Uncovering the mysteries of hantavirus infections

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Abstract | Hantaviruses are negative-sense single-stranded RNA viruses that infect many species of rodents, shrews, moles and bats. Infection in these reservoir hosts is almost asymptomatic, but some rodent-borne hantaviruses also infect humans, causing either haemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome (HCPS). In this Review, we discuss the basic molecular properties and cell biology of hantaviruses and offer an overview of virus-induced pathology, in particular vascular leakage and immunopathology.

Thrombocytopenia
A low platelet count in the blood.

Cytotoxic CD8$^+$ T cells
A subpopulation of T cells that kills abnormal (infected, cancerous or damaged) cells as part of the adaptive immune system.

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Hantaviruses are enveloped RNA viruses belonging to the family *Bunyaviridae*. The International Committee on Taxonomy of Viruses, 2012 Release, has officially approved 24 hantavirus species divided into three phylogenetic clusters (BOX 1). The geographical distributions of these species reflect those of their reservoir hosts, which are primarily rodents, shrews, moles and bats, and similarly, outbreaks of this virus are associated with the population dynamics of the carrier rodents[1–4].

Although hantaviruses do not cause visible disease in rodents, some can be transmitted via aerosols of rodent excreta to humans, in which they can cause two diseases: haemorrhagic fever with renal syndrome (HFRS), which is primarily caused by Hantaan virus (HTNV) and related viruses in Asia, Puumala virus (PUUV) and Dobrava virus (DOBV) in Europe, and Seoul virus (SEOV) worldwide; or hantavirus cardiopulmonary syndrome (HCPS), which is caused by Sin Nombre virus (SNV) and related viruses in North America, and Andes virus (ANDV) and related viruses in Latin America[1–4]. These diseases are characterized by increased capillary permeability (causing vascular leakage) and thrombocytopenia. These pathologies are thought to be caused by viral infection of endothelial cells, which does not disrupt the endothelium but nonetheless leads to dramatic changes in both the barrier function of the endothelium as a whole and the function of infected endothelial cells. It has also been suggested that cytotoxic CD8$^+$ T cells (CTLs) trigger capillary leakage and that cytokines contribute to the increased capillary permeability. The terminal soluble complement complex can also increase vascular permeability, and complement activation is linked to severity of hantavirus infection[1–4].

The recent outbreak of HCPS in Yosemite National Park in California, USA, had a high case-fatality rate (three deaths among ten infected individuals)[5], which has emphasized the importance of furthering our understanding of hantavirus biology and the infections that these viruses cause in humans. In this Review, we discuss recent progress in our understanding of the molecular and cell biology of hantaviruses and then give an overview of the pathophysiology that these viruses cause, with an emphasis on the effects of viral interactions with host cells and with the immune system.

Molecular and cell biology of hantaviruses

The viral genome and virion structure. Characteristically, as members of the family *Bunyaviridae*, hantaviruses are negative-sense single-stranded RNA viruses with a trisegmented genome[6,7]. The viral RNA (vRNA) of each segment contains an ORF flanked by non-coding regions (NCRs) at the 3′ and 5′ ends of each segment. The ORFs of the genome segments, which are called small, medium and large, respectively encode nucleocapsid (N) protein, glycoprotein precursor (GPC; which eventually matures into the glycoproteins Gn and Gc (previously known as G1 and G2, respectively)) and RNA-dependent RNA polymerase (RdRp) (FIG. 1). The very termini of the NCRs contain complementary nucleotides that are predicted to form a panhandle structure, which functions as the viral promoter and is crucial for transcription and replication[8,9]. In addition to encoding N protein, the small genome segment of the hantaviruses carried by rodents of the family Cricetidae contains an overlapping reading frame that encodes the non-structural protein NSs, which can function as a weak interferon (IFN)

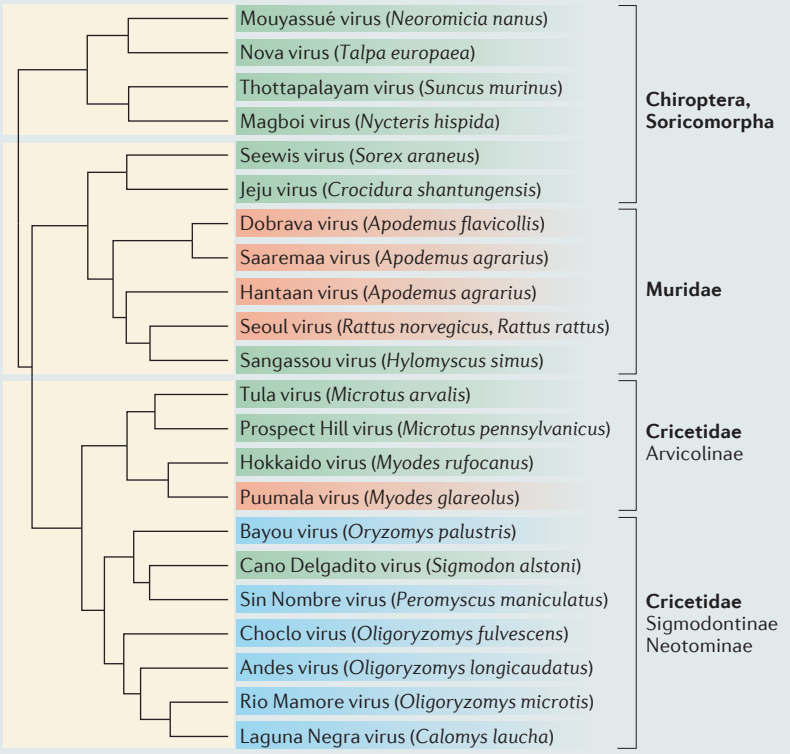
Box 1 | **Hantavirus phylogeny**

Human infections with hantaviruses result from exposure to aerosolized rodent excreta containing pathogenic virus in a suitable environment for transmission, and the exposure risk is usually determined by the population dynamics — primarily, by outbreaks in reservoir species. The pathogenicity of different hantavirus species is associated with the reservoir host taxonomy, and phylogenetically, there is evidence that hantaviruses might have co-evolved with their hosts[119], although increasing numbers of exceptions and host switches have been reported recently[120,121].

Hantaviruses form three major clusters in phylogenetic trees (see the figure; hantaviruses that have not been shown to cause disease are shown in green, haemorrhagic fever with renal syndrome (HFRS)-causing viruses are shown in red, and hantavirus cardiopulmonary syndrome (HCPS)-causing viruses are shown in blue; Tula virus has been associated with a single patient with HFRS[122], who developed neutralizing antibodies against this virus; reservoir host species are given in brackets, and host orders or families are given in bold, with subfamilies beneath). The most ancestral hantaviruses found in soricomorphs and bats form cluster 1; the first ever discovered hantavirus, Thottapalayam virus, belongs to this cluster. The pathogenicity of these soricomorph- or bat-borne hantaviruses is unknown.

