine (23), 3973–3983.

Groen, J., Gerding, M., Koeman, J.P., Roholl, P.J., van Amerongen, G., Jordans, H.G., Niesters, H.G., Osterhaus, A.D., 1995. A macaque model for hantavirus infection. J. Infect. Dis. 172, 38–44.

Hallin, G.W., Simpson, S.Q., Crowell, R.E., James, D.S., Koster, F.T., Mertz, G.J., Levy, H., 1996. Cardiopulmonary manifestations of hantavirus pulmonary syndrome. Crit. Care Med. 24, 252–258.

Hjelle, B., Torrez-Martinez, N., Koster, F.T., 1996. Hantavirus pulmonary syndrome-related virus from Bolivia. Lancet 347, 57.

Hooper, J.W., Kamrud, K.I., Elgh, F., Custer, D., Schmaljohn, C.S., 1999. DNA vaccination with hantavirus M segment elicits neutralizing antibodies and protects against Seoul virus infection. Virology 255 (2), 269–278.

Hooper, J.W., Custer, D.M., Thompson, E., Schmaljohn, C.S., 2001a. 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. 75, 8469–8477.

Hooper, J.W., Larsen, T., Custer, D.M., Schmaljohn, C.S., 2001b. A lethal disease model for hantavirus pulmonary syndrome. Virology 289, 6–14.

Hooper, J.W., Custer, D.M., Smith, J., Wahl-Jensen, V., 2006. Hantaan/Andes virus DNA vaccine elicits a broadly cross-reactive neutralizing antibody response in nonhuman primates. Virology 347, 208–216.

Huggins, J.W., Hsiang, C.M., Cosgriff, T.M., Guang, M.Y., Smith, J.I., Wu, Z.O., LeDuc, J.W., Zheng, Z.M., Meegan, J.M., Wang, Q.N., et al., 1991. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. J. Infect. Dis. 164, 1119–1127.

Huggins, J.W., Kim, G.R., Brand, O.M., McKee Jr., K.T., 1986. Ribavirin therapy for Hantaan virus infection in suckling mice. J. Infect. Dis. 153, 489–497.

Johnson, A.M., Bowen, M.D., Ksiazek, T.G., Williams, R.J., Bryan, R.T., Mills, J.N., Peters, C.J., Nichol, S.T., 1997. Laguna Negra virus associated with HPS in western Paraguay and Bolivia. Virology 238, 115–127.

Johnson, A.M., de Souza, L.T., Ferreira, I.B., Pereira, L.E., Ksiazek, T.G., Rollin, P.E., Peters, C.J., Nichol, S.T., 1999. Genetic investigation of novel hantaviruses causing fatal HPS in Brazil. J. Med. Virol. 59 (4), 527–535.

Johnson, K.M., 2004. The discovery of hantaan virus: comparative biology and serendipity in a world at war. J. Infect. Dis. 190, 1708–1710.

Ketai, L.H., Williamson, M.R., Telepak, R.J., Levy, H., Koster, F.T., Nolte, K.B., Allen, S.E., 1994. Hantavirus pulmonary syndrome: radiographic findings in 16 patients. Radiology 191, 665–668.

Khan, A., Khan, A.S., 2003. Hantaviruses: a tale of two hemispheres. Panminerva Med. 45, 43–51.

Klingstrom, J., Maljkovic, I., Zuber, B., Rollman, E., Kjerrstrom, A., Lundkvist, A., 2004. Vaccination of C57/BL6 mice with Dobrava hantavirus nucleocapsid protein in Freund's adjuvant induced partial protection against challenge. Vaccine 22, 4029–4034.

Kurata, T., Tsai, T.F., Bauer, S.P., McCormick, J.B., 1983. Immunofluorescence studies of disseminated Hantaan virus infection of suckling mice. Infect. Immun. 41 (1), 391–398.

Larson, R.S., Brown, D.C., Ye, C., Hjelle, B., 2005. Peptide antagonists that inhibit Sin Nombre virus and Hantaan virus entry through the beta3-integrin receptor. J. Virol. 79, 7319–7326.

Lee, H.W., 1982. Korean hemorrhagic fever. Prog. Med. Virol. 28, 96–113.

