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Review

The pathogenesis of nephropathia epidemica: New knowledge and unanswered questions

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ABSTRACT

Puumala virus (PUUV) causes an acute hemorrhagic fever with renal syndrome (HFRS), a zoonosis also called nephropathia epidemica (NE). The reservoir host of PUUV is the bank vole (*Myodes glareolus*). Herein we review the main clinical manifestations of NE, acute kidney injury, increased vascular permeability, coagulation abnormalities as well as pulmonary, cardiac, central nervous system and ocular manifestations of the disease. Several biomarkers of disease severity have recently been discovered: interleukin-6, pentraxin-3, C-reactive protein, indoleamine 2,3-dioxygenase, cell-free DNA, soluble urokinase-type plasminogen activator, GATA-3 and Mac-2 binding protein. The role of cytokines, vascular endothelial growth hormone, complement, bradykinin, cellular immune response and other mechanisms in the pathogenesis of NE as well as host genetic factors will be discussed. Finally therapeutic aspects and directions for further research will be handled.

Contents

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1. Introduction

Hantaviruses circulate in a variety of rodent species across Eurasia, occasionally spreading to humans to cause an illness known as hemorrhagic fever with renal syndrome (HFRS), characterized by fever, increased vascular permeability, coagulation defects and acute kidney injury (AKI) (Vaheri et al., 2013b). Severe forms of HFRS caused by Hantaan and Dobrava virus occur in the Far East and in southeastern Europe, while Puumala virus (PUUV) produces a milder disease, nephropathia epidemica (NE) in the Scandinavian countries and other areas of Northern, Eastern and Central Europe. Finland has the highest incidence of recognized cases of NE, with thousands of cases occurring annually, and the case fatality rate of up to 0.4%. No vaccine or specific therapy is available for the disease.

Research on the pathogenesis of NE has been limited by the inability of investigators to reproduce the features of the disease in laboratory animals. However, human clinical studies are now shedding light on its underlying mechanisms and suggesting specific countermeasures to reduce the severity of illness. In this paper, we review current understanding of the pathogenesis of NE and identify gaps in the knowledge that should be targets for future research. We first present basic information on hantaviruses, their rodent reservoirs and routes of transmission to humans. We then summarize the clinical features of NE and biomarkers of disease severity, review the major pathophysiologic mechanisms, focusing in particular on the role of innate and adaptive immune responses in producing vascular leakage, coagulation defects and other features of illness. In the concluding section, we discuss current therapeutic approaches, and focus on the need to develop new treatments that block or neutralize deleterious host responses to infection.

2. Hantaviruses and their reservoir hosts

Puumala virus (PUUV) is a member of the *Hantavirus* genus in the family of Bunyaviridae. Hantaviruses are enveloped viruses (diameter about 120 nm) with a single-stranded RNA genome divided in three segments. They are called L (large) encoding the RNA polymerase, M (medium) encoding the two glycoproteins Gn and Gc, and S (small) encoding the nucleocapsid protein N (Hepojoki et al., 2012). In some hantaviruses, including PUUV, a nonstructural protein NSs is found, which can function as a weak interferon inhibitor (Jääskeläinen et al., 2007) but may have other functions as well (Rönnberg et al., 2012). The reservoir host of PUUV is the bank vole (*Myodes glareolus*), which is common in Europe. Humans get infected from inhaled aerosols of bank vole excreta and secreta (urine, feces, saliva), typically in human dwellings such as woodsheds, summer cottages, barns and cowsheds (Vaheri et al., 2013a; Vapalahti et al., 2003). PUUV is quite stable and may remain infective for two weeks at room temperature (Kallio et al., 2006) and presumably for more time at colder temperatures. PUUV is not transmitted from human-to-human but probably occasionally via platelets or other blood products (Sinisalo et al., 2010). Risk factors to catch PUUV infection include forestry, farming, camping, summer cottages, heating with wood, crises, military activity, male gender and cigarette smoking (Van Loock et al., 1999; Vapalahti et al., 2010).

In the Nordic countries PUUV is the only hantavirus causing HFRS while in central and eastern Europe there are also hantaviruses carried by *Apodemus agrarius* mice (Kurkino and Saaremaa viruses), and *A. flavicollis* and *A. ponticus* mice (Dobrava and Sochi viruses) (Klempa et al., 2013). Rats carrying Seoul virus have been detected also in Europe (Heyman et al., 2011). However, PUUV is by far the most common pathogen, although Dobrava and Sochi infections have considerably higher case-fatality rates. The highest number of human PUUV infections in Europe is reported from Finland; the record was in the year 2008 when 3259 cases were diagnosed. PUUV infections are also quite common in Northern Sweden, the Ardennes regions of Belgium and Northern France, in Southwestern Germany and some parts of European Russia (Vaheri et al., 2013a). PUUV infections are widely distributed in Europe with the exception of the far north and the Mediterranean regions (Heyman et al., 2011).

The underlying causes of varying epidemiological patterns differ among regions: in western and central Europe epidemics of HFRS caused by PUUV infections follow mast years with increased seed production by beech and oak trees followed by increased rodent reproduction. In these regions human infections have a major peak in the summer and a minor peak in January–March (Heyman et al., 2001; Tersago et al., 2011). In the northern regions, hantavirus infections and HFRS epidemics occur in three to four year cycles and are thought to be driven by prey-predator interactions (Olsson et al., 2010). In Northern Europe most cases occur from late autumn to winter and in August 3–5 weeks after the summer holidays. Notably while *Myodes glareolus* is found in large parts of Europe excluding much of Mediterranean regions and the northernmost areas, human PUUV infections have not been detected in e.g. British Isles or Southern Sweden. This may be partly due to lack of diagnostics and the fact that HFRS is not a recognized entity by the local medical community (Vaheri et al., 2013a). While PUUV can experimentally infect rodents, a disease closely mimicking HFRS develops only in *Cynomolgus* ma-

caques (Groen et al., 1995; Klingström et al., 2002; Sironen et al., 2008).

3. Nephropathia epidemica

3.1. Clinical syndrome

3.1.1. General features of illness

Classically, PUUV-induced NE occurs in five distinct phases: febrile, hypotensive, oliguric, polyuric and convalescent phases (Table 1). Typical laboratory findings during acute phase are anemia (sometimes high hemoglobin reflecting hemoconcentration), leukocytosis, thrombocytopenia (low platelet count), elevated liver enzymes and serum creatinine (depressed renal function), as well as proteinuria and hematuria (Vapalahti et al., 2003). The clinical course of NE infection is usually mild and favorable. The case-fatality rate is very low ranging from less than 0.1% in Finland (Brummer-Korvenkontio et al., 1999; Makary et al., 2010) to 0.4% in Sweden (Hjertqvist et al., 2010). In some cases, however, severe complications occur (Vaheri et al., 2013a) (Table 1). Recovery after NE is usually complete without any sequelae. Several long-term consequences, however, have been described (Vaheri et al., 2013a). Most commonly reported are the development of hypertension, hypopituitarism and cardiovascular diseases (CVDs).

3.1.2. Acute kidney injury

Renal involvement in acute NE include transient proteinuria, microscopic hematuria, and oliguric AKI followed by polyuria and recovery (Lähdevirta, 1971). Transient hemodialysis treatment is needed by up to 6% of hospital-treated patients (Braun et al., 2010; Mustonen et al., 1994a; Settergren et al., 1989).

An abrupt decline in glomerular filtration rate (GFR) is commonly observed among hospitalized NE patients. Effective renal plasma flow is decreased, and filtration fraction increased (Ala-Houhala et al., 2002; Settergren et al., 1990). The fall in systemic blood pressure does not explain these functional changes, since renal failure may occur without hypotension and blood pressure levels do not correlate with the severity of AKI. Intrarenal events are suggested to play an important role in the development of AKI (Cosgriff, 1991).

Proteinuria in NE begins abruptly and can be massive. Proteinuria is non-selective (Cosgriff, 1991), indicating a defective glomerular barrier (Ala-Houhala et al., 2002). Concomitant urinary loss of low-molecular-weight proteins such as β2-microglobulin (Cosgriff, 1991; Settergren et al., 1990) and α1-microglobulin (Ala-Houhala et al., 2002) indicates that tubular injury also contributes to the proteinuria.

The characteristic renal histopathological finding in PUUV-induced AKI is acute tubulointerstitial nephritis (Fig. 1a). Infiltrating cells include lymphocytes, plasma cells, monocytes, macrophages and polymorphonuclear leukocytes (Collan et al., 1991; Mustonen et al., 1994b; Temonen et al., 1996). Histological findings associate with the clinical severity of AKI but not with the amount of proteinuria (Mustonen et al., 1994b). Medullary hemorrhages are found in 20–60% of acute-phase biopsies (Fig. 1b) (Collan et al., 1991; Mustonen et al., 1994b). This finding links the renal damage to the hemorrhagic abnormalities. Abnormal renal ultrasound and magnetic resonance findings, such as enlargement of the kidneys, are common during the acute phase of NE, but the findings are not specific and have only limited value in assessing the clinical severity of the disease (Paakkala and Mustonen, 2007).

Hantaviruses can infect tubular epithelial cells, glomerular endothelial cells and podocytes of human kidney, and disrupt cell-to-cell contacts in all of these cell types (Krautkrämer et al., 2011, 2013). This is suggested to decrease the barrier functions of the kidney, and therefore to be the direct cause of proteinuria (Krautkrämer et al., 2011). Hantaviruses replicate predominantly in endothelial cells of capillaries, but there seems to be little if any cytopathic effects to the infected endothelium (Kanerva et al., 1998a; Vaheri et al., 2013a). The absence of obvious endothelial damage suggests that barrier breakdown is caused directly by infection and/or by the release of cytokines rather than by endothelial cell death. This is corroborated by the increased expression of cytokines in the peritubular areas in biopsy specimens of infected kidneys (Temonen et al., 1996) and by the correlation of urinary excretion of interleukin-6 with the amount of proteinuria (e.g. severity of damage) (Mäkelä et al., 2004).

3.1.3. Increased vascular permeability

Pathological changes in hantavirus infections are characterized by increased capillary permeability in the affected organs (Cosgriff, 1991; Kanerva et al., 1998a; Zaki et al., 1995). Increased capillary permeability and vascular leakage explain many signs and symptoms in hantavirus infections, such as hemoconcentration, hypotension and shock, abdominal pains due to retroperitoneal edema, and pleural effusion (Cosgriff, 1991; Kanerva et al., 1998a). Pulmonary edema, which is a typical finding in hantavirus cardiopulmonary syndrome (HCPS) but a rare complication in PUUV infection, is also accounted for vascular leakage. The exact pathogenetic mechanisms behind this central phenomenon in hantavirus infections are currently largely unknown. The endothelium of the small vessels in various organs is the primary target in hantavirus infection (Kanerva et al., 1998a; Zaki et al., 1995) (see above). However, as there does not appear to be widespread cytopathic effects to the endothelial cells, host immune or inflammatory mechanisms are probably important in the development of capillary leakage (Cosgriff, 1991; Kanerva et al., 1998a).

3.1.4. Coagulopathy, thrombocytopenia and hemorrhage

Thrombocytopenia is encountered in the majority of patients with NE. The platelet count nadir is typically around 100 ($\times 10^9$/l), occurs 4–5 days after the onset of fever and usually normalizes within 7 days from the onset of initial symptoms (Braun et al., 2010; Lähdevirta, 1971; Mustonen et al., 1994a; Settergren et al.,

Table 1
Typical clinical phases, symptoms and complications of Puumala hantavirus induced NE.

Phase of illness	Febrile	Hypotensive	Oliguric	Polyuric	Convalescence
Time of occurrence	1–7 days	1–3 days	2–7 days	1–2 weeks	2–8 weeks
Principal features	Fever	Hypotension	Urine output decreased	Urine output increased	
Signs and symptoms	Headache Vomiting Abdominal pains Back pains Visual disturbances	Capillary leakage Pulmonary symptoms	Anuria (urine output < 100 ml/day) Fluid retention	Weight decrease	Weakness Fatigue
Complications	Acute encephalomyelitis, Bleedings, Multiorgan failure, Need of dialysis, Perimyocarditis, Pituitary hemorrhage, Pulmonary edema, Shock, Lethal outcome				Glomerulonephritis

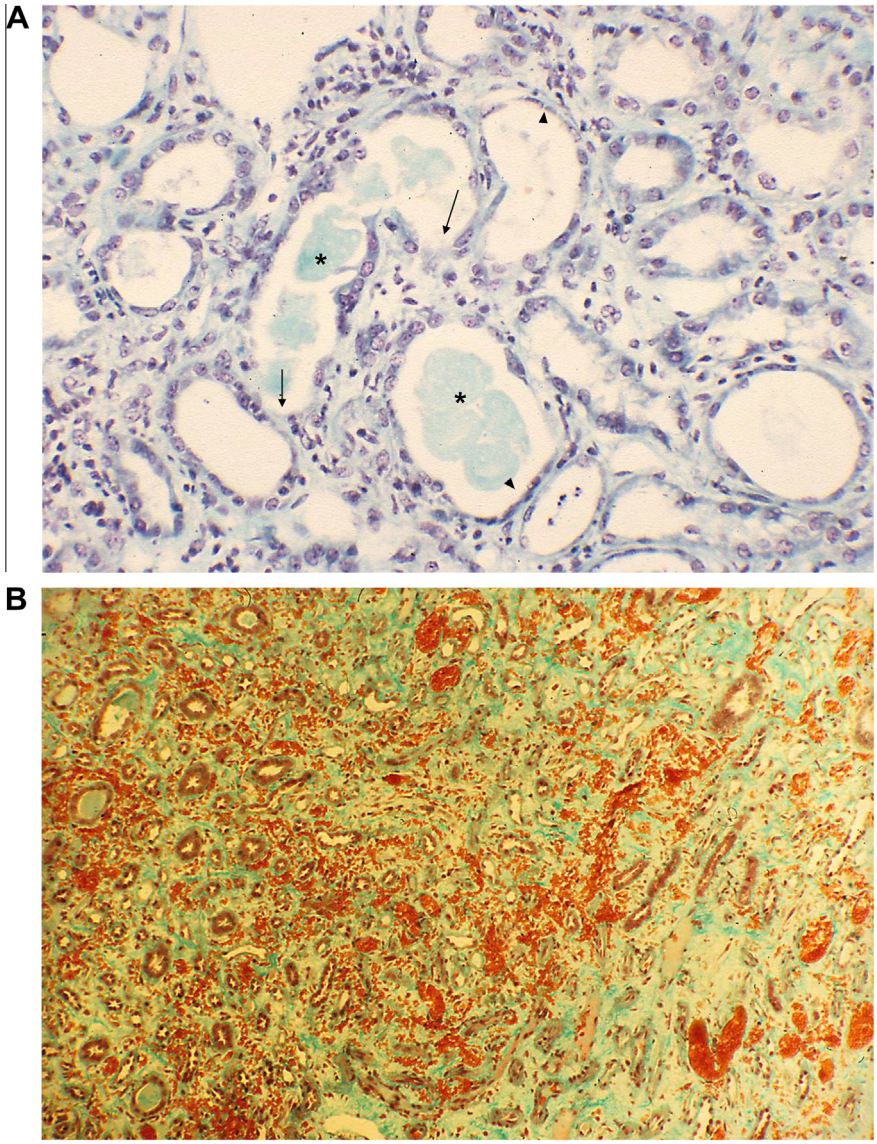

Fig. 1. A renal biopsy sample obtained during the acute phase of PUUV infection reveals interstitial edema and a mild inflammatory cell infiltrate associated with tubular epithelial necrosis (arrows) and flattening (arrowheads), and distended lumina (asterix) (a) (hematoxylin and eosin staining). Hemorrhage in corticomedullary junction (b) (Masson's trichrome staining). The pictures are provided by Professor Heikki Helin, Helsinki University Hospital, Finland.

