REVIEW

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Future VIROLOGY

Hantaviruses: past, present and future

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ABSTRACT: Hantaviruses productively infect endothelial cells in their rodent reservoirs and humans, but the infection only causes disease in humans – hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome. Despite the enormous progress that has been made in understanding the pathogenesis and immune responses of hantavirus infection, there is a large gap in our molecular-based knowledge of hantaviral proteins in their structures, functions and the mechanisms that facilitate their entry, replication and assembly. Importantly, we know little about the specific viral determinants and viral protein–host interactions that drive differences noted in immune responses between the reservoir and humans. This review discusses our current understanding and future work needed for unraveling the biology of these viruses in their reservoirs and in humans.

KEYWORDS

• hantaviruses • hemorrhagic fever with renal syndrome • nephropathia epidemica • persistent infection • rodent reservoirs • vascular leakage

Introduction to the discovery of hantaviruses & their diseases

Historical gretrospectives of medical reports suggest that trench nephritis in the first World War, nephropathia epidemica (NE) in Scandinavia, Song-go fever in Manchuria and hemorrhagic nephrosonephritis in the Soviet Union were all potentially caused by hantaviruses [1–4]. However, it was not until Hantaan virus (HTNV) was isolated in 1977 that two human diseases were attributed to hantaviruses; hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and NE, a mild form of HFRS, in northern Europe. Four *Hantavirus* species are now recognized to cause HFRS (HTNV harbored by *Apodemus agrarius* [1]; Dobrava–Belgrade virus, harbored by *A. agrarius, A flavicollis* and *A. ponticus* rodents [5,6]; Seoul virus, SEOV, harbored by *Rattus norvegicus* [7]) and NE (Puumala virus, PUUV, harbored by *Myodes glareolus*). Combined, these viruses have a global public health impact estimated at over 50,000 cases each year, with lethality ranging from <1 to 12% [8].

The wide prevalence of hantaviruses in rodents in Europe and Asia suggested the potential for hantaviruses in New World rodents. In the mid-1980s rodent surveillance efforts discovered Prospect Hill virus (PHV), harbored by *Microtus pennsylvanicus* [9], and crossreactive antibodies were reported in *Peromyscus maniculatus*, *P. difficilis*, *P. californicus*, *Neotoma mexicana* and *N. cinerea* in the USA [10], and in Old World (laboratory) rodents in South America [11]. It was not long after these efforts that an outbreak of hantavirus pulmonary syndrome (HPS) in individuals residing in the Four Corners area in the southwestern USA in 1993 confirmed the presence of disease-causing hantaviruses in the Americas.

In the Spring of 1993, two young, healthy adults living in the Navajo Nation fell ill and died from an unexplained acute respiratory distress syndrome (ARDS) [12]. Unexplained deaths are reported to

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the Office of the Medical Investigator (OMI) in New Mexico. Discussions between the OMI and the Indian Health Service quickly recognized there were at least five cases of ARDS in the region. The outbreak led to collaborative investigations by state health departments in Arizona, Colorado, New Mexico and Utah; the Indian Health Service; and the OMI, the University of New Mexico and the CDC, with the assistance of the Navajo Nation Division of Health to identify cases, rodent reservoirs and develop diagnostic and treatment approaches. Within a year's time, the CDC identified the causative agent as Sin Nombre virus (SNV), harbored by *Peromyscus maniculatus* [13], a New World rodent species, and one of the rodents reported as having antibodies to hantaviral antigens in 1985 [10]. Other groups also reported the identification of SNV [14] and SN-like viruses from patients and a number of other rodent reservoirs [8,15].

Just 2 years after the outbreak in the USA, outbreaks of HPS caused by Andes virus (ANDV) were recognized in Argentina and Chile [16–18], and caused by Laguna Negra virus (LANV) in Paraguay [19,20]. In Paraguay, the outbreak was associated with those living in the agricultural communities within the Chaco. In contrast to the severity of disease and high mortality (50%) caused by ANDV, the disease caused by LANV shows a lower mortality (<15%) [21]. In the third outbreak in El Bolson (Argentina) in 1996 [22], one physician in Buenos Aires fell ill 27 days after taking care of an HPS patient from El Bolson [23]. This was the first recognition that these viruses may cause person-to-person transmission of the illness. Since that report, additional studies have shown that person-to-person transmission can occur between couples, individuals who sleep in the same bed or room of index patients with ANDV infection, or via sustained contact during travel (e.g., on a bus) [23–26].

