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Hantavirus-induced immunity in rodent reservoirs and humans

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Summary: Hantaviruses are predominantly rodent-borne pathogens, although recently novel shrew-associated hantaviruses were found. Within natural reservoir hosts, hantairuses do not cause obvious pathogenetic effects; transmission to humans, however, can lead to hemorrhagic fever with renal syndrome or hantavirus cardiopulmonary syndrome, depending on the virus species involved. This review is focussed on the recent knowledge on hantavirus-induced immune responses in rodent reservoirs and humans and their impact on susceptibility, transmission, and outcome of hantavirus infections. In addition, this review incorporates a discussion on the potential role of direct cell-virus interactions in the pathogenesis of hantavirus infections in humans. Finally, questions for further research efforts on the immune responses in potential hantavirus reservoir hosts and humans are summarized.

Keywords: *viral hemorrhagic fever, hantavirus cardiopulmonary syndrome, hantaviruses, rodent reservoir, transmission, cell-mediated pathogenesis, immunopathogenesis*

Introduction

Hantaviruses as emerging pathogens

Emerging infectious diseases represent an important threat for public health worldwide. Their emergence is thought to be driven mainly by socio-economic, environmental, and ecological factors (1). Among other factors associated to the pathogen itself, to the environment, and to human activities, the immunity of the reservoir host and the accidental dead end host represent major determinants of emerging infectious diseases in humans (2).

Among zoonotic emerging and reemerging pathogens are hemorrhagic fever-causing viruses of different virus families such as *Arena*-, *Filo*-, *Flavi*-, and *Bunyaviridae* (2–4). Hantaviruses are included in this group due to (i) the first detection of a novel syndrome caused by hantavirus infection in the Americas during the 1990s, (ii) the ongoing process of the discovery of novel hantaviruses, and (iii) the cyclic reemergence of certain hantavirus species in clusters or outbreaks of human infections.

The initial discovery of the prototype hantavirus species dates back to scientific approaches that were initiated after the Korean conflict in 1951–1953, where more than 3000 cases of an acute febrile illness were seen among the UN soldiers

(reviewed in 5). This disease was originally termed Korean hemorrhagic fever but is now referred to as hemorrhagic fever with renal syndrome (HFRS). These investigations resulted in the identification of the *Hantaan virus* (HTNV), becoming the prototype member of the genus *Hantavirus*, and its rodent reservoir host *Apodemus agrarius* (6) (*Table 1*). The discovery of this novel virus triggered a lot of research efforts that identified additional HFRS-causing hantaviruses: *Seoul virus* (SEOV) in Asia, and *Puumala virus* (PUUV) in Europe (7, 8). SEOV, which transmitted by different rat species, is thought to be distributed worldwide, as its reservoir host is abundant in almost all parts of the world. Indeed, SEOV infections have been detected not only in Asia but also in some European and American countries. PUUV, associated to the bank vole *Myodes glareolus* (previously *Clethrionomys glareolus*), causes in humans a disease designated nephropathia epidemica (NE), a less severe form of HFRS (*Table 1*). The first indigenous American virus, the *Prospect Hill virus* (PHV), which is closely related to European Arvicolinae-associated hantaviruses, was found to be non-pathogenic to humans (9).

The next important milestone in the history of hantavirus research was the discovery of a novel hantavirus disease in the Americas. After a cluster of acute respiratory distress syndrome (ARDS) deaths in the Four Corners region of the southwestern United States, a novel disease, hantavirus pulmonary syndrome, was first reported in May 1993 (10, 11). Because of the inclusion of a myocardial depression, the designation hantavirus cardiopulmonary syndrome (HCPS) was suggested (12, 13). Within a very short period a novel virus was found to be the cause of this disease and was named initially as Four Corners virus and later renamed *Sin Nombre virus* (SNV). The common deer mouse (*Peromyscus maniculatus*) was demonstrated to be the rodent reservoir of SNV (14). A potential reason for the discovery of this novel disease at that time was an increased rodent population density, possibly due to an El Niño southern oscillation that increased the food resources for rodents and the ratio of SNV-infected rodents (15). Later on it became clear that SNV and similar Neotominae- and Sigmodontinae-associated hantaviruses, like *Andes virus* (ANDV), are present all over the Americas (*Table 1*).

Thottapalayam virus (TPMV), associated with the shrew *Suncus murinus*, was the first virus isolated from a non-rodent reservoir (16). Recent investigations on the experimental host range, genetics, and molecular phylogeny indicates that TPMV is a real shrew-borne hantavirus that evolved within a non-rodent reservoir host (17). Moreover, an increasing number of shrew-associated hantaviruses suggested a potential role of shrews as reservoirs for a novel group of hantaviruses (18–22). However, it is unclear whether these novel viruses can be transmitted to humans and cause disease.

Taxonomically, hantaviruses represent a separate genus *Hantavirus* in the family *Bunyaviridae*. This family contains four genera of viruses infecting human and animals (genera *Orthobunyavirus*, *Phlebovirus*, *Nairovirus*, *Hantavirus*) and one genus of plant-infecting viruses (genus *Tospovirus*) (23). Besides the genus *Hantavirus*, the genera *Orthobunyavirus*, *Phlebovirus*, and *Nairovirus* also contain zoonotic viruses that are highly pathogenic to humans, namely La Crosse virus (LACV), *Rift Valley fever virus* (RVFV), and *Crimean-Congo hemorrhagic fever virus* (CCHFV), respectively. Currently 22 hantavirus species, with 21 being associated to rodents, are accepted by the International Committee of Virus Taxonomy (23) (*Table 1*). The novel shrew-associated hantaviruses still await their taxonomic classification (*Table 1*).

Structure, genome organization, and replication

Hantavirus particles are of spherical morphology, but elongated forms also have been observed by electron microscopy. The lipid bilayered envelope of 80–120 nm in diameter carries surface glycoprotein projections representing heterodimers formed by the glycoproteins G1 or Gn (of 68–76 kDa) and G2 or Gc (of 52–58 kDa). The genome within the virion consists of three single-stranded RNA segments of negative polarity (vRNA) complexed with viral nucleocapsid (N) protein (of 50–54 kDa). In addition to the three ribonucleocapsids virions contain the RNA-dependent RNA polymerase (RdRp) of about 240 kDa required for primary transcription and replication. According to size, the viral RNA segments are designated S (small), M (medium), and L (large) and code for N protein, two glycoproteins (G1/Gn and G2/Gc), and RdRp, respectively. The genera *Orthobunyavirus* and *Phlebovirus* contain an additional open reading frame (ORF) in the S and/or M segment suggested to be involved in mechanisms to escape antiviral responses (24, 25, reviewed in 26). In almost all Arvicolinae-[PUUV, PHV, and *Tula virus* (TULV)], Neotominae-, and Sigmodontinae-[SNV, ANDV, and *New York virus* (NYV)], but not in Murinae-associated hantaviruses [HTNV, *Dobrava-Belgrade virus* (DOBV)], a second, highly conserved ORF is found within the N-ORF. This second ORF (NSs-ORF) of PUUV or TULV might encode a non-structural protein that was suggested to play a role in pathogenicity or alternatively in the adaptation of the virus to the rodent host (27–30). The detection of a stop codon in this ORF of a cell culture-adapted PUUV strain suggested this region to be non-essential for replication, at least in type I interferon (IFN)-deficient Vero cells (31). The genomic RNA

Table 1. Selected hantavirus species and their rodent hosts, geographical distribution, and human disease caused by them

Virus species*	Reservoir host†				Geographical distribution	Human disease	Case fatality ratio	Reference
	Order	Family	Subfamily	Species				
Hantaan virus (HTNV)	Rodentia	Muridae	Murinae	*Apodemus agrarius*	Asia	HFRS	up to 15%	(6, 297)
Seoul virus (SEOV)				*Rattus norvegicus*, *R. rattus*, *R. losea*	Asia, America, Europe	HFRS	1–2%	(8, 298–301)
Dobrava-Belgrade virus (DOBV)				*A. flavicollis*, *A. agrarius*, *A. ponticus*	Europe	HFRS	9–12%, <1% 5.6%	(100, 302–306)
Thailand virus (THAIV)				*Bandicota indica*	South-East Asia (Thailand)	HFRS	?	(307–309)
Sangassou virus (SANGV)				*Hylomyscus alleni (simus)*	Africa (Guinea)	?	?	(310)
Puumala virus (PUUV)		Cricetidae	Arvicolinae	*Myodes glareolus*	Europe	HFRS/NE	< 1%	(7, 311)
Tula virus (TULV)				*Microtus arvalis*, *M. rossiaemeridionalis*, *M. agrestis*	Europe	?	?	(312–315)
Prospect Hill virus (PHV)				*Microtus pennsylvanicus*, *M. ochrogaster*	USA	–	–	(9)
Isla vista virus (ISLAV)				*Microtus californicus*	USA	–	–	(82)
Sin Nombre virus (SNV)			Neotominae	*Peromyscus maniculatus*, *P. leucopus*	North America	HCPS	35%	(10, 11, 14)
New York virus (NYV)				*Peromyscus leucopus*	North America	HCPS	?	(316, 317)
Bayou virus (BAYV)			Sigmodontinae	*Oryzomys palustris*	North America	HCPS	?	(318–320)
Black Creek Canal virus (BCCV)				*Sigmodon hispidus*	North America	HCPS		(321, 322)
Maporal virus (MAPV)			Sigmodontinae	*Oligoryzomys fulvescens*	Venezuela	?	?	(323)
Andes virus (ANDV)				*Oligoryzomys* longicaudatus, and other *Oligoryzomys* spp.	South America	HCPS	43–56%	(90, 324–326)
Thottapalayam virus (TPMV)	Eulipotyphla/ Soricomorpha		Crocidurinae	*Suncus murinus*	India	?	?	(16, 17)
Tanganya virus (TGNV)				*Crocidura theresae*	Guinea	?	?	(20)
Seewis virus (SWSV)			Soricinae	*Sorex araneus*	Switzerland	?	?	(327)
Camp Ripley virus (RPLV)				*Blarina brevicauda*	USA	?	?	(18)
Ash River virus (ARRV)				*Sorex cinereus*	USA	?	?	(19)
Jemez Springs virus (JMSV)				*Sorex monticolus*	USA	?	?	(19)
Cao Bang virus (CBNV)				*Anourosorex squamipes*	Vietnam	?	?	(21)

HFRS, haemorrhagic fever with renal syndrome; NE, nephropathia epidemica; HCPS, Hantavirus cardiopulmonary syndrome; ?, unknown
*Taxonomy according to Nichol *et al.* (23); virus species are given in Italics;
†Taxonomy according to Wilson & Reeder (22) and Douady *et al.* (328).

segments contain highly conserved complementary 3′- and 5′-terminal nucleotides, which can form panhandle structures, thought to be required for initiation of transcription and replication by the RdRp (26, 32, 33). Detailed analysis of the molecular mechanisms involved in initiation of transcription and replication is hampered, because currently no suitable reverse genetics system for production of recombinant infectious virions has been established.