1989; Sundberg et al., 2011). In one study thrombocytopenia appeared predictive of a severe course of AKI (Rasche et al., 2004).

The bleeding manifestations in NE usually remain mild. Petechiae and epistaxis are the most commonly reported clinical bleeding symptoms (12% and 11–21%, respectively). Conjunctival bleeding, metrorrhagia, macroscopic hematuria, melena and hematemesis occur as well (Lähdevirta, 1971; Settergren et al., 1989). Interestingly, hemorrhagic gastropathy was observed in all 10 consecutive patients in whom gastroscopy was carried out in the acute phase of the disease (Nuutinen et al., 1992). Hemorrhage in the pituitary gland has been detected both by MRI (Hautala et al., 2002) and in autopsy (Hautala et al., 2002; Valtonen et al., 1995). In fatal cases hemorrhage has been documented in the kidneys and other organs; heart, liver, lungs and peritoneal cavity (Valtonen et al., 1995).

Decreased platelet count is the most frequently observed abnormality in the coagulation profile during acute hantavirus infection. So far, the evidence does not imply deficient platelet production. Bone marrow examination has disclosed an increase in the number and size of megakaryocytes and active formation of platelets from the megakaryocytes during Hantaan virus-infection (Lee, 1987). A previous Finnish report of bone marrow aspirations taken during acute NE included five patients and the findings consisted of plasma cell reaction together with increased megakaryocyte formation and immature megakaryocytes (Lähdevirta, 1971). In order to investigate the platelet production less invasive methods, such as an assessment of platelet volume, should be applied.

Recent studies support the idea of increased platelet consumption as a mechanism of thrombocytopenia observed in acute hantavirus infection. A 24-fold increase in D-dimer was noted in our study of patients with NE (Laine et al., 2010). Thrombin formation was not proportionate to fibrinolysis, as the increase in F1 + 2 level was only 3-fold. Other procoagulant changes observed during the acute phase of the disease include the diminished activities of the natural anticoagulants antithrombin, protein C and protein S free antigen (Laine et al., 2010). Since only few or minor bleeding problems have been noted, these procoagulant changes are likely to contribute to the maintenance of hemostasis. Genetic polymor-

phism of plasminogen activator inhibitor (PAI-1), the main physiological regulator of plasminogen activators and fibrinolysis, associates with the severity of AKI in NE (Laine et al., 2012). However, there are no published data on the plasma levels and role of PAI-1 in hantavirus infections.

Disseminated intravascular coagulopathy (DIC) has traditionally been considered common during acute hantavirus infection (Cosgriff, 1991; Lee, 1987), but the diagnostic criteria applied vary considerably. As for PUUV, a Swedish study of 106 patients reported 28% of the patients to meet the criteria for overt DIC based on the modified scoring system of the International Society of Thrombosis and Haemostasis (ISTH) (Sundberg et al., 2011). In line with that is our recent report of 26% incidence of DIC according to the ISTH score during NE (Laine et al., 2010). The Swedish study also reported three patients having a radiologically verified thrombosis (Sundberg et al., 2011), a finding very rarely being described in NE patients. In that study, the ISTH score for DIC seemed to provide a clinical tool for predicting a severe disease when fibrinogen/C-reactive protein (CRP)-ratio was included in the scoring model (Sundberg et al., 2011).

Endothelial activation is thought to play a role in the pathogenesis of hantavirus infection (Cosgriff, 1991) and endothelial cell injury may contribute to the increase of thrombin formation and fibrinolysis and increased platelet consumption during NE (Laine et al., 2011; Lippi et al., 2008). Von Willebrand factor (VWF), fibrinogen and fibronectin are all adhesive platelet ligands related to endothelial function with altered plasma levels during acute PUUV-infection, a finding that might be interpreted as a sign of endothelial cell injury promoting thrombin formation and platelet activation with granule release (Laine et al., 2011). Interestingly, the increase in coagulation factor VIII (FVIII) level does not quite follow VWF levels during acute PUUV infection. This might, again, be explained by increased thrombin formation as FVIII degrades rapidly after release from its carrier molecule VWF by the action of thrombin (Sadler, 1998). FVIII could also bind to platelet-derived microparticles stimulated by thrombin or the complement protein C5b-9 (Gilbert et al., 1991; Wiedmer et al., 1986), both being increased during PUUV-infection (Laine et al., 2010; Sane et al., 2012). The finding of modestly reduced ADAMTS13 level (an enzyme specifically involved in the cleavage of high-molecular-weight VWF) (Laine et al., 2011) further indicates the role for endothelial injury in the pathogenesis of enhanced coagulation, fibrinolysis and platelet consumption in NE. Since hemolysis and thrombotic occlusions in the arterioles are, however, not typical findings of PUUV-infection, the mechanism of thrombocytopenia might not be consistent with that encountered in thrombotic microangiopathies.

There are no reports on platelet function tests performed during acute NE. In Hantaan virus infection, there are some data implying decreased platelet aggregation and release (Lee, 1987; Xiang et al., 1990). In addition to platelet dysfunction, the "exhausted platelet syndrome" (the continued circulation of platelets, that have already been activated) and uremia have been suggested to have some role in bleeding tendency observed during acute hantavirus infection (Cosgriff, 1991). The interaction between hantaviruses, platelets and endothelial cells has been discussed by Gavrilovskaya and coworkers (2010) based on their observation of hantaviruses binding quiescent platelets via β_3-integrin and platelets then binding to the endothelial cell. The platelet layer on the infected endothelial cells may alter cellular immune responses, platelet activation and endothelial cell functions (Gavrilovskaya et al., 2010).

Recently we observed that the spleen measured by magnetic resonance imaging (MRI) is clearly enlarged during acute NE (Koskela et al., 2013, abstract P4-7 of IX International Conference on HFRS, HPS & Hantaviruses, June 5–7, 2013, Beijing, China). The degree of enlargement, however, did not associate with the minimal platelet count and the concept of increased platelet destruction in the spleen as a major mechanism of thrombocytopenia is thus not supported.

3.1.5. Other clinical manifestations

Pulmonary, cardiac, endocrinological, central nervous system and ocular findings are also important manifestations of NE.

Abnormal findings on chest radiographs have been reported in 16–35% of patients with hospital-treated NE (Kanerva et al., 1996; Linderholm et al., 1992; Lähdevirta, 1971; Mustonen et al., 1994a; Paakkala and Mustonen, 2007). Pleural effusion, atelectasis, and interstitial infiltration are the most common X-ray findings (Kanerva et al., 1996). Radiological pulmonary manifestations associate with the degree of AKI and fluid volume overload, as well as with leukocytosis and thrombocytopenia (Kanerva et al., 1996; Paakkala and Mustonen, 2007). The endobronchial mucosal biopsies from patients with NE have revealed increased numbers of CD8+ T cells in both epithelium and submucosa, along with an increase in submucosal CD4+ T cells indicating a local immune response (Rasmuson et al., 2011b). Cytotoxic lymphocytes are suggested to be involved in the pathogenesis of pulmonary involvement in NE (Rasmuson et al., 2011b). PUUV antigen has been detected in lung capillary vascular endothelium and in bronchoalveolar lavage fluid of NE patients (Gizzi et al., 2013; Rasmuson et al., 2011a). Complement activation during the acute stage of the disease may also contribute to the pathogenesis of vascular leakage in the lungs (Sane et al., 2012) (see above).

Occasional cases of clinical myocarditis have been reported in NE (Lähdevirta, 1971; Mustonen et al., 1994a; Puljiz et al., 2005; Valtonen et al., 1995). Transient and unspecific alterations in electrocardiography are common findings (Mäkelä et al., 2009; Puljiz et al., 2005). AKI with fluid retention, abnormal plasma electrolyte levels, fever, and cytokine release are possible pathogenetic mechanisms for the myocardial involvement (Mäkelä et al., 2009). IL-6 has been suggested to be important in inhibiting cardiac function (Jonsson et al., 2010). In our previous study, impaired left ventricular contraction in echocardiography was also observed in some patients implying functional abnormality (Mäkelä et al., 2009). Hantaviral antigen has been detected in the cardiac endothelium in association with a typical histological myocarditis in cases with fatal HCPS (Saggioro et al., 2007). The structural changes, thus, might be responsible for myocardial depression at least in HCPS.

There are case reports of pituitary hemorrhages and hypopituitarism in patients with NE (Forslund et al., 1992; Hautala et al., 2010, 2002; Settergren et al., 1992; Valtonen et al., 1995). Hormonal abnormalities of the gonadal and/or thyroid axis are present in more than half of the patients during the acute phase (Mäkelä et al., 2010). Recent studies have also revealed that patients who had apparently recovered from HFRS may be at high risk of developing hypopituitarism (Mäkelä et al., 2010; Stojanovic et al., 2008). The acute-phase hormonal alterations of central origin associate with the severity of AKI and the degree of inflammation, but the chronic hormonal defects cannot be predicted by the severity of acute disease (Mäkelä et al., 2010). The increased vascular permeability and release of pro-inflammatory cytokines during the acute infection, as well as the tight interaction between the immune and endocrine systems could be involved in the pathogenesis of the hormonal defects (Mäkelä et al., 2010). PUUV antigen has been detected in hypophyseal neuroendocrine and endothelial cells of a patient with fatal NE (Hautala et al., 2002). It is, thus, possible that this PUUV invasion during the acute phase of the illness could injure the neuroendocrine cells, e.g. through inflammatory mechanisms, and consequently in some patients lead to a slowly developing hormonal deficiency.

Central nervous system (CNS)-related symptoms are usual in acute PUUV infection (Alexeyev and Morozov, 1995; Bergmann et al., 2002; Hautala et al., 2002, 2010, 2011b, 2013; Launes and Hautanen, 1988; Toivanen et al., 2002). Typical manifestations include headache, insomnia, as well as somnolence, dizziness, restlessness, anxiety, and amnesia. Young male patients have an elevated risk for serious CNS complications (Hautala et al., 2011b). Cerebrospinal fluid analysis show positive PUUV immunoglobulin M, elevated protein level, or leukocyte count in half of the samples obtained during infection (Hautala et al., 2010). It thus seems possible that CNS symptoms are attributable to true infection of the CNS rather than increased tissue permeability.

Ocular symptoms and disturbances are common in NE, and the symmetry of the ocular changes reflects the systemic nature of the disease (Hautala et al., 2011a). A decrease in intraocular pressure and myopic shift mainly due to thickening of the lens are evident in acute disease (Hautala et al., 2011a). The level of systemic inflammation rather than CNS involvement appears to account for the ocular changes during acute PUUV infection, and the severity of AKI may also have a significant role (Hautala et al., 2012). An osmotic process in the vitreous may explain the increase in vitreous volume and osmotic changes the increase in the thickness of the lens (Eckerle et al., 2012).

3.1.6. Resolution of infection, convalescence and sequelae

Patients mostly experience a total recovery after NE. The convalescence phase usually lasts for some weeks (Table 1). It was recently reported that viremia in PUUV infection can last up to three to four weeks (Korva et al., 2013; Pettersson et al., 2013). Well-controlled prospective clinical and epidemiological studies are needed to find out the possible role of PUUV infection in the development of hypertension (Miettinen et al., 2006; Mäkelä et al., 2000), CVDs (Connolly-Andersen et al., 2013) or chronic endocrinological abnormalities (Mäkelä et al., 2010).

3.2. Diagnostic methods

In humans PUUV has a 2–6 week incubation period. The level of viremia in PUUV infection, as judged by the detection of PUUV RNA, is considerably lower than in the more severe hantavirus infection (Hantaan, Dobrava, Sin Nombre, Andes) (Korva et al., 2013; Pettersson et al., 2013). Nevertheless, also in PUUV-HFRS RT-PCR usually detects viral RNA in the circulation of patients provided that early enough samples (within the first seven days after the onset of symptoms) are available. Both traditional nested and real-time RT-PCRs have been used to detect viremia in the patients (Vaheri et al., 2008). An early high viral load may predict unfavorable outcome in Dobrava and Sin Nombre virus infections (Saksida et al., 2008; Terajima et al., 1999) but possibly not in PUUV infection (Pettersson et al., 2013). RT-PCR appears to be a useful tool for the early diagnosis. Notably, hantaviral RNA can be demonstrated before IgM-response and thus also provides the possibility of genotyping the infecting hantavirus (Evander et al., 2007).

The first clinical diagnostic test was indirect immunofluorescence assay (IFA) based on acetone-fixed tissue sections of PUUV-infected bank voles. The adaptation of PUUV to Vero E6 cells in the early 1980s represented a major advance, also for diagnostics. Many laboratories still use IFA on PUUV-infected Vero E6 cells. Antibodies to PUUV N and glycoproteins are regularly seen in the course of PUUV infection. N protein is the immunodominant protein, especially during the acute stage, and gives a granular pattern in IFA and antibodies of both IgM and IgG classes are present as a rule in the first serum sample taken but in 2–4% of PUUV infections, seroconversion may take up to 5 days after onset of illness. Antibodies to the glycoproteins develop somewhat later and give a diffuse pattern in IFA. IgM-IFA cannot be recommended since it is subject to false positives due to interference by rheumatoid factor (for Refs. see Vaheri et al., 2008). Most laboratories today use PUUV-IgM enzyme immunoassay based on recombinant N protein (Elgh et al., 1997; Sjölander et al., 1997; Vapalahti et al., 1996). Immunochromatographic rapid tests have been developed for detection of PUUV IgM antibodies and are commercially available from Reagena Ltd (Hujakka et al., 2003). Although antibodies have been raised experimentally both to the L protein and the NSs protein, there are no studies to indicate that antibodies to them appear in human hantavirus infections. A low PUUV-specific IgG-response was recently found to associate with a more severe clinical course of NE (Pettersson et al., 2013).

Based on seroprevalence and numbers of diagnosed PUUV infections it has been estimated that only 30% of PUUV-infected individuals manifest with such severe symptoms that the patient seeks medical care and the physician sends a serum sample for diagnosis (Brummer-Korvenkontio et al., 1999). Thus, a large proportion of infections are clinically mild and even subclinical cases have been described (Clement et al., 1996).