Coding & replication strategy of the *Hantavirus* genome

Hantaviruses, family *Bunyaviridae*, are negative-sense, ssRNA viruses with three gene segments (or viral RNAs [vRNAs]): small (S), medium (M) and large (L) [27]. The S-segment encodes for the nucleocapsid (N) protein, the M-segment encodes for the Gn and Gc glycoproteins, and the L-segment encodes for the RNA-dependent RNA polymerase (RdRp). In the S-segment of the hantaviruses carried by *Arvicolinae* and *Sigmodontinae*, but not *Muridae* rodents, the N protein has an overlapping (+1) open reading frame for a small, nonstructural protein (NSs) [28,29]. The ends of each segment of the genome have conserved, complementary 5′ and 3′ termini structures that can form a panhandle [30,31], which has been shown with other viruses in the *Bunyaviridae* [32,33]. This region of the genome may contain *cis*-acting elements that promote replication of the vRNA, complementary RNAs (cRNAs) and mRNA transcription by the RdRp [30,31]. The N protein packages each of the three vRNAs into three ribonucleoprotein (RNP) complexes, which are contained within the virus [8]. By cryoelectron microscopy (Cryo-EM), the virion contains three rod-like RNP structures [34,35], which presumably contain each vRNA wrapped in N proteins. The viral polymerase would be expected to be part of the RNP.

Reverse genetic approaches have been successful for other members of the *Bunyaviridae* [36–39], but success in applying these strategies to hantaviruses has been limited [40]. At present there has been no progress in the creation of systems for the generation of infectious, recombinant hantaviruses. The challenges in generating recombinant systems for the study of hantaviruses may be due to the inability to produce the correct structures of the tripartite genomes, which have a 5′-prime monophosphate [41,42] and perhaps additional unknown structural features.

Structure & function of hantaviral proteins

Hantavirus virions are asymmetric, pleomorphic particles, and until recently by electron microscopy were thought to have an average diameter of approximately 80–120 nm [8,43]. Recent Cryo-EM studies of the HTNV and Tula virus (TULV) virions now show that the particles range in size from 120 to 154 nm [34,35]. The surface rendering of the virion suggests an unusual square, grid-like pattern distinct from other genera in the *Bunyaviridae* and a lack of icosahedral symmetry typical of most viruses. The square spike on the outer surface reflects the glycoprotein projections, which extend from 0 to 12 nm from the lipid bilayer and comprise four molecules of Gn and Gc [34].

Hantaviruses bind epithelial and endothelial cells via interaction of Gn with the host's cell surface receptor(s); β1 integrin for apathogenic and β3 integrin for pathogenic hantaviruses **(Figure 1A)** [44,45]; although additional receptors or coreceptors may also promote entry such as

