Abstract: Hantaviruses, similar to several emerging zoonotic viruses, persistently infect their natural reservoir hosts, without causing overt signs of disease. Spillover to incidental human hosts results in morbidity and mortality mediated by excessive proinflammatory and cellular immune responses. The mechanisms mediating the persistence of hantaviruses and the absence of clinical symptoms in rodent reservoirs are only starting to be uncovered. Recent studies indicate that during hantavirus infection, proinflammatory and antiviral responses are reduced and regulatory responses are elevated at sites of increased virus replication in rodents. The recent discovery of structural and non-structural proteins that suppress type I interferon responses in humans suggests that immune responses in rodent hosts could be mediated directly by the virus. Alternatively, several host factors, including sex steroids, glucocorticoids, and genetic factors, are reported to alter host susceptibility and may contribute to persistence of hantaviruses in rodents. Humans and reservoir hosts differ in infection outcomes and in immune responses to hantavirus infection; thus, understanding the mechanisms mediating viral persistence and the absence of disease in rodents may provide insight into the prevention and treatment of disease in humans. Consideration of the coevolutionary mechanisms mediating hantaviral persistence and rodent host survival is providing insight into the mechanisms by which zoonotic viruses have remained in the environment for millions of years and continue to be transmitted to humans.

Hantaviruses are negative sense, enveloped RNA viruses (family: Bunyaviridae) that are comprised of three RNA segments, designated small (S), medium (M), and large (L), which encode the viral nucleocapsid (N), envelope glycoproteins (G\text{N} and G\text{C}), and an RNA polymerase (PoL), respectively. More than 50 hantaviruses have been found worldwide [1]. Each hantavirus appears to have co-evolved with a specific rodent or insectivore host as similar phylogenetic trees are produced from virus and host mitochondrial gene sequences [2]. Spillover to humans causes hemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome (HCPS), depending on the virus [3–5]. Although symptoms vary, a common feature of both HFRS and HCPS is increased permeability of the vasculature and mononuclear cell infiltration [4]. Pathogenesis of HFRS and HCPS in humans is hypothesized to be mediated by excessive proinflammatory and CD8+ T cell responses (Table 1).

In contrast to humans, hantaviruses persistently infect their reservoir hosts, presumably causing lifelong infections [6]. Hantaviruses are shed in saliva, urine, and feces, and transmission among rodents or from rodents to humans occurs by inhalation of aerosolized virus in excrement or by transmission of virus in saliva during wounding [7,8]. Although widely disseminated throughout the rodent host, high amounts of hantaviral RNA and antigen are consistently identified in the lungs of their rodent hosts, suggesting that the lungs may be an important site for maintenance of hantaviruses during persistent infection [9–18]. Hantavirus infection in rodents is characterized by an acute phase of peak viremia, viral shedding, and virus replication in target tissues, followed by a persistent phase of reduced, cyclical virus replication despite the presence of high antibody titers (Figure 1) [12–16,18–20]. The onset of persistent infection varies across hantavirus–rodent systems, but generally the acute phase occurs during the first 2–3 weeks of infection and virus persistence is established thereafter (Figure 1).

Hantavirus infection alone does not cause disease, as reservoir hosts and non-natural hosts (e.g., hamsters infected with Sin Nombre virus [SNV] or Choclo virus) may support replicating virus in the absence of overt disease [12,14,16,18,21,22]. Our primary hypothesis is that certain immune responses that are mounted in humans during hantavirus infection are suppressed in rodent reservoirs to establish and maintain viral persistence, while preventing disease (Table 2). During the coevolution of hantaviruses with their reservoir hosts, the viruses may have evolved mechanisms to enhance persistence, including immune evasion, direct suppression or alteration of host antiviral, proinflammatory, and cellular immune responses, and induction of host regulatory responses. Alternatively, hosts may have evolved adaptations, including immunological responsiveness to steroid hormones and pre-existing host genetic factors, to regulate the detrimental effects of infection, which also may affect persistence of hantaviruses.