The major disease burden in the Old World, HFRS, is caused by cluster 2 hantaviruses borne by Old World mice and rats (family Muridae): Hantaan virus (HTNV) and related viruses in Asia, Dobrava virus in Europe, and Seoul virus (SEOV) worldwide. Of these, HTNV has been the focus of research as the prototype virus and that causing the major disease burden. Research on SEOV (carried primarily by Norway rats) has yielded significant findings on the virus–host interactions, especially concerning the immunological response of the host to infection. Interestingly, some of the soricomorph-borne hantaviruses are phylogenically related to pathogenic hantaviruses in rodents of the family Muridae, suggesting that these viruses have switched hosts from rodents to shrews[123].

Phylogenetic cluster 3 consists of viruses borne by rodents of the family Cricetidae. The viruses that are carried by rodents of the subfamilies Sigmodontinae and Neotominae in the New World include the causative agents of HCPS: Sin Nombre virus and related viruses in North America, and Andes virus (ANDV) and related viruses in Latin America. Both of these groups have been studied intensively, and the only widely used animal model of hantavirus disease is the ANDV infection of Syrian hamsters. The viruses carried by voles and lemmings (in the subfamily Arvicolinae) in the Holarctic are mainly non-pathogenic in humans, except for Puumala virus (PUUV), which causes mild HFRS (called nephropathia epidemica) in Europe, including the European part of Russia. PUUV is one the best studied hantaviruses, and research on this virus has revealed many aspects of both virus–cell and virus–host interactions[1–3].



inhibitor[10] but is likely to have other functions[11,12]. Non-structural proteins encoded by the small segment are common in viruses of the *Bunyaviridae* family and are typically associated with IFN antagonism[13].

The hantavirus genome segments, each encapsidated by N protein, are stored within the virus particle (FIG. 1), which is round or pleiomorphic (that is, the size and shape varies) and has a size range of 120–160 nm in diameter[14]. The virion comprises a lipid envelope that is 5 nm thick and covered with spikes which protrude approximately 10 nm from the membrane[15]. Four Gn and four Gc units form each spike, which has four-fold symmetry (that is, when viewed from above at 90° rotations, the appearance of the spike is identical)[14,15]. Such symmetry of the spike complex is considered unique, at least among enveloped viruses. The virions consist of >50% protein, 20–30% lipid, 7% carbohydrate and 2% RNA[6,7], and are unexpectedly stable, as they can survive for more than 10 days at room temperature and more than 18 days at +4 °C and at −20 °C[16]. This feature is necessary for hantavirus transmission, which (unlike transmission of other bunyaviruses), does not involve an arthropod vector.

Virus entry. In patients, hantaviruses replicate primarily in the endothelium[17], and endothelial cell cultures are therefore used as *in vitro* models for hantavirus infection. Several host cell surface proteins have been suggested to mediate the entry of hantaviruses into cells[8]. Currently, evidence strongly suggests that integrins are the main receptors for hantaviruses, at least *in vitro*[17], although there has been little evidence in support of this role *in vivo*, and it is possible that the natural hantavirus receptor is not an integrin. Integrins are a family of heterodimeric transmembrane proteins (comprising an α-chain and a β-chain) that promote cell–cell adhesion as well as adhesion of cells to the extracellular matrix[18]. Notably, *in vitro* work has indicated that pathogenic and non-pathogenic hantaviruses use different integrins for entry (αVβ3 being the receptor for pathogenic viruses, and α5β1 for non-pathogenic viruses)[19,20]. Complement decay-accelerating factor (DAF), a glycosylphosphatidylinositol (GPI)-anchored protein of the complement system[21], and GC1QR (globular heads of complement C1q receptor; also known as C1QBP) can also mediate hantavirus infection in cultured cells[22].

After binding to a cell surface receptor, the invading hantavirus is taken up by the cell (FIG. 2). The entry of HTNV (an Old World hantavirus) proceeds via clathrin-dependent endocytosis[23], during which an invagination known as a clathrin-coated pit forms around the receptor-bound virion. This leads to uptake of the virus in a clathrin-coated vesicle (CCV) that is formed of cellular membrane covered with clathrin. By contrast, ANDV (a New World hantavirus) does not use CCVs during entry[24], and increasing evidence suggests that hantaviruses, similarly to other bunyaviruses, in fact use more than one pathway for cellular entry[25]. Alternative entry pathways might include macropinocytosis, clathrin-independent receptor-mediated endocytosis, caveolae- or cholesterol-dependent endocytosis, or other, currently

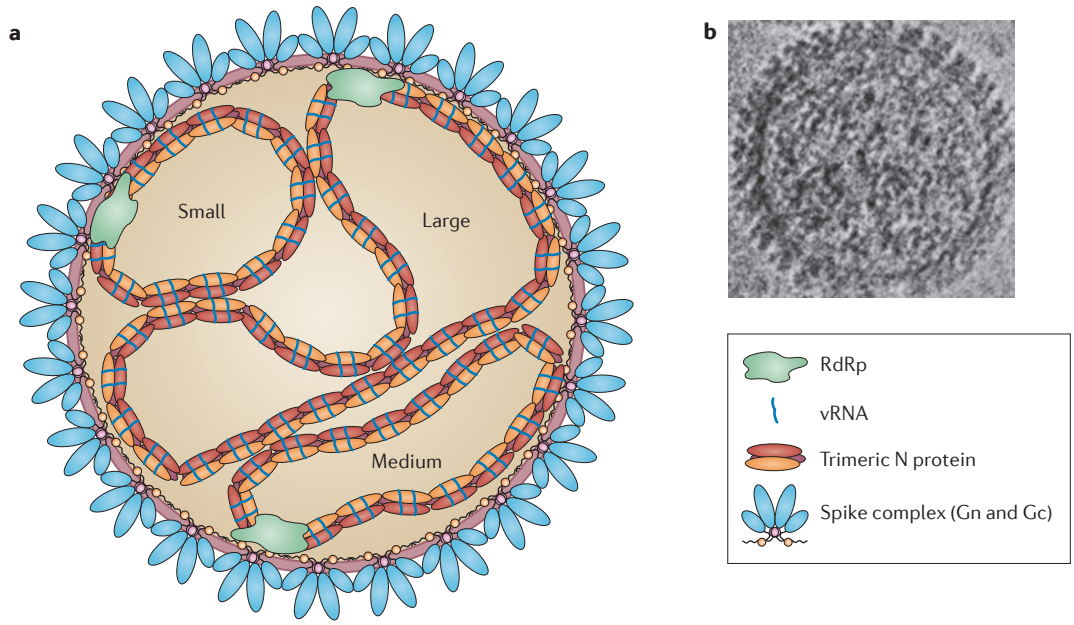


Figure 1 | **Hantavirus particles, genes and proteins.** **a** | Schematic representation of the hantavirus virion. The hantavirus particle contains the trisegmented viral RNA (vRNA) genome, comprising the small, medium and large ORFs. These are encapsidated by nucleocapsid (N) protein. The outer part of the virion consists of spikes comprising four units of each glycoprotein, Gn and Gc. The viral genome is replicated and transcribed by RNA-dependent RNA polymerase (RdRp). **b** | A hantavirus particle viewed by cryoelectron microscopy. The spike height is invariably 12 nm, and the median diameter of the virion is 135 nm. Part **b** image is courtesy of P. Laurinmäki and S. Butcher, University of Helsinki, Finland.

unknown routes. After internalization, the virions are trafficked to early endosomes and possibly to late endosomes, where they detach from the cellular receptor owing to a decrease in pH (early endosomes are about pH 6.0–6.5, and late endosomes about pH 5.0–6.0). This low pH of endosomes triggers a change in the conformation of the Gc glycoprotein that allows binding of the Gc fusion loop to the endosomal membrane, leading to further conformational changes and eventual fusion of viral and cellular membranes. The genetic material released into the cytoplasm is then presumably transported to the putative site of viral replication via interactions with the cellular transport machinery[26]. It is also possible that the initial rounds of transcription and replication take place directly after fusion with the endosomal membrane.

