Hantavirus Nephropathy

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ABSTRACT

Pathogenic rodent-borne hantaviruses cause in humans generalized infections that involve the peripheral vascular bed and severely affect their permeability. We describe a 30-yr-old male patient with clinical symptoms characterizing five conventional phases of hemorrhagic fever with renal syndrome after an uncommonly severe hantavirus infection with the Puumala strain. Renal biopsy in this situation typically demonstrates acute hemorrhagic interstitial nephritis, particularly pronounced in the outer medulla. Hantaviruses are not cytopathic for most cells, and their interactions with endothelial cells that activate immune mechanisms play a key role in the pathogenesis of vascular dysfunction characterizing this disease.


A 30-yr-old man was admitted to hospital with a 5-d history of flu-like symptoms: Headache, shivers, nausea, vomiting, backache, arthralgias, myalgias, and blurred vision. In the previous month, he had been in close contact with rodents in the village of Dobrava, which is endemically infected with two hantavirus strains, Puumala and Dobrava. On admission, he was hypotensive with a BP of 85/55 mmHg and a serum creatinine level of 382 μmol/L (normal range 44 to 97 μmol/L). On day 6, he became oliguric, somnolent, tachypnic, and tachycardic. Physical examination revealed eyelid edema and conjunctival injections with scattered, predominantly retinal and in the outer medulla, histomorphologic changes were much more pronounced and characterized by extensive interstitial hemorrhage (Figure 1A). Compressed floating tubuli and barely visible outlines of congested peritubular capillaries could be seen in a sea created mostly by massive extravasation of erythrocytes, with only a sparse mix of inflammatory cells (Figure 1A, inset). Focal tubular dilation and discrete damage to tubuli and peritubular capillaries were occasionally observed. Immunophenotyping showed that most of the sparse interstitial inflammatory cells were CD8+ cytotoxic T lymphocytes (CTL) and CD68+ macrophages (Figure 1C). Biopsy findings of acute hemorrhagic interstitial nephritis with characteristic distribution of the most pronounced lesions support the diagnosis of hantavirus nephropathy.

PATHOPHYSIOLOGY of the RENAL BIOPSY

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EPIDEMIOLOGY AND CLINICAL FINDINGS

More than 30 different strains of hantaviruses have co-evolved over millennia worldwide, each with a specific rodent host linked to a particular geographic area.3–6 Human infection by inhalation of aerosolized rodent excreta hosting >20 pathogenic hantaviruses have been identified to date, resulting in two life-threatening clinical syndromes.5 All Old World pathogenic hantaviruses cause hemorrhagic fever with renal syndrome (HFRS), a generalized infection of varying severity, having in common acute renal failure caused by hantavirus nephropathy (Table 1).

Prototype Hantaan virus causes HFRS in approximately 150,000 people annually in China, Far East Russia, and

Figure 1. Needle biopsy showing histomorphologic changes in the kidney of a 30-yr-old male patient in the oliguric phase of acute Puumala hantavirus infection. (A) On low magnification, massive interstitial hemorrhage is present in the outer medulla (left cylinder), whereas only mild acute tubular injury, interstitial edema, and inflammatory cell infiltration is seen in the cortex (right cylinder). High magnification (inset) of the renal outer medulla shows barely visible outlines of dilated, blood-engorged peritubular capillaries (arrows) as well as abundant interstitial hemorrhage appearing as a sea of erythrocytes with floating separated and compressed tubuli (periodic acid silver methenamine-Azan). (B) Electron micrograph of the renal cortex showing interstitial edema and extravasated fresh, well-preserved erythrocytes (*), as well as two dysmorphic red blood cells affected by hemolysis (arrows; OsO₄, uranyl acetate and lead citrate). (C) In the hemorrhagic medulla, fairly sparse irregular interstitial inflammatory cell infiltrates, composed predominantly of brown-stained CD8⁺ T lymphocytes and red-stained CD68⁺ monocytes/macrophages (sequential double immunostaining using Ventana mAb against CD8 [clone C8/144B] and ultraView DAB detection kit and subsequently mAb against CD68 [clone KP-1] and ultraView Red detection kit, with hematoxylin counter-stain).
Korea, with a mortality rate of 5 to 15%.

The genetically and serologically closely related Dobrava virus, first isolated in Slovenia, causes similarly severe HFRS predominantly in central and southeast Europe. Puumala virus, which belongs to another, second lineage of the hantavirus phylogenetic tree, affects approximately 5000 people annually throughout Europe, with a low mortality (<1%) and milder form of HFRS. There are exceptions, such as the case presented here and other reported fatal autopsied cases. All pathogenic New World hantaviruses, including prototype Sin Nombre virus isolated in 1993, belong to the second lineage and cause hantavirus pulmonary syndrome (HPS), affecting primarily the lungs, with a mortality rate >50%. Five chronological phases have been clinically studied and reported in patients with severe HFRS (Figure 2). The mechanism of the often severe proteinuria in HFRS remains unclear. Tubular proteinuria may only partly contribute, and our hypothesis of a causal relationship with glomerular endothelial cell injury is speculative.