Virus-Mediated Responses to Hantaviruses

Immune evasion. Mechanisms of immune evasion, including viral mutation and segment reassortment, may contribute...
**Table 1. Summary of Immune Responses in Humans during Hantavirus Infection.**

| Categorical Response | Immune Marker | Effect of Infection | Virus Species* | In Vitro/In Vivo | Tissue or Cell Typeb, Phase of Infectionc | References |
|----------------------|---------------|---------------------|----------------|-----------------|-----------------------------------------|-------------|
| **Innate**           |               |                     |                |                 |                                         |             |
| RIG-I                | Elevated      | SNV                 | In vitro       | HUVEC, ≤24 h p.i. | [79]                                   |             |
|                      | Reduced       | NY-1V               | In vitro       | HUVEC, ≤24 h p.i. | [37]                                   |             |
| TLR3                 | Elevated      | SNV                 | In vitro       | HUVEC, ≤24 h p.i. | [79]                                   |             |
| IFN-β                | Elevated      | PUUV, PHV, ANDV     | In vitro       | HSVEC, HMVEC-L, ≤24 h p.i. | [36,80]                               |             |
|                      | Reduced       | TULV, PUUV NSs      | In vitro       | COS-7 and MRC5 cells, ≤24 h p.i. | [32,33]                               |             |
| IFN-α                | Elevated      | PUUV, HTNV          | In vitro       | MΦ, DCs, 4 days p.i. | [30]                                   |             |
|                      | No change     | HTNV                | In vivo        | Blood, acute     | [81]                                   |             |
| IRF-3, IRF-7         | Elevated      | SNV, HTNV, PHV, ANDV| In vitro       | HMVEC-L, ≤24 h p.i. | [33,38]                               |             |
| MxA                  | Elevated      | HTNV, NY-1V, PHV, PUUV, ANDV, SNV, TULV | In vitro | MΦ, HUVEC, HMVEC-L, 6 h–4 days p.i. | [36,39–41,79] |             |
| MHC I and II         | Elevated      | HTNV                | In vitro       | DCs, 4 days p.i. | [30]                                   |             |
| CD11b                | Elevated      | PUUV                | In vivo        | Blood, acute     | [82]                                   |             |
| CD40, CD80, CD86     | Elevated      | HTNV                | In vitro       | DCs, 4 days p.i. | [30,83]                               |             |
| NK cells             | Elevated      | PUUV                | In vivo        | BAL, acute       | [84]                                   |             |
| **Proinflammatory/ Adhesion** |       |                     |                |                 |                                         |             |
| IL-1β                | Elevated      | SNV, HTNV           | In vivo        | Blood, lungs, acute | [85,86]                               |             |
| IL-6                 | Elevated      | SNV, PUUV           | In vivo        | Blood, lungs, acute | [85,87,88] |             |
| TNF-α                | Elevated      | PUUV, SNV, HTNV     | In vivo        | Blood, lungs, kidney, acute | [85,86,88,89] |             |
|                      | Elevated      | HTNV                | In vitro       | DCs, 4 days p.i. | [30]                                   |             |
| CCL5                 | Elevated      | SNV, HTNV           | In vitro       | HMVEC-L, HUVEC, 12 h–4 days p.i. | [38,39,90] |             |
| CXCL8                | Elevated      | PUUV                | In vivo        | Blood, acute     | [82]                                   |             |
|                      | Elevated      | PUUV                | In vivo        | Men, blood, acute | [62]                                   |             |
|                      | Elevated      | TULV, PHV, HTNV     | In vitro       | HUVEC, MΦ, 2–4 days p.i. | [39,91]                               |             |
| CXCL10               | Elevated      | SNV, HTNV, PHV      | In vitro       | HMVEC-L, HUVEC, 3–4 days p.i. | [38,39]                               |             |
|                      | Elevated      | PUUV                | In vivo        | Men, blood, acute | [62]                                   |             |
| IL-2                 | Elevated      | SNV, HTNV, PUUV     | In vivo        | Blood, lungs, acute | [82,86]                               |             |
| Nitric oxide         | Elevated      | PUUV                | In vivo        | Blood, acute     | [92]                                   |             |
| GM-CSF               | Elevated      | PUUV                | In vivo        | Women, blood, acute | [62]                                   |             |
| ICAM, VCAM           | Elevated      | PUUV                | In vivo        | Kidney, acute    | [87]                                   |             |
|                      | Elevated      | HTNV, PHV           | In vitro       | HUVEC, 3–4 days p.i. | [30,39]                               |             |
| E-selectin           | Elevated      | PUUV                | In vivo        | Blood, acute     | [82]                                   |             |
| **CD8+ and CD4+ T cells** |       |                     |                |                 |                                         |             |
| IFN-γ                | Elevated      | HTNV, SNV           | In vivo        | Blood, CD4+,CD8+, lungs, acute | [81,86] |             |
| CDB+                 | Elevated      | DOBV, PUUV, HTNV    | In vivo        | Blood, BAL, acute | [52,84,93] |             |
| Virus-specific IFN-γ+CD8+ | Elevated | PUUV, SNV | In vivo | PBMC, acute | [45,94] |             |
| Perforin, Granzyme B | Elevated      | PUUV                | In vivo        | Blood, acute     | [95]                                   |             |
| CD4+CD25+ "activated" | Elevated | DOBV, PUUV | In vivo | PBMC, acute | [89,93] |             |
| IL-4                 | Elevated      | SNV                 | In vivo        | Lungs, acute     | [86]                                   |             |
| **Regulatory**       | Reduced       | HTNV                | In vivo        | Blood, acute     | [52]                                   |             |
| IL-10                | Elevated      | PUUV                | In vivo        | Blood, acute     | [86]                                   |             |
| TGF-β                | Elevated      | PUUV                | In vivo        | Kidney, acute    | [99]                                   |             |
| **Humoral**          | Elevated      | All hantaviruses    | In vivo        | Blood, acute     | [4]                                    |             |