Like many other viruses, hantaviruses were shown to enter cells by recruitment of integrins (34–37). Most interestingly, pathogenic and non-pathogenic hantaviruses use different integrin receptors. Human integrins αIIaβ3, expressed on platelets, and αvβ3, expressed on endothelial cells, can mediate cellular entry of HFRS- and HCPS-causing hantaviruses (38). In contrast, non-pathogenic or low pathogenic PHV or TULV were indicated to enter the cell via integrin α5β1 (39). The RGD-motif, present in the natural ligands, is not required for binding of hantaviruses by either β1 or β3 integrins (32). This observation is not unique for hantaviruses, because rotaviruses and West Nile virus strain Sarafend were also shown to enter cells via αvβ3 integrin in a RGD-independent manner (34, 40). Furthermore, decay-accelerating factor (DAF/CD55) was identified as a coreceptor for entry of HTNV and PUUV (41).

After binding to the receptor, the virus was indicated to enter the cell by clathrin-dependent endocytosis, and upon internalization, the three separate ribonucleocapsids are released into the cytoplasm together with the virion-associated RdRp (42). Subsequently, primary transcription and production of viral proteins are initiated. Viral transcripts were found to be about 100 nucleotides shorter compared with the genomic template vRNA, lack a poly A tail, and contain capped heterologous host cell mRNA-derived extensions at the 5′ end (43). By biochemical approaches, almost all mRNAs were found to contain a G residue downstream of the heterologous host-derived regions, followed by the conserved terminal region (44). Based on these findings, a 'prime-and-realign' mechanism for initiation of transcription and replication by the viral RdRp was suggested. Because almost all RNA replication intermediates contained U residues at the 5′-termini, the initial guanosine triphosphate (GTP) indicated to prime replication was suggested to be cleaved after elongation by the RdRp.

Bunyaviruses were thought to assort and mature within the cell. However, in contrast to HTNV and other bunyaviruses, which were shown to mature in characteristic intracellular inclusion bodies (45), SNV and Black Creek Canal virus (BCCV) seem to mature at the cell surface, suggesting that these New World hantaviruses use special maturation pathways (46, 47). After budding into the Golgi, mature HTNV is transported and released from the cell via vesicular secretory pathways.

Reservoir hosts, virus evolution, and transmission

Hantaviruses are the only members of the family *Bunyaviridae* that are not transmitted by arthropods. Instead, hantaviruses are carried and transmitted to humans by persistently infected rodents. Hantaviruses are shed by the persistently infected rodents in urine, feces, and saliva. The major route of transmission to humans is indirect by inhalation of virus-contaminated aerosols. In line with this assumption, hantaviruses were found to be highly stable outside the reservoir host, implicating an indirect transmission (48). Rodent biting represents an alternative but rare transmission route to humans (49). Infectious PUUV and ANDV RNA were detected in saliva of the respective rodent reservoirs (50, 51). A human-to-human transmission has been described exclusively for the South American ANDV (52, 53). Interestingly, PUUV-specific RNA was found in the saliva of NE patients, although no infectious particles were detected in the saliva (54).

Rodents of the family Muridae with the subfamily Murinae and the family Cricetidae with the subfamilies Arvicolinae, Neotominae, and Sigmodontinae have been described as reservoir hosts (*Table 1*). The usually observed close association of each hantavirus species to a certain rodent species or closely related species of the same genus is explained by co-evolution of the virus with the host. Moreover, parallel phylogenetic analysis of hantavirus sequences and mitochondrial sequences from the corresponding rodent reservoirs showed congruent tree topologies and positioning of the corresponding virus and rodent sequences. This observation suggests that hantaviruses and Muridae or Soricidae hosts have evolved and dispersed in parallel and that biological boundaries exist to prevent switching between these hosts (55). However, deeper investigation into their phylogenies reveal a mismatch of hosts and hantavirus distribution at the species level (56), suggesting that specific biological boundaries can be overcome, leading to spillover and new host ranges in the past and present times (29).

Experimental infections and field surveys have provided strong evidence for an exclusively horizontal hantavirus transmission among rodents (57–61). Inhalation of infected aerosols, biting, and other aggressive behavioral interactions are among the main modes of this horizontal transmission. Low transmission between naive and hantavirus-infected rodents housed together has been reported (57), suggesting that inhalation and ingestion of excreta from experimentally infected animals may not be highly efficient means of contracting infection. In the wild, immunosuppression, stresses,

or seasonal signals are likely to induce an increase in hantavirus replication, shedding, and consequently transmission. Moreover, bite wounds might be more productive routes of transmission than inhalation (62–67). Urine aerosols generated during aggression behavior could also explain the correlation between wounding and hantavirus infection in natural reservoirs.

No sexual transmission of hantavirus among rodents has been reported, despite the finding of viral antigens in gonads of SEOV-infected rats (64), BCCV-infected cotton rats *Sigmodon hispidus* (58), and SNV-infected deer mice (68). Moreover, there is no evidence of vertical transfer of these viruses in wildlife or experimental settings (61).

Spillover of hantaviruses occurs with a substantially lower frequency than intraspecies transmission but seems common in many sympatric murid rodents (69–73). They have been reported from Europe, e.g. for *Ondatra zibethicus*, *Apodemus sylvaticus*, *Apodemus flavicollis*, and *Arvicola terrestris* (74–77). Spillover infections have also been documented in numerous studies in rodents in the New World (14, 78–83). There are even reports about probable hantavirus infections of moose (84) and carnivores as red fox (74) and domestic cat and dog (85, 86).

Hantavirus-associated clinical syndromes

Human hantavirus infection can result in different clinical syndromes depending on the causative hantavirus species, namely HCPS in the Americas and HFRS with its more benign form NE in Europe and Asia (87–89). Common to all hantavirus-induced disease forms are increased vascular permeability leading to hypotension, hemoconcentration, and vasodilatation. In addition, acute thrombocytopenia, activation of $CD8^+$ T lymphocytes in the peripheral blood, and an increased leukocyte count (leukocytosis) with an enhanced number of immature leukocytes (left shift) is generally found. The differences between the hantavirus-associated clinical syndromes are caused by the fact that different vascular beds are mainly affected: pulmonary capillaries during HCPS and renal medulla capillaries during HFRS and NE.

The clinical manifestations occurring in HCPS can vary from mild hypoxemia to respiratory failure with cardiogenic shock (90). In comparison with HFRS, the case fatality rates are higher and range from about 30–50% (*Table 1*). The prodromal symptoms last for 3–6 days and include fever, chills, myalgia, nausea, headache, vomiting, abdominal pain, and diarrhea. The gastrointestinal symptoms can be mistaken as acute abdomen. Abrupt onset of progressive cough, shortness of breath (tachypnea), tachycardia, and hypotension are harbingers that precede the cardiopulmonary phase. In many patients, pulmonary edema and respiratory failure occur rapidly, making mechanical ventilation mandatory (91). This cardiopulmonary phase lasts on average 2–4 days and is complicated by cardiogenic shock, lactic acidosis, and massive hemoconcentration. Patients can die within hours of hospitalization. Surviving patients enter the polyuric phase, which is accompanied by the resolution of the pulmonary edema. The convalescent phase proceeds slowly, and patients complain about weakness, fatigue, and impaired exercise tolerance.

The clinical presentations of HFRS ranges from febrile disease to fulminant hemorrhagic shock and death (92). It is estimated that the case fatality rate of infection with HTNV infection or *A. flavicollis*-associated DOBV (DOBV-Af) infection is between 5% and 15% (*Table 1*). In typical HFRS cases, there are five distinct disease stages: febrile phase (3–5 days), hypotensive phase (few hours to 2 days), oliguric phase (few days to 2 weeks), polyuric phase, and convalescent phase. After an incubation period of 2–3 weeks, the disease starts with an abrupt onset of flu-like symptoms including high fever, chills, anorexia, myalgia, prostration, dizziness, nausea, headache, and vomiting. An early sign of HFRS is flushing of the face and conjuntival injections. Frequently, patients have blurred vision. In all cases during the early disease phase, the platelet count decreases abruptly (acute thrombocytopenia), reaching the lowest number at the end of the febrile phase (93). Moreover, platelet function can be disturbed (94). The manifestations resulting thereof are so impressive that the word 'hemorrhagic' was used to allude to this striking clinical feature (5). They include petechiae on the skin and mucosa, ecchymoses, conjunctival suffusion, hematemesis, epistaxis, hematuria, melena, and fatal intracranial hemorrhages. In addition, disseminated intravascular coagulation is commonly observed in HFRS patients (95). The hypotensive phase includes hemoconcentration and falling blood pressure due to vascular leakage, which can finally result in fatal shock syndrome. The kidney function is transiently decreased, leading to oliguria or even anuria, proteinuria, abnormal urinary sediment, including microscopic hematuria, and azotemia. During this oliguric phase, which is often accompanied by abdominal or back pain, patients with severe symptoms have to be treated by hemodialysis. Manifestations of the central nervous system, such as coma, can develop as a direct consequence of the disease process, i.e. intracranial hemorrhages, or due to metabolic disorder (96). Within a few days to 2 weeks after disease onset, renal function starts to recover resulting in the polyuric phase (97, 98). The convalescent phase can last from weeks to several months until full recovery is reached.

Milder forms of HFRS caused by SEOV in Asia and *A. agrarius*-associated DOBV (DOBV-Aa)/Saaremaa virus (SAAV) in Europe or NE caused by PUUV show a limited spectrum of symptoms resulting in lower case fatality rates (99, 100). In NE, the five phases of HFRS are not easily distinguishable. Instead of full-blown shock syndrome, hypotension is observed. One-third of the patients develop hemorrhagic manifestations that are less severe and in general become apparent as petechiae. Most patients have signs of kidney function failure, which usually is less prominent than in HFRS caused by more virulent hantaviruses. Transient ophthalmic involvement including myopia is found in about 50% of NE cases (101–103). Interestingly, in 10–20% of NE patients, pulmonary involvement is observed, including pulmonary infiltrates and pleural fluid accumulation (104, 105). Taken together, the clinical course of NE is often uncharacteristic and resembles more a febrile disease with abdominal pain. For this reason, it is quite often not diagnosed as NE.

In vitro cell culture systems

A major drawback for studying pathogenesis caused by hantaviruses is the lack of suitable animal disease models and/or suitable immunological tools for these animal species. Therefore, most studies are based on established cell lines or primary cells infected with cell culture-adapted hantaviruses. Isolation of the Korean hemorrhagic fever agent has been tried by several groups since its identification in 1952. But it was not until 1981 that French *et al*. (106) described adaptation and propagation of HTNV strain 76–118 isolated from *A. agrarius coreae* in *vitro*. Subsequently, several cell culture systems were established for propagation of hantaviruses (107–111). However, after cell culture adaptation, PUUV did not reproducibly infect bank voles anymore in contrast to the parental wildtype (112). For PUUV as well as SNV, cell culture adaptation-associated mutations were found preferentially in the non-coding region or did not affect the amino acid sequence, except one serine versus phenylalanine at position 2053 of the PUUV L protein (112–114). Based on these data, alterations of regulatory elements in the genome were discussed to be decisive for high-level replication in cell culture. To produce genetically homogenous hantavirus stocks in cell culture, a focus purification method for non-cytolytic viruses was established recently, according to the classical plaque purification method used for cytolytic viruses (31). Interestingly, all hantavirus species investigated produced substantially higher titers after focus purification. Furthermore, most hantavirus species subjected to this procedure replicated with a similar efficiency in Vero E6 cells and stocks with comparable titers were produced. The reason for increased replication level after focus purification is not clear, but removal of defective particles that are produced at the expense of replication competent hantaviruses could explain this finding.