Lee, H.W., Johnson, K.M., 1982. Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. J. Infect. Dis. 146, 645–651.

Lee, H.W., van der Groen, G., 1989. Hemorrhagic fever with renal syndrome. Prog. Med. Virol. 36, 62–102.

Lee, B.H., Yoshimatsu, K., Araki, K., Okumura, M., Nakamura, I., Arikawa, J., 2006. A pseudotype vesicular stomatitis virus containing Hantaan virus envelope glycoproteins G1 and G2 as an alternative to hantavirus vaccine in mice. Vaccine 24, 2928–2934.

Levis, S., Rowe, J.E., Morzunov, S., Enria, D.A., St. Jeor, S., 1997. New hantaviruses causing hantavirus pulmonary syndrome in central Argentina. Lancet 349, 998–999.

Liang, M., Chu, Y.K., Schmaljohn, C., 1996. Bacterial expression of neutralizing mouse monoclonal antibody Fab fragments to Hantaan virus. Virology 217, 262–271.

Lindkvist, M., Lahti, K., Lilliehook, B., Holmstrom, A., Ahlm, C., Bucht, G., 2007. Cross-reactive immune responses in mice after genetic vaccination with cDNA encoding hantavirus nucleocapsid proteins. Vaccine 25, 1690–1699.

Lopez, N., Padula, P., Rossi, C., Lazaro, M.E., Franze-Fernandez, M.T., 1996. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. Virology 220, 223–226.

Maes, P., Clement, J., Gavrilovskaya, I., Van Ranst, M., 2004. Hantaviruses: immunology, treatment, and prevention. Viral Immunol. 17, 481–497.

Maes, P., Clement, J., Groeneveld, P.H., Colson, P., Huizinga, T.W., Van Ranst, M., 2006. Tumor necrosis factor-alpha genetic predisposing factors can influence clinical severity in nephropathia epidemica. Viral Immunol. 19, 558–564.

Martinez, V.P., Bellomo, C., San Juan, J., Pinna, D., Forlenza, R., Elder, M., Padula, P.J., 2005. Person-to-person transmission of Andes virus. Emerg. Infect. Dis. 11, 1848–1853.

McClain, D.J., Summers, P.L., Harrison, S.A., Schmaljohn, A.L., Schmaljohn, C.S., 2000. Clinical evaluation of a vaccinia-vectored Hantaan virus vaccine. J. Med. Virol. 60, 77–85.

McElroy, A.K., Bray, M., Reed, D.S., Schmaljohn, C.S., 2002. Andes virus infection of cynomolgus macaques. J. Infect. Dis. 186, 1706–1712.

McKee Jr., K.T., Kim, G.R., Green, D.E., Peters, C.J., 1985. Hantaan virus infection in suckling mice: virologic and pathologic correlates. J. Med. Virol. 17 (2), 107–117.

Medina, R.A., Mirowsky-Garcia, K., Hutt, J., Hjelle, B., 2007. Ribavirin, human convalescent plasma and anti-beta3 integrin antibody inhibit infection by Sin Nombre virus in the deer mouse model. J. Gen. Virol. 88, 493–505.

Mertz, G.J., Hjelle, B., Crowley, M., Iwamoto, G., Tomicic, V., Vial, P.A., 2006. Diagnosis and treatment of new world hantavirus infections. Curr. Opin. Infect. Dis. 19, 437–442.

Mertz, G.J., Miedzinski, L., Goade, D., Pavia, A.T., Hjelle, B., Hansbarger, C.O., Levy, H., Koster, F.T., Baum, K., Lindemulder, A., Wang, W., Riser, L., Fernandez, H., Whitley, R.J., 2004. Placebo-controlled, double-blind trial of intravenous ribavirin for the treatment of hantavirus cardiopulmonary syndrome in North America. Clin. Infect. Dis. 39, 1307–1313.

Milazzo, M.L., Eyzaguirre, E.J., Molina, C.P., Fulhorst, C.F., 2002. Maporal viral infection in the Syrian golden hamster: a model of hantavirus pulmonary syndrome. J. Infect. Dis. 186, 1390–1395.

Moolenaar, R.L., Breiman, R.F., Peters, C.J., 1997. Hantavirus pulmonary syndrome. Semin. Respir. Infect. 12, 31–39.