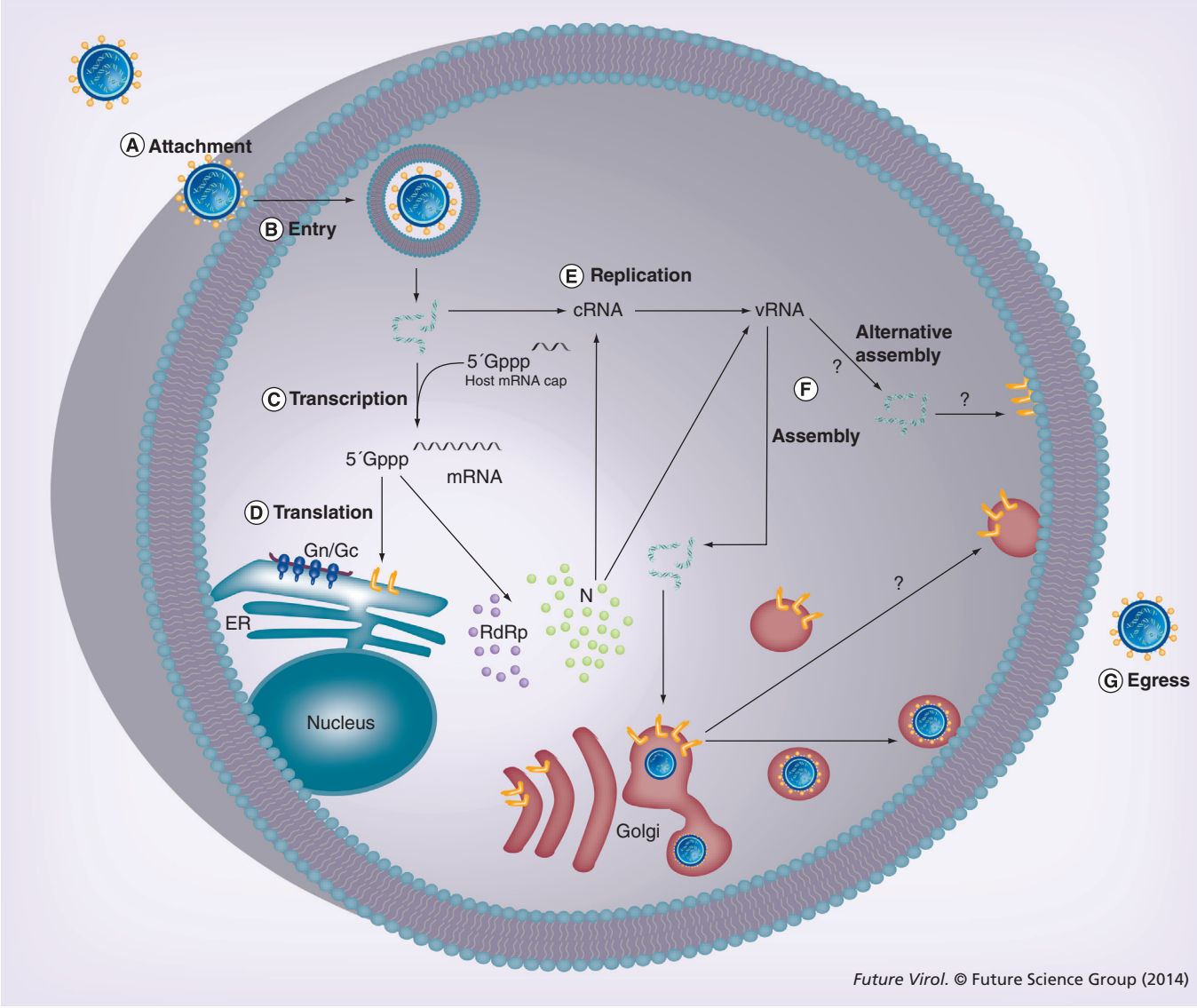

Figure 1. Hantavirus life cycle. The virus life cycle includes: **(A)** attachment to either β1 or β3 integrin of the host cell surface using the viral Gn protein; **(B)** entry of the virus through clathrin receptor-mediated endocytosis; **(C)** transcription via the RdRp cleaves host cell mRNA caps as primers for the vRNA; **(D)** translation of the N and RdRp proteins on free ribosomes and M-segment through rough ER; **(E)** replication of vRNA requires N protein to form the RNP; **(F)** assembly of the virion at the Golgi or possibly for New World at the plasma membrane; and **(G)** egress from the Golgi through the plasma membrane.

DAF (CD55) [46] and the globular head domain of complement C1q [47]. However, interactions between Gn and these receptors have not been shown directly. In addition, hantaviruses infect macrophages, follicular dendritic cells and lymphocytes [46,48–51]. In Vero E6 cells, HTNV has been shown to enter through clathrin-coated pits and traffic to late endosomes **(Figure 1B)** [52]. ANDV do not enter via clathrin; the pathway for entry is not known [53]. Furthermore, early entry events are distinct for HTNV and ANDV given their differences in dependence on an intact actin (ANDV) versus microtubule (HTNV) cytoskeleton for viral replication [53]. Release of the RNPs into the host cell cytoplasm from endosomal compartments is pH dependent [52].

Homology modeling first suggested that the has a structure similar to the alphavirus E1, which, like Gc, harbors the fusion domain [54,55]. The alphavirus E1 is a class II fusion protein that forms a pH-dependent, trimeric configuration to mediate fusion with the host cell membrane within endosomes. A class II fusion peptide maps to the Gc protein, and has been

shown to promote fusion using ANDV Gn/Gc-pseudotyped lentiviral particles [54,56]. With the recent Cryo-EM of HTNV and TULV, elucidation of the structure of the glycoproteins and mapping them within the Cryo-EM structures will shed light on how these Gn/Gc proteins change conformationally to promote membrane fusion.

While the precise site(s) for viral transcription and replication are not known, the RdRp protein transcribes viral mRNAs from each vRNA in the cytoplasm using primers derived from host cellular mRNAs **(Figure 1C)** [57]. The RdRp catalyzes the endonucleolytic cleavage of host cell mRNA at 7–18 nucleotides downstream from the 5′; this activity is also termed 'cap snatching' [57]. Transcription results in hantaviral mRNAs with heterogeneous 5′-ends, which are not polyadenylated. Translation of the S and L mRNAs occurs on free ribosomes, resulting in the production of the N and RdRp proteins; and NSs in some hantaviruses **(Figure 1D)**. The N protein, which accumulates in the perinuclear region [58], is the most abundant viral protein synthesized early in infection. The N protein plays structural and functional roles in the virus life cycle, including modulation of host responses, binding to vRNA, cRNA and host mRNA caps, translation initiation, and assembly [31]. The timing and location at which the N protein apparently commandeers the host mRNA caps is not known, but the N protein has been proposed to retain the caps in P bodies [59]. How these complexes traffic from the P body to the replication complex(es) is not known, but they may traffic on microtubules [60]. It is highly likely that different oligomeric states or conformations of the N protein occur during the life cycle, as demonstrated by their ability to form trimeric structures [61,62]. While not much is yet known about the NSs, the TULV NSs have been reported to localize within the perinuclear region [63]. The M-segment is cotranslationally cleaved in the rough endoplasmic reticulum (RER; **Figure 1D**). Following translation into the ER, the precursor protein is proteolytically cleaved at a WAASA-conserved amino acid motif located from amino acids 264 to 268 into Gn and Gc [64]. A small portion of the c-terminus of the Gn and Gc extend into the cytoplasm (referred to as the Gn and Gc cytoplasmic tails [CTs]). The Gn and Gc proteins are glycosylated in the RER [65] and traffic through the Golgi complex until they assemble into particles. The Gn-CT has been shown to bind to nucleic acid [66] and N protein [67]*in vitro*, and is suggested to act as a matrix protein [68]. At some point following mRNA transcription, the RdRp begins replication of the cRNAs and vRNAs **(Figure 1E)**. The signals that initiate replication are not known; however, it has been suggested that some level of N protein in the cell could drive the switch. The N protein traffics by microtubule dynein to the ER–Golgi intermediate complex (ERGIC), where they may begin to complex with newly synthesized vRNAs to form RNPs [60].

