Hantavirus Infection

Walter Muranyi,* Udo Bahr,* Martin Zeier,* and Fokko J. van der Woude†

*Klinikum der Universität Heidelberg, Sektion Nephrologie, Heidelberg, Germany; and †Medizinische Universitätsklinik (Nephrologie, Endokrinologie, Rheumatologie), Klinikum Mannheim, Mannheim, Germany

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Hantavirus has attracted more and more attention as an emerging pathogenic virus in the past decades. It causes two distinct human diseases: Hemorrhagic fever with renal syndrome (HFRS) and human pulmonary syndrome (HPS).

Reports on clinical entities possibly caused by hantaviruses in China and England backdate into the first millennium and the Middle Age, respectively (1,2). However, it lasted until 1951 to 1953 during the Korean War before hantaviruses found global attention. More than 3000 United Nations and US soldiers experienced an acute febrile illness with acute renal failure and shock and a mortality rate of 7% close to a small river called Hantaan (3,4; G. Schreiner, personal communication, Washington, 1993). The causative agent, Hantaan virus (HTNV), was identified in 1978 by Lee *et al*. (5). Until now, 21 different hantavirus species have been described, and more than 30 genotypes are characterized and can be found all over the world (6,7).

Biology and Epidemiology

Morphology

Hantaviruses comprise one of five genera of the virus family *Bunyaviridae* (8). They replicate in the cytoplasm of host cells and are composed of a spherical lipid envelope; four viral proteins; and three single-stranded, negative-sensed RNA segments designated S (small), M (medium), and L (large) that are coding for the nucleocapsid protein (NP), the surface envelope glycoproteins G1 and G2, and the RNA-dependent RNA polymerase, respectively (Figure 1). Additional minor open reading frames are present in the genomes of hantaviruses, but to date, no corresponding proteins were identified. NP, the main structural protein, is complexed with the viral RNA genome segments that form helical nucleocapsids (9).

Host Range

The main natural reservoir of hantaviruses is murid rodents (order *Rodentia*; family *Muridae*; subfamilies *Murinae, Arvicolinae,* and *Sigmodontinae*). Virus and host share a long period of co-evolution characterized by the absence of any hantavirus-caused disease in infected rodents (10,11). Originally, it was thought that one rodent species is the predominant host for one hantavirus species, but recently more and more studies reveal that there might be multiple rodent hosts for individual virus species and multiple viruses in a single host species (12–14). In addition, numerous studies have reported hantavirus infections to be present in animal species other than rodents, for example, in cattle, moose, cat, dog, *etc.* However, the question of whether these animals are infected accidentally or represent further natural reservoirs has not yet been answered (15). The distribution of single hantavirus species correlates with the geographic extension of their hosts (Table 1), and hantavirus genotypes of the same geographic area are phylogenetically related (15–17).

Humans do not belong to the natural host range of hantaviruses, and infection occurs accidentally *via* virus-containing, aerosolized rodent excretions such as urine, feces, or saliva. People who live or work in close contact with infected rodents are at increased infection risk, and studies usually show higher percentages of seropositive individuals in such groups as compared with control subjects (18,19).

Old World and New World Hantaviruses

The genus *Hantavirus* is roughly composed of two main groups: Old World and New World hantaviruses. HFRS in humans is caused by pathogenic Old World hantaviruses that include Amur virus, Seoul virus, and HTNV, the epidemiologically most important species, with lethality rates up to 15% in Asia, as well as Dobrava virus (DOBV), Tula virus (TULV), and Puumala virus (PUUV) in Europe; the last one is the main hantavirus species in Europe and induces Nephropathia epidemica (NE), a milder variant of HFRS, with mortality rates of 0.1% (10,20). HFRS affects approximately 200,000 people each year predominantly in Asia. In 2004, 235 cases were reported in Germany according to a recent epidemiologic bulletin of the Robert-Koch Institute.

The first pathogenic New World hantavirus (Sin Nombre virus) was discovered in the early 1990s in the Four Corners region of the United States (21). From this time on, numerous additional pathogenic New World hantaviruses were identified and characterized (Table 1). New World hantaviruses are the causative agent of approximately 300 cases of HPS each year in North and South America, with lethality rates up to 50%.