Transcription and replication. The synthesis of viral RNAs from the hantavirus genome (FIGS 2,3a) involves transcription (to produce mRNAs encoding the viral proteins) and replication (to produce viral genomic RNA). Both of these activities are attributed to the viral RdRp[8]. Characterization of the RNA synthesis mechanisms in hantaviruses has been hindered by the lack of suitable reverse genetics systems, although some attempts have been reported[27]. A unifying theme in the transcription of negative-sense segmented RNA viruses (first described for influenza virus[28]) is a process called cap snatching for the initiation of viral mRNA transcription. In the case of hantaviruses, it has been suggested that this involves the localization of viral proteins (N protein and RdRp) to cytoplasmic processing bodies (P bodies), where they can bind the caps of host mRNAs that are destined for degradation[29]. One possibility is that the N- or RdRp-bound mRNA primers are then transported to the putative site of viral replication, the ER–Golgi intermediate compartment (ERGIC). Another option is that P bodies are the site of viral RNA synthesis, and assembled viral ribonucleoproteins (RNPs) are transported to the site of viral assembly. This transport could be mediated by the known interactions between N protein and host cell actin or microtubules[24,26]. It has been suggested that the host mRNAs are processed for viral use at the replication site by the endonuclease activity of RdRp[30,31], thus producing capped primers of 10–15 bases for the transcription of viral mRNAs (see below). An alternative possibility is that cellular endonucleases (residing in the P bodies) generate the capped primers from host mRNAs[29]. The host cell-derived primers have a G residue in their 3′ end that pairs with the first C of the AUC repeats at the vRNA terminus (FIG. 3b). After successive addition of bases, the nascent RNA slips back a few bases and realigns with the complementary nucleotides at the 3′ end of the vRNA. Repetition of this 'prime-and-realign' cycle finally produces the repeated terminal sequences of the vRNA in the newly formed mRNA[32].

In the case of replication, the vRNA first needs to be transcribed into complementary RNA (cRNA), which is then used as the template for the multiplication of vRNA. The cRNA differs from viral mRNA in at least two ways: first, its synthesis is thought to start *de novo* without the need for capped primers; and second, the cRNA is encapsidated by N protein, similarly to

Complement system
Serum proteins that can protect against infection as part of innate immunity. This efficiently regulated system has three major pathways, namely the classical, alternative and the lectin-dependent pathways.

Clathrin-dependent endocytosis
A pathway for the internalization of plasma membrane proteins. Receptors cluster in membrane domains containing a polymeric clathrin coat and a complex of adaptor proteins and GTPases, leading to membrane invagination and scission to form a clathrin-coated vesicle containing the internalized receptors.

Early endosomes
Intracellular vesicular structures that are precursors of mature endosomes and have an important role in endocytosis.

P bodies
Cytoplasmic foci that are thought to store and degrade translationally repressed RNA.