It is unclear where or how the assembly of the RNP takes place; however, at least in the case of the Old World hantaviruses, the RNPs must traffic to the Golgi, as this is the compartment where Gn and Gc glycoproteins are directed and virions have been visualized **(Figure 1F)** [69]. The RNP may interact with the Gn-CT [68] and buds into the Golgi to produce the virion. A Golgi vesicle forms around newly formed particles and transports the virion to be released from the host cell plasma membrane. Alternatively, for the New World hantaviruses, it has been suggested that assembly could also take place at the host cell plasma membrane **(Figure 1F)**. This prediction was initially based on the absence of virions within the Golgi for SN and Black Creek Canal viruses (BCCV). There is still limited evidence for where assembly takes place; however, the glycoproteins of BCCV have been shown to be expressed at the plasma membrane on the apical surface of polarized Vero C1008 cells [70]. Furthermore, studies by Rowe *et al.* have shown that the ANDV associates with the recycling endosome and the Rab 9/11 proteins, and this may serve as an important pathway for trafficking from the Golgi to the plasma membrane [71].

Differential immune responses in rodents & humans

The survival of hantaviruses in nature depends on maintenance of persistent infections within its specific rodent reservoir. Hantaviruses infect and persist only in the rodent reservoir in which the virus has coevolved, and the infection is believed to last the life of the animal [72]. Notably, persistent infection of rodent reservoirs by hantaviruses show continuous virus replication, without complete clearance by the immune system, and no pathological changes [8]. Humans are not a natural reservoir and therefore become infected when they come into contact with excreta from

the rodent reservoir. In humans, infection can result in severe disease, although outcomes vary with different hantaviral species. There are also a number of hantaviruses that do not appear to replicate in human endothelial cells (i.e., PHV [73]) and/or cause disease in humans (i.e., TULV and PHV). The molecular basis for this has been attributed to differences in receptor preferences of apathogenic and pathogenic hantaviruses, which will be discussed later in this review.

The clinical course and pathology of HFRS and HPS has been the subject of several excellent recent reviews [74,75]. It is recognized that both HFRS- and HPS-causing hantaviruses cause systemic vascular leakage of blood vessels without apparent damage to the endothelial cells, even though the target organs for HFRS and HPS differ – kidneys and lungs, respectively. In severe cases, this can lead to hypotension and shock. Mechanisms proposed for the increase in capillary leakage include viral infection (direct increase in VEGF) and immunopathology (indirect through cytokines released by T cells). It has been hypothesized that the immune response to hantavirus infection in human cases causes the severe disease symptoms (e.g., acute thrombocytopenia and increased leukocytes) [76]. A recent study reports a third mechanism for the promotion of vascular leakage and disease. Using a novel *in vitro* capillary blood vessel model, HTNV or ANDV infection has been shown to increase the release of bradykinin (BK) through activation of the kallikrein–kinin system, which correlates with an increase in endothelial cell permeability [77]. The capillary blood vessel model cocultured human umbilical vein endothelial cells with human mesenchymal stem cells or human pulmonary artery smooth muscle cells, which generate blood vessel-like capillary structures. BK is an inflammatory peptide that can cause vasodilation and vascular permeability in the vasculature upon binding of its receptor. Interestingly, the model showed an increase in VEGF following infection, but no loss in vascular integrity. The immune responses in humans following infection with HPS- and HFRS-causing viruses have been extensively reviewed [74,76,78–82]; therefore, in the following text we will highlight those responses that distinguish between infections of the rodent reservoir and humans.

In *Hantavirus* infection, the differences in immune responses between the rodent reservoir and humans are evident in composition, magnitude and kinetics of cytokine/chemokine responses and T cells. Generally, longitudinal studies of cases of hantaviral infection show elevated TNF-α, IL-6, IL-2, IL-1, IL-10, IL-12 (for an example, see [83–85] and references cited) and cytotoxic T lymphocyte (CTL) responses **(Figure 2)** [86,87]. Levels of secreted cytokines and chemokines in deer mice infected with SNV cannot be compared directly, as many antibodies are not yet available. However, longitudinal studies of RNA levels of key cytokines and chemokines in lung and spleen have been reported in SNV-infected deer mice [88] and SEOV-infected rats [89]. While most responses noted in humans were very low in SNV-infected deer mice (<twofold above uninfected mice) and variable, IL-12 rose at 7 days postinfection (d.p.i.) in the spleen. In addition, immune responses included increased GM-CSF (10 d.p.i.) and TGF-β (biphasic peaks at 5 and 15 d.p.i.) in the spleen. The lack on inflammatory signals was similar in SEOV-infected rats; however, TGF-β was elevated in the lungs, not spleen [89].