Human hantavirus infections are assumed to occur accidentally, and men represent a dead end for the hantavirus life

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Address correspondence to: Dr. Martin Zeier, Klinikum der Universität Heidelberg, Sektion Nephrologie, Im Neuenheimer Feld 162, D-69120 Heidelberg, Germany. Phone: +49-6221-91120; Fax: +49-6221-9221-229; E-mail: martin_zeier@med.uni-heidelberg.de

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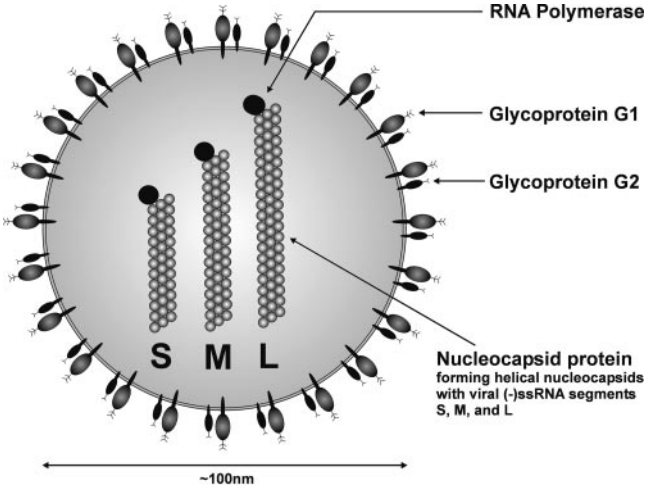

Figure 1. Schematic of hantavirus morphology. The (-)ssRNA segments S (small), M (medium), and L (large) are encoded for the nucleocapsid protein, the glycoproteins G1 and G2, and the RNA-dependent RNA polymerase, respectively.

cycle. Transfer of virus particles from infected to uninfected people normally does not occur. One exception is the Andes hantavirus strain Sout in Argentina, of which sporadical person-to-person transmissions were reported (22,23). This finding reveals a worrying risk potential of hantaviruses for human health.

Clinical Course of NE, HFRS, and HPS

HFRS and HPS are partly overlapping clinical syndromes. In Europe, the hantavirus serotype Puumala causes NE, a milder variant of HFRS. Viremia occurs after initial infection of alveolar macrophages and life-threatening acute-phase symptoms are induced primarily by infection of vascular endothelial cells of the lung and the kidneys with concomitant loss of barrier function resulting in severely increased endothelial permeability.

NE

NE is characterized by a sudden onset with high fever, headache, backache, and abdominal pain. Transient thrombocytopenia is a typical finding in the early phase of the disease. The occurrence of conjunctival hemorrhages, palatine petechiae, and a truncal petechial rash after 3 or 4 d is possible. Approximately 1% of patients experience severe neurologic manifestations, *e.g.*, seizures or bladder paralysis. The hemorrhages are accompanied by oliguria, azotemia, proteinuria, and hematuria. Within 3 d, the rash disappears and the patients develop polyuria. The convalescent phase extends over several weeks, and sequelae are rare. Severe courses of NE with acute renal failure and lethal outcome range between 0.1 and 1% (24).

HFRS

The incubation period of HFRS is 7 to 36 d. Only 10 to 15% of cases have a severe course, with lethality rates between 6 to 15%. HFRS is characterized by systemic involvement of capillaries and venules. It induces various hemorrhagic manifestations and circulation disorders. Renal involvement is characterized by acute renal failure as a result of interstitial hemorrhage and interstitial infiltrates (24,25).

The clinical course is subdivided into five phases: Febrile, hypotensive, oliguric, diuretic, and convalescent. The onset of HFRS resembles NE with high fever, backache, abdominal pain, chills, myalgia, malaise, and bradycardia over 3 to 4 d. Photophobia, pharynx enanthema, and a diffuse reddening of the face are also observed. On the third to fifth days, petechia develop initially on the palate. At the same time, conjunctival hemorrhages may appear and a temporary impairment of the visual function is reported. The urinary sediment reveals hematuria and atypical gross proteinuria (in some cases >3 g/24 h). The hypotensive phase ranges 3 to 6 d after onset of fever. Shock or hypotension may occur. Laboratory findings in this phase are leukocytosis and thrombocytopenia. Patients show a wide range of renal conditions, including acute tubulointerstitial nephritis, necrotizing glomerulonephritis, and IgA nephropathy. The oliguric phase starts at approximately day 8, and hemorrhagic manifestations become more prominent. The diuretic phase starts at approximately day 11, and the convalescent phase lasts approximately 3 wk to 6 mo.

Sequelae are rare but include chronic renal failure and hypertension. In a series of 46 patients in Tampere (Finland) who had NE 3 to 7 y ago, the patients had higher GFR and filtration fraction, more proteinuria, and a higher ambulatory systolic BP compared with healthy control subjects (26). Furthermore, we studied 42 patients after NE from several areas in Germany and found hypertension or elevated serum creatinine (1.5 mg/dl) (27).