3.3. Biomarkers of disease severity

Several immunoinflammatory markers have been shown to serve as indicators of the severity or some other characteristics of NE. However, because the detailed pathogenic mechanisms of the disease are not understood, it is unclear to what extent the

Table 2
The associations of different elevated biomarkers with acute kidney injury, thrombocytopenia and the length of hospital stay in Puumala hantavirus infection.

	AKI	Thrombocytopenia	Length of hospitalization*	Reference
CRP	Protective	No	No	Outinen et al. (2010)
IL-6	Yes	Yes	Yes	Outinen et al. (2010)
PTX3	Yes	Yes	Yes	Outinen et al. (2012b)
IDO	Yes	No	Yes	Outinen et al. (2011)
cf-DNA	No	Yes	Yes	Outinen et al. (2012a)
suPAR	Yes	Yes	Yes	Outinen et al. (2013)
GATA-3	Yes	NS	NS	Libraty et al. (2012)

AKI = acute kidney injury.
CRP = C-reactive protein.
IL-6 = interleukin-6.
PTX3 = pentraxin-3.
IDO = indoleamine 2,3-dioxygenase.
cf-DNA = cell-free DNA.
suPAR = soluble urokinase-type plasminogen activator receptor.
NS = not studied.
* Reflects the overall severity of the disease.

marker concentrations reflect the inflammatory activation that aims at eliminating the virus and to what extent the elevation is due to the response to tissue damage. Nevertheless, even though the production of immunoinflammatory mediators is often co-regulated, it appears that different markers can reflect different immunopathogenic aspects of the disease. Associations between certain biomarkers and clinical findings observed in Finnish NE patients are shown in Table 2.

3.3.1. Interleukin 6

Interleukin-6 (IL-6) is a pleiotropic cytokine that is produced by various immune cells, endothelial cells and fibroblasts upon infection or trauma (Kishimoto et al., 1995). It induces the production of acute phase reactants by the liver and expands the inflammatory responses by activating T cells and promoting the differentiation of B cells (Kishimoto et al., 1995). In addition to being involved in microbial immune responses, high production of IL-6 has been attributed to acute and chronic conditions, such as AKI (Himmelfarb et al., 2004), CVDs (Danesh et al., 2008), and various autoimmune diseases (Ishihara and Hirano, 2002). Although IL-6 is typically considered to be a proinflammatory cytokine with potentially harmful effects, it has also vital anti-inflammatory functions in stimulating the synthesis of anti-inflammatory cytokines and limiting inflammation. For example, in septic shock, IL-6 has been shown to suppress acute neutrophil accumulation induced by intratracheal endotoxin administration (Ulich et al., 1991).

High plasma IL-6 level associates with severe AKI in NE (Linderholm et al., 1996; Outinen et al., 2010). Urinary IL-6 excretion, in turn, correlates with urinary albumin excretion (Mäkelä et al., 2004). However, urinary IL-6 excretion does not correlate with the plasma IL-6 level indicating that local IL-6 production possibly takes place in the kidneys (Mäkelä et al., 2004). High plasma IL-6 levels also associate with a more severe thrombocytopenia in NE patients (Outinen et al., 2010; Takala et al., 2000). Furthermore, plasma IL-6 levels predict the overall clinical severity of PUUV infection and thus, can be used as a marker of the severity of the disease (Outinen et al., 2010).

3.3.2. Pentraxin-3

Pentraxin-3 (PTX3) acts as a soluble pattern recognition receptor (PRR) participating in the innate host defense against certain bacteria and fungi (Daigo and Hamakubo, 2012). Besides mediating inflammatory activation, PTX3 can act as a negative feedback regulator, limiting excessive neutrophil recruitment (Deban et al., 2010). Unlike CRP, PTX3 is produced locally at the site of inflammation; the main producers are dendritic cells (DCs), neutrophils, mononuclear phagocytes, vascular endothelial and smooth muscle cells and fibroblasts (Daigo and Hamakubo, 2012). Intriguingly, PTX3 has been demonstrated to possess direct antiviral activity against human and murine cytomegalovirus (Bozza et al., 2006) and influenza viruses (Reading et al., 2008). Moreover, protective role of PTX3 has been observed in murine hepatitis virus-induced acute lung injury (Han et al., 2012), and in mouse models of myocardial infarction (Salio et al., 2008), and in atherosclerosis (Norata et al., 2009).

In PUUV infection, plasma PTX3 levels are elevated during the acute phase (Outinen et al., 2012b). High PTX3 level associates with a clinically severe course of the disease judged e.g. by the severity of AKI, thrombocytopenia and the length of hospitalization (Outinen et al., 2012b). Most of all, high PTX3 level predicts significant thrombocytopenia. PTX3 level also correlates with the activation of the complement system in NE (Outinen et al., 2012b). These findings have brought up the possibility that PTX3 is involved in the pathogenesis of thrombocytopenia in PUUV infection through the complement cascade interacting with the coagulation system and leading to platelet consumption. On the other hand, plasma PTX3 determinations offer a potential diagnostic tool for assessing the severity and outcome of acute PUUV infection.

3.3.3. CRP

C-reactive protein (CRP) is a prototype acute phase reactant and is produced mainly by the liver in response to IL-6. CRP has the ability to activate complement via the classic pathway and facilitate the functions of phagocytic cells; hence, it serves as an opsonin for infectious agents and damaged cells. CRP levels rise rapidly, even up to 1000-fold, in various conditions such as infections, inflammatory diseases, trauma and myocardial infarction (Volanakis, 2001). In clinical routine, CRP assessment has been shown to have a predictive value in various acute and inflammatory conditions and it can also be used to discriminate between bacterial and viral infections; viral infections typically display lower CRP levels compared to invasive bacterial infections (ten Oever et al., 2012). Current data on the potential protective role of CRP in inflammatory conditions are limited to its role in the clearance of debris and infectious agents – a process that is vital for preventing autoimmune reactions (Szalai, 2004).

Plasma CRP is elevated in almost all patients with NE (Settergren et al., 1989). However, the level does not associate with the severity of the disease (Outinen et al., 2010). In contrast, high CRP is a possible protective factor against AKI in NE (Outinen et al., 2010). High CRP could reduce deposition and increase clearance of immune complexes in the renal cortex and thus help in preserving kidney function. Nevertheless, in evaluating the severity of acute PUUV infection, CRP level is not informative.

3.3.4. Indoleamine 2,3-dioxygenase

Indoleamine 2,3-dioxygenase (IDO) is an interferon-induced, tryptophan-catabolizing enzyme that is produced by DCs, macrophages, B cells and endothelial cells. Although tryptophan starvation by IDO was initially considered a mechanism of local innate host defense against infections, it is now known that via mechanisms involving the activation of regulatory T cells, IDO limits tissue inflammation and autoimmunity. Likewise, IDO can dampen excessive immune reactivity to chronic infections and cancer (Munn and Mellor, 2013). In infectious diseases, IDO possesses a dualistic role. It can directly downmodulate bacterial replication or attenuate progression of a viral infection, but it can also suppress the necessary host immune responses. For example, in mice infected with influenza virus, IDO expression during the infection modifies the inflammatory response in a way that predisposes the animals to a secondary bacterial infection (van der Sluijs et al., 2006).

Serum IDO levels are elevated in acute NE (Outinen et al., 2011). High levels are associated with increased disease severity and especially with the severity of AKI (Outinen et al., 2011). Whether IDO is just a marker of severe disease in NE, or has a pathogenetic role in it, is not known. However, it is possible that IDO is involved in the pathogenesis of AKI. It has been demonstrated that increased IDO activity promotes tubular epithelial cell (TEC) apoptosis and inhibition of IDO enhances TEC survival (Mohib et al., 2007). IDO could promote AKI in PUUV infection by inducing TEC apoptosis. Another possibility is that by inducing immunosuppression through T cell suppression and apoptosis, IDO enhances the infection.

3.3.5. Cell-free DNA

Circulating plasma cell-free DNA (cf-DNA) has recently emerged as a novel marker of the extent of damage in conditions involving cellular death. cf-DNA is most likely released from apoptotic and necrotic cells, although active secretion by living cells has also been proposed as a source of cf-DNA (Wagner, 2012). Markedly in-

creased cf-DNA levels have been reported in patients with acute CVD, trauma and sepsis (Huttunen et al., 2011a; Wagner, 2012), whereas moderately elevated concentrations have been attributed to autoimmune diseases (Wagner, 2012) and very old individuals (Jylhävä et al., 2013). In contrast to the other immunoinflammatory markers described above, elevation of cf-DNA in pathological states is likely due to increased cell death rate rather than immunological activation. As follows, cf-DNA is a very sensitive, but at the same time somewhat unspecific marker of tissue damage.

The plasma levels of cf-DNA are elevated in NE (Outinen et al., 2012a). This probably reflects apoptosis occurring during the acute phase of the disease as the plasma cf-DNA level correlates with the intensity of the apoptotic band in qualitative analysis of cf-DNA. The total plasma cf-DNA level also correlates with leukocytosis, thrombocytopenia and the length of hospital stay (Outinen et al., 2012a). The urinary excretion of cf-DNA, in turn, is not elevated in acute PUUV infection and does not correlate with the severity of AKI. Hence, the urinary cf-DNA does not reflect the degree of inflammation in the kidneys (Outinen et al., 2012a).

3.3.6. Soluble urokinase-type plasminogen activator

Urokinase-type plasminogen activator receptor (uPAR) is a multifunctional glycoprotein released during infection and inflammation (Ossowski and Aguirre-Ghiso, 2000). It interacts with several molecules mediating immune system signals and promotes the migration and adhesion of leukocytes by binding to β-integrins (Ossowski and Aguirre-Ghiso, 2000). Soluble form of the receptor (suPAR) can activate β_3-integrin within the kidney podocyte leading to podocyte dysfunction and effacement and proteinuria (Wei et al., 2011). Plasma suPAR levels are believed to represent the degree of immunoactivation and have been found to be increased as well as predict disease severity in various infections, e.g. bacteremia and HIV infection (Huttunen et al., 2011b; Koch et al., 2011; Sidenius et al., 2000). Interestingly, contact of endothelial cells with either peripheral blood mononuclear cells or thrombocytes strongly promotes release of suPAR (Mustjoki et al., 2000). In NE, plasma suPAR levels are increased in the acute phase and associate with a severe course of the disease (Outinen et al., 2013). It is possible that increased levels of suPAR activate β_3-integrin in NE and are thus involved in the pathogenesis of the disease.

3.3.7. GATA-3

GATA family transcription factors play multiple vital roles in hematopoiesis in many cell lineages. GATA-3 is a factor necessary for the production of type 2 T cells (Hosoya et al., 2010). GATA-3 is also expressed in human kidney embryogenesis and by distal renal tubular and collecting duct cells in the adult kidney (Labastie et al., 1995; Obara et al., 2008). Elevated GATA-3 mRNA levels in circulating mononuclear cells have been reported in patients with minimal change nephrotic syndrome (Komatsuda et al., 2009). We have recently shown that the maximum urinary sediment GATA-3 mRNA level is an independent risk factor for severe PUUV-induced AKI (Libraty et al., 2012). This possibly reflects injury in distal tubular or collecting duct cells in PUUV-infected kidneys.

3.3.8. Mac-2 binding protein

Increased levels of Mac-2 binding protein (Mac-2BP; also known as galectin-3 binding protein) have been detected in the circulation of patients with chronic virus infections (HIV-1, HBV, and HCV) in which the levels correlate with the severity of the disease (for review on Mac-2BP, see Hepojoki J, PhD thesis, available at http://ethesis.helsinki.fi). When purifying Tula hantavirus, we found that it copurifies and binds to Mac-2BP. The results indicated that hantavirus binds Mac-2BP. We found high Mac-2BP levels in acute-stage PUUV–HFRS and that the levels correlate with disease severity and increased complement activation (Hepojoki et al., 2013, abstract O3–6 in IX International Conference on HFRS, HPS & Hantaviruses, June 5–7, 2013, Beijing, China). The physiological functions of Mac-2BP are linked with immune defense against invading microbes and tumors, but this is the first time Mac-2BP has been shown to bind a microbe. The results suggest a role for Mac-2BP in the recognition of an invading virus and activation of the innate-immune response.

4. Pathogenesis of NE

The recent progress in studying of the hantavirus molecular and cellular biology, as discussed recently (Vaheri et al., 2013b), forms the basis for our understanding of the pathogenesis of hantavirus infections. This in turn, since direct antiviral therapy has limited potential as discussed in chapter 6, forms the basis for other forms of therapy such as inhibition of vascular leakage and complement-mediated pathology. New knowledge about the role of innate and cellular immune responses as well as other pathogenetic mechanisms in NE will be discussed below.

4.1. Role of innate immune response

4.1.1. Cytokines

Cytokines are thought to play an important role in the development of the vascular leakage in hantavirus infections. Cytokines are produced by a number of different cell types, such as monocytes, macrophages, and lymphocytes, in response to inflammatory signals and participate in the regulation of the inflammatory response. They can be functionally divided into pro-inflammatory and anti-inflammatory cytokines. In addition to acute-phase protein induction, cytokines, especially tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6, are mediators responsible for fever and septic shock. TNF-α is known to be able to increase vascular permeability (Tracey and Cerami, 1994). Increased cytokine levels have been found in the plasma, urine and kidneys of NE patients (Linderholm et al., 1996; Mäkelä et al., 2004; Outinen et al., 2010; Sadeghi et al., 2011; Saksida et al., 2011; Takala et al., 2000; Temonen et al., 1996). An imbalance in the production of pro-inflammatory and regulatory cytokines is probably important in the pathogenesis of hantavirus infection (Borges et al., 2008; Saksida et al., 2011). In concordance with this suggestion, it has been shown in PUUV infected patients that the disease severity correlates with high pro-inflammatory cytokine levels but low anti-inflammatory transforming growth factor (TGF)-β1 levels (Sadeghi et al., 2011). Furthermore, after the acute phase, the levels of the pro-inflammatory cytokines decrease, whereas TGF-β1 levels increase (Sadeghi et al., 2011). Thus, also delayed induction of the protective immune mechanism to downregulate the early pro-inflammatory immune response possibly contributes to the pathogenesis of PUUV infection.