Patients with severe HPS or HFRS/NE show strong $CD8^+$T cell responses with high levels of perforin and granzyme B [86,87]. From these studies it has been hypothesized that the strong $CD8^+$ T-cell responses will eliminate virus, but that such intensive T-cell responses might also result in an excessive amount of cytokines, which promote capillary leakage and endothelial cell dysfunction [76,80]. In contrast to these findings, a recent study of HFRS patients shows that the prevalence of N-antigen-specific $CD8^+$ T cells correlated with the early, acute stages of infection and declined thereafter [90]. Hence, the $CD8^+$T cells would be expected to have a protective effect rather than promote immune pathology. In support of these findings, T-cell-deficient Rowett nude rats infected with SEOV succumb rapidly to infection and disease, suggesting that cell-mediated immunity may play an important role in controlling infection [91]. More recently, the depletion of T cells from hamsters did not alter the progression of HPS following ANDV challenge, which suggests that vascular permeability does not involve T-cell-mediated immunopathology [92]. In summary, the present literature reports a role for CTLs in promoting disease or protection, hence a more comprehensive analysis of the CTL response is needed in many more patients. However, these studies may be confounded in that most of the hantavirus-specific CTLs may

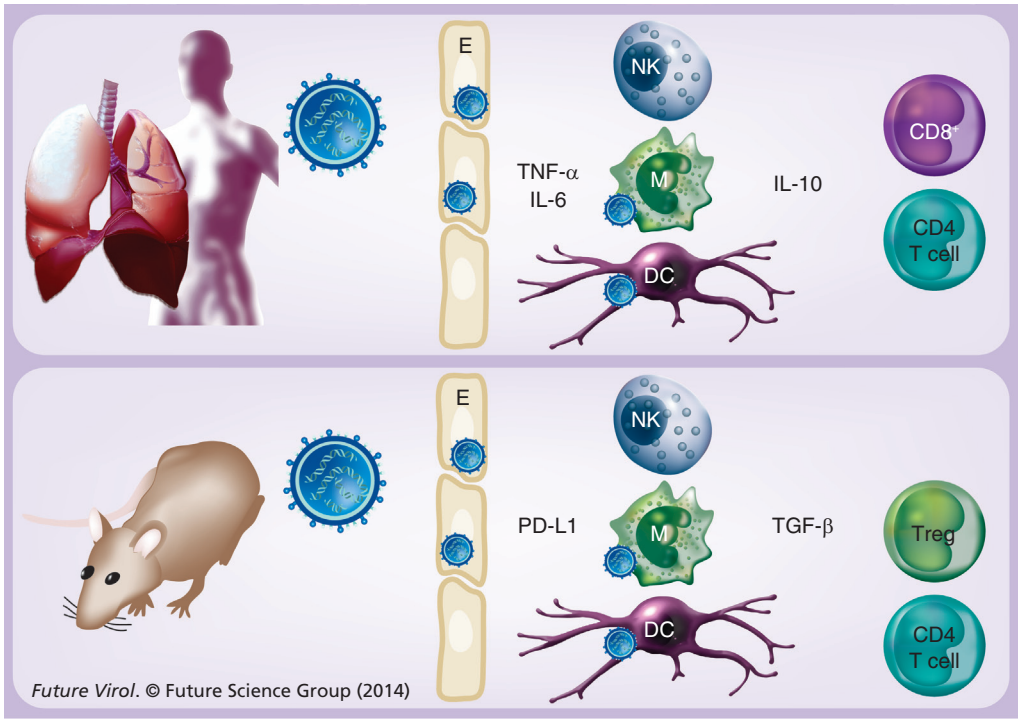

Figure 2. Cellular players and responses in rodent reservoir and human infections with hantaviruses. While there are many gaps in our understanding of the role of the key immune cells and their function, recent studies suggest several key immune responses. During a persistent infection, reservoir species have upregulated PD-L1 and TGF-β resulting Treg response, which results in suppressed immune state IgG antibodies are produced, suggesting a CD^+4 T-cell response. However, in humans, proinflammatory cytokines such as TNF-α, IL-6 and IL-10 are induced, as well as CD^+8 and CD^+4 T cells.
DC: Dendritic cell; E: Endothelial cell; M: Macrophage; NK: NK cell.

be present in organs and not accounted for during analyses of CTLs from PBMCs. An additional complexity is in the recent finding that endothelial cells infected *in vitro* with ANDV and HTNV are protected from CTL- and NK-cell-mediated apoptosis [93].

Mouse models of transient and persistent infection for HTNV have been used to analyze the immune response of virus-specific $CD8^+$ T cells with MHC tetramers [94,95]. In persistently infected mice, N-specific CTLs are strongly regulated and were suppressed in the model by an unknown mechanism [95]. Viral replication in immune cells such as monocytes, macrophages or T cells can interfere with or actively suppress immunity and cause persistence. Hence Taruishi *et al.* proposed that the infection of the spleen early in infection may result in the infection of immune cells that suppress this response [95]. In their persistent animal model experiments, infection of the spleen correlates with changes in CTL response. Consequently, due to the downregulation of the CTLs, some of the endothelial cells may remain infected, resulting in a persistent infection in the natural reservoir.

