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REVIEW

Pathogenetic and Clinical Aspects of the Renal Involvement in Hemorrhagic Fever with Renal Syndrome

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ABSTRACT

Hemorrhagic fever with renal syndrome is the most common clinical manifestation of hantavirus infection. The main target organ is the kidney, resulting in an interstitial hemorrhagic nephritis and sometimes acute tubular necrosis. The pathogenesis is still largely unknown, but several recent studies indicate an important role for immune mechanisms including increased expression of cytokines, for example, tumor necrosis factor. Immunohistochemical studies of kidney biopsies have revealed deposits of IgG, IgM, and C3, but deposits were significantly less numerous than in chronic immune complex disease. Since hantaviruses are not cytolytic, a direct detrimental effect of the infecting virus is less likely. The long-term prognosis of hemorrhagic fever with renal syn-

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drome seems to be favorable, but there are reports that previous hantavirus infection is associated with an increased risk of hypertensive renal disease. Prospective longitudinal studies addressing this issue are underway.

INTRODUCTION

Hantaviruses occur worldwide and cause at least two different clinical syndromes; hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) (1-3). Since hantaviruses were isolated only during the last two decades they can be regarded as true emerging infections. Such hantaviruses associated with HFRS have been found in Asia and Europe (Table 1). Rodents are the main animal reservoir and the hantaviruses are shed in secretions from infected animals. Humans are most probably infected by the airborne route. The major clinical findings are fever, headache, lumbar pain, and abdominal pain (4-7). However, a wide variety of additional symptoms can occur since most organ systems—for example, the kidneys, the gastrointestinal tract, the respiratory tract, and the central nervous system—are involved in HFRS. The kidney involvement is prominent in most patients and manifests as, for example, azotemia, proteinuria, and hematuria with histological evidence of glomerular, interstitial, and tubular impairment. Initial oliguria and subsequent polyuria reflect glomerular and tubular dysfunction. Common complications associated with HFRS are bleeding and hypotension, and the case fatality rate is in the range of 1-10% (4-9). The most severe form of HFRS is caused by Hantaan virus, which occurs mainly in Southeastern Asia (5). Puumala virus (PUV) is the most prevalent hantavirus in Europe and the associated clinical syndrome is known as nephropathia epidemica (NE). The clinical severity of NE varies widely and fatal cases occur (10). Sin Nombre virus is a newly discovered hantavirus in North America causing HPS, a severe pulmonary syndrome without significant renal involvement (3,11). The antiviral drug ribavirin has shown some efficacy for treatment of HFRS cases in China (12). As for prophylaxis of hantavirus infections, recombinant based vaccines are currently being developed.

Table 1

Geographic Distribution of Hantaviruses Associated with HFRS

Virus	Geographic Distribution	Type of HFRS[a]
Hantaan	Asia, Europe	KHF/EHF
Seoul	Asia, Europe, USA	EHF/KHF
Puumala	Europe	NE
Dobrava	Europe	EHF/NE

[a]KHF, Korean hemorrhagic fever; EHF, epidemic hemorrhagic fever; NE, nephropathia epidemica.

TROPISM OF HFRS VIRUSES (HANTAVIRUSES)

Hantaviruses can in vitro infect a wide range of both primary human cell lines—such as glomerular cells, endothelial cells, and peripheral blood mononuclear cells (monocytes/macrophages)—and established human cell lines, for example, kidney (adenocarcinoma cell line A-704), lung, and liver cells (13). It seems, however, that certain cell lines such as primary vole kidney cells and Vero E6 (an established monkey kidney cell line) propagate hantaviruses most efficiently. This infective capacity is shared by all hantaviruses including, for example, Prospect Hill virus (14), which is *not* known to cause human disease. Moreover, hantavirus-infected cell cultures do *not* show cytopathic effects. These biological properties of hantaviruses indicate that mechanisms other than the virus infection per se are of pathogenetic importance in HFRS. Hantaviruses have been cultured from B cells and monocytes/macrophages from HFRS patients, but not from T cells (15). Recently, hantavirus RNA was demonstrated in neutrophils of patients with HPS (16). Hantaviral antigens have been demonstrated in the endothelium of small blood vessels in kidneys, lungs, liver, heart, and brain of HFRS patients (17). Using monoclonal antibodies against Hantaan virus a specific cytoplasmic reaction was observed in tubular epithelial cells of the distal nephron of HFRS patients (18,19). This may indicate that hantaviruses actually infect tubular cells in vivo and thereby directly contribute to the pathogenesis of the acute renal failure. It should be noted, however, that the actual site of hantavirus replication in man is as yet unknown.