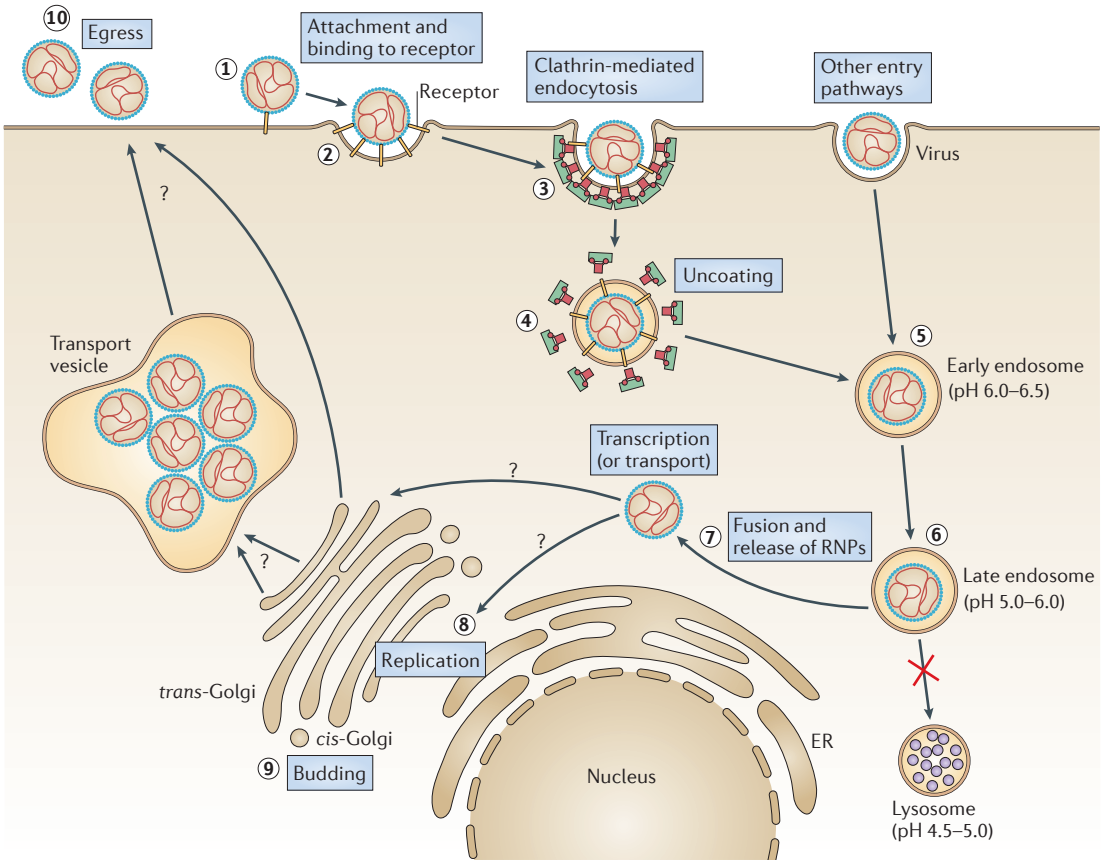


Figure 2 |**The life cycle and replication of hantaviruses.** The hantavirus virion attaches to a receptor on the cell surface (step 1). This binding event induces endocytosis signalling (step 2), after which the virion enters the cell in clathrin-coated vesicles (step 3). Other entry pathways have also been observed for some hantaviruses. In the case of clathrin-mediated endocytosis, the clathrin coat of the vesicle is disassembled (step 4), and the virion-harbouring vesicle enters the early endosome (step 5), which matures into a late endosome (step 6). Fusion between the viral and endosomal membranes is driven by acid-induced conformation changes in the viral fusion protein in the late endosome. This results in release of the viral ribonucleoproteins (RNPs) (step7). Initial transcription might take place at the site of release; alternatively, the RNPs might be transported to the ER–Golgi intermediate compartment (ERGIC) for transcription. It is also possible that the virus is directly transported to the Golgi complex from the late endosome, either before or after fusion. Viral replication is thought to occur in viral factories that might be located at the ERGIC or the *cis*-Golgi (step 8). The nascent viruses are thought to bud into the *cis*-Golgi (step 9), from where they are transported to the plasma membrane for release, presumably via recycling endosomes. The egress of progeny virions takes place at the plasma membrane (step 10).

vRNA[8]. cRNA synthesis also proceeds via a prime-and-realign mechanism, in this case through initial binding of a triphosphorylated G (pppG) to a C residue in the vRNA; the pppG is then cleaved by RdRp to produce the monophosporylated 5′ terminus of cRNA[32]. The same mechanism is used to multiply vRNA using cRNA as a template. In contrast to the proposed *de novo* initiation of replication, it is also possible that the cap-containing primer used for mRNA transcription is instead cleaved by RdRp to produce the 5′ terminus of the cRNA. During the course of vRNA synthesis, there might be a switch from transcription to replication, which might be facilitated by the increased expression of N protein to be used for the encapsidation of cRNA and vRNA.

It is currently not entirely clear where these processes take place. Another member of the *Bunyaviridae* family, Bunyamwera virus (BUNV; the prototype orthobunyavirus), exploits the membranes of the Golgi complex for viral replication and budding by establishing a viral factory around the Golgi or the ERGIC[33]. The viral factory, which is stabilized by the host cell cytoskeleton, is formed of ER and Golgi membranes, which together provide all the components required for transcription, replication and production of viral proteins[33]. N protein and RdRp of hantaviruses are peripheral membrane proteins that associate with the membranes of the Golgi complex during infection and when expressed individually[34,35]. Thus, even though it has not been experimentally demonstrated, the replication of hantaviruses might occur similarly to that of BUNV.

Assembly. When the viral genome has been replicated, it is encapsidated by N protein[8,14]. The encapsidation of vRNA and cRNA is thought to begin by the formation of N protein trimers via interactions between its

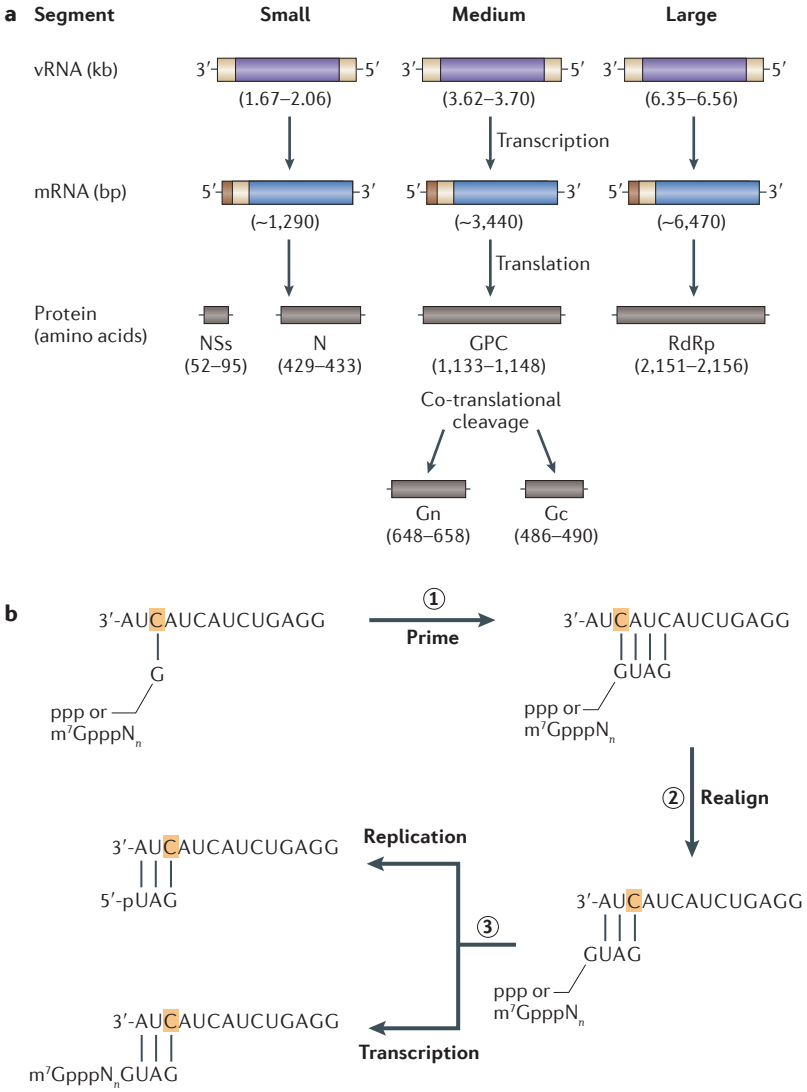


Figure 3 | **Viral genome replication and transcription.** **a** | To produce the viral proteins, viral RNA (vRNA) (in which beige regions represent the non-coding regions) first needs to be transcribed to viral mRNA (in which the brown region represents the host-derived 5′-cap plus 10–20 bases from a host mRNA). The mRNA transcribed from the small ORF of the viral genome gives rise to nucleocapsid (N) protein, and for some hantaviruses a non-structural protein, NSs, is also derived from an overlapping reading frame. The medium ORF gives rise to glycoprotein precursor (GPC), which is further cleaved to the mature glycoproteins Gn and Gc. The large ORF gives rise to RNA-dependent RNA polymerase (RdRp). **b** | The prime-and-realign mechanism as the initiation mechanism for hantavirus RNA synthesis. Either a guanosine triphosphate (pppG), in the case of replication, or a host-derived capped primer (m^7GpppN_n) with a guanine at the 3′ end, in the case of transcription, hybridizes to a cytosine in the 3′ terminus of the vRNA (step 1); the consensus sequence for the hantavirus vRNA 3′ end is shown. After successive addition of bases, the nascent RNA slips back a few bases and realigns backwards by virtue of the terminal sequence repeats (step 2), and elongation can proceed (step 3).

amino- and carboxy-terminal residues[36]. The NCRs in the vRNA are complementary to each other and form a panhandle structure that is unique for each hantavirus genus. Although the actual encapsidation signal in hantaviruses is unknown, it has been suggested that the trimeric N protein complex specifically recognizes the panhandle structure of each hantavirus genus[9]. Binding of N protein to the vRNA has been proposed to alter its conformation, allowing the binding of further N proteins and eventually forming an RNP complex containing a single vRNA segment[9]. In BUNV RNPs, the vRNA bases are sequestered away from solvent by N protein[37], so it has been hypothesized that the termini of bunyavirus vRNA cannot be encapsidated by N protein, as this would not allow panhandle formation or binding of RdRp. This is contradictory to the aforementioned mechanism of RNP assembly for hantaviruses, whereby N protein binds the panhandle with high affinity. However, hantaviruses differ from most bunyaviruses in that their N protein is larger and forms trimers rather than tetramers (as in the case of BUNV[37]) or hexamers (as in the case of another bunyavirus, Rift Valley fever virus[38]). Therefore, it is possible that the mechanism of RNP assembly varies among bunyaviruses.