PATHOLOGY

Knowledge of the pathomorphology of hantavirus nephropathy in the severe form of HFRS comes from detailed autopsy studies during the Korean war, when the cause was still unknown. Biopsy studies have been mostly limited to a series of cases affected by the milder form of HFRS caused by Puumala virus. Although peripheral vascular injury with severely affected permeability seems generalized, the lungs are the major target in HPS, and hantavirus nephropathy dominates in both severe and milder forms of HFRS. Hantaviruses are endotheliotropic and Zaki et al. using all available techniques including immunohistochemistry with a widely effective in-house mAb GB04-BF07, provided evidence that at least in HPS, hantavirus is present in the endothelial cells of small blood vessels in many organs, particularly lungs and to some extent kidneys, predominantly in the interstitial capillaries of the medulla, and rarely in tubular epithelial cells. There have been less convincing studies with other mAb suggesting that pathogenic changes in HFRS appear exclusively in tubular epithelial cells, particularly in the medulla. Electron microscopy studies of HFRS, including our analysis, fail to provide convincing evidence of the presence of hantavirus particles in kidney tissue. This may be due to a low amount of hantaviruses in the kidney: In our fatal cases of HFRS, Dobrava hantavirus RNA was detected only in autopsy samples of kidney medulla by highly sensitive reverse transcriptase–PCR and identified by nucleotide sequence analysis. Our study of low-level infection provides evidence that more or less the whole peripheral vascular bed of the kidney, including glomerular and cortical peritubular capillaries, is involved in hantavirus nephropathy; however, by far the most severe vascular affection is in the outer medulla, particularly the outer stripe, presenting in early phases with congestion and permeability disturbances, and from transition to the oliguric phase with severe blood stasis accompanied by leakage, extensive interstitial hemorrhage, severe endothelial degenerative changes, and occasionally anemic necrosis in the deeper medulla. In line with our results of cell immunophenotyping, CTL and macrophages are the predominant interstitial inflammatory cells.

The histomorphologic features of hantavirus nephropathy are characteristic and diagnostic by renal biopsy when an adequate sample of outer medulla is available. There are only a few other viral

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**Figure 2.** Major clinical manifestations during five phases of HFRS. Data referring to the severe form of HFRS as caused by Hantaan and Dobrava viruses. In the milder form of HFRS, also called nephropathia epidemica, mostly caused by Puumala virus, the clinical phases are generally less apparent, and hemorrhaging, severe hypotension, and shock occur rarely. Adapted from reference.
hemorrhagic fevers and viral interstitial nephritides in which hemorrhages in the kidney have been reported, and they do not have the same histotopography as HFRS. Vasomotor acute tubular injury and/or hemostatic disturbances may also contribute to the pathogenesis.

We performed a comparative study of 10 renal biopsies and four autopsies of patients who had HFRS from infection with Dobrava hantavirus and 10 renal biopsies of patients who had HFRS from infection with Puumala virus without fatality (unpublished data). There were no qualitative differences, but there were quantitative differences in histopathology, with kidneys of patients infected with Dobrava virus being on average more severely affected, in particular with regard to the intensity and extent of medullary in-
terstitial capillary injury, congestion, hemorrhages, and tubular necrosis (Figures 1 and 3).

**PATHOPHYSIOLOGY**

It is well documented that hantaviruses are not cytopathic for most cells. They replicate in macrophages and endothelial cells, entering the host cell via β₃ integrin surface molecules. Both pathogenic and nonpathogenic hantaviruses have the same tissue tropism, but recent studies showed an important difference in endothelial cell gene responses elicited by pathogenic or nonpathogenic hantaviruses, as well as the induction of unique genes by HPS- and HFRS-producing hantaviruses.²⁴

The association of a mild or severe clinical course of disease with certain HLA alleles suggests a role for the host immune response.⁵,²⁵ Hantaviruses induce an innate immune response, as well as humoral and cellular adaptive immune responses.⁷ Elevated titers of virus-specific IgM, IgA, IgG, and IgE antibodies and immune complexes are detected in serum during hantavirus infections. Mechanisms of immune complex deposition were once suggested to play a part in pathogenesis, but this was not confirmed by later studies, including our results.¹⁴,¹⁵,²⁶ Antibody-dependent cellular cytotoxicity and complement activation are most likely induced as effector mechanisms by antibodies against hantaviruses. The degree of activation of the classical complement pathway correlates with the clinical course of HFRS.²⁵

CTL predominate in kidney interstitial inflammatory cell infiltrates in hantavirus disease. They seem to have an important role in viral clearance associated with apoptosis of infected cells, and also in tissue injury. Furthermore, CTL as well as infected endothelial cells, macrophages, and dendritic cells produce various cytokines and chemokines, which contribute to the pathogenesis of hantavirus disease (Figure 3). Significantly elevated plasma levels of IFN-γ, TNF-α, IL-2, and IL-6 are detected in the acute phase of hantavirus disease, and levels of TNF-α, one of the most important proinflammatory cytokines, correlates with severity of disease.²⁵,²⁷ Increased expression of TNF-α, as well as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and plateletendothelial cell adhesion molecule-1, are present in the peritubular areas of the distal nephron in kidney biopsies of patients with Puumala virus–induced HFRS.²¹ In vivo studies also confirm the important role of TNF-α, showing that exposure to high dosages of this cytokine induce shock, increased capillary leakage, and mortality.²⁵ Some studies indicate that increased vascular permeability is also associated with elevated levels of vascular endothelial growth factor produced by infected endothelial cells and macrophages.²⁸

**CONCLUSIONS**

Infection with hantavirus causes harm to endothelial cell function and, through the induction of cytokines, chemokines, and cellular receptors, modulates the immune response, which seems to have a key role in the pathogenesis of hantavirus disease.

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