4.1.2. Vascular endothelial growth factor

β_3-integrins have an important role in regulating vascular integrity, endothelial cell permeability, and platelet functions (Mackow and Gavrilovskaya, 2009). Pathogenic hantaviruses inhibit these functions, thus interfering with the endothelial permeability (Mackow and Gavrilovskaya, 2009). Vascular endothelial growth factor (VEGF) is expressed on angiogenic endothelium and is able to induce vascular permeability (Dvorak, 2006). β_3-integrins regulate vascular permeability through restricting VEGF-directed permeability caused by the internalization of vascular endothelial (VE)-cadherin, a predominant structural component of endothelial cell adherens junctions (Gavrilovskaya et al., 2008; Mackow and Gavrilovskaya, 2009). Pathogenic hantaviruses inhibit this normal regulation by β_3-integrins (Gavrilovskaya et al., 2008). As a conse-

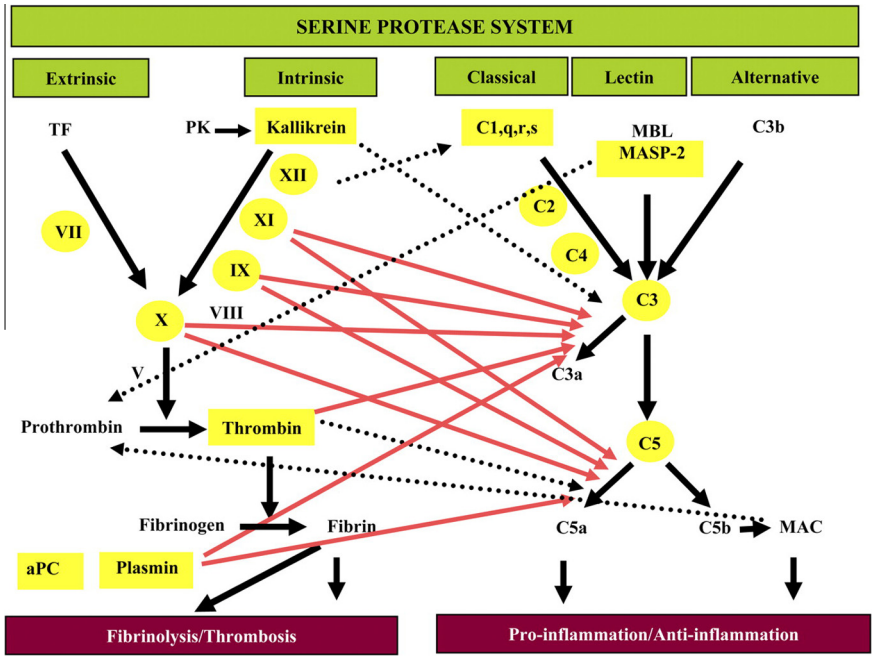

Fig. 2. Simplified model of the serine protease system depicting the complex interplay between the coagulation/fibrinolysis cascade and the complement system. The serine proteases of the complement, coagulation, and fibrinolysis systems are all high-lighted in yellow. The black dotted arrow bars show previously known interactions of these systems. The red arrows identify the new paths of complement activation by the coagulation/fibrinolysis factors resulting in the generation of C3a and C5a, aPC, activated protein C; MAC, membrane attack complex; MBL, mannan-binding lectin; PK, prekallikrein. (Amara et al., 2010. Copyright 2010. The American Association of Immunologists, Inc.)

quence, hantavirus infected endothelial cells are hyperresponsive to the permeabilizing effects of VEGF leading to vascular hyperpermeability (Gavrilovskaya et al., 2008). VEGF has not been studied in PUUV infection but, in HCPS patients, VEGF levels are elevated in pulmonary edema fluid and peripheral blood mononuclear cells, so that the more severe cases present the highest levels (Gavrilovskaya et al., 2012). Hantavirus-directed permeability has been inhibited by antibodies against VEGF receptor-2 (Gavrilovskaya et al., 2008; Gorbunova et al., 2011). Targeting VEGF responses may offer a potential therapeutic approach in hantavirus infections.

4.1.3. Complement

The complement system consists of the classical, the alternative and the lectin-dependent pathway, all of them converging on complement component C3, which holds a key position in the function of the complement system (Walport, 2001). When the complex is formed in the absence of a target membrane in the fluid phase, C5b-9 binds to S-protein (vitronectin) and a non-lytic soluble SC5b-9 terminal complement complex is formed. The final product of the complement cascade is the cytolytic membrane attack complex (MAC) (Podack et al., 1979). The complement system is linked to the coagulation and fibrinolytic system via multiple interactions in terms of a complex serine protease network. Interactions between coagulation and complement system are presented in Fig. 2 (Amara et al., 2010).

In a Finnish study the complement system was found to be activated in 23 out of 25 patients with NE (Paakkala et al., 2000). Activation through the alternative route was a more frequent but the classical pathway activation was associated with more severe clinical disease (Paakkala et al., 2000). The complement activation through the alternative pathway was recently confirmed in a study of 61 patients revealing increased SC5b-9 levels and decreased C3 levels in NE (Sane et al., 2012). The complement activation associated with chest X-ray abnormalities such as accumulation of pleural fluid, a finding implying a relationship between the complement activation and capillary leakage. These findings of complement activation and their association with the clinical outcome in acute PUUV-HFRS are in line with the earlier observations of circulating immune complexes along with decreased C3 levels in patients with Hantaan virus-induced HFRS (Lee, 1987). In that study, the decrease in C3 levels was most evident in patients with most severe abnormalities in coagulation tests which could possibly be a reflection of several molecular mechanisms linking the coagulation and fibrinolysis cascades with the central complement components C3 and C5 (Amara et al., 2010).

Decay-accelerating factor of the complement regulatory system as well as complement receptor gC1qR have been characterized to mediate the entry of hantaviruses into the endothelial cell *in vitro* (Krautkrämer and Zeier, 2008; Spiropoulou, 2011). SC5b-9 is capable of ligating β_3-integrin, which may result in increased endothelial permeability (Tsukada et al., 1995). SC5b-9 has been demonstrated to release bradykinin and platelet-activating factor, thus promoting endothelial permeability (Bossi et al., 2004). In addition, the MAC complex is known to trigger cellular reactions and production of inflammatory cytokines capable of altering endothelial function (Morgan, 1999). These are observations linking complement activation to the loss of endothelial integrity but do not yet provide with an explicit and comprehensive view on the pathogenesis of hantavirus infection.

4.1.4. Bradykinin

Bradykinin (BK) promotes release of inflammatory mediators, vasodilatation and increase vascular permeability in many pathophysiological states (Maurer et al., 2011). It is considered the key mediator in vascular leakage by disrupting inter-endothelial junctions. More than 20 years ago BK was suggested to be a mediator of hypotension in hantavirus infections through its effects on vascular tone and permeability (Cosgriff, 1991). Difficulties in measuring BK in clinical samples may have been the obstacle to further study of

this theory. Interestingly, a recent report from the U.S.A. demonstrated dramatic increases in endothelial cell permeability after kallikrein-kinin system activation and liberation of BK in an experimental model of hantavirus infection (Taylor et al., 2013). Alterations in permeability could be prevented using inhibitors that block BK binding (Taylor et al., 2013).

4.2. Role of cellular immune response

Acute phase PUUV infection is associated with a vigorous T cell response, in which both CD4+ and CD8+ T cells participate; at peak the responding cells can account for 50% of the total peripheral blood CD8+ population (Lindgren et al., 2011; Tuuminen et al., 2007). The cytotoxic response has been tracked by using HLA multimers with a cognate antigen, as well as PUUV antigen-specific IFN-gamma ELISPOT assays. So far, most of the identified HLA class I restricted antigenic peptides have been in the N protein, supporting the view of N protein as the immunodominant antigen (Terajima and Ennis, 2011; Van Epps et al., 2002). No HLA class II restricted antigens have been characterized, so the identification of responding CD4+ T cells is based on phenotypic changes, notably the upregulation of the cell cycle marker Ki-67 (Lindgren et al., 2011). Both approaches show that the response is usually at a peak already at presentation, during the febrile phase of the disease (Lindgren et al., 2011; Tuuminen et al., 2007). The responding cells express high levels of effector molecules, such as perforin and granzyme B, indicating that they are highly active, functional effector cells.

In human patients most of the T cell data is based on analyzing peripheral blood lymphocytes, and little is known of local T cell function in infection foci, such as the kidneys. Cynomolgus macaques infected with PUUV develop symptoms similar to the human disease, and a limited study showed T cell infiltrates in kidneys, colocalizing with viral RNA and antigens (Sironen et al., 2008). The infiltrating cells were mainly CD8+ and were found especially at sites of tissue damage. Similar results have been obtained from kidney biopsies taken from human patients with acute PUUV infection (Temonen et al., 1996; Terajima et al., 2004).

The frequency of responding T cells decreases as the viremia is cleared, influenced probably by both cell-intrinsic negative signals and extrinsic regulation (Lindgren et al., 2011; Tuuminen et al., 2007). Already at presentation the responding T cells express inhibitory molecules linked to down-regulating T cell responses. CD4+ T cells express both CTLA-4 and PD1, while in CD8+ T cells CTLA-4 seems more important. Regulatory T cells expressing the FOXP3 transcription factor are also activated during the acute infection, at least judging by an increased frequency of Ki-67 expression. It is probable that they also contribute to the control of the effector response.

In samples taken during convalescence the frequency of effector T cells is already below the detection threshold of flow cytometric methods. However, antigen-specific responses can still be detected by cytokine ELISPOT, and the CD8+ T cells thus identified probably represent the emerging memory population (Tuuminen et al., 2007). After the acute infection, CD8+ memory T cells specific to N-protein epitopes have been demonstrated in samples taken up to 15 years later, consistent with an effective long-term T cell memory (Van Epps et al., 2002). The acute PUUV infection has also been shown to induce heterologous memory responses against persistent viruses, such as EBV (Tuuminen et al., 2007). It is not known how the reactivation of EBV replication or the EBV-specific T cell response affects the ongoing response against PUUV, or the course of the hantaviral disease.

Natural killer cells are also involved in the cellular immune response to PUUV. A recent study showed that NK cells expand rapidly during the acute infection and remain at increased numbers for more than 2 months (Björkström et al., 2011). The expanded NK cell population expresses activating receptors and is fully functional ex vivo. These findings suggest that the NK cell response may also possess memory-like features, with prolonged persistence of an activated population well after viral clearance.

Cytotoxic T cells may contribute to capillary leakage in NE, either by producing pro-inflammatory cytokines or by directly killing PUUV-infected endothelial cells. This is indirectly supported by findings linking HLA class I alleles to the disease severity (see above). More directly, PUUV-infected patients have increased extracellular levels of perforin, which correlate with the level of serum lactate dehydrogenase, a marker of cell membrane damage (Klingström et al., 2006). The colocalization of CD8+ T cells, viral markers, and tissue damage in biopsy samples is also consistent with T cell-mediated pathology in the infection foci. However, these observations do not prove a causal correlation between cytotoxic T cell responses and endothelial damage; a pronounced T cell response may also be a secondary consequence of the signals associated with a severe infection. Indeed, a recent study showed that hantavirus N-protein can inhibit the function of granzyme B and caspase 3, molecules central to T cell-mediated killing (Gupta et al., 2013). The infected endothelial cells are thus rendered resistant to apoptosis induced by cytotoxic T cells.

Regulatory T cells participate in the acute phase T cell response, and most likely have an inhibitory effect on effector cells. This may be important in limiting the effector response and thus preventing capillary immunopathology, as has been suggested for other hantaviruses (Zhu et al., 2009). On the other hand, they might also hinder efficient viral clearance by cytotoxic T cells and thus prolong the viremia, as has been suggested in studies of hantaviral persistence in rodent hosts (Easterbrook et al., 2007; Schountz et al., 2007). Thus, the role of regulatory T cells in the pathogenesis of PUUV-induced HFRS is still unclear.

4.3. Other disease mechanisms

PUUV causes a generalized infection and PUUV RNA and/or N protein has been detected also in urine (Plyusnin et al., 1997), saliva (Pettersson et al., 2008) and cerebrospinal fluid (Mähönen et al., 2007) and in lethal cases in the hypophysis, brain, kidney, lungs, spleen and liver own unpublished data. PUUV infects many types of cultured human cells: fibroblasts, kidney glomerular cells (epithelial and mesangial), and endothelial cells, monocyte-macrophages (Temonen et al., 1993, 1995). Ordinarily, no cytopathic effects are seen although hantavirus-induced apoptosis is seen in certain experimental conditions (Kang et al., 1999; Li et al., 2004; Markotic et al., 2003) and markers of apoptosis such as caspase-cleaved cytokeratin-18 and DNA fragments corresponding to the size of apoptotic DNA (150–200 bp) (Outinen et al., 2012a) are detected in PUUV-infected patients. Thus, the tissue damage in PUUV infection may be mediated by apoptosis but also by indirect mechanisms such as cytokines, complement activation, and cytotoxic T cells (see above).

Several cell surface proteins have been suggested to act as hantavirus receptors, especially integrins. Integrins are a family of heterodimeric (contain two distinct protein chains, α and β) transmembrane proteins that act as adhesion molecules (cell–cell and cell-matrix contacts). Pathogenic and apathogenic hantaviruses have been suggested to differ in their receptor usage; $\alpha_V\beta_3$ being the receptor for pathogenic (such as PUUV) and $\alpha_5\beta_1$ for apathogenic viruses. This is probably true at least in cell culture conditions; however, decay-accelerating factor, a glycosylphosphatidylinositol (GPI)-anchored protein of the complement regulatory system (Krautkrämer and Zeier, 2008) and complement C1q receptor gC1qR can also mediate hantavirus infection in cell culture (Choi et al., 2008).

5. Host genetic factors affecting disease outcome

The clinical course of NE is clearly influenced by host-related factors. In Finland, the individuals who had HLA alleles B8, C4A*Q0, and DRB1*0301 were likely to have the most severe form of the PUUV infection (Mustonen et al., 1996; Mäkelä et al., 2002), and those with HLA-B27 had a benign clinical course (Mustonen et al., 1998). Interestingly, individuals with the haplotype HLA-B8 DRB1*0301 are prone to normal or increased humoral immune response and a low T cell immune responsiveness (Candore et al., 1994). Carriers of this haplotype have an altered immune response against hepatitis B vaccine (Alper et al., 1989; Egea et al., 1991) and in HIV infection (Steel et al., 1988).

In Slovenian population, PUUV-infected patients, and especially those who had a severe form of the disease, had more frequently HLA-DRB1*13 haplotype than DOBV-infected patients (Korva et al., 2011). HLA-B*07, in turn, showed to have a possible protective role in PUUV infection. Furthermore, DOBV-infected patients had a significantly higher frequency of HLA-B*35 than PUUV-infected patients (Korva et al., 2011). Thus, different hantaviruses may be presented differently through the same HLA molecules. The association between HLA alleles and severity implies an involvement of T-cell-mediated effects in hantavirus pathogenesis.

A high-producing genotype of TNF-α gene (polymorphism at position -308) associates with the severe clinical course of NE in Finnish patients (Kanerva et al., 1998b; Mäkelä et al., 2001). It is part of the susceptibility HLA haplotype for severe infection, but a less important risk factor than the HLA-B8-DR3 haplotype (Mäkelä et al., 2002). On the other hand, Belgian patients with the low producer genotype of TNF-α gene (polymorphism at position -238) had a more severe clinical course (Maes et al., 2006). Additional genes contributing to susceptibility to PUUV infection include non-carriage of the interleukin-1 receptor antagonist (IL-1RA) allele 2 and IL-1α (-511) allele 2 (Mäkelä et al., 2001).