Animal models

Animal models for hantaviruses are important tools for two different research applications, i.e. studies on the mechanisms of hantavirus persistence and transmission in natural rodent reservoirs (see Reservoir hosts, virus evolution, and transmission) on one hand and appropriate disease models on the other.

Disease models mimicking human pathogenesis have been developed using small mammals and non-human primates. Small mammal models are based on Syrian hamsters (*Mesocricetus auratus*) and laboratory mice. Thus, ANDV, but not SNV, was found to be highly lethal in adult Syrian hamsters (115, 116). The characteristics of the disease in hamsters, including the incubation period, symptoms of rapidly progressing respiratory distress, and pathologic findings of pulmonary edema, pleural effusion, and hypotension, closely resemble HCPS in humans (115–117). Similarly, Maporal virus (MAPV), another South American hantavirus, can cause in the Syrian hamster a disease that is clinically and pathologically remarkably similar to HCPS. These similarities include mononuclear cellular infiltrate in lung and liver, widespread distribution of hantaviral antigen in endothelial cells of the microvasculature of lung and other tissues, and variable lethality (118).

Laboratory mouse models are usually based on newborn suckling or immunologically deficient mice. Newborn mice (*Mus musculus domesticus*) and immunologically deficient animals [nude or severe combined immunodeficiency (SCID) mice] were found to die after infection with hantaviruses (119). Newborn mice infected with HTNV develop a fatal illness with acute systemic infection with high titers of virus in almost all organs. Infected mice develop inflammatory and destructive lesions in various organs and die 2–4 weeks with wasting and neurological damages. Most of these symptoms are similar to the findings in severe human HFRS infection. Several strains of adult mice may also be susceptible to HTNV when infected intraperitoneally (120).

In addition to small mammals, non-human primates have been employed for pathogenicity studies. First, intravenous inoculation of three cynomolgous monkeys (*Macaca fascicularis*) and a chimpanzee (*Pan troglodytes*) with PHV, a hantavirus non-pathogenic to human, surprisingly caused acute nephropathy characterized by mild transient proteinuria and azotemia (121). Experimental intratracheal infection of cynomolgus

macaques with cell culture-adapted PUUV resulted in signs of lethargy followed by mild proteinuria and microhematuria. Histopathologic changes were largely confined to abnormalities in medullary tubular cells of the kidneys, which coincided with the demonstration of viral antigen and viral RNA (122). Infection of cynomolgus macaques with a PUUV strain, which was exclusively replicated in the natural host, induced typical signs of HFRS including lethargy, anorexia, proteinuria, and/or hematuria, in addition to cytokine, C-reactive protein, creatinine, and nitric oxide (NO) responses. Viral RNA was detected in plasma during the acute phase, infectious virus was recovered, and the virus-specific immune responses mimicked those seen in humans (123). For future studies, it is of interest to study animal species other than those mentioned here, where natural spillover infections were detected, for potential disease symptoms and their similarity to those observed in humans.

Antiviral immune mechanisms

Interactions of the host immune system and hantaviruses are of great importance for understanding hantavirus infections, in regard to the susceptibility, transmission, and outcome in rodent reservoirs and spillover infected animals. Moreover, the hantavirus-induced pathogenesis in humans is clearly influenced by immunological factors.

The host immune response to viral pathogens including hantaviruses can be broadly divided into an adaptive and an innate part. The adaptive immune response consists of specific pathogen recognition by clonally distributed T- and B-cell receptors, which are generated randomly to form a highly diverse repertoire of antigen receptors. Before they can contribute to the host defense, specific T- and B-cell clones need to expand and differentiate into effector cells delaying the adaptive responses for 5–7 days. In this early phase, therefore, hantaviruses have to be controlled by the innate immune response, which represents the evolutionarily more ancient and more universal part (124). Pattern recognition receptors (PRRs) expressed by immune cells interact with conserved structural moieties called pathogen-associated molecular patterns (PAMPs) displayed by infectious agents (125). In this way, innate immune responses against quite diverse pathogens are triggered. Generally, four types of virus-associated PAMPs have been found (126): envelope glycoproteins, double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and non-methylated CpG DNA.

Dendritic cells (DCs) bridge the innate and adaptive immune response by sensing pathogens through PRRs and differentiating into the most effective antigen-presenting cells of the immune system (127). As such, they are important for activation of the effector components of the adaptive immune system: $CD4^+$ T-helper (Th) cells, $CD8^+$ cytolytic T lymphocytes (CTLs), and B cells. DCs take up and process microbial antigen and present the resulting peptides through surface human leukocyte antigen (HLA) molecules, which are subsequently recognized by T cells through their T-cell receptor (TCR). DCs determine not only the strength but also the quality of the immune response (128, 129). Through secretion of different cytokines, depending on the type of pathogen-derived stimuli received during differentiation, DCs drive the development of antigen-specific T cells into different types of Th cells (130): Th1, Th2, Th17, or regulatory T (Treg) cells, which can modulate fundamentally the outcome of viral infections. On one hand, Treg cells are important for maintaining host homeostasis by controlling destructive inflammatory reactions and thus prevent immunopathology during antiviral immune responses. On the other hand, suppression of the host antiviral immune response by Treg cells can help viruses to persist in the host (131, 132).

In the following sections, the different aspects of the immune response in reservoirs and humans will be discussed. There are also some important cell-biological parameters that have to be considered briefly.

Cell-biological determinants in pathogenesis

Hantavirus-induced apoptosis

Hantaviruses were found to infect cell lines like Vero from African green monkey but also Huh7 and A549 derived from human carcinoma of the liver and lung, respectively. Furthermore, primary human monocytes/macrophages, DCs, and endothelial cells can be infected with hantaviruses *in vitro* (107–109). In many of these cells, obvious cytopathic effects caused by the infection were not detected (107, 108, 111). However, signs of apoptosis were induced in part by both pathogenic and non- or low pathogenic hantaviruses *in vitro* (133, 134). In an attempt to quantify induction of apoptosis by different HFRS-causing hantaviruses, significant signs of apoptosis were detected only in proliferating but not in growth-arrested cells (135). Thus, hantavirus infection might partially destroy the endothelial vasculature associated with an increased permeability by induction of apoptosis preferentially in proliferating cells.

Interaction of hantaviruses with specific integrins

Pathogenic and non- or low-pathogenic hantaviruses recruit different integrins to enter the cell. The pathogenic NYV and HTNV were shown to interact with the plexin–semaphorin–integrin (PSI) domain located at the N-terminus of β3

integrin. The PSI domain contains important alloantigens and generation of corresponding autoantibodies were indicated to cause immune thrombocytopenias (136). Whether binding of the PSI domain also by hantaviruses causes development of thrombocytopenia in HFRS or HCPS as described by autoantibody-mediated neonatal alloimmune thrombocytopenia or post-transfusion purpura is an interesting open question.

The affinity of integrins for their ligands varies, and currently accepted models suggest that the affinity of integrin αvβ3 is regulated by conformational changes. High-affinity binding to the natural ligands is associated with an extended structure and low affinity or inactivation of integrin αvβ3 with a bent or folded conformation. Treatment of cells with Mn^{2+} ions favors the activated conformation of αvβ3 integrin. Interestingly, pathogenic NYV and HTNV preferentially bind inactive αvβ3 integrin stabilized in the bent or folded conformation. Consistent with this preference, addition of Mn^{2+} ions reduced infectivity of NYV and HTNV by a factor of two compared with the untreated control (137).

Modulation of cell motility and permeability by hantaviruses

Integrins play a pivotal role in regulation of vascular permeability and migration of endothelial cells. Mutations or deficiencies in integrin αIIbβ3 or αvβ3 cause severe bleeding disorders in humans and mice (138, 139). Generally, activation of αIIβ3 and αvβ3 are thought to trigger vasodilation and integrin α5β1 vasoconstriction (140). These correlations suggest that infection of endothelial cells with pathogenic hantaviruses via β3-integrin could be decisive for development of vascular permeability in HFRS and HCPS patients. This theory is supported by the finding that infection of endothelial cells with pathogenic hantaviruses can modulate motility and vascular permeability. Infection with NYV, HTNV, and SEOV significantly reduced motility of endothelial cells on vitronectin coated transwell membranes controlled by β3-integrin. TULV and PHV did not alter migration of endothelial cells in that experimental setting (39). Recently, infection of endothelial cells with HCPS- and HFRS-associated hantaviruses for 3 days was demonstrated to increase the permeability of the cell layer elicited by addition of vascular epithelial growth factor (VEGF) (38). Non-pathogenic hantaviruses or addition of VEGF at earlier time points after infection did not increase VEGF-induced vascular permeability. This temporal requirement suggests that initial binding of integrin β3 is not sufficient to sensitize cells for VEGF-induced vascular permeability.

VEGF was originally described as vascular permeability factor based on its activity to induce vascular permeability, which was about 50 000-fold higher compared with histamine (141). Interestingly, integrin αvβ3 has been demonstrated to form complexes with the VEGF receptor-2 (VEGFR-2), which is specifically expressed on angiogenic endothelium. Other integrins, such as the β1 integrin, do not form complexes with VEGFR-2 (142, 143). These findings suggest that the interaction of pathogenic hantaviruses with β3 integrins in concert with other factors like VEGF might be important determinants for pathogenesis caused by hantaviruses *in vivo*. Most interestingly, as several VEGF-induced inhibitors of vascular permeability are used clinically for other indications, these approaches might provide an option to treat hantavirus patients (38).

Hantavirus glycoproteins with immunoreceptor tyrosine-based activation motif region and degron activity

By amino acid sequence alignments, an immunoreceptor tyrosine-based activation motif (ITAM) was predicted in the C-terminal cytoplasmic region of G1/Gn of several HCPS-associated hantaviruses and of TULV (144). Based on co-immunoprecipitation and kinase assays with G1/Gn peptides and recombinant sub-fragments, respectively, the cytoplasmic tail of NYV-G1/Gn was shown to contain a functional ITAM region. ITAMs are short protein motifs with a $YxxL/Ix_{6-8}YxxI/L$ consensus sequence (x is any amino acid) present in various receptors of the immune system, including TCR-ζ, immunoglobulin α (Igα), Igβ, and FcεRIγ. Generally, activation of an ITAM leads to phosphorylation of the tyrosines by Src-family tyrosine kinases, and recruitment of Syk or related kinases initiates further downstream signaling (145). Recent studies have shown that β2 and β3 integrins can transduce signals via adapter molecules containing ITAM regions (146). This finding implies that the ITAM region of the G1/Gn cytoplasmic tail might trigger intracellular signaling cascades via Src family kinases by recruitment of β3 integrin. Interestingly, the tyrosine residues of this ITAM region were subjected to direct ubiquitination and 26S proteasome-dependent degradation of the cytoplasmic tail of NYV-G1/Gn (147). The authors suggest that the degron activity might downregulate cellular proteins that interact with the hantavirus G1 glycoprotein (144).