Studies of persistent infection of SEOV in the rat [96,97] and SNV in the deer mouse [98] suggest a role for Tregs in establishing persistence. The Tregs are $FoxP3^+CD4^+CD25^+$ and are activated during infection of the reservoir. In contrast, these cells are reduced in HTNV-infected HFRS patients [99], although they show no change in PUUV-infected HFRS patients [87]. The Treg responses can enable a persistent infection by limiting T-helper cell responses (Th1 and Th2 cells) indirectly by modulating antigen-presenting cell (APC) function or directly by cell–cell contact. The production of anti-inflammatory cytokines (e.g., TGF-β and IL-10) by Tregs can suppress innate immunity and proinflammatory responses, and thereby interfere with viral clearance and pathology [100]. Interestingly, in HFRS patients, higher levels of IL-10 correlated with higher viral load [84]. In rat macrophages infected with

SEOV, NF-kB-mediated inflammatory responses noted in patients (TNF-α, IL-6 and IL-10) are suppressed [89,101]. Interestingly, SEOV induces PD-L1 expression in rat endothelial cells and TGF-β in alveolar macrophages **(Figure 2)** [51]. The PD-1–PD-L1 pathway has been correlated with increased Treg activity and has also been shown to play an important role in other chronic viral infections such as HIV, HBV, HCV and lower respiratory infections [102–104].

• Hantaviral mechanisms in regulation of nonreservoir host immune responses

While gaps remain in our understanding of how hantaviruses regulate the immune responses at the molecular level, studies have suggested that viral N and glycoproteins interact with host cellular proteins to modulate the innate immune response **(Figure 3)**. Four different cellular pathways have been implicated as targets of hantaviral antagonism in primate or human cell models of infection; IFN-α/β responses (see reviews [78,105,106]), JAK/STAT, TNF-α receptor-mediated signaling, and apoptosis [107]. Highlights of what are currently known regarding hantaviral N, N proteins s and/or glycoproteins (GPCs) in modulation of these cellular activities will be summarized in the following.

Differences in which proteins are used by hantaviruses to inhibit amplification of IFN