6. Current therapy

6.1. Supportive measures

The clinical disease in NE is in most cases fairly mild, maybe only 30% of patients have symptoms leading to medical attention and diagnostics. In Finland, 52% of all serologically diagnosed patients with PUUV infection need hospital care for the average of seven days (Makary et al., 2010). Of those patients, up to 6% need transient hemodialysis treatment and some need care in the intensive care unit (Vaheri et al., 2013a). Maintaining fluid and electrolyte balance is a crucial component of care. In addition to kidney function, blood pressure and oxygenation may need support. Platelet transfusions have been used in severe thrombocytopenia and bleedings. Pharmacotherapy may be needed for headache and back pains. As there is currently no specific therapy available for hantavirus infections, biomarkers selecting the patients with the need of an early and intensive supportive treatment would be of clinical value.

6.2. Antiviral drugs

The nucleoside analog ribavirin is the only established antiviral drug with some *in vitro*- and in vivo-efficacy against hantavirus replication (Severson et al., 1999). However, ribavirin as well as interferon only have therapeutic effect prophylactically or at very early times postinfection. When the symptoms of HFRS appear, viremia is already fading, and by the point the patients seek medical attention the time has already run out for their effective use. Ribavirin has not been tested in PUUV infection (Vaheri et al., 2013a).

Another recently discovered compound with antiviral activity against Hantaan virus is ETAR (1-beta-D-ribofuranosyl-3-ethynyl-(1,2,4) triazole). It neither decreases viral RNA levels nor increases mutation frequency of Hantaan virus genome and thus different mechanism of action compared with ribavirin may be speculated (Chung et al., 2008). To our knowledge, there are no reports on the use of ETAR in the clinical setting of acute hantavirus infection in humans.

Other antiviral strategies against hantaviruses include the use of cyclic peptides or peptidomimetic compounds similar to cyclic peptides which bind $\alpha_v\beta_3$ integrin as a virus receptor (Hall et al., 2007, 2008; Larson et al., 2005), but so far there are no published reports on their clinical use. As for passive immunization, there are studies demonstrating the ability of polyclonal and monoclonal antibodies to prevent or delay the hantavirus infection in animals (Hammerbeck and Hooper, 2011).

6.3. Other drugs

Since hantaviruses infect endothelial cells and alter their functions, targeting endothelial cell responses to stabilize the vasculature could be a potential therapeutic approach (Gavrilovskaya et al., 2012). It also has the advantage of being useful after the incubation period, at the onset of clinical symptoms. Moreover, there are already several well-studied VEGFR2 and Src inhibitors in human clinical trials or used therapeutically. Angiopoietin-1, sphingosine-1-phosphate, pazopanib and dasatinib all inhibit microvascular leak in hantavirus infection *in vitro* (Gavrilovskaya et al., 2012). The Robo4 receptor that is highly expressed by lung microvascular endothelial cells is currently under evaluation as a therapeutic for a variety of vascular disorders (Jones et al., 2009; Marlow et al., 2010).

Interestingly, our recent report of a patient with an extremely severe NE describes a successful therapeutic use of icatibant, a drug licensed for the treatment of acute episodes of hereditary angioedema (Antonen et al., 2013). Icatibant is a synthetic polypeptide that is structurally similar to BK. It acts as a selective and competitive antagonist of the BK type 2 receptor, reverses increased vascular permeability and inhibits vasodilatation (Cicardi et al., 2010; Cockcroft et al., 1994). In another recent study increased vascular permeability in an experimental hantavirus infection could be prevented with drugs that block BK binding, the activity of coagulation factor XII, or the activity of kallikrein (Taylor et al., 2013). An additional possibility might be to use drugs that prevent the generation of SC5b-9 (MAC) known to release BK (Bossi et al., 2004). As the effective therapy for hantavirus diseases is still missing, the use of currently licensed drugs with therapeutic potential against microvasculatory leak should be considered in patients at high risk of mortality.

7. Gaps in knowledge and directions for future research

Our understanding of the pathogenesis HFRS caused by PUUV has improved in recent years. Much more knowledge, however, is needed of the roles of many factors in the pathogenesis of the main manifestations of the disease: capillary leakage, thrombocytopenia and AKI. The disease is obviously very complex but still it is probable that one factor acts as the primary cause in the pathogenesis and several others come secondary of lesser importance. We think that a central disturbance is increased capillary leakage and further studies should explore the mechanisms of that.

Currently the only animal model for PUUV infection is the Cynomolgus macaque model (Klingström et al., 2002; Sironen et al.,

2008). Human PUUV infection is reproduced in macaques quite accurately but studies on the pathogenesis, therapy and vaccine development would greatly benefit if a rodent model, such as exists for HCPS, infection of Syrian hamsters with Andes virus (Safronetz et al., 2012), would be available.

At the moment no specific drug therapy is in use. More studies of the pathogenesis of increased capillary leakage are of great importance. This may provide the road towards effective therapeutics. An example of this is to test the effects of icatibant, a drug that blocks the binding of bradykinin, in large materials of patients with PUUV and other hantavirus infections.

Further important questions are identification of risk factors, preventive measures and vaccination, use of antiviral drugs and other drugs influencing capillary permeability, treatment of bleedings, prophylaxis of AKI as well as identification of long-term consequences of the disease. Answers should be sought in carefully planned studies performed in close collaboration between scientists of various fields.

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References

Ala-Houhala, I., Koskinen, M., Ahola, T., Harmoinen, A., Kouri, T., Laurila, K., Mustonen, J., Pasternack, A., 2002. Increased glomerular permeability in patients with nephropathia epidemica caused by Puumala hantavirus. Nephrol. Dial. Transplant. 17, 246–252.

Alexeyev, O.A., Morozov, V.G., 1995. Neurological manifestations of hemorrhagic fever with renal syndrome caused by Puumala virus: review of 811 cases. Clin. Infect. Dis. 20, 255–258.

Alper, C.A., Kruskall, M.S., Marcus-Bagley, D., Craven, D.E., Katz, A.J., Brink, S.J., Dienstag, J.L., Awdeh, Z., Yunis, E.J., 1989. Genetic prediction of nonresponse to hepatitis B vaccine. N. Engl. J. Med. 321, 708–712.

Amara, U., Flierl, M.A., Rittirsch, D., Klos, A., Chen, H., Acker, B., Bruckner, U.B., Nilsson, B., Gebhard, F., Lambris, J.D., Huber-Lang, M., 2010. Molecular intercommunication between the complement and coagulation systems. J. Immunol. 185, 5628–5636.

Antonen, J., Leppänen, I., Tenhunen, J., Arvola, P., Mäkelä, S., Vaheri, A., Mustonen, J., 2013. A severe case of Puumala hantavirus infection successfully treated with bradykinin receptor antagonist icatibant. Scand. J. Infect. Dis. 45, 494–496.

Bergmann, F., Krone, B., Bleich, S., Prange, H., Paulus, W., 2002. Encephalitis due to a hantavirus infection. J. Infect. 45, 58–59.

Björkström, N.K., Lindgren, T., Stoltz, M., Fauriat, C., Braun, M., Evander, M., Michaelsson, J., Malmberg, K.J., Klingström, J., Ahlm, C., Ljunggren, H.G., 2011. Rapid expansion and long-term persistence of elevated NK cell numbers in humans infected with hantavirus. J. Exp. Med. 208, 13–21.

Borges, A.A., Campos, G.M., Moreli, M.L., Moro Souza, R.L., Saggioro, F.P., Figueiredo, G.G., Livonesi, M.C., Moraes Figueiredo, L.T., 2008. Role of mixed Th1 and Th2 serum cytokines on pathogenesis and prognosis of hantavirus pulmonary syndrome. Microbes Infect. 10, 1150–1157.

Bossi, F., Fischetti, F., Pellis, V., Bulla, R., Ferrero, E., Mollnes, T.E., Regoli, D., Tedesco, F., 2004. Platelet-activating factor and kinin-dependent vascular leakage as a novel functional activity of the soluble terminal complement complex. J. Immunol. 173, 6921–6927.

Bozza, S., Bistoni, F., Gaziano, R., Pitzurra, L., Zelante, T., Bonifazi, P., Perruccio, K., Bellocchio, S., Neri, M., Iorio, A.M., Salvatori, G., De Santis, R., Calvitti, M., Doni, A., Garlanda, C., Mantovani, A., Romani, L., 2006. Pentraxin 3 protects from MCMV infection and reactivation through TLR sensing pathways leading to IRF3 activation. Blood 108, 3387–3396.

Braun, N., Haap, M., Overkamp, D., Kimmel, M., Alscher, M.D., Lehnert, H., Haas, C.S., 2010. Characterization and outcome following Puumala virus infection: a retrospective analysis of 75 cases. Nephrol. Dial. Transplant. 25, 2997–3003.

Brummer-Korvenkontio, M., Vapalahti, O., Henttonen, H., Koskela, P., Kuusisto, P., Vaheri, A., 1999. Epidemiological study of nephropathia epidemica in Finland 1989–96. Scand. J. Infect. Dis. 31, 427–435.

Candore, G., Cigna, D., Gervasi, F., Colucci, A.T., Modica, M.A., Caruso, C., 1994. In vitro cytokine production by HLA-B8, DR3 positive subjects. Autoimmunity 18, 121–132.

Choi, Y., Kwon, Y.C., Kim, S.I., Park, J.M., Lee, K.H., Ahn, B.Y., 2008. A hantavirus causing hemorrhagic fever with renal syndrome requires gC1qR/p32 for efficient cell binding and infection. Virology 381, 178–183.

Chung, D.H., Kumarapperuma, S.C., Sun, Y., Li, Q., Chu, Y.K., Arterburn, J.B., Parker, W.B., Smith, J., Spik, K., Ramanathan, H.N., Schmaljohn, C.S., Jonsson, C.B., 2008. Synthesis of 1-beta-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole and its in vitro and in vivo efficacy against Hantavirus. Antiviral Res. 79, 19–27.

Cicardi, M., Banerji, A., Bracho, F., Malbran, A., Rosenkranz, B., Riedl, M., Bork, K., Lumry, W., Aberer, W., Bier, H., Bas, M., Greve, J., Hoffmann, T.K., Farkas, H., Reshef, A., Ritchie, B., Yang, W., Grabbe, J., Kivity, S., Kreuz, W., Levy, R.J., Luger, T., Obtulowicz, K., Schmid-Grendelmeier, P., Bull, C., Sitkauskiene, B., Smith, W.B., Toubi, E., Werner, S., Anne, S., Bjorkander, J., Bouillet, L., Cillari, E., Hurewitz, D., Jacobson, K.W., Katelaris, C.H., Maurer, M., Merk, H., Bernstein, J.A., Feighery, C., Floccard, B., Gleich, G., Hebert, J., Kaatz, M., Keith, P., Kirkpatrick, C.H., Langton, D., Martin, L., Pichler, C., Resnick, D., Wombolt, D., Fernandez Romero, D.S., Zanichelli, A., Arcoleo, F., Knolle, J., Kravec, I., Dong, L., Zimmermann, J., Rosen, K., Fan, W.T., 2010. Icatibant, a new bradykinin-receptor antagonist, in hereditary angioedema. N. Engl. J. Med. 363, 532–541.

Clement, J., Underwood, P., Ward, D., Pilaski, J., LeDuc, J., 1996. Hantavirus outbreak during military manoeuvres in Germany. Lancet 347, 336.

Cockcroft, J.R., Chowienczyk, P.J., Brett, S.E., Bender, N., Ritter, J.M., 1994. Inhibition of bradykinin-induced vasodilation in human forearm vasculature by icatibant, a potent B2-receptor antagonist. Br. J. Clin. Pharmacol. 38, 317–321.

Collan, Y., Mihatsch, M.J., Lähdevirta, J., Jokinen, E.J., Romppanen, T., Jantunen, E., 1991. Nephropathia epidemica: mild variant of hemorrhagic fever with renal syndrome. Kidney Int. 35 (Suppl. 35), S62–S71.

Connolly-Andersen, A.M., Ahlm, K., Ahlm, C., Klingström, J., 2013. Puumala virus infections associated with cardiovascular causes of death. Emerg. Infect. Dis. 19, 126–128.

Cosgriff, T.M., 1991. Mechanisms of disease in hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. Rev. Infect. Dis. 13, 97–107.

Daigo, K., Hamakubo, T., 2012. Host-protective effect of circulating pentraxin 3 (PTX3) and complex formation with neutrophil extracellular traps. Front. Immunol. 3, 378.

Danesh, J., Kaptoge, S., Mann, A.G., Sarwar, N., Wood, A., Angleman, S.B., Wensley, F., Higgins, J.P., Lennon, L., Eiriksdottir, G., Rumley, A., Whincup, P.H., Lowe, G.D., Gudnason, V., 2008. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med. 5, e78.

Deban, L., Russo, R.C., Sironi, M., Moalli, F., Scanziani, M., Zambelli, V., Cuccovillo, I., Bastone, A., Gobbi, M., Valentino, S., Doni, A., Garlanda, C., Danese, S., Salvatori, G., Sassano, M., Evangelista, V., Rossi, B., Zenaro, E., Constantin, G., Laudanna, C., Bottazzi, B., Mantovani, A., 2010. Regulation of leukocyte recruitment by the long pentraxin PTX3. Nat. Immunol. 11, 328–334.

Dvorak, H.F., 2006. Discovery of vascular permeability factor (VPF). Exp. Cell Res. 312, 522–526.

Easterbrook, J.D., Zink, M.C., Klein, S.L., 2007. Regulatory T cells enhance persistence of the zoonotic pathogen Seoul virus in its reservoir host. Proc. Natl. Acad. Sci. USA 104, 15502–15507.

Eckerle, I., Jakob, E., Hofmann, J., Schmidt-Bacher, A., Ettinger, J., Schnitzler, P., 2012. Atypical severe puumala hantavirus infection and virus sequence analysis of the patient and regional reservoir host. Zoonoses Public Health 59 (Suppl. 2), 110–115.

Egea, E., Iglesias, A., Salazar, M., Morimoto, C., Kruskall, M.S., Awdeh, Z., Schlossman, S.F., Alper, C.A., Yunis, E.J., 1991. The cellular basis for lack of antibody response to hepatitis B vaccine in humans. J. Exp. Med. 173, 531–538.

Elgh, F., Lundkvist, Å., Alexeyev, O.A., Stenlund, H., Avsic-Zupanc, T., Hjelle, B., Lee, H.W., Smith, K.J., Vainionpää, R., Wiger, D., Wadell, G., Juto, P., 1997. Serological diagnosis of hantavirus infections by an enzyme-linked immunosorbent assay based on detection of immunoglobulin G and M responses to recombinant nucleocapsid proteins of five viral serotypes. J. Clin. Microbiol. 35, 1122–1130.