Immune response to hantaviruses in rodents

Immunity and rodent life-history

Hantavirus-induced immune responses can affect different life-history traits of rodent reservoir hosts such as the hantavirus transmission between rodents and the fitness of infected rodents.

Immune response and transmission

Several infection experiments and field surveys support the hypothesis of maternal hantavirus-specific antibody transmission in rodents (57, 62, 148–152). Both IgG- and IgA-specific antibodies are transferred, either *in utero* or by breastfeeding (149, 153). These maternal antibodies are protective until maturity, as demonstrated in rats (149) or bank voles (59). When challenged again as adults, rodents are susceptible to hantavirus infection (150).

Consequences of hantavirus infection in rodent reservoirs

The ability to isolate or detect hantavirus from wild caught rodents, even in the presence of high levels of serum antibody, has led to the initial belief that hantavirus infection is asymptomatic and has no impact on survival or fecundity in their reservoir hosts (154). However, several more recent experimental and field data do not support these assumptions. There are reports of subtle histopathologic lesions in some of the natural hosts of certain HCPS-causing hantaviruses. Morphologic examination of the white-footed mouse *Peromyscus leucopus* infected with NYV (155) and SNV-infected deer mouse (156) reveals pulmonary edema and periportal hepatitis. Using immunohistochemical analysis, viral particles can be detected in pulmonary endothelium, and immune infiltrates can be observed in portal zones of the liver. A recent field survey of an island bank vole population reveals that PUUV infection has a negative influence on the over-winter but not over-summer survival of bank voles (59, 157). However, the mechanisms causing these effects remain unknown.

Chronic infection with three stages: acute phase, persistence, and clearance

Hantavirus infection is generally described as persistent and lifelong in its reservoir hosts. Persistence is defined as the situation in which the virus is present in host cells, usually at low levels, maintaining the capacity for either continued or episodic reproduction at some future period (158). However, much evidence comes from studies that do not always include measurements of virus shedding, and such generalization deserves further research efforts (reviewed in 159). Experimental infections of striped field mouse *A. agrarius* with HTNV, *R. norvegicus* with SEOV, *M. glareolus* with PUUV, *P. maniculatus* with SNV, and *S. hispidus* with BCCV have shown that these natural reservoirs experience a short transient viremia beginning approximately few days (160, 161) or 2–3 weeks (51, 61, 68) after infection. This acute phase usually ends after 2–3 weeks, when infectious titers, viral antigen expression, or virus RNA levels peak and begin to decline. In parallel, early after infection, infectious virus can be recovered from different organs (including lungs, spleen, kidneys, intestines, and salivary glands) that may serve as sites for virus replication or maintenance (51, 68). Viral shedding in the rodents' saliva, urine, and feces is then detectable and seems persistent during the course of the studies (60–62, 160, 162). When tissues from infected rodents were examined at the latest points post-infection (more than 1 year), only the brain (BCCV), lungs (PUUV, HTNV, SEOV, and SNV), heart (SNV), or liver (PUUV) exhibited markers of infection (51, 58, 61, 156, 161, 162). Some of the tissues that are positive for viral markers during the acute phase can become negative during the persistent phase. Globally, a dramatic decrease in virus titers is observed few months after infection, suggesting that some stages of virus lifecycle are downregulated. This also corresponds to the presence of high titers of circulating hantavirus-specific antibodies that will be detected lifelong. Intermittent excretion/secretion of virus in feces, saliva, and urine has also been described for several natural reservoirs (51, 61, 62). These results indicate periodic episodes of viral recrudescence, suggesting that cyclical bursts of replication may take place during persistent infection. Such regulation could occur through changes in the virus and/or host immune response.

Individual variability in susceptibility to hantavirus

Experimental infections and natural population surveys have highlighted the individual variability of rodent susceptibility to hantaviruses. First, most infections detected during longitudinal studies of reservoir populations occur through age-dependent horizontal routes. A positive correlation is often observed between the age of rodents and the seroprevalence (62, 163–166). Non-immune voles of any age are apparently equally susceptible to hantavirus infection, resulting in an increase of the probability of acquiring infection with age.

Males and females differ in their susceptibility to hantaviruses (167). Longitudinal studies have highlighted that more males are infected than females among rodents that carry hantaviruses (62, 154, 164, 168, 169). Experimentally infected male rats produced stronger circulating antibody responses, shed SEOV for longer times and more consistently, and had more virus copies than did conspecific females (167, 170). Both experimental infection and field surveys showed that SEOV infection increases aggression behavior in male Norway rats (64, 171). It is unlikely that hantavirus can cause more aggressive behavior by infecting brain cells, as only BCCV is known to cross brain barriers (160). Alternatively, sex steroid hormones were hypothesized to underlie the dimorphism in infection, through effects on the behavior or the immune

system (65). The manipulation of sex steroid hormones in adulthood does not affect sex differences in response to hantavirus infection (167), and neonatal hormone manipulation only alters antibody responses and SEOV shedding but not virus replication in target tissues (172). Sex steroids thus only mediate some but not all sex differences in hantavirus infection. It is likely that virus replication is mediated by sex steroid-independent mechanisms such as sex-based differences in gene expression associated with innate immunity (171, 173). Infected females have higher expression levels of genes encoding for pro-inflammatory, antiviral, major histocompatibility complex (MHC), Ig, and T-cell marker proteins, whereas infected males had higher expression of heat shock protein genes. Neonatal hormone manipulation altered anti-SEOV Ig2a responses to infection, suggesting that neonatal sex steroids may affect inflammatory responses associated with cell-mediated immunity (172), which is important for hantavirus clearance. Infestation experiments also suggest the existence of variability in the susceptibility to hantaviruses in natural reservoirs, independently of sex and age (59, 162).

Immune responses during rodent infection

Establishing the immune mechanisms underlying the characteristics of hantavirus infection in natural reservoirs appears essential to explain persistence as well as sex differences and other factors mediating individual variability in susceptibility to infection. These features are expected to strongly affect the hantavirus dynamics in rodent reservoirs and the epidemiology of HFRS and HCPS. Unfortunately, studying hantavirus infection of natural reservoirs has been challenging because of the near absence of immunologic and genetic reagents for non-model rodent species (174).

To establish a persistent infection in a host, a virus must be able to regulate its cytopathic effects on host cells, maintain functionally intact genomes in cells, and avoid elimination by host immune system (159). A mechanism such as replication in a 'privileged' site, not readily accessed by the host immune system, is unlikely, as hantaviruses replicate in many tissues and organs that are targeted by immune system. Prevailing hypotheses to explain hantavirus persistence have thus concerned the suppression or exploitation by hantaviruses of rodent host immune responses that are necessary for clearance of the infection (175).

Adaptive immunity: antibody and B-cell-mediated immunity

The importance of the humoral response for protection of rodents against hantavirus infection was demonstrated by passive transfer of immune sera and subsequent challenge in newborn rats (176), suckling mice (177, 178), or Syrian hamsters (178). All neutralizing monoclonal antibodies protected rodents from lethal hantavirus infection. G1 and G2 envelope glycoproteins are the main inducer of this protective humoral response (179). This humoral response is also important in natural rodent reservoirs, as shown by a recent study of cytokine expression in deer mice infected with SNV. Interleukin-5 (IL-5), an important B-cell stimulating factor that induces the secretion of IgG and IgA from antigen-activated B-cell subsets and likely contributes to IgG persistence during hantavirus infection, is highly expressed by T cells from the acute phase but downregulated during persistence (174).

Animal studies have shown that hantaviruses can persist in hosts despite the presence of high titers of neutralizing antibodies (61, 161). Neutralizing antibodies found in sera of HTNV-infected newborn mice did not affect the amount of HNTV N protein (180). The role of hantavirus-specific antibodies in persistently infected mice seems to be related to the suppression of hantavirus dissemination into the brain and the evasion of fatal disease (177, 181). Thus, the existence of B cells is essential for the establishment of persistent HTNV infection without any signs of disease (181).

Adaptive immunity: T-cell-mediated immunity

T-cell responses play an important role in controlling hantavirus infection and viral replication in rodents. (i) SEOV-infected nude rats, which cannot mount competent T-cell responses, exhibit higher viral titers than their T-cell-competent counterparts. They may even succumb rapidly to SEOV infection (182). (ii) The persistent HTNV infection in nude mice can be prevented by transfer of immune serum or immune T cells from BALB/c mice (183). It seems that T cells expressing either the CD4 or CD8 marker, i.e. helper and cytotoxic T cells, were almost equally effective in protecting nude mice against HTNV infection. In contrast, $CD4^+$ T cells were less effective than $CD8^+$ cytotoxic T cells for viral clearance (183). In particular, cytotoxic splenocytes from HTNV-infected mice have been found to lyse HTNV- and SEOV-infected macrophages (184).

The persistence of hantavirus infection in rodent reservoirs could be caused by escape from $CD8^+$ T-cell immune surveillance (180, 181). HTNV-infected newborn BALB/c mice, which are a model of persistent infection similar to natural reservoirs, transiently induce IFN-γ-producing hantavirus-specific $CD8^+$ T cells during the acute phase. The TNF-α production and cytotoxic activity of these cells are impaired, and they disappear very quickly by functional exhaustion.

Virus antigen loads seem to increase because of this functional impairment of the $CD8^+$ T cells, as HTNV-infected newborn BALB/c mice with large numbers of IFN-γ $CD8^+$ T cells tended to have no or only small amounts of N protein (180). Similar results are observed from experimental infections using adult SCID mice: a strong correlation is observed between HTNV persistence and the lack of HTNV-specific $CD8^+$ T cells (181). This study reveals that the dissemination of hantavirus infection before the induction of immune response is important for the establishment of persistent infection in mice. It also shows that HTNV can establish persistent infection in adult mice that possess mature immune systems. The suppression of HTNV-specific $CD8^+$ T cells would then occur through induction of anergy or clonal deletion in the periphery. These results are of great importance, as natural rodent reservoirs are more likely to become infected when adult. Similar mechanisms of an impaired HTNV-specific $CD8^+$ T-cell response could contribute to hantavirus persistence in natural reservoirs.

$CD8^+$ T cells also contribute to HTNV clearance in BALB/c and SCID mice. N proteins in the brain or lungs of mice disappeared after the appearance (BALB/c model) or transfer (SCID model) of HTNV-specific $CD8^+$ T cells, and a correlation is observed between the decrease of N protein amount and the increase in number of IFN-γ-producing $CD8^+$ T cells (180, 181).