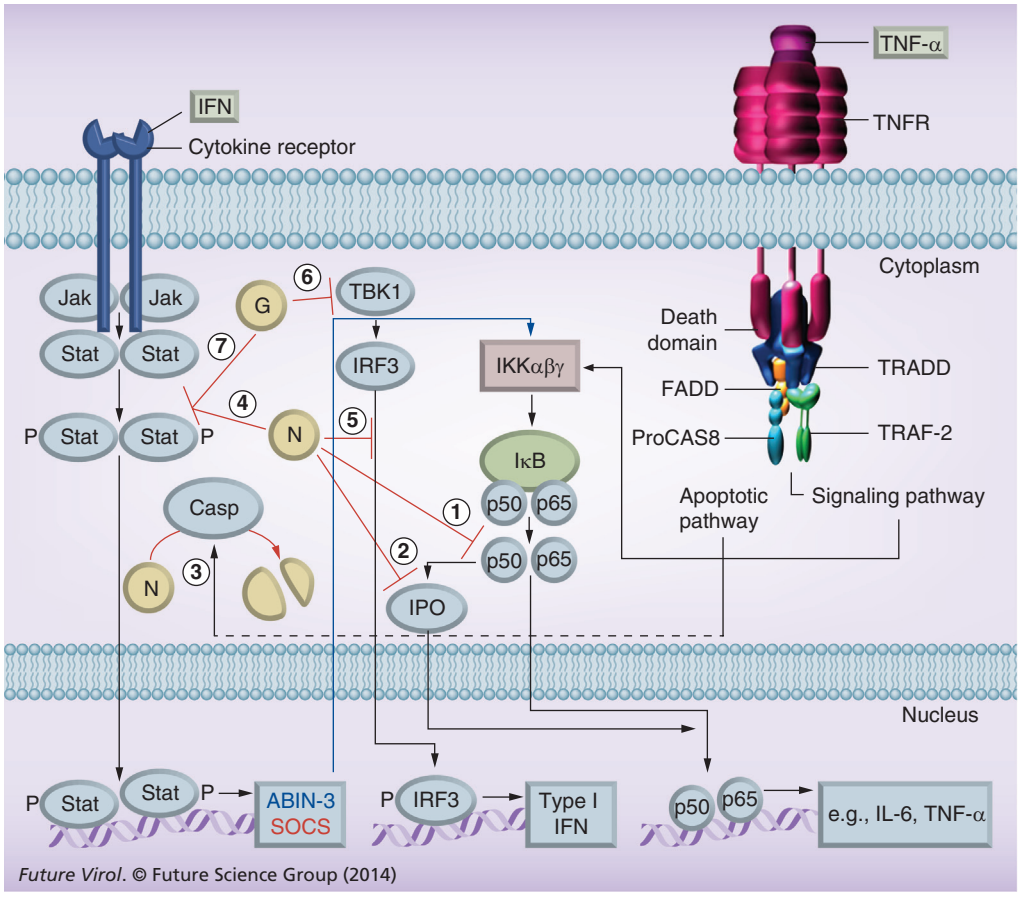

Figure 3. Hantaviral mechanisms in regulation of nonreservoir host immune responses. Generally, suppression of immune responses shown in this figure are from studies of pathogenic hantaviruses and hantaviral proteins in human/primate cell culture models. However, interspecies variation is suggested in the proteins used for immune suppression and pathways targeted. The N protein inhibits NF-κB's transport to the nucleus by binding **(1)** importin-alpha proteins and NF-κB complex; or **(2)** importin-alpha proteins alone. **(3)** The N protein can be cleaved by caspase 3. Moreover, the N protein is required for suppression of **(4)** JAK/STAT signaling and **(5)** Type I IFN induction for some hantaviruses. For some viruses, the glycoprotein is also involved in suppression of Type I IFN induction through **(6)** TBK1 and **(7)** suppression of JAK/STAT signaling.

responses have been reported. Moreover, differences in induction of IFN-β RNA and protein have been shown following infection of human microvascular endothelial cells by ANDV (pathogenic) or PHV (nonpathogenic) [108]. ANDV suppressed IFN-β induction while PHV induced its activation; however, both viruses suppressed STAT1/2 phosphorylation and translocation [108]. Levine *et al.* showed that ANDV uses GPC and N protein to suppress IFN-β induction and IFN-dependent JAK/STAT signaling [82]. In that same report, SNV uses the GPC, but not N protein, to suppress IFN-β induction. In studies of the New York virus (NYV), a pathogenic, New World hantavirus closely related to SNV, the Gn-CT blocks RIG-I/TBK1 activation of IFN sequence regulatory element (ISRE) transcriptional responses, but the PUUV Gn-CT did not [73]. The NYV Gn-CT coprecipitates the N-terminal domain of TRAF3 [109]. The interaction of the NYV Gn-CT with TRAF3 is suggested to disrupt the formation of TRAF3–TBK1 complexes and inhibit induction of IFN-β [109]. Interestingly, the Gc-CT TULV, but not PHV, inhibit IFN-β and ISRE induction through TBK1, but not via TRAF3 [110]. Finally, the N protein of TULV has also been reported to be a weak antagonist of IFN-β induction [28]. Finally, HTNV has been shown to activate the Type III IFN, IFN-λ1, through a mechanism independent of Type I IFN [111].

In addition to suppression of IFN and JAK/STAT, the N protein downregulates TNF-α receptor-mediated signaling by inhibiting activation of NF-κB [112–114]. Studies in 293T cells suggest that the HTNV N, but not ANDV or PUUV N, can block activation of NF-κB by binding the importin-alpha proteins, which are responsible for NF-κB's transport into the nucleus [113]. In a study by Ontiveros *et al.*, it was suggested that N may bind to both NF-κB and importin as a complex to prevent its nuclear translocation [114]. Both studies also show that TNF-α induces degradation of IκB, implicating the block at NF-κB's transport.

Inhibition of signaling pathways normally leading to activation of caspases and apoptosis is evident in cells expressing HTNV N protein [114] and ANDV N protein [93]. Furthermore, ANDV and HTNV-infected endothelial and epithelial cells are protected against staurosporine-induced apoptosis [93,114] and against cytotoxic granule-mediated induction of apoptosis [93]. Intriguingly, the suppression of caspase activity in HTNV N mapped to a highly nonconserved region from amino acids 270 to 330. In a study by Gupta *et al.*, it was shown that the ANDV N interacts with caspase 3 and granzyme B, resulting in inhibition of these apoptosis-inducing enzymes and cleavage of the N protein. Hence, hantavirus inhibits both granzyme B-mediated activation of caspase 3 and inhibits activated caspase 3 in infected endothelial cells targeted by NK cells, thereby protecting infected cells from being killed by cytotoxic lymphocytes [93]. In ANDV N, the caspase cleavage site mapped to DLID285, a site that is not conserved across New and Old World hantaviruses [93]. *In silico* prediction using GraBCas 1.0 [115] suggests potential caspase and granzyme B cleavage sites in N from other hantaviruses as well.

Conclusion

Diseases caused by hantaviruses cause a spectrum of vascular leakage in endothelial cells within the lungs or kidneys that can lead to shock and death. At present, there are no US FDA-approved treatments, and hence continued efforts to determine the mechanisms hantaviruses use to persist in their reservoirs and which cause disease in humans are essential for the discovery of effective therapeutics. A number of recent studies show differences among the Old and New World hantaviruses in several aspects of their life cycle. Furthermore, studies show that there are striking differences in the immune responses following infection of hantaviruses between the reservoirs and humans. Despite the enormous progress that has been made in understanding the pathogenesis and immune responses of hantaviruses in humans and rodents, there is a large gap in our molecular-based knowledge of hantaviral proteins in their structures, functions and the mechanisms that facilitate the differences in the immune responses. Importantly, we know little about the specific viral determinants and viral protein–host interactions that drive these responses.