Evander, M., Eriksson, I., Pettersson, L., Juto, P., Ahlm, C., Olsson, G.E., Bucht, G., Allard, A., 2007. Puumala hantavirus viremia diagnosed by real-time reverse transcriptase PCR using samples from patients with hemorrhagic fever and renal syndrome. J. Clin. Microbiol. 45, 2491–2497.

Forslund, T., Saltevo, J., Anttinen, J., Auvinen, S., Brummer-Korvenkontio, M., Korhonen, A., Poutiainen, M., 1992. Complications of nephropathia epidemica: three cases. J. Intern. Med. 232, 87–90.

Gavrilovskaya, I.N., Gorbunova, E.E., Mackow, N.A., Mackow, E.R., 2008. Hantaviruses direct endothelial cell permeability by sensitizing cells to the vascular permeability factor VEGF, while angiopoietin 1 and sphingosine 1-phosphate inhibit hantavirus-directed permeability. J. Virol. 82, 5797–5806.

Gavrilovskaya, I.N., Gorbunova, E.E., Mackow, E.R., 2010. Pathogenic hantaviruses direct the adherence of quiescent platelets to infected endothelial cells. J. Virol. 84, 4832–4839.

Gavrilovskaya, I., Gorbunova, E., Koster, F., Mackow, E., 2012. Elevated VEGF levels in pulmonary edema fluid and PBMCs from patients with acute hantavirus pulmonary syndrome. Adv. Virol. 2012, 674360.
Gilbert, G.E., Sims, P.J., Wiedmer, T., Furie, B., Furie, B.C., Shattil, S.J., 1991. Platelet-derived microparticles express high affinity receptors for factor VIII. J. Biol. Chem. 266, 17261–17268.
Gizzi, M., Delaere, B., Weynand, B., Clement, J., Maes, P., Vergote, V., Laenen, L., Hjelle, B., Verroken, A., Dive, A., Michaux, I., Evrard, P., Creytens, D., Bulpa, P., 2013. Another case of "European hantavirus pulmonary syndrome" with severe lung, prior to kidney, involvement, and diagnosed by viral inclusions in lung macrophages. Eur. J. Clin. Microbiol. Infect. Dis. 32, 1341–1345.
Gorbunova, E.E., Gavrilovskaya, I.N., Pepini, T., Mackow, E.R., 2011. VEGFR2 and Src kinase inhibitors suppress Andes virus-induced endothelial cell permeability. J. Virol. 85, 2296–2303.
Groen, J., Gerding, M., Koeman, J.P., Roholl, P.J., van Amerongen, G., Jordans, H.G., Niesters, H.G., Osterhaus, A.D., 1995. A macaque model for hantavirus infection. J. Infect. Dis. 172, 38–44.
Gupta, S., Braun, M., Tischler, N.D., Stoltz, M., Sundström, K.B., Bjorkström, N.K., Ljunggren, H.G., Klingström, J., 2013. Hantavirus-infection confers resistance to cytotoxic lymphocyte-mediated apoptosis. PLoS Pathog. 9, e1003272.
Hall, P.R., Malone, L., Sillerud, L.O., Ye, C., Hjelle, B.L., Larson, R.S., 2007. Characterization and NMR solution structure of a novel cyclic pentapeptide inhibitor of pathogenic hantaviruses. Chem. Biol. Drug Des. 69, 180–190.
Hall, P.R., Hjelle, B., Brown, D.C., Ye, C., Bondu-Hawkins, V., Kilpatrick, K.A., Larson, R.S., 2008. Multivalent presentation of antihantavirus peptides on nanoparticles enhances infection blockade. Antimicrob. Agents Chemother. 52, 2079–2088.
Hammerbeck, C.D., Hooper, J.W., 2011. T cells are not required for pathogenesis in the Syrian hamster model of hantavirus pulmonary syndrome. J. Virol. 85, 9929–9944.
Han, B., Ma, X., Zhang, J., Zhang, Y., Bai, X., Hwang, D.M., Keshavjee, S., Levy, G.A., McGilvray, I., Liu, M., 2012. Protective effects of long pentraxin PTX3 on lung injury in a severe acute respiratory syndrome model in mice. Lab. Invest. 92, 1285–1296.
Hautala, T., Sironen, T., Vapalahti, O., Pääkkö, E., Särkioja, T., Salmela, P.I., Vaheri, A., Plyusnin, A., Kauma, H., 2002. Hypophyseal hemorrhage and panhypopituitarism during Puumala virus infection: magnetic resonance imaging and detection of viral antigen in the hypophysis. Clin. Infect. Dis. 35, 96–101.
Hautala, T., Mähönen, S.M., Sironen, T., Hautala, N., Pääkkö, E., Karttunen, A., Salmela, P.I., Ilonen, J., Vainio, O., Glumoff, V., Rytky, S., Plyusnin, A., Vaheri, A., Vapalahti, O., Kauma, H., 2010. Central nervous system-related symptoms and findings are common in acute Puumala hantavirus infection. Ann. Med. 42, 344–351.
Hautala, N., Kauma, H., Vapalahti, O., Mähönen, S.M., Vainio, O., Vaheri, A., Hautala, T., 2011a. Prospective study on ocular findings in acute Puumala hantavirus infection in hospitalised patients. Br. J. Ophthalmol. 95, 559–562.
Hautala, T., Hautala, N., Mähönen, S.M., Sironen, T., Pääkkö, E., Karttunen, A., Salmela, P.I., Vainio, O., Rytky, S., Plyusnin, A., Vaheri, A., Vapalahti, O., Kauma, H., 2011b. Young male patients are at elevated risk of developing serious central nervous system complications during acute Puumala hantavirus infection. BMC Infect. Dis. 11, 217.
Hautala, N., Kauma, H., Rajaniemi, S.M., Sironen, T., Vapalahti, O., Pääkkö, E., Karttunen, A., Ilonen, J., Rytky, S., Vainio, O., Vaheri, A., Hautala, T., 2012. Signs of general inflammation and kidney function are associated with the ocular features of acute Puumala hantavirus infection. Scand. J. Infect. Dis. 44, 956–962.
Hautala, T., Partanen, T., Sironen, T., Rajaniemi, S.-M., Hautala, N., Vainio, O., Vapalahti, O., Kauma, H., Vaheri, A., 2013. Elevated cerebrospinal fluid neopterin concentration is associated with disease severity in acute Puumala hantavirus infection. Clin. Dev. Immunol. 634632. http://dx.doi.org/10.1155/2013/634632. [Epub 2013 Jul 31].
Hepojoki, J., Strandin, T., Lankinen, H., Vaheri, A., 2012. Hantavirus structure–molecular interactions behind the scene. J. Gen. Virol. 93, 1631–1644.
Heyman, P., Vervoort, T., Escutenaire, S., Degrave, E., Konings, J., Vandenvelde, C., Verhagen, R., 2001. Incidence of hantavirus infections in Belgium. Virus Res. 77, 71–80.
Heyman, P., Ceianu, C.S., Christova, I., Tordo, N., Beersma, M., Joao Alves, M., Lundkvist, Å., Hukic, M., Papa, A., Tenorio, A., Zelena, H., Essbauer, S., Visontai, I., Golovljova, I., Connell, J., Nicoletti, L., Van Esbroeck, M., Gjeruldsen Dudman, S., Aberle, S.W., Avsic-Zupanc, T., Korukluoglu, G., Nowakowska, A., Klempa, B., Ulrich, R.G., Bino, S., Engler, O., Opp, M., Vaheri, A., 2011. A five-year perspective on the situation of haemorrhagic fever with renal syndrome and status of the hantavirus reservoirs in Europe, 2005–2010. Euro Surveill 16, http://dx.doi.org/pii:19961.
Himmelfarb, J., Le, P., Klenzak, J., Freedman, S., McMenamin, M.E., Ikizler, T.A., 2004. Impaired monocyte cytokine production in critically ill patients with acute renal failure. Kidney Int. 66, 2354–2360.
Hjertqvist, M., Klein, S.L., Ahlm, C., Klingström, J., 2010. Mortality rate patterns for hemorrhagic fever with renal syndrome caused by Puumala virus. Emerg Infect. Dis. 16, 1584–1586.
Hosoya, T., Maillard, I., Engel, J.D., 2010. From the cradle to the grave: activities of GATA-3 throughout T-cell development and differentiation. Immunol. Rev. 238, 110–125.
Hujakka, H., Koistinen, V., Kuronen, I., Eerikäinen, P., Parviainen, M., Lundkvist, Å., Vaheri, A., Vapalahti, O., Närvänen, A., 2003. Diagnostic rapid tests for acute hantavirus infections: specific tests for Hantaan, Dobrava and Puumala viruses versus a hantavirus combination test. J. Virol. Methods 108, 117–122.
Huttunen, R., Kuparinen, T., Jylhävä, J., Aittoniemi, J., Vuento, R., Huhtala, H., Laine, J., Syrjänen, J., Hurme, M., 2011a. Fatal outcome in bacteremia is characterized by high plasma cell free DNA concentration and apoptotic DNA fragmentation: a prospective cohort study. PLoS One 6, e21700.
Huttunen, R., Syrjanen, J., Vuento, R., Hurme, M., Huhtala, H., Laine, J., Pessi, T., Aittoniemi, J., 2011b. Plasma level of soluble urokinase-type plasminogen activator receptor as a predictor of disease severity and case fatality in patients with bacteraemia: a prospective cohort study. J. Intern. Med. 270, 32–40.
Ishihara, K., Hirano, T., 2002. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev. 13, 357–368.
Jääskeläinen, K.M., Kaukinen, P., Minskaya, E.S., Plyusnina, A., Vapalahti, O., Elliott, R.M., Weber, F., Vaheri, A., Plyusnin, A., 2007. Tula and Puumala hantavirus NSs ORFs are functional and the products inhibit activation of the interferon-beta promoter. J. Med. Virol. 79, 1527–1536.
Jones, C.A., Nishiya, N., London, N.R., Zhu, W., Sorensen, L.K., Chan, A.C., Lim, C.J., Chen, H., Zhang, Q., Schultz, P.G., Hayallah, A.M., Thomas, K.R., Famulok, M., Zhang, K., Ginsberg, M.H., Li, D.Y., 2009. Slit2-Robo4 signalling promotes vascular stability by blocking Arf6 activity. Nat. Cell Biol. 11, 1325–1331.
Jonsson, C.B., Figueiredo, L.T., Vapalahti, O., 2010. A global perspective on hantavirus ecology, epidemiology, and disease. Clin. Microbiol. Rev. 23, 412–441.
Jylhävä, J., Nevalainen, T., Marttila, S., Jylhä, M., Hervonen, A., Hurme, M., 2013. Characterization of the role of distinct plasma cell-free DNA species in age-associated inflammation and frailty. Aging Cell 12, 388–397.
Kallio, E.R., Klingström, J., Gustafsson, E., Manni, T., Vaheri, A., Henttonen, H., Vapalahti, O., Lundkvist, Å., 2006. Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. J. Gen. Virol. 87, 2127–2134.
Kanerva, M., Paakkala, A., Mustonen, J., Paakkala, T., Lahtela, J., Pasternack, A., 1996. Pulmonary involvement in nephropathia epidemica: radiological findings and their clinical correlations. Clin. Nephrol. 46, 369–378.
Kanerva, M., Mustonen, J., Vaheri, A., 1998a. Pathogenesis of Puumala and other hantavirus infections. Rev. Med. Virol. 8, 67–86.
Kanerva, M., Vaheri, A., Mustonen, J., Partanen, J., 1998b. High-producer allele of tumour necrosis factor-alpha is part of the susceptibility MHC haplotype in severe Puumala virus-induced nephropathia epidemica. Scand. J. Infect. Dis. 30, 532–534.
Kang, J.I., Park, S.H., Lee, P.W., Ahn, B.Y., 1999. Apoptosis is induced by hantaviruses in cultured cells. Virology 264, 99–105.
Kishimoto, T., Akira, S., Narazaki, M., Taga, T., 1995. Interleukin-6 family of cytokines and gp130. Blood 86, 1243–1254.
Klempa, B., Avsic-Zupanc, T., Clement, J., Dzagurova, T.K., Henttonen, H., Heyman, P., Jakab, F., Kruger, D.H., Maes, P., Papa, A., Tkachenko, E.A., Ulrich, R.G., Vapalahti, O., Vaheri, A., 2013. Complex evolution and epidemiology of Dobrava-Belgrade hantavirus: definition of genotypes and their characteristics. Arch. Virol. 158, 521–529.
Klingström, J., Plyusnin, A., Vaheri, A., Lundkvist, Å., 2002. Wild-type Puumala hantavirus infection induces cytokines, C-reactive protein, creatinine, and nitric oxide in cynomolgus macaques. J. Virol. 76, 444–449.
Klingström, J., Hardestam, J., Stoltz, M., Zuber, B., Lundkvist, Å., Linder, S., Ahlm, C., 2006. Loss of cell membrane integrity in puumala hantavirus-infected patients correlates with levels of epithelial cell apoptosis and perforin. J. Virol. 80, 8279–8282.
Koch, A., Voigt, S., Kruschinski, C., Sanson, E., Duckers, H., Horn, A., Yagmur, E., Zimmermann, H., Trautwein, C., Tacke, F., 2011. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Crit. Care 15, R63.
Komatsuda, A., Wakui, H., Iwamoto, K., Togashi, M., Masai, R., Maki, N., Sawada, K., 2009. GATA-3 is upregulated in peripheral blood mononuclear cells from patients with minimal change nephrotic syndrome. Clin. Nephrol. 71, 608–616.
Korva, M., Saksida, A., Kunilo, S., Vidan Jeras, B., Avsic-Zupanc, T., 2011. HLA-associated hemorrhagic fever with renal syndrome disease progression in slovenian patients. Clin. Vaccine Immunol. 18, 1435–1440.
Korva, M., Saksida, A., Kejzar, N., Schmaljohn, C., Avsic-Zupanc, T., 2013. Viral load and immune response dynamics in patients with haemorrhagic fever with renal syndrome. Clin. Microbiol. Infect. 19, E358–E366.
Krautkrämer, E., Zeier, M., 2008. Hantavirus causing hemorrhagic fever with renal syndrome enters from the apical surface and requires decay-accelerating factor (DAF/CD55). J. Virol. 82, 4257–4264.
Krautkrämer, E., Grouls, S., Stein, N., Reiser, J., Zeier, M., 2011. Pathogenic old world hantaviruses infect renal glomerular and tubular cells and induce disassembling of cell-to-cell contacts. J. Virol. 85, 9811–9823.
Krautkrämer, E., Zeier, M., Plyusnin, A., 2013. Hantavirus infection: an emerging infectious disease causing acute renal failure. Kidney Int. 83, 23–27.
Labastie, M.C., Catala, M., Gregoire, J.M., Peault, B., 1995. The GATA-3 gene is expressed during human kidney embryogenesis. Kidney Int. 47, 1597–1603.
Lähdevirta, J., 1971. Nephropathia epidemica in Finland. A clinical, histological and epidemiological study. Ann. Clin. Res. 3 (Suppl. 8), S1–S154.
Laine, O., Mäkelä, S., Mustonen, J., Huhtala, H., Szanto, T., Vaheri, A., Lassila, R., Joutsi-Korhonen, L., 2010. Enhanced thrombin formation and fibrinolysis during acute Puumala hantavirus infection. Thromb. Res. 126, 154–158.
Laine, O., Mäkelä, S., Mustonen, J., Helminen, M., Vaheri, A., Lassila, R., Joutsi-Korhonen, L., 2011. Platelet ligands and ADAMTS13 during Puumala hantavirus