Extrarenal manifestations include acute impairment of visual function, acute myopia, CNS complications with seizures, sometimes myocarditis, and severe gastrointestinal hemorrhages. In addition, thyroid, liver, and pancreas may be affected. Lung involvement but to a lesser extent than in HPS is also observed during HFRS (24,28–30).

HPS

The onset of HPS is characterized by flu-like symptoms such as high fever, myalgia, and headache. The patients develop acute noncardiac pulmonary edema and hypotension within 2 to 15 d. Bilateral infiltrates develop rapidly, sometimes associated with pleural effusions. Neutrophilic leukocytosis, hemoconcentration, thrombocytopenia, and circulating immunoblasts are observed. Severe courses of HPS are associated with increased lactate levels. The mortality rates of HPS are approximately 50%. Patients who survive the acute phase of the disease recover normally within 5 to 7 d without any sequelae (24). Acute renal failure is secondary as a result of shock and respiratory failure.

The medical knowledge about HFRS and HPS has increased substantially in the past years, resulting in the conclusion that both syndromes are partly overlapping. The number of reports on HFRS cases with lung involvement and HPS cases with renal involvement is continuously growing, and it is conceivable that the descriptions of the clinical courses of both syndromes will further converge in the near future.

Table 1. Main natural reservoirs and geographic distribution of pathogenic hantaviruses[a]

	Pathogenic Hantavirus Serotype	Human Disease	Main Natural Reservoir	Geographic Distribution of Host and Virus
Old World Hantaviruses	Amur	HFRS	*Apodemus peninsulae* (*Murinae*) Korean field mouse	Southeast Siberia and northeast China south through Korea, and east Mongolia to southwest China; also on Japanese islands of Sakhalin and Hokkaido
	Dobrava-Af	HFRS	*Apodemus flavicollis* (*Murinae*) yellow-necked field mouse	England and Wales, northwest Spain, France, south Scandinavia, European Russia, south Italy, Balkans, Syria, Lebanon, and Israel
	Dobrava-Aa Hantaan	HFRS	*Apodemus agrarius* (*Murinae*) striped field mouse	Central Europe south to Thrace, Caucasus, and Tien Shan Mountains (Dobrava-Aa); Amur River through Korea to China and Taiwan (Hantaan)
	Puumala	NE	*Clethrionomys glareolus* (*Arvicolinae*) red bank vole	West Palearctic from France and Scandinavia to Lake Baikal, south to North Spain, north Italy, Balkans, west Turkey, north Kazakhstan, Altai and Sayan Mountains; Britain and southwest Ireland
	Seoul	HFRS	*Rattus norvegicus Rattus rattus* (*Murinae*) Norway rat, black rat	Worldwide
	Tula	HFRS	*Microtus arvalis* (*Arvicolinae*) common vole	From central and north Spain throughout Europe to the Black Sea in the south and northeast to the Urals in Russia; also on the Orkney Islands, Guernsey (Channel Islands), and Yeu (France)
New World Hantaviruses	Andes Oran	HPS	*Oligoryzomys longicaudatus* (*Sigmodontinae*) long-tailed pygmy rice rat	North-central to south Andes, to approximately 50° southern latitude of Chile and Argentina
	Araraquara	HPS	—	Brazil
	Bayou	HPS	*Oryzomys palustris* (*Sigmodontinae*) marsh rice rat	Southeast Kansas to east Texas, eastward to south New Jersey and peninsular Florida
	Bermejo	HPS	*Oligoryzomys chacoensis* (*Sigmodontinae*) Chacoan pygmy rice rat	West Paraguay, southeast Bolivia, west-central Brazil, and north Argentina
New World Hantaviruses	Black Creek Canal Muleshoe	HPS	*Sigmodon hispidus* (*Sigmodontinae*) Hispid cotton rat	Southeast United States; south Nebraska to central Virginia, south to southeast Arizona and peninsular Florida; interior and east Mexico through middle America to central Panama; in South America to north Colombia and north Venezuela
	Castelo dos Sonhos	HPS	—	Brazil
	Choclo	HPS	*Oligoryzomys fulvescens* (*Sigmodontinae*) Fulvous pygmy rice rat	West and east versants of south Mexico, through Mesoamerica, to Ecuador, northernmost Brazil, and Guianas in South America
	Hu39694	HPS	—	Argentina
	Juquitiba	HPS	—	Brazil
	Laguna Negra	HPS	*Calomys laucha* (*Sigmodontinae*) vesper mouse	North Argentina and Uruguay, southeast Bolivia, west Paraguay, and west-central Brazil
	Lechiguanas	HPS	*Oligoryzomys flavescens* (*Sigmodontinae*) yellow pygmy rice rat	Southeast Brazil, Uruguay, and Argentina (south to Chubut Province)
	Maciel	HPS	*Necromys benefactus* (*Sigmodontinae*) dark field mouse	Argentina
	Sin Nombre Monongahela	HPS	*Peromyscus maniculatus* (*Sigmodontinae*) deer mouse	Panhandle of Alaska and across north Canada, south through continental United States, excluding the southeast and east seaboard, to southernmost Baja California Sur and to north-central Oaxaca, Mexico
	New York	HPS	*Peromyscus leucopus* (*Sigmodontinae*) white-footed mouse	Central and east United States to south Alberta and south Ontario, Quebec, and Nova Scotia, Canada; to north Durango and along Caribbean coast to Isthmus of Tehuantepec and Yucatan Peninsula, Mexico
	Rio Mamore	HPS	*Oligoryzomys microtis* (*Sigmodontinae*) small-eared pygmy rice rat	Central Brazil south of Rios Solimoes-Amazon and contiguous low lands of Peru, Bolivia, Paraguay, and Argentina