Nevertheless, hantavirus infection in laboratory mouse models differs from infection in natural rodent reservoirs. To investigate whether a potential suppression of $CD8^+$ T cells is involved in persistent infection of reservoir hosts still requires the development of a method for detecting hantavirus-specific T cells in non-model organisms. For the natural reservoirs *R. norvegicus* and *P. maniculatus*, methods for investigating the role of $CD4^+$ T-cell subsets in hantavirus persistence were developed using functional inactivation of CD25 (175) and molecular assays (174). The cytokine expression pattern is different in T cells derived from acutely or persistently infected deer mice. In particular, T cells from persistently infected deer mice were demonstrated to exhibit diminished types 1 and 2 cytokine expression levels compared with acutely infected ones and increased expression level of tissue growth factor-β1 (TGF-β1) in Treg cells (174). Increased transcription of TGF-β1 and forkhead box protein 3 (FoxP3) as well as an increased percentage of $CD4^+CD25^+FoxP3^+$ Treg cells was reported in persistently SEOV-infected *R. norvegicus* (175). Furthermore, inactivation of Treg cells reduces genomic SEOV RNA in target organs. These results suggest that the activation of Treg cells may be a common mechanism mediating persistence of hantaviruses in natural rodent reservoirs. This is corroborated by the capacity of Treg cells to alter the function and chemotaxis of immune cell populations such as $CD8^+$ T cells and macrophages. Because Treg cells also suppress inflammatory responses that are necessary to eliminate pathogens, the presence of TGF-β1 and IL-10 early in infection suggests that these cytokines may limit inflammatory responses and consequently immunopathology during acute infection. It is thus likely that early events during the innate response drive the development of Treg cells (174).

Innate immunity

Little attention has been given to innate immunity in natural rodent reservoirs, and current knowledge mainly comes from the studies of sex differences in the expression of genes encoding for proteins associated with innate antiviral defenses in SEOV-infected rats (171, 173).

The induction of the supposed PRRs of hantavirus differs between the sexes, with SEOV-infected females showing higher expression of Toll-like receptor-7 (TLR-7), retinoic acid-inducible gene I (RIG-I), and IFN regulatory factor-7 (IRF-7), a transcriptional factor involved in induction of type 1 IFNs, than SEOV-infected males (173). Consequently, higher production of IFNα/β, which results in the increased expansion and survival of $CD8^+$ T cells, and downstream expression of IFN-stimulated genes (ISGs), which are critical for restricting virus replication, are observed in SEOV-infected females (171, 173). In particular, the expression of Mx2 genes, encoding for cytoplasmic Mx2 proteins which confer resistance to HTNV *in vitro* (185), is suppressed in male rats during SEOV infection and may contribute to increased virus shedding and viral RNA levels in lung tissues observed among males compared with females (167). These differences in the expression of Mx and IFN genes may underlie male-biased susceptibility to hantaviruses, and further investigations are required to test their impact on variability in susceptibility to infection or reservoir competence.

Elevated pro-inflammatory cytokine levels are not systematically detected in hantavirus infected rodents. Considering NO, laboratory mice that die after DOBV-Af inoculation exhibit high levels of nitrites, whereas no evidence of elevated NO is found in SAAV-infected laboratory mice or SNV-infected deer mice (186, 187). Furthermore, no replicating virus is detected in inducible NO synthase knockout mice ($iNOs^{-/-}$) after HTNV infection, suggesting that NO is not involved in hantavirus clearance. NO seems to be a mediator of the hantavirus pathogenesis in rodents, probably in combination with virus replication rate and the Th-type of immune response mounted (187).

Immunogenetics and variability in immune response

Immunogenetics, the analysis of genetic polymorphism in specific recognition and immune regulation, remains scarcely investigated to explain the variation of immune responses observed across natural rodent reservoirs during hantavirus infection. Only MHC haplotypes have been explored for their influence on immune responses against hantavirus, both in laboratory mice and in natural populations of bank voles. MHC-specific differences in humoral response magnitude to recombinant PUUV N-antigens have been found in inbred strains of laboratory mice (H-2^b, H-2^d, and H-2^k) (188). Recent preliminary work in natural French populations of *M. glareolus* also suggests potential links between MHC class II characteristics and hantavirus infection status (189). Other candidate genes potentially involved in immune responses against hantaviruses could be selected from the recent microarray analyses performed on Norway rats. They could next be applied to the study of wild rodents. For example, a general pattern of upregulation of about 200 immune-related genes is detected in females infected with SEOV compared with infected males (171, 173). Such differences of immune gene expression could explain variability in susceptibility. Sequence polymorphism could also be considered. No conspicuous differences were observed in the amino acid sequences of deer mouse cytokines (IL-2, IL-6, IL-12a, IL-13, IL-21, and IL-23a) or chemokines (CCL2, CCL3, CCL4, and CXCL2) compared with rat or house mouse orthologs that would suggest a functional difference in the mechanism by which these molecules exert their effects in response to hantaviruses (190). The TNF promoter is a promising candidate gene for hantavirus susceptibility as variations are associated with high and low levels of TNF production in deer mouse (191).

Hantavirus escape mechanisms, quasi-species and persistence of rodent infection

Most viruses have developed sophisticated escape mechanisms to impair induction of the antiviral response at least in part to win time for efficient replication and establishment of an infection. The talent to escape or block the innate IFN system is a key determinant for the virulence of many pathogenic viruses (192–194). The mechanisms by which viral replication is regulated during hantavirus infection are still not clearly understood, especially in natural rodent reservoirs. Many viruses evade sterilizing immune response with virally encoded proteins that modulate the host response in a favorable manner to the virus. However, size constraints for RNA viruses limit their ability to establish persistence through the expression of immune evasion genes, and there was no polypeptide with immunomodulating activities described in hantaviruses, except the recently described putative function of NSs-ORF of Arvicolinae-associated hantaviruses (27–29, 195).

It seems unlikely however that hantavirus persistence and infectivity are regulated solely through immune mechanisms. Hantavirus escape from host immune system could also occur via antigenic variation. Several groups have studied these genetic changes and virus quasi-species have been described in naturally SNV-, TULV-, or PUUV-infected rodents (195–197). Using mark-recapture studies of deer mice, Feuer *et al.* (195) observed high genetic diversity in both the segments coding for the G1 region (mutation frequency 3.2×10^{-3}) and in the 3′ S segment non-coding variable region (SVAR) (mutation frequency 5.1×10^{-3}) of SNV, with about 60% of the mutations being non-synonymous in the G1-encoding region. This mostly corresponds to the diversity reported for TULV (1×10^{-3}) (197) and PUUV (3×10^{-3}) (196) S segments in wild common and bank voles. Many of the amino acid exchanges were found in protein regions of high surface probabilities and antigen index values (195).

Viral clones isolated before seroconversion indicate that hantavirus diversity existed before the generation of an active antibody response, indicating that host immune responses were not influencing the extention of the SNV quasi-species at this time (195). Moreover, higher percentages of A → G or U → C mutations were observed in viral sequences originating from certain tissues (195, 197), suggesting a role for the cellular RNA-editing enzyme, double-stranded RNA adenosine deaminase (dsRAD), in generating hantavirus genetic variation. Interestingly, this enzyme has been found to be IFN inducible and may form a cellular antiviral defense mechanism which could degrade SNV dsRNA.

Considering SNV-infected deer mice, G1-encoding and SVAR sequences exhibited higher diversity in the spleen than in the lung and liver, especially when including only A → G or U → C mutations (195). The reasons for these differences may lie in the fact that viral immunological escape through the generation of viral diversity may be more critical in major sites of immune responses to antigens such as the spleen and lymph nodes. Hantavirus variants may thus be contributing to persistence by yielding analogous peptide epitopes which can still interact with hantavirus-specific TCRs without delivering a full T-cell stimulatory signal.

Immunity and interspecies transmission

Immune mechanisms inducing the clearance of hantavirus spillover infection in non-reservoir hosts still remain unknown. Comparing sympatric deer mice and wood rats exhibiting

circulating antibodies specific for SNV, Feuer *et al.* (195) observed similar exchange frequencies at the nucleotide and amino acid levels for the G1 region of the genome. This finding refutes the hypothesis that higher hantavirus genetic diversity in specific reservoirs than in non-reservoir species mediates the persistence rather than the clearance of hantavirus infection in rodents.

The observation of a predominant viral strain in the variant SNV genomes detected in SNV-infected rodents and the lack of progressive temporal shifts in SNV variants both suggest the importance of a genetic bottleneck, i.e. the transfer of a minor variant of the virus population, for hantavirus transmission into new hosts. The fact that hantavirus genetic diversity exists in saliva gland and bladder highlights the likelihood that emergence of new viruses is favored by viral diversity and genetic plasticity of hantaviruses.

Immune response to hantaviruses in humans

Human immune responses to hantaviruses cannot only provide a defense against to hantaviruses but can also contribute to virus dissemination and virus-induced pathogenesis.

Role of the human immune system in dissemination of hantaviruses

It is not yet defined how hantaviruses disseminate in the human body after inhalation, but immature DCs play a pivotal role, as they express β3 integrin and DAF/CD55. In the airways and alveoli of the lung, a network of immature DCs is located in the vicinity of epithelial cells that can take up pathogens (198). Hantaviruses can infect DCs from different sources, including monocyte-derived immature DCs (*Fig. 1*) and mature DCs, without causing cell death (108). These hantavirus-infected DCs could serve as vehicles for the transport of virions through the lymphatic vessels to the regional lymph nodes, where they could infect other immune cells such as macrophages and monocytes. After further replication, cell-bound and free virions could reach endothelial cells, which represent the main target cells for pathogens causing viral hemorrhagic fever (199). DCs have also been implicated in the dissemination of DNA viruses as well as plus-strand and other minus-strand RNA viruses (200–202). Besides DCs, human alveolar macrophages are susceptible to hantavirus infection and could contribute to viral dissemination (203). In any route of transmission, immature DCs located in the skin and mucosal surfaces are probably among the first cells infected and facilitate hantaviral dissemination.

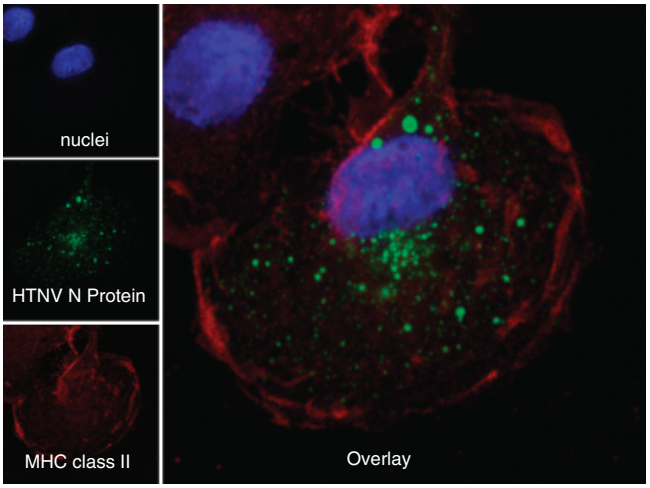

***Fig. 1.* HTNV infection of immature DCs.** DCs generated from monocytes by incubation with GM-CSF and IL-4 for 6 days were infected with HTNV at a MOI of 1. Four days post-infection, the cells were fixed and stained for nuclei (Dapi/Blue), HTNV N protein (FITC/Green), and MHC class II (Texas red/Red). The overlay is shown on the right side.