infection and associated thrombocytopenia. Blood Coagul. Fibrinolysis 22, 468–472.
Laine, O., Joutsi-Korhonen, L., Mäkelä, S., Mikkelsson, J., Pessi, T., Tuomisto, S., Huhtala, H., Libraty, D., Vaheri, A., Karhunen, P., Mustonen, J., 2012. Polymorphisms of PAI-1 and platelet GP Ia may associate with impairment of renal function and thrombocytopenia in Puumala hantavirus infection. Thromb. Res. 129, 611–615.
Larson, R.S., Brown, D.C., Ye, C., Hjelle, B., 2005. Peptide antagonists that inhibit Sin Nombre virus and hantaan virus entry through the beta3-integrin receptor. J. Virol. 79, 7319–7326.
Launes, J., Hautanen, A., 1988. Nephropathia epidemica encephalitis. Acta Neurol. Scand. 78, 234–235.
Lee, M., 1987. Coagulopathy in patients with hemorrhagic fever with renal syndrome. J. Korean Med. Sci. 2, 201–211.
Li, X.D., Kukkonen, S., Vapalahti, O., Plyusnin, A., Lankinen, H., Vaheri, A., 2004. Tula hantavirus infection of Vero E6 cells induces apoptosis involving caspase 8 activation. J. Gen. Virol. 85, 3261–3268.
Libraty, D.H., Mäkelä, S., Vlk, J., Hurme, M., Vaheri, A., Ennis, F.A., Mustonen, J., 2012. The degree of leukocytosis and urine GATA-3 mRNA levels are risk factors for severe acute kidney injury in Puumala virus nephropathia epidemica. PLoS One 7, e35402.
Linderholm, M., Billström, A., Settergren, B., Tärnvik, A., 1992. Pulmonary involvement in nephropathia epidemica as demonstrated by computed tomography. Infection 20, 263–266.
Linderholm, M., Ahlm, C., Settergren, B., Waage, A., Tärnvik, A., 1996. Elevated plasma levels of tumor necrosis factor (TNF)-alpha, soluble TNF receptors, interleukin (IL)-6, and IL-10 in patients with hemorrhagic fever with renal syndrome. J. Infect. Dis. 173, 38–43.
Lindgren, T., Ahlm, C., Mohamed, N., Evander, M., Ljunggren, H.G., Björkström, N.K., 2011. Longitudinal analysis of the human T cell response during acute hantavirus infection. J. Virol. 85, 10252–10260.
Lippi, G., Franchini, M., Targher, G., Favaloro, E.J., 2008. Help me, Doctor! My D-dimer is raised. Ann. Med. 40, 594–605.
Mackow, E.R., Gavrilovskaya, I.N., 2009. Hantavirus regulation of endothelial cell functions. Thromb. Haemost. 102, 1030–1041.
Maes, P., Clement, J., Groeneveld, P.H., Colson, P., Huizinga, T.W., Van Ranst, M., 2006. Tumor necrosis factor-alpha genetic predisposing factors can influence clinical severity in nephropathia epidemica. Viral Immunol. 19, 558–564.
Mähönen, S.M., Sironen, T., Vapalahti, O., Paakko, E., Hautala, N., Ilonen, J., Glumoff, V., Vainio, O., Kauma, H., Vaheri, A., Plyusnin, A., Hautala, T., 2007. Puumala virus RNA in cerebrospinal fluid in a patient with uncomplicated nephropathia epidemica. J. Clin. Virol. 40, 248–251.
Makary, P., Kanerva, M., Ollgren, J., Virtanen, M.J., Vapalahti, O., Lyytikäinen, O., 2010. Disease burden of Puumala virus infections, 1995–2008. Epidemiol. Infect. 138, 1484–1492.
Mäkelä, S., Ala-Houhala, I., Mustonen, J., Koivisto, A.M., Kouri, T., Turjanmaa, V., Vapalahti, O., Vaheri, A., Pasternack, A., 2000. Renal function and blood pressure five years after Puumala virus-induced nephropathy. Kidney Int. 58, 1711–1718.
Mäkelä, S., Hurme, M., Ala-Houhala, I., Mustonen, J., Koivisto, A.M., Partanen, J., Vapalahti, O., Vaheri, A., Pasternack, A., 2001. Polymorphism of the cytokine genes in hospitalized patients with Puumala hantavirus infection. Nephrol. Dial. Transplant. 16, 1368–1373.
Mäkelä, S., Mustonen, J., Ala-Houhala, I., Hurme, M., Partanen, J., Vapalahti, O., Vaheri, A., Pasternack, A., 2002. Human leukocyte antigen-B8-DR3 is a more important risk factor for severe puumala hantavirus infection than the tumor necrosis factor-alpha(-308) G/A polymorphism. J. Infect. Dis. 186, 843–846.
Mäkelä, S., Mustonen, J., Ala-Houhala, I., Hurme, M., Koivisto, A.M., Vaheri, A., Pasternack, A., 2004. Urinary excretion of interleukin-6 correlates with proteinuria in acute Puumala hantavirus-induced nephritis. Am. J. Kidney Dis. 43, 809–816.
Mäkelä, S., Kokkonen, L., Ala-Houhala, I., Groundstroem, K., Harmoinen, A., Huhtala, H., Hurme, M., Paakkala, A., Pörsti, I., Virtanen, V., Vaheri, A., Mustonen, J., 2009. More than half of the patients with acute Puumala hantavirus infection have abnormal cardiac findings. Scand. J. Infect. Dis. 41, 57–62.
Mäkelä, S., Jaatinen, P., Miettinen, M., Salmi, J., Ala-Houhala, I., Huhtala, H., Hurme, M., Pörsti, I., Vaheri, A., Mustonen, J., 2010. Hormonal deficiencies during and after Puumala hantavirus infection. Eur. J. Clin. Microbiol. Infect. Dis. 29, 705–713.
Markotic, A., Hensley, L., Geisbert, T., Spik, K., Schmaljohn, C., 2003. Hantaviruses induce cytopathic effects and apoptosis in continuous human embryonic kidney cells. J. Gen. Virol. 84, 2197–2202.
Marlow, R., Binnewies, M., Sorensen, L.K., Monica, S.D., Strickland, P., Forsberg, E.C., Li, D.Y., Hinck, L., 2010. Vascular Robo4 restricts proangiogenic VEGF signaling in breast. Proc. Natl. Acad. Sci. USA 107, 10520–10525.
Maurer, M., Bader, M., Bas, M., Bossi, F., Cicardi, M., Cugno, M., Howarth, P., Kaplan, A., Kojda, G., Leeb-Lundberg, F., Lotvall, J., Magerl, M., 2011. New topics in bradykinin research. Allergy 66, 1397–1406.
Miettinen, M.H., Mäkelä, S.M., Ala-Houhala, I.O., Huhtala, H.S., Kööbi, T., Vaheri, A.I., Pasternack, A.I., Pörsti, I.H., Mustonen, J.T., 2006. Ten-year prognosis of Puumala hantavirus-induced acute interstitial nephritis. Kidney Int. 69, 2043–2048.
Mohib, K., Guan, Q., Diao, H., Du, C., Jevnikar, A.M., 2007. Proapoptotic activity of indoleamine 2,3-dioxygenase expressed in renal tubular epithelial cells. Am. J. Physiol. Renal Physiol. 293, F801–F812.
Morgan, B.P., 1999. Regulation of the complement membrane attack pathway. Crit. Rev. Immunol. 19, 173–198.
Munn, D.H., Mellor, A.L., 2013. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. Trends Immunol. 34, 137–143.
Mustjoki, S., Sidenius, N., Vaheri, A., 2000. Enhanced release of soluble urokinase receptor by endothelial cells in contact with peripheral blood cells. FEBS Lett. 486, 237–242.
Mustonen, J., Brummer-Korvenkontio, M., Hedman, K., Pasternack, A., Pietilä, K., Vaheri, A., 1994a. Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand. J. Infect. Dis. 26, 7–13.
Mustonen, J., Helin, H., Pietilä, K., Brummer-Korvenkontio, M., Hedman, K., Vaheri, A., Pasternack, A., 1994b. Renal biopsy findings and clinicopathologic correlations in nephropathia epidemica. Clin. Nephrol. 41, 121–126.
Mustonen, J., Partanen, J., Kanerva, M., Pietilä, K., Vapalahti, O., Pasternack, A., Vaheri, A., 1996. Genetic susceptibility to severe course of nephropathia epidemica caused by Puumala hantavirus. Kidney Int. 49, 217–221.
Mustonen, J., Partanen, J., Kanerva, M., Pietilä, K., Vapalahti, O., Pasternack, A., Vaheri, A., 1998. Association of HLA B27 with benign clinical course of nephropathia epidemica caused by Puumala hantavirus. Scand. J. Immunol. 47, 277–279.
Norata, G.D., Marchesi, P., Pulakazhi Venu, V.K., Pasqualini, F., Anselmo, A., Moalli, F., Pizzitola, I., Garlanda, C., Mantovani, A., Catapano, A.L., 2009. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. Circulation 120, 699–708.
Nuutinen, H., Vuoristo, M., Färkkilä, M., Kahri, A., Seppälä, K., Valtonen, V., Joutsiniemi, T., Miettinen, T., 1992. Hemorrhagic gastropathy in epidemic nephropathy. Gastrointest. Endosc. 38, 476–480.
Obara, N., Suzuki, N., Kim, K., Nagasawa, T., Imagawa, S., Yamamoto, M., 2008. Repression via the GATA box is essential for tissue-specific erythropoietin gene expression. Blood 111, 5223–5232.
Olsson, G.E., Leirs, H., Henttonen, H., 2010. Hantaviruses and their hosts in Europe: reservoirs here and there, but not everywhere? Vector Borne Zoonotic Dis. 10, 549–561.
Ossowski, L., Aguirre-Ghiso, J.A., 2000. Urokinase receptor and integrin partnership: coordination of signaling for cell adhesion, migration and growth. Curr. Opin. Cell Biol. 12, 613–620.
Outinen, T.K., Mäkelä, S.M., Ala-Houhala, I.O., Huhtala, H.S., Hurme, M., Paakkala, A.S., Pörsti, I.H., Syrjänen, J.T., Mustonen, J.T., 2010. The severity of Puumala hantavirus induced nephropathia epidemica can be better evaluated using plasma interleukin-6 than C-reactive protein determinations. BMC Infect. Dis. 10, 132.
Outinen, T.K., Mäkelä, S.M., Ala-Houhala, I.O., Huhtala, H.S., Hurme, M., Libraty, D.H., Oja, S.S., Pörsti, I.H., Syrjänen, J.T., Vaheri, A., Mustonen, J.T., 2011. High activity of indoleamine 2,3-dioxygenase is associated with renal insufficiency in Puumala hantavirus induced nephropathia epidemica. J. Med. Virol. 83, 731–737.
Outinen, T.K., Kuparinen, T., Jylhävä, J., Leppänen, S., Mustonen, J., Mäkelä, S., Pörsti, I., Syrjänen, J., Vaheri, A., Hurme, M., 2012a. Plasma cell-free DNA levels are elevated in acute Puumala hantavirus infection. PLoS One 7, e31455.
Outinen, T.K., Mäkelä, S., Huhtala, H., Hurme, M., Meri, S., Pörsti, I., Sane, J., Vaheri, A., Syrjänen, J., Mustonen, J., 2012b. High pentraxin-3 plasma levels associate with thrombocytopenia in acute Puumala hantavirus-induced nephropathia epidemica. Eur. J. Clin. Microbiol. Infect. Dis. 31, 957–963.
Outinen, T.K., Tervo, L., Mäkelä, S., Huttunen, R., Mäenpää, N., Huhtala, H., Vaheri, A., Mustonen, J., Aittoniemi, J., 2013. Plasma levels of soluble urokinase-type plasminogen activator receptor associate with the clinical severity of acute puumala hantavirus infection. PLoS One 8, e71335.
Paakkala, A., Mustonen, J., 2007. Radiological findings and their clinical correlations in nephropathia epidemica. Acta Radiol. 48, 345–350.
Paakkala, A., Mustonen, J., Viander, M., Huhtala, H., Pasternack, A., 2000. Complement activation in nephropathia epidemica caused by Puumala hantavirus. Clin. Nephrol. 53, 424–431.
Pettersson, L., Klingstrom, J., Hardestam, J., Lundkvist, A., Ahlm, C., Evander, M., 2008. Hantavirus RNA in saliva from patients with hemorrhagic fever with renal syndrome. Emerg. Infect. Dis. 14, 406–411.
Pettersson, L., Thunberg, T., Rocklöv, J., Klingström, J., Evander, M., Ahlm, C., 2013. Viral load and humoral immune response in association with disease severity in Puumala hantavirus-infected patients-implications for treatment. Clin. Microbiol. Infect. http://dx.doi.org/10.1111/1469-0691.12259 [Epub ahead of print].
Plyusnin, A., Hörling, J., Kanerva, M., Mustonen, J., Cheng, Y., Partanen, J., Vapalahti, O., Kukkonen, S.K., Niemimaa, J., Henttonen, H., Niklasson, B., Lundkvist, Å., Vaheri, A., 1997. Puumala hantavirus genome in patients with nephropathia epidemica: correlation of PCR positivity with HLA haplotype and link to viral sequences in local rodents. J. Clin. Microbiol. 35, 1090–1096.
Podack, E.R., Biesecker, G., Muller-Eberhard, H.J., 1979. Membrane attack complex of complement: generation of high-affinity phospholipid binding sites by fusion of five hydrophilic plasma proteins. Proc. Natl. Acad. Sci. USA 76, 897–901.
Puljiz, I., Kuzman, I., Markotic, A., Turcinov, D., Matic, M., Makek, N., 2005. Electrocardiographic changes in patients with haemorrhagic fever with renal syndrome. Scand. J. Infect. Dis. 37, 594–598.
Rasche, F.M., Uhel, B., Kruger, D.H., Karges, W., Czock, D., Hampl, W., Keller, F., Meisel, H., von Muller, L., 2004. Thrombocytopenia and acute renal failure in Puumala hantavirus infections. Emerg. Infect. Dis. 10, 1420–1425.
Rasmuson, J., Andersson, C., Norrman, E., Haney, M., Evander, M., Ahlm, C., 2011a. Time to revise the paradigm of hantavirus syndromes? Hantavirus pulmonary syndrome caused by European hantavirus. Eur. J. Clin. Microbiol. Infect. Dis. 30, 685–690.