The medium genomic segment of hantaviruses encodes a glycoprotein precursor, GPC, a polypeptide that is co-translationally cleaved by the cellular signal peptidase complex[8] (FIG. 3a). Specifically, GPC contains two signal sequences, one at the very 5′ end of the mRNA, which targets the transcript to the ER for translation, and the other in the precursor protein itself, between glycoproteins Gn and Gc, which mediates cleavage of the nascent GPC into N- and C-terminal portions that mature together to yield the two glycoproteins[8,14]. It is not known how the glycoproteins form the spike complex, but it is evident that both proteins are required for translocation to the Golgi[8]. It has been suggested that Gn initially forms tetramers in the ER and that interaction with Gc alters the Gn conformation, revealing a Golgi-targeting signal[14]. When the spike complex, consisting of four Gn and four Gc molecules[39], is assembled and transported to the Golgi, the cytoplasmic tails of Gn and Gc interact with the newly formed RNPs through protein–protein (N) and protein–RNA (vRNA) interactions[40,41], possibly through the formation of a curved lattice on the Golgi membrane[15]. It is still unclear how RdRp is packaged into progeny virions. The polymerase is thought to be associated with the RNPs and could be packaged together with this complex; however, no direct interaction between RdRp and the RNPs has been demonstrated for hantaviruses. Alternatively, RdRp might bind to Gn cytoplasmic tails independently of the RNPs, like in Rift Valley fever virus[42]. It is currently unclear how the virion ensures that three different vRNA-containing RNPs are packed inside each virion. It is possible that the Gn cytoplasmic tail, which is known to have RNA-binding activity[41], specifically recognizes vRNA from cRNA (for instance, through its zinc-finger domain) and facilitates the specific packaging of vRNA (as opposed to cRNA) into RNPs[43]. In addition, interactions between the different vRNA segments, as shown for influenza viruses[44], could promote the specific assembly of vRNA into progeny virions. This mechanism has also been suggested for Rift Valley fever virus[45].

The virion buds inside the Golgi, and the newly formed virion is released to the extracellular milieu from the Golgi, probably via exocytosis[46], but the details of virion egress are still largely unknown.

Hantavirus infections in reservoir hosts

Rodents, shrews, moles and bats act as reservoir hosts for hantaviruses, and their infection is chronic and nearly asymptomatic[47,48]. However, hantavirus infection has been shown to impair the survival of some reservoir animals in the wild[49,50]. Each hantavirus is associated characteristically with one reservoir species, and spillover to other rodent species seems to result in antibody production and clearance of the virus[51]. The prevalence of hantavirus-infected animals can vary greatly, from 0% to 100% in host populations, and this may depend on the season and population age structure (older animals are more often infected than younger animals, because older animals have had more chance, in terms of time, for exposure to the virus)[52]. Viral replication persists in infected host animals, which excrete the virus in urine, faeces and saliva, and the earliest reported detection of shedding is day 5 after infection[53]. Shedding can continue for 50–60 days in laboratory experiments[54,55] and, at least in bank voles, possibly for up to 8 months in the wild[56].

An intriguing question is why infection of reservoir hosts is nearly asymptomatic, and some clues have come from the analysis of their immune responses following hantavirus infection. Unlike humans (who show high levels of inflammation following infection; see below), reservoir animals mount an immune response characterized by high titres of neutralizing antibodies and suppression of innate immunity owing to the upregulation of transforming growth factor β1 (TGFβ1)-producing regulatory T cells (T_{Reg} cells)[57]. This anti-inflammatory phenotype is associated with the persistence of hantaviruses in their reservoir hosts, as shown for SEOV in rats[58] and SNV in deer mice[59]. Moreover, CTLs are absent in infected rodents, possibly owing to immunosuppression. Immunosuppression is evident in the lungs, which are the main site of viral replication in the host; by contrast, inflammation has been observed in the spleen[57]. Notably, the levels of basal pro-inflammatory tumour necrosis factor (TNF) production correlate inversely with PUUV prevalence in voles originating from different parts of Europe, with low TNF production in voles in Finland (where the virus is endemic), but high levels of TNF in animals from Central Europe (where the virus is less endemic)[60]. This corroborates the idea that the absence of an overt pro-inflammatory response in reservoir hosts has a major role in hantavirus persistence.

The differences between the immune responses mounted by humans and reservoir hosts in response to hantavirus infection can also be seen in cell culture models. Specifically, SEOV (a rat hantavirus) can suppress nuclear factor-κB (NF-κB)-mediated pro-inflammatory responses in rat antigen-presenting cells[61] and can enhance the regulatory phenotype of T cells in rat lung endothelial cell co-cultures[62]. Hantaviruses trigger the activation of an innate immune response in human endothelial cells, and this response is characterized by the upregulation of IFNβ and subsequent activation of IFN-inducible genes (for example, *MX1* and retinoic acid-inducible gene I (*RIG-I*))[63]; however, the response is not triggered in the cells of reservoir hosts[57]. This indicates that pattern recognition receptors of reservoir host cells either do not detect the vRNAs or are inhibited by an unknown mechanism. One possibility is inhibition of IFNβ production[10] by the non-structural protein NSs, which is expressed by hantaviruses of rodents in the family Cricetidae; however, this cannot be the common molecular mechanism of hantavirus persistence, as other hantaviruses (those that infect rodents of the family Muridae), such as SEOV, do not encode this protein. The cytoplasmic tail of Gn was shown to inhibit IFNβ production[63], but this study was carried out using human cells, so whether such inhibition also occurs in reservoir host cells is not known.

Pathology of human infection

Humans are mostly infected via inhaled aerosols of rodent excreta. However, person-to-person transmission of the South American ANDV has been reported[64]. In addition, transmission of PUUV via transfusion of platelets or other blood products has been documented[65]. Risk factors for catching hantavirus infections include forestry work, farming, camping, smoking and burning wood for heating[1,2,66,67].

