Virus Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

# Old World hantaviruses: Aspects of pathogenesis and clinical course of acute renal failure

Ellen Krautkrämer*, Martin Zeier

*Department of Nephrology, University of Heidelberg, Heidelberg, Germany*

ARTICLE INFO

*Article history:*
Available online xxx

*Keywords:*
Hantavirus
Pathogenesis
Receptor
Endothelial progenitor cells

ABSTRACT

Hantavirus-associated diseases represent emerging infections that are ranked in the highest priority group of communicable diseases for surveillance and epidemiological research. In the last years, several novel hantavirus species were described and the number of host reservoir species harboring hantaviruses is also increasing. Reports of cases with severe or atypical clinical courses become also more frequent. These facts raise more and more questions concerning host reservoir specificity, pathogenicity and molecular mechanism of pathogenesis. Hantavirus disease is characterized by vascular leakage due to increased capillary permeability. The infection manifests often in the lung (hantaviral cardiopulmonary syndrome; HCPS) or in the kidney (hemorrhagic fever with renal syndrome, HFRS). The underlying mechanisms of both syndromes are probably similar despite the difference in organ tropism. Characterization of hantaviral replication cycle and of patient-specific determinants will help to identify factors responsible for the clinical symptoms and course.



## 1. Determinants of hantaviral pathogenicity

### 1.1. Target cells and receptors

The epidemiology and clinical course of hantavirus infection is complex. The pathogenicity of hantaviruses differs enormously between hantavirus species. Men are more affected than women and behavioral factors seem to be responsible for the unequal male-to-female ratio (Krautkrämer et al., 2013b). However, case fatality rates (CFRs) are higher for women as reported from different countries (Hjertqvist et al., 2010; Klein et al., 2011; Martinez et al., 2010). The reason for the higher CFR for women is not known so far. The broad spectrum of hantavirus disease ranging from asymptomatic infection and severe courses does not seem to depend on gender-specific differences since laboratory parameters, renal, cardiac or pulmonary findings were similar in both sexes. In contrast, young male patients suffer more often from severe central nervous system complications during Puumala virus (PUUV) infection (Hautala et al., 2011).

Hantavirus disease demonstrates various symptoms and may affect different organs. Endothelial and epithelial cells of several organs as well as immune cells represent targets of hantavirus infection. Identification of target cells and the characterization of replication steps and its effects on cellular function play an important role in the understanding of pathogenesis of hantavirus infection. Entry and spread of pathogens are major determinants in pathogenicity. The abundance and use of receptors determine the susceptibility of target cells. The use of certain receptors may also activate signaling pathways and thereby contributing to cellular dysfunction. The localization of receptors at the basolateral face of polarized cells may require the crossing of the monolayer and is often associated with the disruption of epithelial and endothelial barriers. In vitro analysis of susceptibility and permissiveness identified different cell types as target cells for hantaviruses. Human cell lines of kidney, lung, liver, primary renal cells and peripheral blood monocytes/macrophages were permissive for hantavirus infection (Guhl et al., 2010; Krautkrämer et al., 2011; Raftery et al., 2002; Temonen et al., 1993). Viral antigen is detectable in epithelial cells of different organs of patients with hantavirus infection (Groen et al., 1996; Hautala et al., 2002; Hung et al., 1992; Kim et al., 1993). Endothelial and epithelial cells represent highly specialized cells and differ enormously between different organs. The determinants of organ manifestation of hantavirus disease are not completely understood. The organ manifestation of different pathogenic hantaviruses is not restricted to a certain organ as demonstrated by cases of PUUV infection with cardiopulmonary involvement or central nervous system complications (Gizzi et al., 2013; Hautala et al., 2002, 2010; Kanerva et al., 1996; Rasmuson et al., 2011, 2013).