Hantavirus-associated immunopathogenesis in humans

The mechanisms leading to increased vascular permeability and decreased platelet count, the hallmarks of hantavirus-associated pathogenesis in humans, are poorly understood. Endothelial cells, which regulate vascular permeability by forming a barrier at the interface of blood and tissue (204), are infected with hantaviruses *in vitro* without showing any cytopathic effect (107, 109, 111, 205). In addition, infection with pathogenic hantaviruses *per se* is not sufficient to increase the permeability of endothelial cell monolayers *in vitro* (38, 203, 206). In contrast, other hemorrhagic fever viruses such as Ebola virus (EBOV) are cytopathic and can cause directly vascular leakage of endothelial cells (207). Thus, hantaviruses are non-cytopathic in endothelial cells and do not enhance *per se* vascular permeability pointing toward the host immune system as an important player in hantaviral pathogenesis. An inadequate and harmful antiviral immune response may result from the fact that hantaviruses are very well adapted to specific rodents but not to the human immune system, which did not undergo coevolution with these pathogens. To understand hantavirus-induced immunopathology, analysis of the interaction between hantaviruses and the human antiviral defense is obligatory.

Innate immune response against hantaviruses in humans

Viruses invading a host are detected early during infection by non-immune cells as well as DCs located at the host-pathogen interface (127, 208). The host virus-sensing PRRs have been discovered, including TLRs and RIG-I-like helicases (RLHs), which comprise RIG-I and melanoma differentiation antigen 5 (MDA5) (209). RIG-I has been demonstrated to be required

for detection of members of the families *Orthomyxoviridae*, *Paramyxoviridae*, *Rhabdoviridae*, and *Flaviviridae* (210–212). RIG-I recognizes uncapped ssRNA 5′-triphosphate ends and blunt ended or 5′-overhang dsRNA ends (213). Recently, it has been discovered that RIG-I is unable to detect genomic RNA derived from HTNV particles (214), because the first nucleotide of the genome 5′ end is cleaved off by viral RdRp activity resulting in a monophosphorylated 5′-end (44). There is evidence that NYV can interfere with RIG-I-mediated signaling, suggesting that RIG-I is involved in detection of hantaviral components other than genomic RNA (215). However, ultraviolet-irradiated SNV is not recognized by any of the known PRRs and induces an innate response that is independent of IRF-3 (216). In conclusion, the human PRRs that sense hantaviruses remain to be identified.

As a consequence of PRR signaling, type I IFN and other pro-inflammatory cytokines are produced, which are crucial for inducing a variety of innate antiviral effector mechanisms (193, 217). These cytokines induce cellular resistance to virus infection and activate innate immune cells like natural killer (NK) cells or NKT cells (218). In this way, the host is able to limit viral spread in the lag phase before the adaptive response is ready to strike. In Vero E6 cells, treatment with IFN-α, -β, and -γ inhibited replication of HTNV, PUUV, and TULV (219), with IFN-β showing the strongest antiviral effect. Pre-treatment of A549 cells, a human lung epithelial cell line, with type III IFN (IFN-λ1 -λ2, and -λ3, also called IL-29) has been demonstrated to weakly reduce the level of HTNV replication (220). A type I IFN-induced mechanism that interferes with hantavirus replication is mediated by members of the Mx gene family, which encodes large guanosine triphosphatases that are related to dynamin (193). The human ortholog of the mouse Mx1 protein, called MxA, has antiviral activity against different genera of the family *Bunyaviridae*, including *Hantavirus*, *Orthobunyavirus* (LACV), *Nairovirus* (Dugbe virus, CCHFV), and *Phlebovirus* (RVFV) (221–226), by recognizing and sequestering the ribonucleoprotein complex (225). Stable transfection of the MxA gene into Vero E6 cells renders these cells resistant to infection with PUUV, TULV, and HTNV (224, 227). Although in general hantaviruses are relatively poor inducers of type I IFN, induction of ISGs including the MxA protein has been observed in endothelial cells after hantavirus infection (228–231), and MxA has been shown to colocalize with hantaviral N protein (229). The induction of ISGs by NYV, HTNV, ANDV, and PHV has been demonstrated to require viral replication and was associated at least in part with activation of IRF-3 (215, 232). It was also reported that replication of HTNV can be prevented by IFN-induced antiviral mechanisms that are independent of MxA (233). It is well established that treatment of cells with type I IFN induces the expression of several hundred ISGs, with many of them possibly showing antiviral activity (194). Most likely not a single antiviral protein but multiple and redundant IFN-induced antiviral components are required to efficiently eliminate invading hantaviruses.

Most if not all pathogenic viruses have evolved strategies to subvert IFN-induced responses (194). It has been shown in primary endothelial cells (228, 231) that the type I IFN response including MxA expression is delayed in cells that are infected with pathogenic hantaviruses (HTNV, NYV) in comparison with non-pathogenic or low pathogenic hantaviruses (PHV, TULV). In line with this observation, a major difference in the initial type I IFN induction between pathogenic (ANDV) and non-pathogenic (PHV) hantaviruses after infection of primary endothelial cells was observed (232). Consistent with a delayed induction of the antiviral state, replication of pathogenic hantaviruses resulted in higher titers than that of non- or low-pathogenic hantaviruses. Thus, pathogenic hantaviruses could interfere with PRR signaling, as suggested by the finding that the G1 cytoplasmic tail of NYV but not PHV contains a motif that inhibits RIG-I-mediated responses (215). Moreover, hantaviruses cannot only down-regulate PRR-mediated type I IFN production but also interfere with IFN signaling by yet undefined mechanisms (220, 232). Finally, the NSs-ORF of PUUV and TULV was found to function as a weak inhibitor of the IFN response (27, 28). Interestingly, a recently described NSs-ORF-deficient PUUV variant produces about 10-fold lower amounts of infectious titers in the IFN-competent A549 cells compared with the parental virus, while both viruses replicated with a similar efficiency in Vero cells (A. Rang, 2008, unpublished work). Collectively, these studies indicate that by detaining the IFN-mediated antiviral state, pathogenic hantaviruses could open a window of opportunity in which they efficiently replicate and spread within the endothelial cell layer.

Inflammatory cytokines/chemokines produced by the antiviral innate response represent a double-edged sword. On one hand, they can eliminate viruses and contribute to viral elimination directly or indirectly by inducing and amplifying innate effector functions and antigen presentation of viral epitopes to T cells. On the other hand, if not properly regulated, inflammatory cytokines/chemokines could facilitate immunopathological processes in virus-associated diseases. For example, cytokine-induced reactive nitrogen intermediates (RNI), like NO and peroxynitrite, have been shown to inhibit *in vitro* the replication of DNA viruses as well as plus- and

minus-strand RNA viruses (194, 234) including hantaviruses (187). Increased amounts of stable end-products of RNI have been detected in patients with hantavirus-associated disease (186, 194, 235, 236) and in cynomolgus macaques infected with PUUV (123) but not in the natural rodent reservoir host of SNV (186). This observation suggests a role for RNI in hantavirus-associated pathogenesis in humans.

Another example is TNF-α, which can purge viruses from infected cells without cell lysis (237) but at the same time modulate endothelial cell barrier function by increasing leukocyte adhesion, transendothelial migration, and promoting vascular leakage and thrombosis (238). In accordance with the latter property, TNF-α and other pro-inflammatory cytokines are suspected to play a major role in septic shock during viral hemorrhagic fever caused by filoviruses like EBOV (239). TNF-α and other inflammatory cytokines are produced by macrophages and DCs after activation (240). Histopathologic analyses of tissues from HCPS patients suggest that monocytes/macrophages represent a target for hantaviruses (205, 241). Monocytes/macrophages isolated from human peripheral blood were susceptible to infection with PUUV (109, 242), SEOV (243), and TULV (244). Moreover, it has been demonstrated that SNV targets human alveolar macrophages (203). However, the supernatant from SNV-infected macrophages contained only low amounts of TNF-α and failed to induce endothelial cell leakage. Infection of DCs at a multiplicity of infection (MOI) of 1 induced the production of substantial amounts of TNF-α (108), which could contribute to the increased plasma level of this cytokine found in the acute phase of HFRS patients (245, 246). However, there is no evidence that such a harmful and abnormally sustained innate immune response producing a 'cytokine storm,' as observed for the 1918 strain of influenza virus (247), contributes to hantaviral immunopathogenesis.

After infection of human lung microvascular endothelial cells with either HTNV or SNV at a low MOI of 0.1, no virus-specific modulation of either cellular adhesion molecules or HLA molecules was found (206). In contrast, after hantavirus infection with a MOI of 1, a strong upreglation of HLA class I molecules on endothelial cells was observed (228, 230, 231) (*Fig.* 2). Interestingly, the time course of this upregulation is faster in endothelial cells infected with non- or low-pathogenic hantaviruses (PHV, TULV) compared with endothelial cells infected with highly virulent hantaviruses (HTNV, NYV). As HLA class I molecules represent the recognition structures for antiviral CTLs, endothelial cells infected with an non-pathogenic hantavirus are most likely eliminated at an earlier time point than those infected with pathogenic hantavirus.

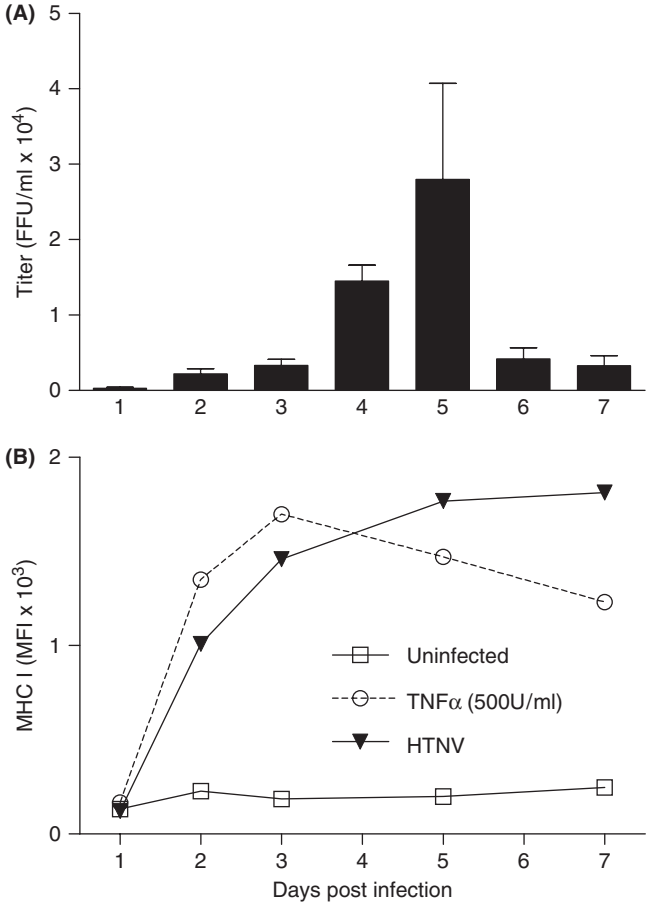

***Fig.* 2. Upregulation of HLA class I molecules on HTNV-infected endothelial cells.** Human umbilical vein endothelial cells (HUVECs) infected with HTNV at a MOI of 1.5 were analyzed over 7 days for (A) HTNV production (titer) by a chemiluminescence detection assay measuring focus-forming units (FFU) (296) (N = 2) and (B) MHC I expression by FACS analysis. TNF-α added at day 0 served as a positive control for MHC I upregulation.