Rasmuson, J., Pourazar, J., Linderholm, M., Sandström, T., Blomberg, A., Ahlm, C., 2011b. Presence of activated airway T lymphocytes in human puumala hantavirus disease. Chest 140, 715–722.
Reading, P.C., Bozza, S., Gilbertson, B., Tate, M., Moretti, S., Job, E.R., Crouch, E.C., Brooks, A.G., Brown, L.E., Bottazzi, B., Romani, L., Mantovani, A., 2008. Antiviral activity of the long chain pentraxin PTX3 against influenza viruses. J. Immunol. 180, 3391–3398.
Rönnberg, T., Jääskeläinen, K., Blot, G., Parviainen, V., Vaheri, A., Renkonen, R., Bouloy, M., Plyusnin, A., 2012. Searching for cellular partners of hantaviral nonstructural protein NSs: Y2H screening of mouse cDNA library and analysis of cellular interactome. PLoS One 7, e34307.
Sadeghi, M., Eckerle, I., Daniel, V., Burkhardt, U., Opelz, G., Schnitzler, P., 2011. Cytokine expression during early and late phase of acute Puumala hantavirus infection. BMC Immunol. 12, 65.
Sadler, J.E., 1998. Biochemistry and genetics of von Willebrand factor. Annu. Rev. Biochem. 67, 395–424.
Safronetz, D., Ebihara, H., Feldmann, H., Hooper, J.W., 2012. The Syrian hamster model of hantavirus pulmonary syndrome. Antiviral Res. 95, 282–292.
Saggioro, F.P., Rossi, M.A., Duarte, M.I., Martin, C.C., Alves, V.A., Moreli, M.L., Figueiredo, L.T., Moreira, J.E., Borges, A.A., Neder, L., 2007. Hantavirus infection induces a typical myocarditis that may be responsible for myocardial depression and shock in hantavirus pulmonary syndrome. J. Infect. Dis. 195, 1541–1549.
Saksida, A., Duh, D., Korva, M., Avsic-Zupanc, T., 2008. Dobrava virus RNA load in patients who have hemorrhagic fever with renal syndrome. J. Infect. Dis. 197, 681–685.
Saksida, A., Wraber, B., Avsic-Zupanc, T., 2011. Serum levels of inflammatory and regulatory cytokines in patients with hemorrhagic fever with renal syndrome. BMC Infect. Dis. 11, 142.
Salio, M., Chimenti, S., De Angelis, N., Molla, F., Maina, V., Nebuloni, M., Pasqualini, F., Latini, R., Garlanda, C., Mantovani, A., 2008. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. Circulation 117, 1055–1064.
Sane, J., Laine, O., Mäkelä, S., Paakkala, A., Jarva, H., Mustonen, J., Vapalahti, O., Meri, S., Vaheri, A., 2012. Complement activation in Puumala hantavirus infection correlates with disease severity. Ann. Med. 44, 468–475.
Schountz, T., Prescott, J., Cogswell, A.C., Oko, L., Mirowsky-Garcia, K., Galvez, A.P., Hjelle, B., 2007. Regulatory T cell-like responses in deer mice persistently infected with Sin Nombre virus. Proc. Natl. Acad. Sci. USA 104, 15496–15501.
Settergren, B., Juto, P., Trollfors, B., Wadell, G., Norrby, S.R., 1989. Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev. Infect. Dis. 11, 921–927.
Settergren, B., Trollfors, B., Fasth, A., Hultberg, B., Norrby, S.R., 1990. Glomerular filtration rate and tubular involvement during acute disease and convalescence in patients with nephropathia epidemica. J. Infect. Dis. 161, 716–720.
Settergren, B., Boman, J., Linderholm, M., Wistrom, J., Hagg, E., Arvidsson, P.A., 1992. A case of nephropathia epidemica associated with panhypopituitarism and nephrotic syndrome. Nephron 61, 234–235.
Severson, W., Partin, L., Schmaljohn, C.S., Jonsson, C.B., 1999. Characterization of the Hantaan nucleocapsid protein-ribonucleic acid interaction. J. Biol. Chem. 274, 33732–33739.
Sidenius, N., Sier, C.F., Ullum, H., Pedersen, B.K., Lepri, A.C., Blasi, F., Eugen-Olsen, J., 2000. Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. Blood 96, 4091–4095.
Sinisalo, M., Vapalahti, O., Ekblom-Kullberg, S., Laine, O., Mäkelä, S., Rintala, H., Vaheri, A., 2010. Headache and low platelets in a patient with acute leukemia. J. Clin. Virol. 48, 159–161.
Sironen, T., Klingström, J., Vaheri, A., Andersson, L.C., Lundkvist, Å., Plyusnin, A., 2008. Pathology of Puumala hantavirus infection in macaques. PLoS One 3, e3035.
Sjölander, K.B., Elgh, F., Kallio-Kokko, H., Vapalahti, O., Hagglund, M., Palmcrantz, V., Juto, P., Vaheri, A., Niklasson, B., Lundkvist, Å., 1997. Evaluation of serological methods for diagnosis of Puumala hantavirus infection (nephropathia epidemica). J. Clin. Microbiol. 35, 3264–3268.
Spiropoulou, C.F., 2011. Molecular biology of hantavirus infection. In: Plyusnin, A., Elliott, R.M. (Eds.), Bunyaviridae. Molecular and Cellular Biology. Caiser Academic Press, Norfolk, UK, pp. 41–60.
Steel, C.M., Ludlam, C.A., Beatson, D., Peutherer, J.F., Cuthbert, R.J., Simmonds, P., Morrison, H., Jones, M., 1988. HLA haplotype A1 B8 DR3 as a risk factor for HIV-related disease. Lancet 1, 1185–1188.
Stojanovic, M., Pekic, S., Cvijovic, G., Miljic, D., Doknic, M., Nikolic-Djurovic, M., Micic, D., Hrvacevic, R., Nesic, V., Popovic, V., 2008. High risk of hypopituitarism in patients who recovered from hemorrhagic fever with renal syndrome. J. Clin. Endocrinol. Metab. 93, 2722–2728.
Sundberg, E., Hultdin, J., Nilsson, S., Ahlm, C., 2011. Evidence of disseminated intravascular coagulation in a hemorrhagic fever with renal syndrome-scoring models and severe illness. PLoS One 6, e21134.
Szalai, A.J., 2004. C-reactive protein (CRP) and autoimmune disease: facts and conjectures. Clin. Dev. Immunol. 11, 221–226.
Takala, A., Lähdevirta, J., Jansson, S.E., Vapalahti, O., Orpana, A., Karonen, S.L., Repo, H., 2000. Systemic inflammation in hemorrhagic fever with renal syndrome correlates with hypotension and thrombocytopenia but not with renal injury. J. Infect. Dis. 181, 1964–1970.
Taylor, S.L., Wahl-Jensen, V., Copeland, A.M., Jahrling, P.B., Schmaljohn, C.S., 2013. Endothelial cell permeability during hantavirus infection involves factor XII-dependent increased activation of the Kallikrein–Kinin system. PLoS Pathog. 9, e1003470.
Temonen, M., Vapalahti, O., Holthöfer, H., Brummer-Korvenkontio, M., Vaheri, A., Lankinen, H., 1993. Susceptibility of human cells to Puumala virus infection. J. Gen. Virol. 74, 515–518.
Temonen, M., Lankinen, H., Vapalahti, O., Ronni, T., Julkunen, I., Vaheri, A., 1995. Effect of interferon-alpha and cell differentiation on Puumala virus infection in human monocyte/macrophages. Virology 206, 8–15.
Temonen, M., Mustonen, J., Helin, H., Pasternack, A., Vaheri, A., Holthöfer, H., 1996. Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: an immunohistochemical study. Clin. Immunol. Immunopathol. 78, 47–55.
ten Oever, J., Tromp, M., Bleeker-Rovers, C.P., Joosten, L.A., Netea, M.G., Pickkers, P., van de Veerdonk, F.L., 2012. Combination of biomarkers for the discrimination between bacterial and viral lower respiratory tract infections. J. Infect. 65, 490–495.
Terajima, M., Ennis, F.A., 2011. T cells and pathogenesis of hantavirus cardiopulmonary syndrome and hemorrhagic fever with renal syndrome. Viruses 3, 1059–1073.
Terajima, M., Hendershot 3rd, J.D., Kariwa, H., Koster, F.T., Hjelle, B., Goade, D., DeFronzo, M.C., Ennis, F.A., 1999. High levels of viremia in patients with the Hantavirus pulmonary syndrome. J. Infect. Dis. 180, 2030–2034.
Terajima, M., Vapalahti, O., Van Epps, H.L., Vaheri, A., Ennis, F.A., 2004. Immune responses to Puumala virus infection and the pathogenesis of nephropathia epidemica. Microbes Infect. 6, 238–245.
Tersago, K., Verhagen, R., Vapalahti, O., Heyman, P., Ducoffre, G., Leirs, H., 2011. Hantavirus outbreak in Western Europe: reservoir host infection dynamics related to human disease patterns. Epidemiol. Infect. 139, 381–390.
Toivanen, A.L., Valanne, L., Tatlisumak, T., 2002. Acute disseminated encephalomyelitis following nephropathia epidemica. Acta Neurol. Scand. 105, 333–336.
Tracey, K.J., Cerami, A., 1994. Tumor necrosis factor: a pleiotropic cytokine and therapeutic target. Annu. Rev. Med. 45, 491–503.
Tsukada, H., Ying, X., Fu, C., Ishikawa, S., McKeown-Longo, P., Albelda, S., Bhattacharya, S., Bray, B.A., Bhattacharya, J., 1995. Ligation of endothelial alpha v beta 3 integrin increases capillary hydraulic conductivity of rat lung. Circ. Res. 77, 651–659.
Tuuminen, T., Kekäläinen, E., Mäkelä, S., Ala-Houhala, I., Ennis, F.A., Hedman, K., Mustonen, J., Vaheri, A., Arstila, T.P., 2007. Human CD8+ T cell memory generation in Puumala hantavirus infection occurs after the acute phase and is associated with boosting of EBV-specific CD8+ memory T cells. J. Immunol. 179, 1988–1995.
Ulich, T.R., Yin, S., Guo, K., Yi, E.S., Remick, D., del Castillo, J., 1991. Intratracheal injection of endotoxin and cytokines. II. Interleukin-6 and transforming growth factor beta inhibit acute inflammation. Am. J. Pathol. 138, 1097–1101.
Vaheri, A., Vapalahti, O., Plyusnin, A., 2008. How to diagnose hantavirus infections and detect them in rodents and insectivores. Rev. Med. Virol. 18, 277–288.
Vaheri, A., Henttonen, H., Voutilainen, L., Mustonen, J., Sironen, T., Vapalahti, O., 2013a. Hantavirus infections in Europe and their impact on public health. Rev. Med. Virol. 23, 35–49.
Vaheri, A., Strandin, T., Hepojoki, J., Sironen, T., Henttonen, H., Mäkelä, S., Mustonen, J., 2013b. Uncovering the mysteries of hantavirus infections. Nat. Rev. Microbiol. 11, 539–550.
Valtonen, M., Kauppila, M., Kotilainen, P., Lähdevirta, J., Svartbäck, C.M., Kosunen, O., Nurminen, J., Sarkkinen, H., Brummer-Korvenkontio, M., 1995. Four fatal cases of nephropathia epidemica. Scand. J. Infect. Dis. 27, 515–517.
van der Sluijs, K.F., Nijhuis, M., Levels, J.H., Florquin, S., Mellor, A.L., Jansen, H.M., van der Poll, T., Lutter, R., 2006. Influenza-induced expression of indoleamine 2,3-dioxygenase enhances interleukin-10 production and bacterial outgrowth during secondary pneumococcal pneumonia. J. Infect. Dis. 193, 214–222.
Van Epps, H.L., Terajima, M., Mustonen, J., Arstila, T.P., Corey, E.A., Vaheri, A., Ennis, F.A., 2002. Long-lived memory T lymphocyte responses after hantavirus infection. J. Exp. Med. 196, 579–588.
Van Loock, F., Thomas, I., Clement, J., Ghoos, S., Colson, P., 1999. A case-control study after a hantavirus infection outbreak in the south of Belgium: who is at risk? Clin. Infect. Dis. 28, 834–839.
Vapalahti, O., Lundkvist, Å., Kallio-Kokko, H., Paukku, K., Julkunen, I., Lankinen, H., Vaheri, A., 1996. Antigenic properties and diagnostic potential of Puumala virus nucleocapsid protein expressed in insect cells. J. Clin. Microbiol. 34, 119–125.
Vapalahti, O., Mustonen, J., Lundkvist, Å., Henttonen, H., Plyusnin, A., Vaheri, A., 2003. Hantavirus infections in Europe. Lancet Infect. Dis. 3, 653–661.
Vapalahti, K., Virtala, A.M., Vaheri, A., Vapalahti, O., 2010. Case-control study on Puumala virus infection: smoking is a risk factor. Epidemiol. Infect. 138, 576–584.
Volanakis, J.E., 2001. Human C-reactive protein: expression, structure, and function. Mol. Immunol. 38, 189–197.
Wagner, J., 2012. Free DNA – new potential analyte in clinical laboratory diagnostics? Biochem. Med. (Zagreb) 22, 24–38.
Walport, M.J., 2001. Complement. First of two parts. N. Engl. J. Med. 344, 1058–1066.
Wei, C., El Hindi, S., Li, J., Fornoni, A., Goes, N., Sageshima, J., Maiguel, D., Karumanchi, S.A., Yap, H.K., Saleem, M., Zhang, Q., Nikolic, B., Chaudhuri, A., Daftarian, P., Salido, E., Torres, A., Salifu, M., Sarwal, M.M., Schaefer, F., Morath, C., Schwenger, V., Zeier, M., Gupta, V., Roth, D., Rastaldi, M.P., Burke, G., Ruiz, P., Reiser, J., 2011. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat. Med. 17, 952–960.

Wiedmer, T., Esmon, C.T., Sims, P.J., 1986. Complement proteins C5b-9 stimulate procoagulant activity through platelet prothrombinase. Blood 68, 875–880.
Xiang, L.B., Zhang, Z.Y., Chen, J.M., Xiang, J.M., Cosgriff, T.M., 1990. Epidemic hemorrhagic fever. The mechanism of coagulation and fibrinolysis. Chin. Med. J. (Engl.) 103, 391–395.
Zaki, S.R., Greer, P.W., Coffield, L.M., Goldsmith, C.S., Nolte, K.B., Foucar, K., Feddersen, R.M., Zumwalt, R.E., Miller, G.L., Khan, A.S., et al., 1995. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am. J. Pathol. 146, 552–579.
Zhu, L.Y., Chi, L.J., Wang, X., Zhou, H., 2009. Reduced circulating CD4+CD25+ cell populations in haemorrhagic fever with renal syndrome. Clin. Exp. Immunol. 156, 88–96.