The clinical picture of infection. Both HFRS and HCPS are acute infections affecting the renal, cardiac, pulmonary, central nervous and hormonal systems. Moreover, both diseases have significant acute-phase complications and long-term consequences. However, chronic hantavirus-mediated diseases have never been reported[1,2,68–71]. The symptoms of the diseases are characterized by the direct effects of the virus on the endothelium as well as by immunopathology caused by activation of the innate and adaptive immune systems in response to the virus.

The course of HFRS is highly variable, ranging from frequently asymptomatic to a lethal outcome. Host genetic factors influence the clinical outcome (BOX 2). The most common symptoms are high fever, headache, abdominal pains, backache and nausea or vomiting[1,2,4,68]. Proteinuria, haematuria and acute kidney injury are signs of renal involvement[1,2,4,68]. Moreover, the average disease severity depends on the hantavirus genotype; for example, HFRS caused by HTNV is more severe than that resulting from PUUV infection, which generally has a mild clinical course[1,2,4,68]. Classically, HFRS occurs in five distinct phases: febrile, hypotensive, oliguric, polyuric and convalescent[2].

HCPS is a severe acute disease that is often associated with a rapid onset of respiratory failure owing to pulmonary oedema and cardiogenic shock[72]. Myalgia, cough and diarrhoea are more commonly present in HCPS than in HFRS[1,2,4,72–75]. Overt haemorrhagic or renal symptoms are not common in the disease caused by SNV in North America, but they are often reported in HCPS in South America[75]. In the light of recent studies, HFRS and HCPS seem to be the same disease, which we propose should be called hantavirus disease[76]. The case fatality rate for HFRS is 0.08–0.4% for disease caused by PUUV[2], ~5% for HTNV-mediated disease[1] and up to 10% in DOBV-mediated disease[77], whereas for HCPS it is up to 40%[4,72–75].

The extent of viraemia varies between HFRS and HCPS and depends largely on the hantavirus type. In

Regulatory T cells
A subpopulation of T cells that modulates the immune system. These are $CD4^+CD25^+FOX3P^+$ cells that have suppressor activity towards other T cells either by cell–cell contact or by cytokine release.

Pattern recognition receptors
A highly diverse group of soluble and surface-bound proteins that can detect specific molecular surface structures.

Proteinuria
The presence of excess protein in the urine.

Box 2 | **Impact of host genetic factors**

In rodents

In bank voles (*Myodes glareolus*), polymorphism in the major histocompatibility complex (MHC) class II gene *DQA*[124] and in the tumour necrosis factor gene (*TNF*)[60] is associated with susceptibility to Puumala virus (PUUV) infection.

In humans

The clinical course of hantavirus infections is influenced by host genes. Human leukocyte antigens (HLAs) are major cell-surface antigens, the role of which is to present pathogen-derived antigens to T cells and to initiate adaptive immune responses. In a study in Finland, individuals with HLA alleles *HLA-B8*, *HLA-C4A*Q0* and *HLA-DRB1*0301* were likely to have the most severe form of PUUV infection[125], whereas those with *HLA-B27* were likely to have a benign clinical course[126]. By contrast, in patients with Hantaan virus-induced haemorrhagic fever with renal syndrome (HFRS) in China, *HLA-DRB1*09* and *HLA-B*46-DRB1*09* haplotypes were significantly more frequent than in controls[127]. In the United States, the *HLA-B*3501* allele was associated with increased risk of severe Sin Nombre virus-induced hantavirus cardiopulmonary syndrome (HCPS)[90]. In Slovenia, patients infected with PUUV more frequently had an *HLA-DRB1*13* haplotype than patients infected with Dobrava virus (DOBV)[128]. *HLA-B*07*, in turn, seemed to have a possible protective role in PUUV infection. Furthermore, patients infected with DOBV had a significantly higher frequency of *HLA-B*35* than patients infected with PUUV[128]. Thus, different hantaviruses might be presented differently through the same HLA molecules.

A human genotype that leads to high levels of *TNF* transcription (an allele with a polymorphism at position –308) has been found to be associated with a severe clinical course of PUUV infection in Finnish patients[126]. This polymorphism is part of the susceptibility HLA haplotype in severe PUUV infection, but is a less important risk factor than the *HLA-B8-DR3* haplotype[129]. A severe clinical course has also been associated with a genotype associated with low *TNF* transcription (a polymorphism at position –238) in Belgian patients[130]. In Brazil, an allele resulting in high levels of TNF production (a G-to-A switch at position –308) was more frequently found in patients with HCPS than in individuals with positive serology for hantavirus but without a history of HCPS illness[131].

Additional genotypes that contribute to susceptibility to PUUV infection include non-carriage of the interleukin-1 receptor antagonist (*IL1RA*) allele 2 and *IL1B* allele 2 with a polymorphism at position –511 (REF. 132). Polymorphisms in the genes encoding plasminogen activator inhibitor 1 and platelet membrane glycoprotein 1A (also known as integrin α2) have also been associated with severe PUUV infection[133].

general, vRNA is readily detected or a high viral load is found in severe hantavirus infections (HTNV, DOBV, SNV or ANDV infections), but the level of viraemia is considerably lower in the mild form of HFRS caused by PUUV. An early high viral load might be an indicator for an unfavourable outcome both in HCPS and in HFRS (reviewed REF. 78). In addition, the levels of cell-free DNA (probably from apoptotic or necrotic cells) found in plasma are likely to reflect the amount of cellular damage and to correlate with the severity of infection. For example, in acute PUUV infection, levels of cell-free DNA correlate with leukocytosis, thrombocytopenia and the length of patient hospitalization[79].

Virus-induced pathology. Increased capillary permeability is characteristic of various types of hantavirus infection and is thought to be of fundamental pathophysiological importance. This effect might explain many of the symptoms and features of human hantavirus infections, such as hypotension and abdominal pain, as well as the extravasation of fluid to alveolar spaces and the pulmonary oedema that occur in both HFRS and HCPS[80–82]. Hantaviruses replicate predominantly in endothelial cells of the capillaries of various organs, but this seems to have little, if any, cytopathic effect on the infected endothelium[1,2,17], and alternative explanations for hantavirus pathogenesis have been widely sought after. As mentioned above, integrins have been proposed to act as the cellular receptors for hantaviruses, at least *in vitro*, and it has been suggested that the use of distinct integrins could relate to hantavirus pathology[17].

In vitro studies have shown that hantavirus infection of vascular endothelial cells (FIG. 4) increases monolayer permeability, and that this effect might be exerted through upregulation of the cytokine vascular endothelial growth factor A (VEGFA), which has been shown to downregulate VE-cadherin (a major component of endothelial cell adherens junctions) in infected cells *in vitro*[17,83,84]. It has been proposed that the VEGFA-induced vascular hyperpermeability which is facilitated by hantavirus infection is due to inactivation of β3 integrins by the virus and subsequent deregulation of VEGF receptor 2 (VEGFR2) on the cell surface[17].