Differences in the receptor usage do not seem to be responsible for the variation in organ tropism. Pathogenic hantaviruses causing HFRS or HCPS enter cells via integrin $\alpha_v\beta_3$ and non-pathogenic

* Corresponding author at: Department of Nephrology, University of Heidelberg, Im Neuenheimer Feld 162, 69120 Heidelberg, Germany. Tel.: +49 6221 91120; fax: +49 6221 9112209.
*E-mail addresses:* ellen.krautkraemer@med.uni-heidelberg.de (E. Krautkrämer), martin.zeier@med.uni-heidelberg.de (M. Zeier).

0168-1702/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.virusres.2013.12.043

viruses use integrin $\beta_1$ (Buranda et al., 2010; Gavrilovskaya et al., 1998, 1999; Klempa et al., 2012; Krautkrämer and Zeier, 2008). In addition, the entry of hantaviruses is mediated by CD55 (Krautkrämer and Zeier, 2008). gC1qR/p32 was also described to mediate hantavirus entry (Choi et al., 2008). CD55 and gC1qR/p32 are components of the complement system. The glycosyl-phosphatidyl inositol (GPI)-anchored protein CD55 (also called decay accelerating factor, DAF) is a negative regulator of the complement system and protects tissue from injury mediated by complement activation (Quigg et al., 1989). The glycoprotein gC1qR/p32 is a complement binding protein, which interacts with the subunit C1q of the classical complement pathway. In addition, gC1qR/p32 binds several other proinflammatory proteins and bacterial and viral antigens (Peerschke and Ghebrehiwet, 2007; Yin et al., 2007). Whether the entry via these receptors interferes with their function within the complement cascade and contributes to hantaviral pathogenesis is so far not elucidated.

Pathogenic hantaviruses differ enormously in severity and case fatality rate. Patient-specific genetic factors were identified to be associated with severe courses. However, differences between infections with PUUV, Dobrava-Belgrade (DOBV) and Hantaan virus (HTNV) may not be explained by genetic diversity in the infected population. The search for hantaviral pathogenicity factor(s) was so far not successful. The virulence of viruses is often determined by replication kinetics and is influenced by entry strategies or the modulation of the host cell machinery. Sequence variations of receptor proteins or structural and accessory proteins may influence viral fitness. Several interactions and effects of hantaviral proteins in the host cell have been identified. The L protein of ANDV suppresses mRNA and protein expression in the infected cell. The hantaviral N protein binds and interacts with several factors of the infected cell, such as importin α, mRNA cap, ribosomal protein S19 and influences cellular processes (Cheng and Mir, 2012; Haque and Mir, 2010; Heinemann et al., 2013; Mir et al., 2010; Ronnberg et al., 2012; Taylor et al., 2009). For HCPS-causing hantaviruses a functional immunoreceptor tyrosine-based activation motif (ITAM) was identified in the glycoprotein Gn that may interfere with the signaling in the infected cell (Alff et al., 2006). This motif is conserved in New World hantaviruses, but is absent from Gn sequences of HFRS-causing hantaviruses. The identification of differences in amino acid sequences and motifs may help to recognize determinants of hantaviral pathogenesis. The characterization of viruses of the genetic lineage of DOBV and related viruses may help in establishing the association between clinical characteristics and molecular determinants. DOBV is characterized by a complex epidemiology and may be subdivided in four related genotypes that cause hantavirus disease of different severity (Klempa et al., 2013). Genotypes Dobrava, Kurkino and Sochi are associated with mild to severe clinical courses and case fatality rates between 0.3 and 12%. In contrast, no fatal outcome has been observed for Saaremaa virus (SAAV). Infections with SAAV seem to be mainly clinical inapparent. The classification of DOBV in four genotypes represents an alternative classification that does not correspond to the current taxonomy which classifies SAAV as own virus species (Plyusnin et al., 2006; Sjolander et al., 2002). The discussion reflects the difficulties to categorize and characterize such a heterogeneous group of viruses that differ in molecular determinants, pathogenicity, symptoms, geographical distribution and host reservoir species. Nevertheless, molecular analysis of the Dobrava-Belgrade virus and its related virus species together with their replication cycles may provide useful insights in the mechanism of hantavirus pathogenesis.