[a]HFRS, hemorrhagic fever with renal syndrome; HPS, human pulmonary syndrome; NE, nephropathia epidemica; —, unknown.

Pathology of NE, HFRS, and HPS

NE and HFRS

Immunohistochemistry analysis of hantavirus-infected renal tissue reveals interstitial infiltrates with immune cells and interstitial hemorrhage. The most common histopathologic lesion are acute tubulointerstitial nephritis. Tubular epithelial and luminal alterations are present. Intertubular capillaries are congested, and the interstitium is broadened by edema, indicative of a generalized capillary damage. Occasionally, glomerular pathology, *e.g.*, hypercellularity and expansion of the mesangium, are observed, and this is probably the underlying cause of gross proteinuria. Tubular, interstitial, and glomerular histologic damage are associated with the clinical severity of renal failure (Figure 2). It is of note that urinary sediment contains tubular cells with extremely enlarged nucleoli. These cells resemble uroepithelial tumor cells and spontaneously disappear after the disease has subsided (29,31). Recent work has shown that these tubular cells contain hantavirus antigen (32).

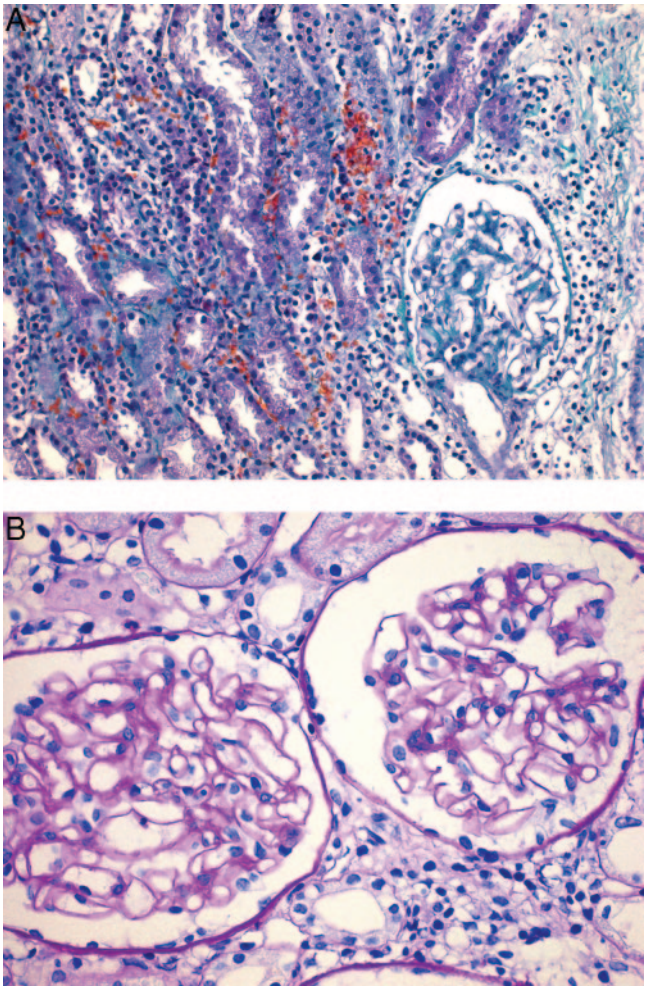

Figure 2. (A) Focal mononuclear interstitial infiltration, capillary congestion, and interstitial hemorrhage at the corticomedullary junction. (B) Normal glomeruli. Focal interstitial edema with a mild mononuclear infiltrate and prominent endothelial cells of peritubular capillaries. Magnification, ×100 in A, Masson trichrome stain; ×160 in B, PAS stain.