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*aSNV, Sin Nombre virus; NY-1V, New York-1 virus; PUUV, Puumula virus; PVH, Prospect Hill virus; ANDV, Andes virus; TULV, Tula virus; HTNV, Hantaan virus; DOBV, Dobrava virus.  
bHUVEC, human umbilical vascular endothelial cells; HSVEC, human saphenous vein endothelial cells; HMVEC-L, human lung microvascular endothelial cells; COS-7, African green monkey kidney fibroblasts transformed with Simian virus 40; MRC5, human fetal lung fibroblasts; MΦ, macrophages; DCs, dendritic cells; BAL, bronchoalveolar lavage, PBMC, human peripheral blood mononuclear cells.  
cAcute infection is during symptomatic disease in patients.  
dSuppressor T cells likely represent cells currently referred to as regulatory T cells.  
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Table 2. Summary of Immune Responses in Rodents during Hantavirus Infection.

| Categorical Response | Immune Marker | Effect of Infection | Virus Species\(^a\) | Host, Tissue or Cell Type\(^b\) | Phase of Infection\(^c\) | References |
|----------------------|--------------|---------------------|----------------------|-------------------------------|--------------------------|------------|
| Innate               | TLR7         | Reduced             | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [19]       |
|                      |              | Elevated            | SEOV                 | Female Norway rats, lungs     | Acute, Persistent        | [19]       |
|                      | RIG-I        | Elevated            | SEOV                 | Female Norway rats, lungs     | Acute, Persistent        | [19]       |
|                      |              | Elevated            | Newborn rats, thalamus | Acute                         |                         | [96]       |
|                      | TLR3         | Elevated            | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [19]       |
|                      | IFN-β        | Reduced             | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [19,61]    |
|                      | Mx2          | Reduced             | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [19,60]    |
|                      |              | Elevated            | Female Norway rat lungs | Acute                         |                         | [19,61]    |
|                      |              | Elevated            | HTNV, SEOV           | Mice\(^d\), fibroblasts transfected with Mx2 | 3–4 days p.i. | [97]       |
|                      | JAK2         | Elevated            | SEOV                 | Female Norway rats, lungs     | Acute                    | [60]       |
|                      | MHC II       | Elevated            | PUUV                 | Bank voles                    | Genetic susceptibility   | [74]       |
| Proinflammatory/     | IL-1β        | Reduced             | SEOV                 | Male Norway rats, lungs       | Persistent               | [29]       |
| Adhesion             |              | Elevated            | SEOV                 | Male and female Norway rats, lungs | Acute, Persistent    | [29,61]    |
|                      | IL-6         | Reduced             | SEOV                 | Male rats, spleen             | Acute                    | [29]       |
|                      |              | Elevated            | SEOV                 | Male Norway rats, lungs       | Acute                    | [29]       |
|                      | TNF-α        | Reduced             | HTNV                 | Newborn mice\(^e\), CD8\(^+\), spleen | Acute                  | [49,50]    |
|                      |              | Elevated            | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [29,42,61] |
|                      | CX3CL1, CXCL10 | Reduced             | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [29]       |
|                      |              | Elevated            | SEOV                 | Male Norway rats, spleen      | Acute                    | [29]       |
|                      | CCL2, CCL5   | Elevated            | SEOV                 | Male Norway rats, spleen      | Acute                    | [29]       |
|                      | NOS2         | Reduced             | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [29,61]    |
|                      |              | Elevated            | SEOV                 | Male Norway rats, spleen      | Acute                    | [29]       |