Adaptive immune response against hantaviruses in humans

Owing to their pivotal role in initiating and orchestrating antiviral immune responses, DCs represent prime targets for viruses to evade immune control (248, 249). Many viruses including DNA viruses, such as human cytomegalovirus (250) and herpes simplex virus (251), impair the function of DCs and induce apoptosis of DCs by a variety of different mechanisms (252, 253). Filoviruses such as EBOV and Marburgvirus infect human immature DCs and inhibit their transition to the mature antigen-presenting stage as well as secretion of T-cell-stimulatory cytokines (254, 255). In contrast, hantaviruses do not seem to subvert the adaptive immune response. Immature DCs infected with HTNV do not undergo apoptosis but instead mature, thereby upregulating HLA, costimulatory, and adhesion molecules, which are important for efficient antigen presentation (108). On the functional level, this mature DC phenotype after HTNV infection was reflected by an increased

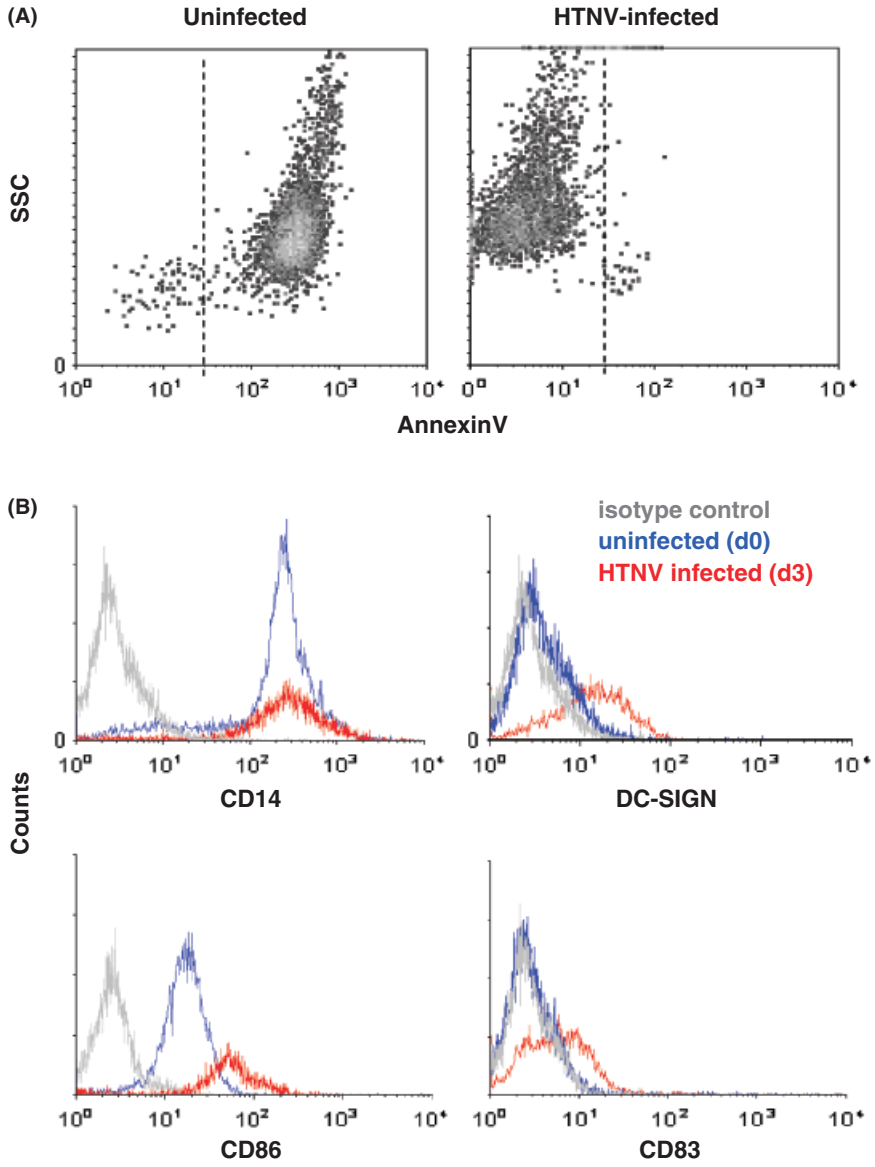

***Fig.* 3. Development of HTNV-infected monocytes into DC-like cells.** Monocytes were isolated from peripheral blood mononuclear cells (PBMCs) derived from healthy human donors by using the Monocyte Isolation Kit II (Miltenyi Biotec) and infected with HTNV at a MOI of 1. Staining and FACS analysis were performed 3 days post-infection. (A) Uninfected monocytes died due to apoptosis, whereas HTNV-infected monocytes did not undergo apoptosis and survived. The *x*-axis shows detection of apoptosis by AnnexinV staining, whereas the *y*-axis depicts the side scatter (SSC) profile. (B) Expression of surface markers on HTNV-infected monocytes 3 days post-infection. Infected monocytes (red curves) still expressed CD14 and differentiated toward a DC/macrophage phenotype as upregulation of CD86, CD83, and DC-SIGN could be observed. For comparison surface expression on uninfected monocytes (blue curves) right after isolation (d0) is depicted as uninfected monocytes do not survive until day 3. The isotype control (gray curves) for staining of HTNV-infected monocytes is also shown. The results shown are representative of three separate experiments with cells derived from different donors.

T-cell-stimulatory capacity. Moreover, our experiments revealed that after infection with HTNV *in vitro*, monocytes survive and develop into DC-like cells, although the T-cell stimulatory function of these cells has not been determined (N. Lütteke, 2008, unpublished work) (*Fig.* 3). A similar observation was reported recently by other investigators (256). The lack of viral immune evasion mechanisms impairing DC function and the development of monocytes into DC-like cells after infection can explain the observation that hantaviruses elicit a vigorous T-cell response during acute infection (257), leading to a robust and longlasting hantavirus-specific CTL memory (258).

Although epitopes were defined for all three hantaviral structural proteins (N, G1, and G2), the hantaviral N protein is the major viral target antigen recognized by antiviral T cells. This could be due to the fact that the N protein is the most conserved structural protein of hantaviruses and probably the

most abundant viral protein produced during infection (259). From three donors who had previously experienced laboratory-acquired infections with HTNV, CTL clones could be generated that were reactive to a limited number of epitopes on the HTNV N protein (260). Similarly, $CD8^+$ and $CD4^+$ T-cell clones isolated from the blood of patients with acute HCPS recognized epitopes on the SNV N protein (261). Additional $CD8^+$ T-cell epitopes in the SNV G2 protein have been reported (257).

In recent years, indirect evidence supporting the concept that CTL responses directed against hantavirus-infected endothelial cells could trigger increased capillary permeability has accumulated (262). The T-cell-attracting chemokines CCL5 (RANTES) and CXCL10 [the 10 kDa IFN-inducible protein (IP-10)] are secreted by human lung microvascular endothelial cells after infection with either HTNV or SNV *in vitro* (206). In addition, *in vitro* lysis of SNV-infected human endothelial cells by CTL was shown to lead to increased vascular permeability (263). The idea that CTLs directed against hantavirus infected cells damage the endothelial cell barrier is also in line with a recent report describing increased serum levels of perforin, granzyme B, and the epithelial cell apoptosis marker caspase-cleaved cytokeratin-18 during acute PUUV infection (264). Compatible with this view, the CTL response in PUUV-infected patients peaked at the onset of clinical disease and decreased in the following weeks (265) and strongly enhanced numbers of activated $CD8^+$ T cells and a reversed $CD4^+CD8^+$ T-cell ratio were detected in patients with acute HFRS (266, 267). Moreover, analysis of individuals previously infected with PUUV revealed a high frequency of memory CTLs against the PUUV N protein (258). In fact, the frequencies of PUUV-specific CTLs were comparable to those found in some other virus infections in which CTL responses are boosted by periodic re-exposure or viral persistence as observed for herpesviruses (268). However, there is no evidence for persistence of hantaviruses in humans, and presence of antigen is not required for the maintenance of long-lived memory T lymphocytes (269). Conceivably, an unusual strong primary response can account for the high frequency of hantavirus-specific memory CTLs. Intriguingly, significantly higher frequencies of SNV-specific $CD8^+$ T cells were detected in patients with severe acute HCPS than in patients with less severe symptoms (257). Consistent with this view, it has been reported that the G1 cytoplasmic tails of pathogenic hantaviruses (NYV, ANDV, and HTNV) are more easily degraded by the cellular proteasome than that of non-pathogenic hantaviruses, possibly resulting in more efficient processing and presentation of G1-derived epitopes to CTLs (147). Thus, the strength of the antiviral CTL response and the number of available CTL targets in the endothelial cell layer could determine the damage to the integrity of the vascular bed and, therefore, the severity of hantaviral pathogenesis. This differs fundamentally from severe EBOV infection which is accompanied by lack of CTL activation and peripheral T-cell numbers below the nomal range (270, 271). In contrast to the situation in the natural rodent reservoirs, in humans immune control mechanisms that downregulate hantavirus-specific CTL activity and check viral replication non-cytolytically could be missing, leading to efficient and rapid viral elimination at the cost of 'collateral' damage to the hantavirus-infected endothelial barrier (*Fig.* 4). Thus, hantaviruses induce a different quality of immune reponse in humans as compared with rodents. The quality of the ensuing antiviral T-cell response is determined by DCs, which are programmed through PRRs in the early phase of infection (130). In the future it will be important, therefore, to define the hantavirus-detecting PPRs in the natural reservoir host as compared with humans and analyze their respective signaling pathways.