Significant gaps in knowledge remain in the entry, replication and assembly strategies used by hantaviruses. Furthermore, structural studies have been challenging due to difficulty in the purification of hantaviral proteins and the lack of a reverse genetics system, which have limited our current ability to gain insight into function. Additionally, the majority of the studies that characterize the structure and function of hantaviral proteins have been conducted in Vero E6 cells or with viruses produced from Vero E6 cells.

In the past decade, *in vitro* primary endothelial and immune cell models have emerged to study the host responses elicited by hantaviruses in humans and in a few cases in rodent reservoirs. It is assumed that the structure and function of hantaviral proteins are the same within the Vero E6, human and rodent reservoir, but further work to confirm similarities and differences remain. New insight into the virion structure suggests novel class II mechanisms for binding to its receptor and assembly based on the tetrameric conformation. How the two hantaviruses with Cryo-EM structures interact with different receptors – β1 integrin for TULV and β3 integrin for HTNV, remains to be elucidated. In addition, distinct requirements for entry and trafficking of New and Old World hantaviruses suggest differences in these mechanisms. Whether these differences also extend to rodent reservoir endothelial cells is not known. Finally,

EXECUTIVE SUMMARY

Background

- The genus *Hantavirus* includes species from Old and New World rodents that may cause two human diseases – hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and nephropathia epidemica (NE), a mild form of HFRS, in northern Europe.
- Andes virus, a New World species, has been shown to have the ability to transmit from person to person.

Overview of hantaviral infection rodents & humans

- Hantaviruses infect and persist in their rodent reservoirs, and do not elicit any pathology.
- Persistent infection of reservoir species increases PD-L1 and TGF-β, leading to a Treg response.
- Accidental infection of humans by HFRS- and HPS-causing hantaviruses cause systemic vascular leakage of blood vessels without apparent damage to the endothelial cells, even though the target organs for HFRS and HPS differ – the kidneys and lungs, respectively.
- Vascular leakage has been attributed to VEGF, T-cell cytokines and bradykinin.

Replication strategies

- Hantaviruses are negative-sense, ssRNA viruses with three gene segments (or viral RNAs): small, medium and large. However, some hantaviruses have an additional protein, NS.
- Recapitulation of replication through reverse genetics has been limited and challenging.

Hantaviral proteins: structure & function

- New cryoelectron microscopy studies reveal novel virion topology.
- Recent studies show that the Gc protein mediates fusion following entry through clathrin-mediated endocytosis.
- The plasma membrane may be the location of assembly for New World hantaviruses versus the Golgi for the Old World hantaviruses.

Host immune responses

- The N protein and Gn tail have been shown to modulate immune responses, although differences in Old and New World hantaviral proteins, and even differences in regulatory mechanisms between pathogenic and apathogenic hantaviruses, have been reported.
- Hantavirus N protein inhibits the translocation of NF-κB into the nucleus, and inhibits granzyme B and caspase 3.
- Hantavirus infection of endothelial cells inhibits NK-cell-mediated and chemically induced apoptosis.

Future perspective

- Future efforts that define the cellular components that interact with viral proteins may reveal potential therapeutic targets.
- Unraveling the differences in immune responses in their reservoirs and humans may shed important light into the biology of these viruses and novel approaches for their treatment.

recent findings suggest that hantaviruses regulate TNF-α and IFN-induced responses as well as apoptosis within infected endothelial cells and nearby immune cells. These studies also underscore interspecies differences in strategies among the hantaviruses in the use of the N, NSs and/or Gn-CT in modulating host response. While some viral protein–host protein interactions have been uncovered, additional studies are needed to define the precise mechanisms across hantaviruses. Finally, studies show the potential importance of the CTL responses in causing disease and also in protection, depending on the virus. Clear answers await further analysis of the CTL response in many more patients across the major hantaviral diseases.

Future perspective

Design and development of vaccines and antivirals for treatment of hantaviral infections remains challenging. Continued advancement of vaccines and antivirals would be greatly accelerated with knowledge gained from future research focused on the structure and function of hantaviral proteins during entry, fusion, replication and assembly. For example, knowledge of the glycoprotein spike structure will enable insight into neutralization epitopes that can be incorporated into vaccination technology. Knowledge of viral sites of replication and assembly of hantaviruses within cells will benefit the discovery of new targets for antiviral drug discovery. Furthermore, future efforts that define the cellular components that interact with viral proteins may reveal potential therapeutic targets. Using current and newer approaches in structural and molecular virology, one can begin to unravel sites and mechanisms of binding, replication and assembly of hantaviruses within cells. These types of studies will be important in revealing unique aspects of the viral life cycle that have presumably thwarted the field's ability to generate recombinant viruses to study the function of viral proteins.

In addition to understanding the structure and function of hantaviral proteins, it is clear that unraveling the differences in immune responses in their reservoirs and humans may shed important light into novel approaches for the treatment of these serious diseases [107]. Breakthroughs in the study of immune responses of hantaviruses in rodent models will require the active development of new reagents in lethal models of disease (e.g., hamster) and persistence (e.g., deer mouse). The recent sequencing of hamster and deer mouse genomes is an important new development in that regard.

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