|                      | VCAM, VEGF   | Elevated            | SEOV                 | Male Norway rats, spleen      | Acute                    | [29]       |
|                      |              | Elevated            | HTNV                 | Mouse M6\(^f\), in vitro      | 6 h p.i.                 | [98]       |
| CD8\(^+\) and CD4\(^+\) T cells | CD8\(^+\) | Reduced             | HTNV                 | Newborn mice\(^g\), spleen   | Persistent               | [50]       |
|                      |              | Elevated            | HTNV                 | SCID mice\(^e\), CD8\(^+\) transferred, spleen | Persistence  | [49]       |
|                      | IFN-γ        | Elevated            | SEOV                 | Female Norway rats, lungs     | Persistent               | [61]       |
|                      |              | Elevated            | SEOV                 | Male Norway rats, spleen      | Acute                    | [29]       |
|                      |              | Elevated            | SEOV                 | Male and Female Norway rats, spleen | Acute                  | [20]       |
|                      | IFN-γR       | Elevated            | SEOV                 | Female Norway rats, lungs     | Persistent               | [61]       |
|                      |              | Reduced             | SEOV                 | Male Norway rats, lungs       | Acute                    | [29]       |
|                      | T cells      | Elevated            | SEOV                 | Nude rats                     | Persistence              | [47]       |
|                      |              | Elevated            | HTNV                 | Nude mice\(^g\)                | Persistence              | [100]      |
|                      | IL-4         | Reduced             | SEOV                 | Male Norway rats, lungs       | Acute, Persistent        | [61]       |
|                      |              | Elevated            | SNV                  | Deer mice, CD4\(^+\) T cells  | Acute                    | [48]       |
|                      |              | Elevated            | SEOV                 | Male and female Norway rats, spleen | Acute                  | [20]       |
|                      | Regulatory T cells | Elevated          | SEOV                 | Male Norway rats, lungs       | Persistent               | [42,61]    |
|                      | FoxP3        | Elevated            | SEOV                 | Male Norway rats, lungs       | Persistent               | [29,42,61] |
|                      | TGF-β        | Elevated            | SEOV                 | Male Norway rats, lungs       | Persistent               | [29]       |
|                      | IL-10        | Reduced             | SEOV                 | Male Norway rats, lungs and spleen | Acute, Persistent    | [29]       |
|                      |              | Elevated            | SNV                  | Deer mice, CD4\(^+\) T cells  | Acute                    | [48]       |
| Humoral              | IgG          | Elevated            | SNV                  | Deer mice                     | Persistent               | [12,57]    |
|                      |              | Elevated            | SEOV                 | Norway rats                   | Persistent               | [16,17]    |
to viral persistence. Cyclical rises of Seoul virus (SEOV) L segment deletions and S segment insertions in regions necessary for initiation of transcription are observed in vitro [6]; whether mutated RNAs can self-repair and whether this occurs in vivo remains to be determined. Quasispecies have been identified in vivo in deer mice (Peromyscus maniculatus) and European common voles (Microtus arvalis) infected with SNV and Puumala virus (PUUV), respectively, with nucleotide and amino acid mutations identified in immunodominant regions of the GN protein [23,24]. Segment reassortment of hantaviruses has been identified in Vero E6 cells that are co-infected with related strains of SNV and in vivo in deer mice infected with SNV [25,26]. Recent data also suggest that a newly discovered hantavirus in Paraguay may be a reassortment between Laguna Negra or Rio Mamoré viruses with Pergamino or Maciel viruses [27]. Quasispecies and reassortants likely contribute to the evolution of new species of hantaviruses, but also may be involved in immune evasion.