Moolenaar, R.L., Dalton, C., Lipman, H.B., Umland, E.T., Gallaher, M., Duchin, J.S., Chapman, L., Zaki, S.R., Ksiazek, T.G., Rollin, P.E., et al., 1995. Clinical features that differentiate hantavirus pulmonary syndrome from three other acute respiratory illnesses. Clin. Infect. Dis. 21, 643–649.

Nichol, S.T., Spiropoulou, C.F., Morzunov, S., Rollin, P.E., Ksiazek, T.G., Feldmann, H., Sanchez, A., Childs, J., Zaki, S., Peters, C.J., 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 262, 914–917.

Padula, P.J., Edelstein, A., Miguel, S.D., Lopez, N.M., Rossi, C.M., Rabinovich, R.D., 1998. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology 241, 323–330.

Padula, P.J., Rossi, C.M., Della Valle, M.O., Martinez, P.V., Colavecchia, S.B., Edelstein, A., Miguel, S.D., Rabinovich, R.D., Segura, E.L., 2000. Development and evaluation of a solid-phase enzyme immunoassay based on Andes hantavirus recombinant nucleoprotein. J. Med. Microbiol. 49, 149–155.

Peters, C.J., Khan, A.S., 2002. Hantavirus pulmonary syndrome: the new American hemorrhagic fever. Clin. Infect. Dis. 34, 1224–1231.

Peters, C.J., Simpson, G.L., Levy, H., 1999. Spectrum of hantavirus infection: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Annu. Rev. Med. 50, 531–545.

Plyusnin, A., Kruger, D.H., Lundkvist, A., 2001. Hantavirus infections in Europe. Adv. Virus Res. 57, 105–136.

Plyusnin, A., Morzunov, S.P., 2001. Virus evolution and genetic diversity of hantaviruses and their rodent hosts. Curr. Top. Microbiol. Immunol. 256, 47–75.

Ravkov, E.V., Compans, R.W., 2001. Hantavirus nucleocapsid protein is expressed as a membrane-associated protein in the perinuclear region. J. Virol. 75, 1808–1815.

Saggioro, F.P., Rossi, M.A., Duarte, M.I., Martin, C.C., Alves, V.A., Moreli, M.L., Figueiredo, L.T., Moreira, J.E., Borges, A.A., Neder, L., 2007. Hantavirus infection induces a typical myocarditis that may be responsible for myocardial depression and shock in hantavirus pulmonary syndrome. J. Infect. Dis. 195, 1541–1549.

Schmaljohn, C., Hjelle, B., 1997. Hantaviruses: a global disease problem. Emerg. Infect. Dis. 3, 95–104.

Schmaljohn, C.S., Chu, Y.K., Schmaljohn, A.L., Dalrymple, J.M., 1990. Antigenic subunits of Hantaan virus expressed by baculovirus and vaccinia virus recombinants. J. Virol. 64, 3162–3170.

Schmaljohn, C.S., Hasty, S.E., Harrison, S.A., Dalrymple, J.M., 1983. Characterization of Hantaan virions, the prototype virus of hemorrhagic fever with renal syndrome. J. Infect. Dis. 148, 1005–1012.

Schmaljohn, C.S., Hooper, J.W., 2001. Bunyaviridae: the viruses and their replication. In: Knipe, P.M.H.D.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology, vol. 2. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 1581–1633.

Tanishita, O., Takahashi, Y., Okuno, Y., Tamura, M., Asada, H., Dantas Jr., J.R., Yamanouchi, T., Domae, K., Kurata, T., Yamanishi, K., 1986. Persistent infection of rats with haemorrhagic fever with renal syndrome virus and their antibody responses. J. Gen. Virol. 67, 2819–2824.

Vapalahti, O., Mustonen, J., Lundkvist, A., Henttonen, H., Plyusnin, A., Vaheri, A., 2003. Hantavirus infections in Europe. Lancet Infect. Dis. 3, 653–661.

Vial, P.A., Valdivieso, F., Mertz, G., Castillo, C., Belmar, E., Delgado, I., Tapia, M., Ferres, M., 2006. Incubation period of hantavirus cardiopulmonary syndrome. Emerg. Infect. Dis. 12, 1271–1273.