One of the hallmarks of hantavirus disease (for both HFRS and HCPS) is thrombocytopenia[2,68,80,81], which might contribute to symptoms involving bleeding, such as petechiae, epistaxis, gastrointestinal bleeding and, in severe cases, fatal intracranial haemorrhage. The exact mechanism of thrombocytopenia is unknown, but the interaction of platelets with the infected endothelium is probably important. It has been shown that endothelial cell cultures infected with pathogenic hantaviruses adhere to the surface of quiescent platelets through the interaction between hantavirus glycoproteins and the platelet integrin αIIβ3 (REF. 85). Thus, the use and regulation of β3 integrins might contribute to the pathogenesis of hantavirus-mediated disease[17].

However, there are other explanations for the cause of thrombocytopenia that might not be directly linked to viral infection. von Willebrand factor, fibrinogen and fibronectin can all act as adhesive ligands for platelet binding when endothelial function is defective. The plasma levels of these proteins are altered in acute PUUV-induced HFRS, a finding which might imply that endothelial cell injury promotes platelet activation[86]. Hantavirus infection also leads to increased thrombin formation and fibrinolysis[87], suggesting that hantavirus-induced thrombocytopenia results from the increased consumption of platelets owing to coagulation.

Hantaviruses can also infect tubular epithelial cells, glomerular endothelial cells and podocytes of human kidneys, and can disrupt cell-to-cell contacts in all of these cell types[88]. This disruption could potentially decrease the barrier function of the kidneys and might therefore be the underlying cause of the renal manifestations observed during HFRS (for example, proteinuria).

Immunopathology. Recognition of vRNA by cell receptors induces the activation of signalling cascades that ultimately lead to the production of type I IFNs. These proteins can then signal the activation of both innate and adaptive immune cells[63]. This is later characterized by the activation of antigen-specific cells such as CTLs, $CD4^+$ T cells and antibody-producing B cells. In SNV- and ANDV-induced HCPS, there is evidence that the presence of high levels of virus-neutralizing antibodies in the

Leukocytosis
A condition in which the number of white blood cells is above the normal range. Frequently associated with inflammation.

Fibrinolysis
A process whereby fibrin clots (the products of blood coagulation) are broken down.

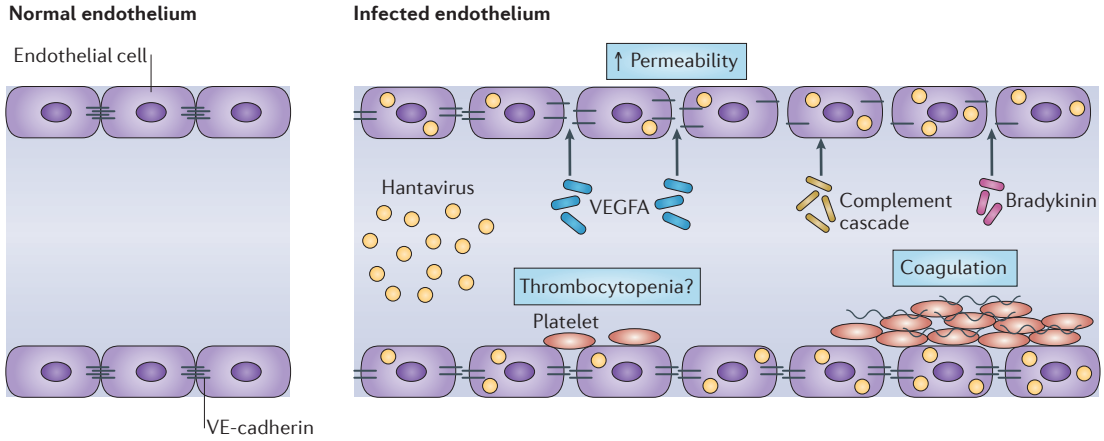


Figure 4 | **Vasculopathy in hantavirus-mediated diseases.** Hantaviruses productively infect endothelial cells of the vasculature. Various hypotheses have been presented to explain the commonly observed thrombocytopenia, vascular permeability and intravascular coagulation in hantavirus-mediated diseases. An infected endothelial monolayer shows increased permeability, possibly owing to upregulation of vascular endothelial growth factor A (VEGFA), which downregulates the adherens junction protein VE-cadherin. *In vitro* studies indicate that platelets interact with infected endothelial cells and hantavirus glycoproteins, and this might be the cause of thrombocytopenia. Other factors might also be involved (not shown). Complement activation correlates with disease severity in haemorrhagic fever with renal syndrome and could induce vascular permeability either directly or by indirect mechanisms. Vascular permeability might also be increased by the secretion of bradykinin, as shown in a cell culture model and by successful treatment of a PUUV-infected patient with a bradykinin receptor antagonist. Hantavirus infection might lead to systemic intravascular coagulation, which may also contribute to thrombocytopenia. The temporal order of the phenomena shown is not known.

acute stage is associated with a favourable outcome[89]. At the onset of HFRS and HCPS, increased amounts of circulating CTLs are observed[90]. CTLs are the predominant cell type in the infiltrate of the kidneys during the acute phase of PUUV infection, as well as in the lungs in lethal HCPS cases[80,81]. Endobronchial mucosal biopsies from patients with PUUV infection have revealed increased numbers of both $CD8^+$ and $CD4^+$ T cells[91]. These findings indicate that there is a local immune response in the lungs. Memory T cells, which develop during the convalescent phase of PUUV-induced HFRS[92], have been shown to persist for several years after infection with PUUV[93] and ANDV[94], and might thus offer long-lasting immunity after hantavirus infection. Hantavirus-specific antibodies persist for life, so re-infections with the virus have not been observed.

Similarly to the effects of many other pathogenic microorganisms, the disease caused by hantaviruses is largely mediated by the efforts of the immune system, both innate and adaptive, to clear the infection. Cytokines are thought to be one of the major causes of the symptoms in hantavirus infection. These molecules are produced by various cells, such as monocytes, macrophages and lymphocytes, in response to pro-inflammatory signals and participate in the regulation of inflammation; cytokines, especially TNF, interleukin-1 (IL-1) and IL-6, have been linked to fever, septic shock and the induction of acute-phase proteins[95]. Increased cytokine levels have been found in the plasma, urine and tissues of patients with hantavirus infections[80,96–99]. High plasma IL-6 levels are associated with severe renal failure and thrombocytopenia in PUUV-induced HFRS and can be used as a marker of disease severity[97,98,100]. Moreover, plasma IL-6 concentrations and urinary IL-6 excretion are markedly increased in patients with acute PUUV-induced HFRS; however, there is no correlation between plasma and urinary IL-6 levels in individual patients[100]. The high urinary levels might reflect local IL-6 production in the kidneys.

Hantaviruses have been shown to induce IFNβ secretion, although the viral species differ in both the extent and duration of this induction, which in turn affects the subsequent magnitude of the innate immune response. For example, according to *in vitro* evidence, the non-pathogenic Prospect Hill virus (PHV) differs from other hantaviruses in that it does not hinder early type I IFN production, which leads to early restriction of PHV replication in immune-competent cells[63]. This suggests that the ability of hantaviruses to inhibit innate immunity actually relates to their various degrees of pathogenicity. However, hantavirus infection of cells in the respective reservoir hosts does not trigger activation of the IFN response but instead induces immunosuppressive signals, possibly explaining the persistence of hantaviruses in reservoir hosts (see above)[57]. Consistent with this, in the Syrian hamster animal model of pathogenic ANDV infection, in which T cell depletion was applied, upregulation of type I IFN-responsive genes correlates with the development of disease[101], supporting the role of innate immunity in the development of hantavirus pathology.