**Direct alteration of host cellular responses.** Hantaviruses can infect macrophages and endothelial cells in rodents and humans [13,14,16,28]. These cell types have been identified primarily by morphological analyses or by non-specific cell separation. Recently, SEOV N protein was definitively identified in macrophages and endothelial cells in lung tissue of infected rats using cell-specific antibodies [29]. Hantaan virus (HTNV) infects human dendritic cells (DCs) in vitro and induces DC activation and maturation [30]. Whether rodent DCs are infected by hantaviruses and whether rodent macrophage and DC activity is suppressed by hantavirus infection to cause persistence remains unknown. Several viruses in the Bunyaviridae family encode a non-structural protein (NSs) that suppresses antiviral immune responses in infected cells [31]. The S segment of PUUV and Tula virus (TULV) has an open reading frame (ORF) for NSs, a protein that reduces the expression of IFN-β in human lung fibroblasts and contributes to virus survival in vitro [32,33]. Immunoreceptor tyrosine-based activation motifs (ITAMs) that bind kinases to regulate immune and endothelial cell function have been identified in the Gp protein of hantaviruses [34,35]. Additionally, the cytoplasmic tails of HCPS-causing hantavirus G proteins inhibit the expression of type I IFN responses in human umbilical vein endothelial cells (HUVECs) and human lung microvascular endothelial cells (HMVEC-Ls) in vitro [36,37]. Whether hantavirus proteins and RNA have immunomodulatory activity in rodent reservoirs remains unknown.

### Host-Mediated Responses to Hantaviruses

**Innate antiviral and proinflammatory responses.** Infection of HMVEC-Ls and HUVECs indicate that at least some of the hantaviruses that cause disease in humans (e.g., HTNV, Andes virus [ANDV], SNV, New York-1 virus [NY-1V], PUUV, and SEOV) delay induction of factors in the type I IFN pathway (e.g., production of IFN-α, IFN-β, and MxA) as compared with hantaviruses that cause no known disease in humans (e.g., TULV and Prospect Hill virus [PHV]) [36,38–41]. Delayed production of antiviral responses in humans may contribute to more efficient replication of pathogenic hantaviruses than non-pathogenic hantaviruses. Despite delayed induction of type I IFNs, human cells mount innate antiviral responses that also may contribute to viral clearance.

In the lungs of rats with high amounts of virus (i.e., male rats), the expression of pattern recognition receptors (i.e., RIG-I and TLR7) is reduced or remains unchanged throughout SEOV infection, suggesting that inhibition of viral recognition may contribute to the establishment of persistent
infection [19]. Furthermore, antiviral (e.g., IFN-β, Mx2, and IFN-γ) and proinflammatory (e.g., IL-1β, TNF-α, and NOS2) responses are reduced or unaltered during infection in the lungs of male rats, which also may contribute to hantavirus persistence [19,29,42]. In contrast, in the spleen, a peripheral immune organ that supports low amounts of virus, the expression and production of proinflammatory and antiviral factors are elevated during acute SEOV infection and subsequently return to baseline [20,29]. Thus, rats infected with SEOV do not appear to be globally immunosuppressed, but rather have a site-specific reduction of proinflammatory responses. There is no evidence that male rats that are naturally infected with SEOV are more likely to acquire additional pathogens, further illustrating that infected rats are not immunocompromised [43]. Conversely, natural populations of deer mice that have antibody against SNV elicit a lower response to phytohemagglutinin (i.e., a measure of immunocompetence) than their uninfected counterparts, suggesting that SNV causes some degree of immunosuppression in deer mice [44]. Administration of exogenous IL-1β, which elevates circulating IL-1β and IL6 and Tnfα expression in the lungs within physiological ranges, does not affect SEOV persistence in male rats nor does it cause observable disease [29]. Thus, extremely high proinflammatory responses observed during acute infection in humans may be necessary for viral clearance at the expense of causing potentially fatal proinflammatory-mediated disease.