The identification of novel hantavirus genotypes (e.g. Sangassou virus, SANGV; Maporal virus, MPRLV) opens new questions regarding their receptor usage and pathogenicity. SANGV uses integrin $\beta_1$ for entry while MPRLV uses integrin $\beta_3$ suggesting that they represent a non-pathogenic and a pathogenic virus, respectively (Table 1). Epidemiological and clinical studies in the endemic areas will clarify if the receptor usage applies to the pathogenicity of the respective novel hantavirus species (Buys et al., 2011; Klempa et al., 2013). Whether the use of integrin $\beta_3$ as entry receptor leads to the activation of signaling cascades and accounts solely for disease in human remains to be investigated. Other, not yet identified, differences which could influence replication kinetics and immune response may also contribute to pathogenesis and may be responsible for the broad range of severity within hantavirus infections.

### *1.2. Infection of polarized cells and disruption of cell-to-cell contacts*

The infection of polarized cells requires complex entry mechanisms to overcome the barrier of cell-to-cell contacts. PUUV and HTNV enter and release target cells via the apical surface. Integrin $\alpha_v\beta_3$ is localized at the basolateral site of polarized cells, whereas the GPI-anchored protein CD55 is exposed at the apical face of the monolayer. The interplay interaction of both receptors in the entry process of hantavirus infection is not completely understood. The receptor use may also play a role in the pathogenesis of the infection. It was shown that pathogenic hantaviruses block migration of endothelial cells on the integrin $\beta_3$ ligand vitronectin (Gavrilovskaya et al., 2002). Functional effects on gC1qR/p32 and CD55 signaling in hantavirus infected cells were not shown so far. Both proteins are part of the complement system and may be associated with the clinical picture.

The localization of receptors and their density on the surface influence the susceptibility of target cells. The conformation of integrin $\alpha_v\beta_3$ plays also a role in the infection. Hantaviruses bind the inactive bent conformation of integrin $\alpha_v\beta_3$ (Raymond et al., 2005). The susceptibility of cells depends on the cellular environment and activation state. To understand the replication cycle and its effects, it is important to analyze the infection in relevant cell culture models of organs that were affected in the infection.

ANDV infects airway epithelial cells that express integrin $\beta_3$ and entry and release occurs at the apical and basolateral membrane (Rowe and Pekosz, 2006). It is hypothesized that the infection of pulmonary cells may be responsible for the person-to-person transmission observed exclusively for ANDV (Martinez et al., 2005; Rowe and Pekosz, 2006). In contrast, HTNV and PUUV infect and release cells via the apical side (Krautkrämer et al., 2012; Krautkrämer and Zeier, 2008). Both Old and New World viruses are transmitted via inhalation. It would be of interest if differences between HFRS- and HCPS-causing viruses exist in the ability to infect epithelial cells of the airway. Susceptibility of cells and mode of entry and release may contribute to the clinical picture. The infection with PUUV and HTNV is often characterized by acute renal failure. The often massive proteinuria is non-selective indicating a possible involvement of the glomerular and tubular apparatus (Ala-Houhala et al., 2002; Cosgriff and Lewis, 1991). Histological analysis of kidney tissue by light microscopy reveals tubulointerstitial nephritis but demonstrates no obvious cellular damage in the glomeruli. Glomeruli consist of highly specialized and differentiated cell types. Three layers are responsible for the filtration capacity of the kidney: glomerular endothelial cells, basal membrane, and podocytes are necessary for renal function. Barrier function of endothelial and epithelial cells depends on the integrity of monolayers and their cell-to-cell contacts. The disruption of cell-to-cell contacts leads to increased permeability. Damage to cell-to-cell contacts is often the consequence of infection and is directly caused by the pathogen to facilitate entry and spread and/or indirectly via cytokines.

Our in vitro studies revealed that PUUV and HTNV infect tubular epithelial, glomerular endothelial cells and podocytes. The infection leads to the redistribution and decrease of tight

Please cite this article in press as: Krautkrämer, E., Zeier, M., Old World hantaviruses: Aspects of pathogenesis and clinical course of acute renal failure. Virus Res. (2014), http://dx.doi.org/10.1016/j.virusres.2013.12.043