Vincent, M.J., Quiroz, E., Gracia, F., Sanchez, A.J., Ksiazek, T.G., Kitsutani, P.T., Ruedas, L.A., Tinnin, D.S., Caceres, L., Garcia, A., Rollin, P.E., Mills, J.N., Peters, C.J., Nichol, S.T., 2000. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. Virology 277, 14–19.

Vitek, C.R., Breiman, R.F., Ksiazek, T.G., Rollin, P.E., McLaughlin, J.C., Umland, E.T., Nolte, K.B., Loera, A., Sewell, C.M., Peters, C.J., 1996. Evidence against person-to-person transmission of hantavirus to health care workers. Clin. Infect. Dis. 22, 824–826.

Wahl-Jensen, V., Chapman, J., Asher, L., Fisher, R., Zimmerman, M., Larsen, T., Hooper, J.W., 2007. Temporal analysis of Andes virus and Sin Nombre virus infections of Syrian hamsters. J. Virol. 81, 7449–7462.

Wells, R.M., Young, J., Williams, R.J., Armstrong, L.R., Busico, K., Khan, A.S., Ksiazek, T.G., Rollin, P.E., Zaki, S.R., Nichol, S.T., Peters, C.J., 1997. Hantavirus transmission in the United States. Emerg. Infect. Dis. 3, 361–365.

Williams, R.J., Bryan, R.T., Mills, J.N., Palma, R.E., Vera, I., De Velasquez, F., Baez, E., Schmidt, W.E., Figueroa, R.E., Peters, C.J., Zaki, S.R., Khan, A.S., Ksiazek, T.G., 1997. An outbreak of hantavirus pulmonary syndrome in western Paraguay. Am. J. Trop. Med. Hyg. 57, 274–282.

Wong, T.W., Chan, Y.C., Yap, E.H., Joo, Y.G., Lee, H.W., Lee, P.W., Yanagihara, R., Gibbs Jr., C.J., Gajdusek, D.C., 1988. Serological evidence of hantavirus infection in laboratory rats and personnel. Int. J. Epidemiol. 17, 887–890.

Xiao, R., Yang, S., Koster, F., Ye, C., Stidley, C., Hjelle, B., 2006. Sin Nombre viral RNA load in patients with hantavirus cardiopulmonary syndrome. J. Infect. Dis. 194, 1403–1409.

Xu, X., Ruo, S.L., McCormick, J.B., Fisher-Hoch, S.P., 1992. Immunity to Hantavirus challenge in Meriones unguiculatus induced by vaccinia-vectored viral proteins. Am. J. Trop. Med. Hyg. 47 (4), 397–404.

Xu, Z., Wei, L., Wang, L., Wang, H., Jiang, S., 2002. The in vitro and in vivo protective activity of monoclonal antibodies directed against Hantaan virus: potential application for immunotherapy and passive immunization. Biochem. Biophys. Res. Commun. 298, 552–558.

Yanagihara, R., Amyx, H.L., Lee, P.W., Asher, D.M., Gibbs Jr., C.J., Gajdusek, D.C., 1988. Experimental hantavirus infection in nonhuman primates. Arch. Virol. 101 (1–2), 125–130.

Yao, Z.Q., Yang, W.S., Zhang, W.B., Bai, X.F., 1989. The duration of viremia patients with epidemic hemorrhagic fever. Chin. Med. J. (Engl.) 102, 116–119.

Young, J.C., Hansen, G.R., Graves, T.K., Deasy, M.P., Humphreys, J.G., Fritz, C.L., Gorham, K.L., Khan, A.S., Ksiazek, T.G., Metzger, K.B., Peters, C.J., 2000. The incubation period of hantavirus pulmonary syndrome. Am. J. Trop. Med. Hyg. 62, 714–717.

Zaki, S.R., Greer, P.W., Coffield, L.M., Goldsmith, C.S., Nolte, K.B., Foucar, K., Feddersen, R.M., Zumwalt, R.E., Miller, G.L., Khan, A.S., et al., 1995. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am. J. Pathol. 146, 552–579.

Zhang, X.K., Takashima, I., Hashimoto, N., 1989. Characteristics of passive immunity against hantavirus infection in rats. Arch. Virol. 105, 235–246.