ANIMAL MODELS OF HANTAVIRUS INFECTIONS

Experimentally hantavirus infected rodents never show severe symptoms, except suckling mice who develop fatal CNS infection (20). However, renal disease mimicking HFRS cannot be induced in animals. For example, cynomolgus macaques infected with hantavirus intratracheally did show symptoms such as anorexia, lethargy, and skin rash, but no signs of renal disease (21). Postmortem examination of the animals revealed histopathologic alterations only in the renal tubuli. Using immunoperoxidase staining hantavirus antigen could be demonstrated in the cytoplasm of renal tubular epithelial cells. In dot-spot hybridization experiments using specific oligonucleotide probes, hantavirus-specific RNA could be demonstrated in extracts from kidney cells (21). Thus, laboratory findings only indicate slight renal involvement in experimental animals and so far there are no reports that they develop overt kidney disease. The optimal animal model of HFRS therefore still has to be looked for.

RENAL PATHOPHYSIOLOGY IN HFRS

The acute renal failure in HFRS usually develops within the first week after onset of disease (1,4,5,6,8,22). Most HFRS patients have initial oliguria and, in severe cases, anuria, which is generally followed by polyuria. Early in the course of HFRS both the glomerular filtration rate (GFR) and renal plasma flow are normal or even increased (23). Increased vascular resistance has been noted in the acute phase, in spite of concomitant dilatation of the renal artery including the interlobular arteries (24). This could indicate a primary vascular lesion in the glomerular or postglomerular capillaries. During the oliguric phase

plasma renin concentrations are increased (25,26), while the onset of polyuria is associated with decreased renin levels and increased levels of atrial natriuretic hormone (26,27). A significant increase of both systemic blood pressure and pulse rate is seen in the oliguric phase, and it was suggested that the increased levels of atrial natriuretic hormone may have resulted from increased circulatory volume. Increased plasma concentrations of the very potent vasoconstrictor endothelin, probably originating from endothelial cells, have been demonstrated in HFRS patients (27,28). Endothelin-induced vasoconstriction may reduce the renal plasma flow and thus be of pathophysiological importance in HFRS. It was even suggested that symptoms such as vomiting and mental confusion encountered in HFRS patients could be explained by reduced blood flow mediated by endothelin (27). The major pathophysiological factor in HFRS is vascular dysfunction, mainly due to impaired vascular tone and increased vascular permeability early in the course of disease (29-32). The vascular dysfunction leads to a decrease of peripheral vascular resistance and a compensatory increase of cardiac output (33). Blood volume determinations using tracer disappearance rates showed decreased plasma volumes and total blood volumes in HFRS patients with severe shock (29). According to Cosgriff (31), dysregulation of the renal vasculature appears to be central in the pathophysiology of the renal failure in HFRS. Congestion and dilatation of medullary vessels give rise to edema and packing of red blood cells in the renal interstitium, which in turn leads to tubular compression and cell injury with ultimate degeneration, necrosis, and formation of tubular casts. Thrombocytopenia, platelet dysfunction, circulating heparin-like activity, and disseminated intravascular coagulopathy (34,35) lead to impairment of hemostasis, which might result in subsequent bleeding in, for example, the renal medulla. It has previously been shown that virus infection of endothelial cells markedly increases the adherence of platelets (36,37). Interestingly, despite the thrombocytopenia found, thrombocytes may be strongly activated as indicated by increased expression of a unique platelet-activation antigen (α-granule membrane protein) on the platelet surface and also by enhanced spontaneous luminol-dependent chemiluminescence (38,39). An increased oxidative burst of neutrophils from HFRS patients has been demonstrated, possibly stimulated by proinflammatory cytokines (40). The increased production of oxygen radicals in HFRS may contribute to tissue destruction by causing alterations of the vascular endothelium and the microvascular permeability, as reported in other types of kidney disease (41). Oxygen radicals might also induce aggregation of platelets. Increased production of nitric oxide, as indicated by high serum levels of nitrate, was recently reported in HFRS patients (42). In addition, the serum nitrate levels of those patients were shown to correlate significantly with scores on the acute physiological and chronic health evaluation (APACHE II) scale and with serum creatinine, and inversely with platelet counts.