**CD4+ and CD8+ T cell responses.** Cellular immune responses, in particular CD8+ T cells, contribute to clearance of hantaviruses in humans at the expense of causing disease [45,46]. Following inoculation with SEOV, nude (i.e., T cell deficient) rats have more virus in target tissues and shed more infectious virus than do their immunocompetent counterparts and die 10 weeks after inoculation, indicating that T cells contribute to the control of virus replication and survival in a reservoir host [47]. During the acute phase of SNV infection, deer mice have observable Th1 and Th2 responses (i.e., elevated expression of Ifnγ, Gata3 [i.e., the hallmark Th2 transcription factor], Il4, and Il5) in cultured CD4+ T cells, which are not evident in CD4+ T cells isolated from persistently infected deer mice [48]. In severe-combined immunodeficient (SCID) mice (Mus musculus), the transfer of functional CD8+ T cells is necessary for clearance of HTNV [49]. Persistence of HTNV in newborn BALB/c mice is correlated with a decrease in HTNV-specific CD8+ T cell numbers and activity, as measured by IFN-γ production, further suggesting a role of functional CD8+ T cell responses in viral clearance [49,50]. Not only are laboratory mice non-natural hosts, but SCID and newborn mice do not have fully functional immune systems, so these models do not accurately represent viral persistence in immunocompetent rodent reservoir populations. The effect of infection on hantavirus-specific CD8+ T cells in adult rodent reservoir hosts requires examination.

**Regulatory T cell responses.** Regulatory T cell responses suppress proinflammatory and effector T cell responses locally at the site of infection to allow pathogen persistence, as well as to mitigate proinflammatory-mediated pathogenesis [51]. Recent studies have demonstrated that regulatory T cells contribute to SEOV and SNV persistence in rats and deer mice, respectively [42,48]. Expression of Foxp3 mRNA and proportions of CD4+CD25+FoxP3+ regulatory T cells are elevated locally at a site of elevated SEOV replication (i.e., in the lungs) in male rats during persistent SEOV infection [29,42]. Functional inactivation of regulatory T cells reduces the amount of SEOV RNA present in the lungs and the proportion of animals shedding viral RNA in saliva [42]. In the lungs, the expression and production of TGF-β is elevated and TNF-α is suppressed during persistent infection; both cytokine expression patterns are dependent on the presence of functional regulatory T cells [42]. Similarly, CD4+ T cells isolated from deer mice during the persistent phase of SNV infection have higher expression of Tgfβ than do CD4+ T cells isolated from deer mice during the acute phase of infection [48]. The production of IL-10 is consistently reduced throughout SEOV and during the persistent phase of SNV infection in rats and deer mice, respectively, revealing that IL-10 does not contribute to regulatory T cell-mediated hantaviral persistence [29,42,48]. Because responses to hantavirus infection in humans involve overproduction of proinflammatory cytokines, it is consistent that “regulatory T cell activity” (i.e., T cells which reduce ConA-induced proliferation of PBMCs) is suppressed during symptomatic HTNV infection in humans [52]; whether suppressed regulatory T cell responses contribute to disease in humans requires consideration.

**Antibody responses.** Hantaviruses persist in their rodent hosts despite the presence of neutralizing antibody. Antibody against hantaviruses is usually detectable after the first 2 weeks of infection, rapidly increases for the next 4–6 weeks, and declines, but remains detectable presumably for the lifetime of the rodent (Figure 1) [9,13–16,18,29]. Hantavirus-specific antibody responses, although not capable of eliminating virus, can serve a protective role against infection. Maternal antibody protects offspring of hantavirus-infected dams during the first 2 months of life (i.e., when the immune system is not fully developed) in various rodent reservoirs [53–55]. Not only are young rodents protected from hantavirus infection, but young male and female bank voles with maternal antibody against PUUV also mature earlier, suggesting that reproductive success may be increased in bank voles with, as compared to bank voles without, maternal antibody [54]. How hantaviruses evade antibody responses in their rodent hosts remains to be answered.