HPS

Immunohistochemistry analysis of HPS documents the distribution of viral antigens within the endothelium of capillaries throughout various tissues. Infected endothelial cells lack any morphologic changes and show no visible cytopathic effects (CPE). Accumulations of hantaviral antigens are observed in the pulmonary microvasculature and in dendritic cells within the lymphoid follicles of spleen and lymph nodes. In some autopsy cases, endothelial cells in the capillaries of the myocardium and the endocardium bear hantavirus antigen, contributing substantially to severe courses of HPS.

Gross pathologic findings show that the lungs of patients with HPS are dense, rubbery, and heavy, usually weighing twice as much as the average lung. The pathologic lesions are primarily vascular with variable degrees of generalized capillary dilation and edema. Frequently, the lungs reveal a mild to moderate interstitial pneumonitis with variable degrees of congestion, edema, and mononuclear cell infiltration (33).

Pathophysiology of Hantavirus Infection

The main factor that determines the course and the severity of HFRS and HPS is the degree of increased permeability of infected endothelium that shows no histologic signs of damage and no visible CPE. At present, it is poorly understood how pathogenic hantaviruses induce the capillary leakage during the acute phase of the two syndromes and why some hantavirus species are nonpathogenic.

Genetic Predisposition

Patients with certain HLA antigens seem to have a genetic predisposition for severe courses of HFRS and HPS. Patients who bear HLA-B8, DRB1*0301, C4A*Q0, or DQ2 alleles seem to have a significantly higher risk for a severe course of NE (34–36), and the HLA-B35 allele was associated with severe courses of HPS (37). The mechanisms that are involved in these genetic predispositions are unknown.

Hantavirus Replication Cycle

Hantavirus replication takes place in macrophages and vascular endothelial cells, especially in the lung and the kidney (10,38). For pathogenic hantaviruses, the entry into host cells occurs by attachment to $\alpha_V\beta_3$ integrin on the cellular surface and subsequent endocytosis (39,40). The virion envelope fuses with the endosome membrane in a pH-dependent way, and nucleocapsids are released into the cytoplasm. Thereafter, the viral RNA-dependent RNA polymerase directs transcription of viral genes and replication of the viral RNA genome segments. The viral NP and RNA polymerase mRNA are translated at free ribosomes, whereas the glycoprotein mRNA is translated into the endoplasmic reticulum. G1 and G2 glycoproteins are transported to the Golgi complex for final glycosylation. Large intracellular inclusion bodies, probably composed of NP, are formed in the cytoplasm. It is assumed that hantavirions are formed at the membranes of the Golgi complex, followed by budding into the Golgi cisternae, migration in secretory vesicles to the plasma membrane, and release by exocytosis. Several *in vitro* studies have shown that this hantaviral life cycle does not

induce any visible CPE in endothelial cells. Host cells are not lysed by infection with pathogenic hantaviruses, and no increased permeability is induced in endothelial cell cultures (41,42). Apoptosis and expression of apoptosis-related genes in cells that were infected with pathogenic hantaviruses was reported for cultured VeroE6 and human embryonic kidney cells; however, *in vivo*, there is no evidence for programmed cell death in infected endothelial cells (43–46). These data indicate that increased endothelial permeability during HFRS and HPS might be the result of the infection with pathogenic hantaviruses in combination with additional factors that are specific for the *in vivo* situation and that are not present in *in vitro* cell cultures. In this context, it is assumed that antiviral processes in the infected cells and immune mechanisms may play a key role in the development of vascular dysfunction (10,47).

Innate Antiviral Immune Responses

Infection with hantaviruses induces innate immune responses in host cells, whereas pathogenic hantaviruses seem to be able to evade these responses to a certain degree. Different types of interferons are expressed, and IFN-inducible genes are activated. The expression of the IFN-inducible MxA protein is delayed in cells that are infected with pathogenic hantaviruses in comparison with nonpathogenic hantaviruses. Similarly, levels of antigen-presenting molecules, *e.g.*, HLA class I, are elevated; however, the upregulation proceeds more slowly after infection with pathogenic than with nonpathogenic hantaviruses (48–51). Other innate antiviral mechanisms that are induced during hantavirus infection include activation of the complement system of the classical and alternative route, *e.g.*, with elevated titers of soluble terminal complex SC5b-9 and higher C4d/C4 ratios during NE caused by PUUV (52). Natural killer cells, known as effector and regulatory cells in innate and adaptive immunity in terms of production of cytotoxic molecules and secretion of cytokines and chemokines (10), are assumed to migrate into hantavirus-infected tissues (53,54).