In prospective studies of patients during the acute phase of HFRS in Europe it was shown that, in addition to glomerular dysfunction, there were also signs of injury to both proximal and distal tubuli (22). This was indicated by a transient, but marked, elevation of the urinary content of β_2-microglobulin and *N*-acetyl-β-D-glucosaminidase (NAG), a lysosomatic enzyme synthesized in the proximal tubule. There were also increased serum levels of antibodies against the Tamm-Horsfall (TH) glycoprotein, which is secreted by the ascending limb of Henle and considered to be a major constituent of casts (43). In animal experiments, TH protein has been shown to induce nephritis, which leads to scarring (44). TH protein has been demonstrated in the proximal tubuli and Bowmans' space of HFRS patients (45). Collan et al. (46) suggested that in response to tubular injury there could be an increased production of TH protein in HFRS, which would lead to the formation of casts. It

has been hypothesized that tubular obstruction due to casts or interstitial edema may play a substantial role in the pathophysiology of the acute renal failure of hantavirus infection (45,46). However, no correlation was found between TH IgG antibody levels and severity of HFRS (22). Finally, there are indications that TH protein can induce antibody-dependent cell-mediated cytotoxicity (47), but the exact pathogenic role of TH protein in HFRS remains obscure.

In a prospective study of patients with HFRS caused by PUV, all had normal or only slightly increased levels of urinary β_2-microglobulin and NAG 3 months after discharge (22). At that time desmopressin tests showed impaired tubular concentrating ability in about 20% of the patients. Despite severe renal impairment initially in some patients, all regained normal renal function although the convalescence could be prolonged up to 6-8 months until restitution (22). In a follow-up study performed 5 years after acute HFRS, Lähdevirta et al. (48) showed that the tubular function was still slightly depressed in about half of the patients, as indicated by decreased concentrating and acidifying capacity.

In summary, the renal involvement in HFRS has the pathophysiological features of acute renal failure. The patients have reductions of GFR, urine volumes, and tubular concentrating ability, which may last for months until normalization; generally the tubular functions are the last to recover.

MORPHOLOGICAL AND HISTOLOGICAL ALTERATIONS OF KIDNEYS IN HFRS

Ultrasonography of HFRS patients reveals enlarged kidneys (7,8), while gamma-camera nephrography has been shown to be a more sensitive tool to identify renal impairment (49). Sequential computed tomography (CT) of kidneys of HFRS patients showed a characteristic "cart-wheel" pattern during the washout stage (50). The authors suggested that this pattern represents relief of vasoconstriction and repair of tubular function. Follow-up CT at 2 weeks showed multifocal, "wedge-shaped," nonenhanced areas of the kidney, which were thought to be ischemic zones due to persistent vasoconstriction. Interestingly, CT performed 6 weeks after the onset of disease in one HFRS patient showed scarring of the kidney in the same region that did not enhance on the 2-week CT. Magnetic resonance imaging (MRI) of the kidneys in HFRS has shown low signal intensity along the medulla, possibly representing medullary hemorrhage (51).

Histological examination of kidney biopsies taken early during the disease show an acute tubulointerstitial nephritis with cellular infiltration, interstitial edema, and flattening of epithelial cells in the proximal tubuli (4,8,46,52). Tubular epithelial changes also include luminal dilatation and formation of casts (46,52,53). Interstitial bleeding is a common finding (46,52). The glomeruli characteristically show congestion and mild hypercellularity (46). In one case of HFRS caused by Hantaan virus, kidney biopsy revealed diffuse proliferative glomerulonephritis (54). Immunohistochemical studies of renal biopsies from patients with acute hantavirus infection showed interstitial infiltration of lymphocytes, plasma cells, monocytes/macrophages, and polymorphonuclear leucocytes, mainly eosinophilic granulocyte and neutrophils (53). Immunofluorescence studies of renal biopsies show focal deposition of IgG, IgM, complement factor 3, and fibrin in the tubular basement membrane in about half of the cases, and also focal immunoglobulin and complement deposits along the capillary basement membrane in the glomeruli (46,55). However, deposits were less numerous than in chronic immune complex diseases. Electronmicros-

copy studies have revealed splitting of the tubular basement membrane as well as glomerular deposits under the endothelial cells, in the mesangium, but also inside the thickened basement membrane and occasionally on the epithelial side of the membrane (46,55-57). Virus-like particles (i.e., intraendothelial microtubular inclusions) were seen in glomerular and/or intertubular capillary endothelium in about 30% of cases, but only in kidney biopsies taken during the first 2 weeks of disease (46).