It has also been proposed that the complement system, which promotes opsonization of microorganisms, lysis of Gram-negative bacteria and clearance of immune complexes, is also involved in hantavirus-induced immunopathology. The soluble form of the terminal complement membrane attack complex, SC5b–9, can bind β3 integrin, the suggested receptor for pathogenic hantaviruses, which might result in increased endothelial

Acute-phase proteins
A group of proteins, including C-reactive protein and fibrinogen, the blood concentration of which changes in response to trauma, inflammation or disease. These proteins can be inhibitors or mediators of inflammation.

permeability[102]. SC5b–9 can also increase the permeability of cultured endothelial cells through the release of bradykinin and platelet-activating factor[103]. The complement system becomes activated in the acute phase of PUUV infection, and the levels of complement activation correlate with the severity of disease[104,105]. Complement activation might thus contribute to the development of vascular leakage. Complement activation in the context of hantavirus infection could be mediated by the acute-phase protein pentraxin-related protein 3 (PTX3), which is produced at the site of inflammation[106]. PTX3 levels are increased during the acute phase of PUUV infection[107]; such high plasma PTX3 levels are associated with the overall clinical severity of hantavirus disease, especially with thrombocytopenia, and with activation of the complement system[107]. Galectin-3-binding protein (LGAL3SBP) might also be involved in activating the complement system in PUUV infections. Increased levels of this protein have been observed in infected primary human cells and in the plasma of patients hospitalized owing to acute PUUV infection; in the latter, levels of LGAL3SBP were found to correlate with disease severity and with increased complement activation[108].

Another secreted mediator that is thought to be associated with hantavirus infection is indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme that induces the differentiation of T_{Reg} cells (thus promoting immunosuppression) and inhibits the replication of various bacteria, intracellular parasites and viruses (thus acting as an antimicrobial effector)[109]. However, serum IDO levels are increased during acute PUUV infection, and this is associated with clinically severe PUUV infection[110].

Cell-mediated responses have also been associated with immunopathology. T cell activation has been suggested to be linked with hantavirus pathology, either through the excess secretion of pro-inflammatory cytokines or through CTL-mediated killing of infected cells[90]. During the acute stage of PUUV infection, there is upregulation of perforin 1 and granzyme B, both of which are secreted by CTLs to kill infected cells, and markers for epithelial cell apoptosis can be detected in the serum[111]. The recruitment of CTLs is thought to be mediated by hantavirus antigens, which have been found in renal tubular cells of HFRS-patients[112] and might attract T cells to attack the renal tubular system. CTL accumulation in the kidneys might be associated with epithelial cell leakage and damage, contributing to acute kidney injury[113]. However, recent data suggest that both ANDV and HTNV protect infected endothelial and epithelial cells from CTL-induced apoptosis by blocking caspases through N protein[114], and such protection might explain the absence of endothelial cell damage despite a strong CTL response. Natural killer cells might also contribute to the pathology and the capillary leak syndrome characteristic of hantavirus-mediated disease[115].

Conclusions and perspectives

Hantavirus infections and the diseases that they cause are a growing public health problem, and there is currently no therapy or vaccine in global use. Moreover, by the time the symptoms of HFRS or HCPS appear, viraemia has already started fading, so the administration of antiviral drugs might not be beneficial. Thus, a better understanding of the viral infection and of the involvement of factors such as vascular leakage, cytokine storm and CTL proliferation in disease pathology will be essential to the development of new therapies.

Detailed knowledge about the exact contribution of these different host response factors to hantavirus pathology is currently limited, and it is unclear whether one factor acts as the primary cause of pathogenesis and others are secondary causes. It is possible that insights into the pathogenesis of hantavirus infections will be gained by studying the pathological mechanisms which lead to vascular leakage in infections with other haemorrhagic fever-causing viruses (flaviviruses, filoviruses and arenaviruses).

The difference in pathogenicity of hantaviruses is determined by the rodent host taxonomy: HCPS-causing hantaviruses (for example, SNV and ANDV) are carried by rodents of the subfamilies Sigmodontinae and Neotominae (in the family Cricetidae); hantaviruses that cause severe HFRS (for example, HTNV and DOBV) are carried by rodents of the family Muridae; and non-pathogenic hantaviruses and those that cause only mild HFRS (for example, PUUV, Tula virus (TULV) and PHV), are carried by rodents of the subfamily Arvicolinae (also in the family Cricetidae). However, as opposed to what one might expect, there are no striking differences in the structure or genomic organization of the viruses carried by rodents from different families or subfamilies. Therefore, a better understanding of the relationship between viruses and their reservoir hosts could also provide clues about the pathogenicity factors of hantaviruses.

Monitoring and modelling of rodent dynamics and/or related climatic anomalies could be used to develop early warning systems for human epidemics. Remote sensing technologies can be used to predict the potential environments of various hantaviruses. Human epidemiology is dependent on exposure to rodents and the population dynamics of reservoir species. Of the European hantaviruses carried by *Apodemus* mice, the ICTV has approved DOBV and Saaremaa virus (SAAV). Recently, it was proposed that DOBV and SAAV be subdivided into four related genotypes[77] — Dobrava, Sochi, Kurkino and Saaremaa — that have characteristic differences in their phylogeny, specific host reservoirs, geographical distribution, and pathogenicity for suckling mice and humans. More detailed studies of these closely related hantavirus genotypes that cause either life-threatening (the Dobrava and Sochi genotypes), relatively mild (the Kurkino genotype) or possibly inapparent (the Saaremaa genotype) human infections could reveal the molecular determinants of virus–host interaction mechanisms that lead to virulence.

There is an increasing demand to develop therapy for the deadly hantavirus infections, both HFRS and HCPS. Drugs that are known to influence increased capillary permeability (for example, those that inhibit VEGFR2, SRC family kinases, angiopoietin 1 and sphingosine 1-phosphate) are in clinical trials for other indications, so they could potentially be used to treat

Caspases
A family of cysteine proteases that execute cell death events in the apoptotic pathway.

hantaviruses infections[116,117]. The successful application of icatibant, which blocks the binding of bradykinin to its receptor, is evidence that such drugs can be used in this way[118]. Interestingly, a dramatic increase in endothelial cell permeability and release of bradykinin was recently reported in hantavirus-infected endothelial cells (S. L. Taylor and C. S. Schmaljohn, personal communication)[119]. To conclude, more research is needed in order to fully understand the biology and pathogenesis of the ever-growing Hantavirus genus.

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Competing interests statement
The authors declare no competing financial interests.

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International Committee on Taxonomy of Viruses, 2012 Release:
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