Humoral Immune Response

The adaptive immune system counters a hantavirus infection *via* a humoral and a cellular response. In the course of the humoral immune response, all types of Ig are expressed during HFRS and HPS. Elevated titers of total serum and virus-specific IgA, the main immunologic component of the mucosa, were detected during the acute phase of both syndromes (55,56). Total and virus-specific IgE titers were found to be increased before and during the acute phase of HFRS. It is conceivable that IgE participates in hantavirus pathophysiology by activation of IL-1β and TNF-α secretion that could influence permeability of infected endothelium; however, it was not possible to find a correlation between IgE levels and HFRS severity (10,57,58). High titers of virus-specific IgM against viral NP, G1, and G2 are produced during and after the acute phase of HFRS and HPS, whereas the hantavirus NP is regarded as the major viral antigen (10,55,56,59,60). Virus-specific IgG, the most abundant antibody of total Ig against hantaviruses, is also predominantly directed against viral NP and appears during the acute phase of HFRS and HPS, whereas further increasing titers can be observed during the early convalescent phase (10,56,61).

Cellular Immune Response

Cytotoxic $CD8^+$ T cells (CTL) are the predominant lymphocytes in the course of the cellular immune response to a hantavirus infection and are assumed to play important roles in virus clearing and HFRS/HPS pathogenicity. Increased numbers of CTL were observed at the onset of HFRS and HPS and were also found in the lungs of patients who died from HPS. The severity of disease generally correlates with the number of CTL (10,37,51). CTL epitopes were identified in all three viral structural proteins, whereas NP again seems to be the predominant immunogenic protein (62,63).

Secretion of Cytokines and Chemokines

Various types of chemokines and cytokines are secreted in variable amounts to regulate the immune response during a hantavirus infection. It is assumed that cytokines/chemokines play an important role in vascular dysfunction during HFRS and HPS. Many cytokines/chemokines, such as TNF-α, are known to increase endothelial permeability in the course of natural immune response mechanisms, *e.g.*, during lymphocyte migration through the vascular walls. Significantly elevated plasma levels of IFN-γ, TNF-α, IL-2, and IL-6 were detected at the onset of the acute phase of HFRS and HPS (51,64,65). Increased titers of TNF-α seem to correlate with a severe course of NE (66). Increased expression of cytokines, especially of TNF-α in the peritubular area of the distal nephron, was reported during HFRS (67,68), and in the lungs of patients with HPS, increased numbers of IFN-γ–, IL-1α–, IL-1β–, IL-2–, IL-4–, IL-6–, and TNF-α/β–producing cells were observed (37). Hantaviruses are also able to infect dendritic cells, resulting in secretion of proinflammatory cytokines, *e.g.*, IFN-α and TNF-α that could also contribute to increased endothelial permeability (65,69). *In vitro* infection of human lung microvascular endothelial cells with HTNV or Sin Nombre virus generated increased amounts of RANTES and 10-kD IFN-inducible protein (42). A recent *in vitro* study by Niikura *et al.* (70) showed that TNF-α–induced increased permeability of endothelial cells is significantly prolonged in HTNV-infected cells in comparison with uninfected cells.

Cellular Target Proteins

The cumulative data about hantavirus pathophysiology so far indicate that a hantavirus infection interferes in a thus far unknown way with vascular permeability regulation during inflammation, resulting in endothelial dysfunction. It is conceivable that this interference is mediated by interactions between viral and cellular proteins that participate in permeability regulation. Several studies identified associations of hantavirus NP with small ubiquitin-like modifier-1, with small ubiquitin-like modifier-1–interacting proteins and with the Fas-mediated apoptosis enhancer Daxx (71–74); however, none of these proteins is involved in permeability regulation in endothelial cells.

A much more promising candidate is $\alpha_V\beta_3$-integrin, the cel-

lular surface receptor for pathogenic hantaviruses. $\alpha_V\beta_3$ Integrin participates in the regulation of cell-to-cell adhesion, platelet aggregation, and maintenance of vascular barrier function. The binding of hantaviruses to $\alpha_V\beta_3$ integrin inhibits β_3 integrin–directed endothelial cell migration. Furthermore, it was shown that hantaviruses bind to so-called plexin-semaphorin-integrin domains that are present on the surface of inactive $\alpha_V\beta_3$ integrin molecules. These interactions are assumed to inhibit regular $\alpha_V\beta_3$ integrin functions and probably interfere with endothelial permeability regulation (39,40,74–76). A further study identified immunoreceptor tyrosine-based activation motifs within the G1 cytoplasmic tail of all HPS-causing hantaviruses. These G1 immunoreceptor tyrosine-based activation motifs bind key cellular kinases that regulate immune and endothelial cell functions. The implications of these interactions are not clear, but an influence on permeability regulation is possible (77).