To summarize, the renal histologic alterations in HFRS have the features of both acute tubular necrosis and hemorrhagic interstitial nephritis with glomerular and endothelial damage. Moreover, it seems that the tubular lesion is of the ischemic rather than the toxic type of tubular necrosis (52).

IMMUNE MECHANISMS IN THE PATHOGENESIS OF HFRS

There are several factors that favor immune-mediated mechanisms being of major pathogenetic importance in HFRS. Firstly, specific antibodies to hantaviruses (e.g., of the IgM and IgE isotypes) occur very early in the course of disease (58,59). It is known that virus-specific antibodies can be harmful to the host through mechanisms such as molecular mimicry and the production of anti-idiotypic antibodies (60). Secondly, immune complexes can be demonstrated in serum, urine, glomeruli, tubular interstitium, skin, and on red blood cells and platelets of HFRS patients (31,61-64). Immune complex formation and deposition can lead to tissue injury and is considered the main pathogenetic mechanism of the renal damage in virus infections (65-67). Thirdly, activation of both the classical and alternative complement pathway has been reported (62,68,69). There is also a very strong unspecific immune response manifested by, for example, highly increased concentrations of C-reactive protein, not usually seen in virus infections (7,70,71). Fourthly, there is evidence of T-cell activation; for example, increased numbers of CD8+ cells and a concomitant decrease of CD4+ cells occur very early in the course of HFRS (31,63,72,73). Functional testing of suppressor T cells has shown a decreased activity in spite of increased numbers of CD8+ cells, indirectly indicating a corresponding increase in number of cytotoxic T cells (63). The significance of this finding in terms of HFRS pathogenesis is unclear. However, it is well known that cytotoxic T cells may secrete γ-IFN which can potentiate the cytotoxic effects of macrophages. During the first week of HFRS significantly lowered numbers of natural killer (NK) cells have been observed as compared to the second week (72). In kidney biopsies from HFRS patients, accumulations of mainly CD8-positive cells could be demonstrated in the peritubular areas (53). At the same location, strong accumulations of cells positive for antilysozyme antibodies were seen. A further indication that immune mechanisms are of importance in the pathogenesis of HFRS is the prolonged (up to 8 weeks) incubation period (74), which would argue against a direct cytotoxic effect by the infecting virus as the cause of the symptoms. Finally, HFRS patients may have serum antibodies against several different "self" antigens, such as endothelial cells, glomerular basement membrane, Tamm-Horsfall protein, and rheumatoid factors (22,61,76,76). The occurrence of this type of antibody could be due to amino acid homologues shared between viruses and specific host cell components (60). The same authors suggested that autoantigens may actually appear de novo as a result of virus-induced alterations of the host cell.

Both hantavirus-specific and total IgE levels are increased in HFRS patients (58). The highest levels are seen during the first 2 weeks of disease, but hantavirus-specific IgE can be

demonstrated for up to 18 months. IgE-containing immune complexes have been found in both serum (77) and kidney (78) of HFRS patients. IgE may induce increased expression of cytokines such as TNF-α, resulting in increased vascular permeability (58). Also, it has been shown that viral antigen binds to IgE on basophils and on mast cells in vascular tissue (79). However, IgE levels did not show a significant correlation to clinical or laboratory markers of severity, such as duration of fever, hemorrhagic manifestations, serum creatinine levels, or thrombocyte counts (58).

The very complex and interactive system of cytokines and growth factors are mediators of several important pathophysiological events but have been studied to only a limited degree in HFRS. The cytokines have very delicate feedback systems, for example, interferon (IFN)-γ stimulates macrophages to synthesize both tumor necrosis factor (TNF)-α and interleukin (IL)-1, while IL-2 serves as a "signal" for the generation of TNF-α. TNF is a pluripotent cytokine with effects, including vasodilation and hypotension, thought to be secondary to increased NO synthesis, an event that was recently reported in patients with PUV-induced HFRS (42).

Interestingly, injection of TNF-α leads to acute tubular necrosis in rats (80). TNF-α also augments the production of IL-1 by macrophages and endothelial cells. Both TNF-α and IL-1 have major effects on endothelial and inflammatory cells. Virus infection of endothelial cells can upregulate the expression of certain cytokines, for example, IL-1 (14). TNF-α augments the expression of adhesion molecules—for example, intercellular adhesion molecule (ICAM)-1—on human renal tubular epithelial cells (81). Increased expression of ICAM-1 and vascular cell adhesion molecule (VCAM)-1 are seen in several immunologically mediated renal diseases (82), and was recently also demonstrated in kidney biopsies of HFRS patients (53). In addition, those patients had increased expression of TNF-α, transforming growth factor (TGF)-β, and platelet-derived growth factor (PDGF) in the kidneys. Both TGF-β and PDGF are known to be produced in, for example, mesangial cells in the kidney (83,84). Animal studies have shown that overproduction of TGF-β is associated with accumulation of extracellular matrix in nephritic glomeruli (85).