Similar to the cellular immune response, hantaviruses induce a stable and long lasting humoral immune response involving antibodies of all Ig subclasses (IgA, IgM, IgG) (272). Serum antibodies to the hantaviral N protein, the major target antigen (273–277), are present soon after the onset of disease, whereas neutralizing antibodies against G1 and G2 do appear later during the progress of disease (278). An efficient antibody response in the early phase of disease is important to minimize viral dissemination and limit the number of virus-infected endothelial cells that have to be eliminated by CTLs. It is conceivable that such containment is helpful for prevention of extensive damage of the endothelial barrier. In line with this idea, it has been demonstrated that HCPS patients developed a more benign course, if high titers of neutralizing antibodies were present in the acute phase of the disease (279). Relatively high titers of neutralizing antibodies were found in individuals that have experienced HCPS years ago (280, 281) and have been detected even decades after PUUV infection of humans (282), suggesting that previously infected individuals are protected life-long from reinfection. In contrast, EBOV infection is characterized by little or no activation of B-cell-mediated immunity (283).

Host genetic factors clearly influence the clinical course of viral infections. Genes encoding HLA molecules particularly have been associated with susceptibility or resistance to infectious disease in humans (284, 285). HLA class I genes encode HLA-A, -B, and -C and are expressed in almost all human cells. In contrast, HLA class II genes encode

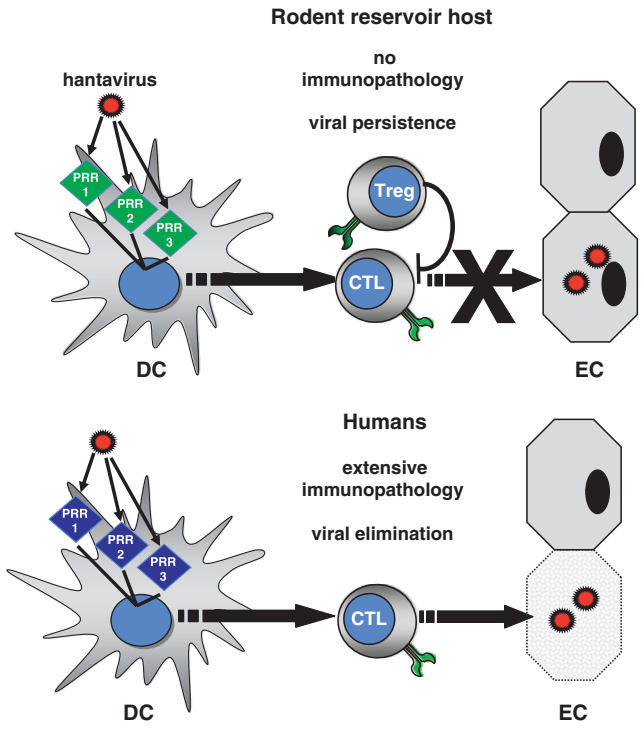

***Fig. 4.* Working hypothesis of differential regulation of hantavirus-specific immune responses in rodent reservoir hosts and humans.** During their encounter with viruses DCs integrate different signals received through several PRRs (PRR 1, PRR 2, PRR3, etc.) which determine the quality of the ensuing T-cell response. (A) In their rodent reservoir host, hantavirus-associated PRR signaling could program DCs to stimulate Treg cells that can suppress virus-specific CTLs, leading to viral persistence and at the same time preventing virus-induced immunopathology. (B) In humans, who are not adapted to hantaviruses, PRR signaling in DCs results in a dominant antiviral CTL response. As a consequence, hantavirus-infected endothelial cells (EC) are immediately eliminated leading to immunopathology.

HLA-DR, -DQ, and -DP molecules that are found only on specialized antigen-presenting cells like DCs. The HLA molecules are extremely polymorphic and can present a huge diversity of peptide epitopes to T cells. An association of certain HLA alleles with a more severe clinical course has been observed for HCPS and NE. Individuals with HLA-B*3501 have an enhanced risk for suffering from severe symptoms of HCPS (257). In the case of NE, the HLA haplotype B8-DRB1*03 is strongly linked with a severe clinical course in adults (286, 287). Moreover, in pediatric NE patients, HLA haplotype B8-DRB1*03 is overrepresented, although significant differences in disease severity between patients with and without HLA haplotype B8-DRB1*03 could not be demonstrated (288). In contrast, HLA-B27 is associated with a mild course of PUUV infection (289). Other genes that could influence the course of viral infections, for example cytokine-encoding genes, also show some limited polymorphism. It was demonstrated that a single nucleotide polymporphism (SNP) in the TNF-α gene promoter at position − 238 leading to low TNF-α production was associated with a more severe clinical course of PUUV infection (290) and the development of chronic active hepatitis B and C (291, 292). TNF-α is especially important for elimination of non-cytopathic viruses like hepatitis B and C virus, as it can purge viruses from infected cells without lysing them and, therefore, without collateral damage (237). This result may implicate that the antiviral role of TNF-α is more important than its possible impact as a pathogenicity factor that may increase vascular permeability.

The pathologic findings in humans that suffered from hantavirus-associated diseases are compatible with the concept of immunopathology. Interstitial pneumonitis with mononuclear cell infiltrates consisting of T cells in a reversed $CD4^+CD8^+$ T-cell ratio and macrophage/monocytic cells was seen in the lungs of HCPS patients (205, 241). In fact, histopathological analysis of specimens derived from HCPS cases revealed that many of the lymphoid cells associated with lung endothelial cells were actually $CD8^+$ T lymphocytes (205). In addition, high numbers of cells producing vasoactive cytokines (IL-1α, IL-1β, IL-6, and TNF-α) and T-cell-derived cytokines (IFN-γ, IL-2) were detected in the lung and spleen of HCPS patients, as compared with patients who died with non-HCPS ARDS or causes other than ARDS (293). This finding supports the view that inflammatory cytokines are involved in the pathogenesis of HCPS. Moreover, immunohistological analyses of kidney biopsies from patients with PUUV infection revealed infiltrating $CD8^+$ T lymphocytes at the peritubular area of the distal nephrons (294, 295). At this site, locally secreted inflammatory cytokines including TNF-α and platelet-derived growth factor (PDGF) and enhanced expression of adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and platelet endothelial cell adhesion molecule-1 (PECAM-1) was observed. Finally, in the Syrian hamster model of human HCPS, large numbers of mononuclear cell infiltrates have been found within the alveoli and in the perivascular interstitium (115).

The following model of hantavirus-induced immunopathogenesis in humans could be envisaged (*Fig.* 5). Pathogenic hantaviruses are able to spread efficiently within endothelial cell layers by delaying IFN-mediated responses but are not cytopathic *per se*. Owing to dysfunctional hantaviral immune evasion and absence of inhibitory Treg cells, a harmful and abnormally strong CTL response is induced that attacks hantavirus-infected endothelial cells and triggers immunopathology. In addition, due to acute thrombocytopenia, there are not sufficient platelets available to repair

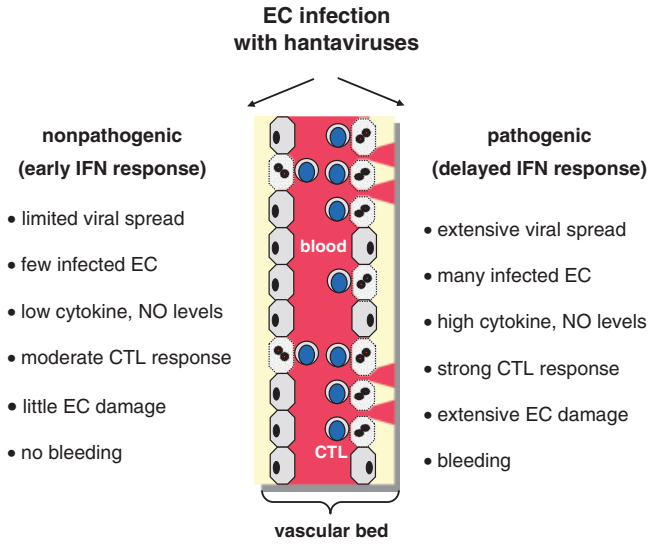

***Fig. 5.* Working hypothesis of hantaviral immunopathogenesis in humans.** Left side: Non-pathogenic hantaviruses induce early type I IFN-dependent antiviral effector components, e.g. MxA, which limit viral spread in the endothelial cell layer. As a consequence only few endothelial cells (ECs) are infected, which are recognized and eliminated by CTLs. In this way, the damage caused by CTLs is minimized and no vascular leakage occurs. Right side: Pathogenic hantaviruses can disseminate in the endothelial cell layer by delaying type I IFN-dependent antiviral effector components. As a result, many virus-infected ECs have to be cleared by virus-specific CTLs leading to vascular damage. Owing to acute thrombocytopenia, there are not sufficient platelets available to repair 'holes' in the EC barrier, resulting in vascular leakage. In addition, cytokines produced during the innate response against pathogenic hantaviruses like TNF-α or cytokine-induced NO could enhance vascular permeability.

'holes' in the endothelial cell barrier, resulting in increased vascular permeability. Finally, cytokines produced during the innate response against pathogenic hantaviruses, like TNF-α or cytokine-induced NO, could aggravate the disease process. Only indirect evidence for the concept of immunopathogenesis has been collected, and this issue should be addressed in a suitable animal model.

Perspective

Many questions concerning the role of the immune response on the susceptibility, transmission, and outcome of hantavirus infections in natural reservoirs and humans are still unsolved (*Table* 2). To answer these questions, it will be necessary to identify differences in the antiviral immune responses and their regulation in rodent reservoirs and humans. In addition, the role of a direct virus-cell interaction in pathogenesis versus an indirect virus-induced immunopathogenesis as the cause of hantavirus-associated syndromes in humans has to be figured out. The recent identification of novel hantaviruses in shrews raised also questions on the role of immunity in these potential reservoirs and its influence on hantavirus infections in these species. These investigations require the development and exploitation of suitable animal disease models and the development of reverse genetics systems. These basic research studies should provide a better understanding of hantavirus-induced pathogenesis to build the basis for developing efficient immunoprophylaxis and possibly immunotherapy measures for hantavirus-associated disease in humans. In addition, studies on the hantavirus-induced immune responses in wild rodents are essential for a better assessment of the (re-) emergence risk of hantavirus-associated diseases.

***Table* 2. Selected questions for further research**

How environmental factors, population density, and interspecific competition influence the susceptibility for and outcome of hantavirus infection in reservoir hosts?
What are the influences of rodent reservoir immunogenetics on hantavirus quasi-species diversity as well as susceptibility, persistence and transmission characteristics of a hantavirus infection in the reservoir?
What are the limiting factors for a spillover infection and are there indications of hantavirus-associated disease in spillover-infected animals?
Are hantavirus infection characteristics and induced immune reponses similar in shrews and in rodents ?
Which PRRs are involved in detection of hantaviruses in reservoirs and humans?
Which are the molecular mechanisms leading to enhanced vascular permeability and thrombocytopenia in hantavirus-associated pathogenesis?
Why do New World hantaviruses and Old World hantaviruses cause HCPS and HFRS or NE, respectively?
What makes PHV non-pathogenic for humans?
Is it possible to prove the concept of hantavirus-associated immunopathogenesis in a suitable animal model?

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