Laboratory Diagnosis of Hantavirus Infections

Diagnosis of hantaviruses is usually made on the basis of clinical and serologic findings. Hantavirus should be performed in a patient with fever, lumbago, renal failure, and recent outdoor activities. In the early course of the disease, thrombocytopenia is detectable. An ELISA-based detection of NP-specific IgM antibodies is usually performed for laboratory diagnosis of an acute hantavirus infection (78). The highest titers are demonstrable between 8 and 25 d after onset of disease. It is important to note for the differential diagnosis of Puumala and Hantaan virus infections that PUUV NP-specific ELISA cross-reacts with HTNV NP, whereas HTNV NP-specific ELISA shows virtually no cross-reaction with PUUV NP (29). In addition, immunochromatographic assays (79) and reverse transcriptase–PCR have been used increasingly in recent years, but they have not yet become widely accepted as standard clinical laboratory tests (80,81).

Therapy for Hantavirus Infections

At present, there are no antiviral drugs that are applicable to cure hantavirus infections. The treatment of patients with HFRS or HPS is restricted to supportive procedures to keep under control the symptoms, which may be life-threatening. Patients are normally supervised in an emergency department or intensive care unit for close monitoring and care until the patient's immune system has cleared the virus and the convalescent phase begins.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a guanosine-analog, was shown to possess anti-hantaviral activity. Controlled trials in the early 1990s reported on decreased virus titers, higher survival rates, and decreased morbidity both in murine models and in patients with HFRS (82,83). However, newer ribavirin trials in patients with HPS did not confirm the promising results (10,84,85). Recently, it was shown that ribavirin inhibits the production of hantavirus progeny *in vitro*. The antiviral activity was due to incorporation of ribavirin into nascent RNA, resulting in high mutation frequencies (9.5/1000 nucleotides) and, hence, in the synthesis of transcriptionally defect viral RNA (86). The study showed that hantavirus RNA-dependent RNA polymerase is susceptible to drugs that lead to error catastrophes during the viral replication cycle. These insights allow new strategies for the development of therapeutic procedures that include the incorporation of lethal mutations during hantavirus replication (87).

Normally, a viral infection induces specific antiviral processes in target cells; among them is the expression of interferons and IFN-inducible genes. In VeroE6 cells, it was shown that pretreatment with human IFN-α, -β, and -γ leads to an inhibition of HTNV, PUUV, and TULV replication (88). The IFN-inducible human MxA protein, an intracytoplasmic GTPase of the dynamin superfamily, shows antiviral activity against a wide range of RNA viruses, including hantaviruses. Viral replication induces the expression of the MxA protein that was shown to interfere with the replication cycle of hantaviruses (46,89,90). However, up to now, no study has indicated how interferons and IFN-inducible proteins could serve as therapeutic agents during hantavirus infections.

Recently, Klingstrom *et al.* (91) passively immunized cynomolgus macaques with neutralizing mAb and subsequently challenged them with wild-type PUUV. A delayed onset of viremia and seroconversion was observed, and one of the immunized monkeys showed neither symptoms nor elevated levels of IL-6, IL-10, and TNF-α. The efficiency of passive immunization was also confirmed in an earlier study in a Syrian hamster model for lethal HPS using antibodies against Andes virus glycoproteins that were induced by DNA vaccines (92). Future clinical trials have to show whether passive immunization could represent a therapeutic instrument for the treatment of acute HFRS or HPS in humans.

In a recent Chinese study, it was reported that intracellularly applied single-chain Fv of mAb against HTNV NP was able to bind to the hantavirus nucleocapsid protein in the cytoplasm of infected cells. The method could represent a new therapeutic approach in the future (93).

Unusual approaches such as the therapeutic assessment of plant compounds directed against phleboviruses (plant viruses of the family *Bunyaviridae*) (94) or the application of integrated traditional Chinese medicine (95) were pursued to identify a treatment for hantavirus infections; however, up to now, none of these studies has provided a crucial breakthrough in HFRS or HPS therapy. It is conceivable that the elucidation of the molecular mechanisms of hantavirus pathophysiology or, more precise, the clarification of cellular processes that participate in endothelial dysfunction during HFRS and HPS in the future will provide new targets for effective therapeutic strategies.