Increased plasma concentrations of TNF-α and IL-6 can be demonstrated early in the acute phase of HFRS (86,87). TNF-α levels remained high up to 1 week after onset of disease, while concentrations of IL-6 had returned to normal at this time (87). Serum levels of soluble TNF receptors (p55, p75) were also elevated and showed a significant correlation with the TNF-α levels. Interestingly, maximal plasma concentrations of both TNF-α and IL-6 showed a significant correlation to maximal serum concentrations of creatinine (87). Another study showed that serum levels of TNF-α were significantly higher in a group of HFRS patients requiring hemodialysis as compared to nondialysed patients (86). Increased plasma concentrations of IL-10, an important downregulator of TNF-α, can be demonstrated early in the course of HFRS (87). Elevated levels of γ-IFN and α-IFN, both of which show antiviral activity, have also been documented (88). Persistently elevated plasma levels of γ-IFN could be demonstrated >3 weeks after onset of disease in HFRS patients, which may indicate prolonged immune activation (88). Increased plasma levels of IL-2 have not been demonstrated, but an increased proportion of cells expressing IL-2 receptors (CD 25) have previously been reported (63,89; Mats Linderholm, unpublished data). In addition, soluble IL-2 receptor levels in plasma are increased during the acute phase of HFRS (89). In a limited investigation of HFRS patients, increased plasma levels of IL-1β were found in about one-third of plasma samples taken within 10 days after onset of disease, while IL-1α could not be demonstrated in any plasma sample (Bo Settergren, unpublished data). Possible immunopathogenetic mechanisms in HFRS are summarised in Figure 1.

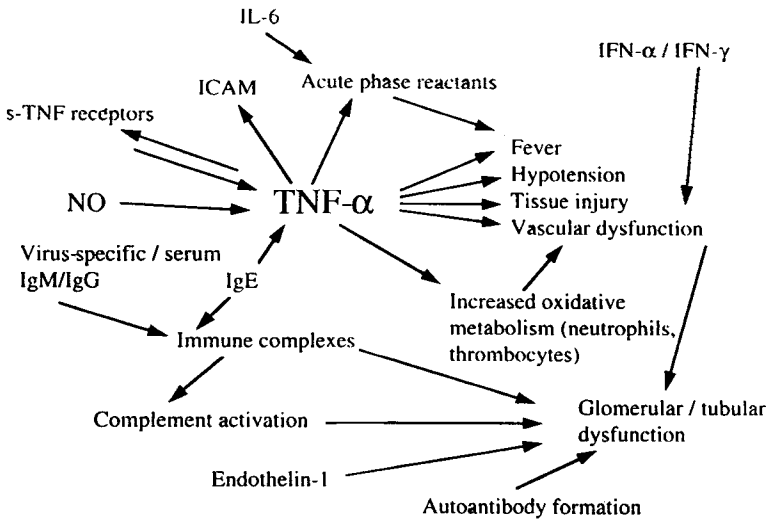

Figure 1. Possible immunopathogenetic mechanisms in hantavirus-induced hemorrhagic fever with renal syndrome (based on Refs. 22, 27, 31, 39, 40, 42, 53, 55, 58, 61, 62, 68, 72, 73, 75–77, 86–89).

CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF THE RENAL DISEASE IN HFRS