Prevention of Hantavirus Infections

Because infection with some hantavirus species results in high morbidity and mortality rates and in view of the present situation of missing effective antiviral drugs, it is of particular importance to try to prevent an infection. On the one hand, it is indicated to avoid places where murid rodents live in large quantities to avert contact with virus-containing rodent excretions. This includes keeping homes and the near surrounding area rodent-free, for example, by eliminating crawl spaces and

debris and removing food sources to make homes and work areas unattractive for rodents. On the other hand, many research efforts have been made in the past years to develop an effective and safe vaccine against hantaviruses applying vaccination techniques varying from killed virus to recombinant DNA technology. In Korea, a formalin-inactivated HTNV vaccine, Hantavax (Korea Green Cross, Seoul, Korea), that is produced from rodent brain–derived virus, is commercially available. Hantavax was shown to induce high titers of IgG-specific antibodies in almost 100% of human volunteers after three vaccinations accompanied by the production of neutralizing antibodies in approximately 80% of test individuals; however, the antibody titers declined very rapidly within months, and boosters yielded no satisfactory protection rates (96–99). Further studies confirmed that Hantavax elicited only protection rates between 30 and 50% for longer time periods (100). In another study, a VeroE6 cell culture–derived, formaldehyde-inactivated HTNV vaccine showed significantly higher antibody titer and protection rates in Balb/c mice in comparison with Hantavax; however, protection rates in humans were also very low (101). To this day, there are no hantavirus vaccines that are based on inactivated viruses and that elicit satisfactory protection rates in humans (10,98,102).

In addition to inactivated whole-virus particles, single viral components (the viral structural proteins NP, G1, and G2) were obtained with recombinant DNA technology, expressed in several cell culture systems and organisms, and tested for their immunogenicity and protective potential. For example, recombinant PUUV NP expressed in yeast induced protective immunity in bank voles (103), and recombinant NP of DOBV expressed in yeast induced high antibody titers in Balb/c and C57BL/6 mice (104). PUUV NP was expressed successfully in transgenic tobacco and potato plants by our group but failed to induce an antibody response in mice when administered as an oral vaccine (105,106). Recently, recombinant NP of DOBV was tested in combination with various adjuvants for immunogenicity and protective efficacy in C57/BL6 mice. The study identified Freund's adjuvant as the additive of choice because mice that were vaccinated with this adjuvant in combination with the DOBV NP showed a protection rate from challenge of 75%, whereas the usage of other adjuvants such as Alum, which induces strong Th2-type immune responses, did not result in protective immunity (107).

Furthermore, known immunogenic epitopes of PUUV, DOBV, and HTNV NP were incorporated into chimeric hepatitis B virus core particles and elicited high antibody titers and protective immunity in bank voles (108,109). In addition, life recombinant viruses that express and carry hantavirus structural proteins were constructed. For example, HTNV NP, G1, and G2 expressed with baculovirus and vaccinia virus vectors were shown to induce protection after a Hantaan virus challenge in hamster and mouse models (110–112). A vaccinia-vectored Hantaan virus vaccine was tested in a Phase II, double-blinded, placebo-controlled clinical trial among 142 volunteers. Neutralizing antibodies to Hantaan virus were detected in 72% of the test individuals (113).

Finally, plasmid-based DNA vaccines, which express hantavirus structural proteins, were tested for their immunogenic and protective potential. Many groups introduced the coding sequences of the structural proteins of various pathogenic hantaviruses into usually CMV-based eukaryotic expression vectors and tested the immunogenic potential of these DNA vaccines in mouse, hamster, and Rhesus macaque models. The DNA vaccines always induced high antibody titers often of the neutralizing type (10,92,114–117). Despite the extensive work of many research groups on the field of hantavirus vaccine development and the presence of promising data in animal models, there is still no worldwide approved and commercially available vaccine against hantaviruses, and it seems unlikely that this situation will change in the near future.

Conclusion

Since the discovery of HTNV as the causative agent of HFRS, much knowledge about the various hantaviruses and their manifestations in animals and humans has been gathered. NE, HFRS, and HPS are human diseases, caused by hantaviruses, which may be encountered by clinical nephrologists. The diagnosis rests on serologic evidence. Supportive therapy is dependent on the Hantavirus strain and clinical symptoms, especially important in HFRS and HPS, for which correction of bleeding, maintenance of BP, and treatment of renal or respiratory insufficiency may be indicated. It is hoped that a better understanding of viral biology and pathophysiology will lead to more effective and specific therapeutic modalities in the future.

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