Oliguria is a very common initial feature in HFRS, usually followed by polyuria. Urinalysis show proteinuria and hematuria in virtually all patients, while urine microscopy reveal casts in 20-50% (4,70,71,74). HFRS has been associated with macroscopic hematuria and kidney rupture (4,90,91), and with a nephrotic syndrome (92). Complications of HFRS include major bleeding, DIC, hypotension, and shock (4,5,71,74,90). Clinical severity of hantavirus infection has been associated with the degree of thrombocytopenia and platelet dysfunction, and to a more vigorous immune response (31), but also to the degree and duration of fever (93), to the degree of leucocytosis (10; Prof. VA Figurnov, Medical Institute, Blagoveshensk, Russia, personal communication), to the serum levels of specific serum IgM (94), and to the magnitude of complement activation (68,69). It has also been claimed that serum levels of albumin and aminotransferases can predict disease outcome in HFRS (32). Peritoneal or hemodialysis have been performed in up to 10% of HFRS patients (4,5,6,95,96). However, the clinical indications for dialysis in those patients were not clearly defined. Since anuria persisting for several days is uncommon in HFRS, there would, in our opinion, very rarely be an absolute need for dialysis. Follow-up studies in HFRS patients up to 5 years after acute disease (48,97) showed slightly impaired urinary concentration capacity in a small fraction of patients. However, there was no evidence of increased risk of progressive renal disease or hypertension. This is in contrast to anecdotal reports of an association between previous hantavirus infection and subsequent hypertensive renal disease (8,98,99,100). Moreover, a large seroepidemiological survey in the United States showed that previous infection with a rat-borne hantavirus consistently was associated with hypertensive renal disease (101). They found that 6.5% of patients with end-stage renal disease due to hypertension were seropositive for a hantavirus. In contrast, screening for hantavirus antibodies in more than 1600 European patients on hemodialysis or

with a functioning renal transplant showed seroprevalence rates of <1%, which is not significantly different from the rate found in the control groups (102-104). Two large seroepidemiological surveys performed in northern Sweden also failed to demonstrate a correlation between previous hantavirus infection and cardiovascular disease or renal dysfunction (105,106). The results of these European seroepidemiological studies would suggest that hantavirus infection does *not* have a major etiologic role in chronic renal disease. Moreover, out of 174 American Indians with renal disease, none had serum antibodies against two tested hantaviruses (107). A possible explanation for these seemingly contradictory data could be that hantaviruses differ in their capacity to induce chronic renal failure. However, it is important to note that the true relationship between hantavirus infection and the development of chronic renal failure can only be evaluated in controlled longitudinal studies.

Very recently it was reported that HFRS patients with a specific genetic setup seem to have an increased risk of severe course of disease (108). Determinations of major histocompatibility complex (MHC) markers showed that patients with the most severe disease had a very high frequency of a specific HLA B8 haplotype. For example, severe renal failure (serum creatinine >500 μmol/L) was significantly more common among HLA B8 positive than in HLA B8 negative patients. Interestingly, the same MHC alleles are overrepresented among patients with various autoimmune diseases (108).

CONCLUDING REMARKS

The results of several recent studies suggest a central role for immune mechanisms in the pathogenesis of HFRS and there is increasing evidence that a combination of cell-mediated mechanisms and humoral responses is involved. The demonstration of increased expression of cytokines (e.g., TNF-α) in both plasma and kidney biopsies of HFRS patients is of special interest since TNF is cytolytic to virus-infected cells, and injection of TNF in humans induces symptoms commonly encountered in HFRS. Although there is a lack of evidence for a direct cytotoxic effect of hantaviruses, they may still induce alterations in the host cell membrane leading to exposure of host antigens to the immune system, and antibodies to several such antigens have been demonstrated in HFRS patients. The capacity of hantaviruses to infect immunocompetent cells (e.g., B cells, monocytes) might hypothetically also have pathogenetic implications. In vitro hantaviruses readily infect different types of renal cells, and the kidneys are also the main target organs in HFRS, although most organ systems are involved. Kidney biopsies of HFRS patients show an interstitial, hemorrhagic nephritis with endothelial and glomerular damage. The tubuli seem to be the most severely affected part of the kidney and there are indications that, in addition to impairment of the proximal tubular reabsorptive capacity, there is also damage to cells of both proximal and distal tubuli and the collecting ducts. Immunohistochemical studies have revealed deposits of both immunoglobulins and complement factors along basement membranes in tubule and glomeruli. The mechanisms of renal tissue damage in HFRS therefore probably include both virus induced production of cytokines, formation and deposition of immune complexes, and possibly also autoimmune antibody reactions. Using monoclonal antibodies, hantaviral antigens have been demonstrated in endothelial cells in the vessels of the tubular, medullary part of the kidney, which could indicate that endothelial cells in the tubular region are an important replicative site of hantaviruses in man. An intriguing recent finding is that HFRS patients with certain HLA haplotypes seem to have an increased risk

of severe course of the disease. Since an adequate animal model of HFRS is still lacking, there is a continuous need for well-designed prospective studies of pathogenesis addressing questions such as: How does the increased expression of cytokines in HFRS patients correlate to symptoms? What are the best laboratory markers of clinical severity? Is the formation of autoantibodies in hantavirus infection associated with pathogenicity?

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