**Sex differences and sex steroids.** In natural populations of rodent reservoirs, males are more frequently infected with hantaviruses and are more likely to engage in aggressive encounters than are females, which may result in elevated exposure and transmission of hantaviruses among males [10,56–59]. In laboratory settings, when given the same challenge, male rats have more SEOV RNA and antigen in target organs and saliva than do females [19,20]. Removal of the testes in males (i.e., reduction of androgens to non-detectable levels) reduces, whereas removal of the ovaries in females (i.e., reduction of estrogens and progesterone to non-detectable levels) increases SEOV RNA loads as compared with their intact counterparts [19]. Consistent with sex differences in SEOV load, the expression of innate antiviral (e.g., Th7, Myd88, Rig-I, Vip3a, Ifnb, and Mx2) and proinflammatory (e.g., Tnfα and Ccl5) factors is higher in the lungs of female than male rats [11,19,60,61]. Similarly, immunocompetence, as measured by swelling in response to PHA, is higher in female than in male deer mice during SNV infection [44]. Conversely, the expression and production of regulatory factors, including Foxp3 and TGF-β, is elevated in the lungs of males as compared with those of females [61]. These sexually dimorphic immune responses may be dependent on estradiol in females and testosterone in males, as gonadectomy reverses these differences [19,60]. It is plausible that reduced innate and proinflammatory defenses and elevated regulatory responses combined with an increased propensity to engage in aggression may contribute to increased maintenance and transmission of
hantaviruses among male as opposed to female rodents. Whether there exists a sexual dimorphism in the risk of zoonotic transmission of hantaviruses should continue to be considered.

Sex differences in response to hantavirus infection in humans are beginning to receive attention. During acute PUUV infection in humans, circulating concentrations of CXCL8 and CXCL10 are higher, whereas concentrations of IL-9 and GM-CSF are lower in men than in women [62,64]. Although a similar proportion of men and women have antibodies against PUUV [63], men are more likely to develop symptoms of disease (i.e., be hospitalized) during PUUV infection than are women [62,64]. Whether sexually dimorphic immune responses during hantavirus infection cause differences in the severity of disease between men and women requires further investigation.

**Glucocorticoids.** Glucocorticoids are potent immunosuppressive steroid hormones that can suppress proinflammatory and cellular responses and have been administered to patients with HFRS or HCPS to reduce immunopathology [65,66]. Infection of humans with either HTNV or PUUV causes pituitary and adrenal necrosis, which may contribute to the reduced concentrations of cortisol and elevated proinflammatory responses in patients during the acute phase of infection [67,68]. In rats, circulating concentrations of corticosterone are reduced during SEOV infection in males, but not in females [61]. Males with reduced concentrations of glucocorticoids have more SEOV RNA in the lungs than do males with elevated concentrations [61]. No such relationship between glucocorticoid concentration and SEOV load is observed in females [61]. Low concentrations of corticosterone correlate with elevated regulatory responses (i.e., expression of Foxp3 and production of TGF-β) and expression of the glycosenase, matrix metalloproteinase (Mmp)-9, in the lungs of male, but not female, rats during SEOV infection [61]. Increased production of Mmp-9 may disrupt the basement membrane and extracellular matrices in tissues to increase virus dissemination in male rats [69]. Whether hantaviruses alter glucocorticoids in other reservoir hosts requires further investigation. Based on our data, administration of corticosteroids to patients with HFRS or HCPS would not be expected to cause chronic hantavirus infection and may even reduce viral dissemination in tissues.

**Genetic factors.** Host genetic factors may contribute to susceptibility to and outcome of hantavirus infection in both humans and rodent hosts. Patients with TNF polymorphisms associated with elevated or reduced TNF-α transcription are predisposed to exhibit more severe disease during PUUV infection [70,71]. Possession of certain HLA haplotypes (i.e., HLA-B8-DR3, C4A*Q0, and DRB1*0301) is a risk factor for severe disease during PUUV infection in humans, illustrating that antigen presentation and T cell responses likely contribute to the severity of disease [72,73]. Several MHC alleles may alter susceptibility in rodent reservoirs, specifically Cgl-DQ4*009, which is positively, and Cgl-DQ4*05 and Cgl-DQ4*12, which are negatively, associated with the likelihood of bank voles being infected with PUUV [74]. Because uninfected bank voles may never have been exposed to PUUV, whether these specific MHC alleles alter susceptibility following a known exposure to PUUV remains to be determined.

**Conclusions**

Hantaviruses and their rodent reservoirs represent highly coevolved systems in which virulence and host responses have been adapted to ensure survival of both the virus and the host. The current literature suggests that hantavirus persistence is mediated by both the virus and the host. Although several potential mechanisms mediating the persistence of hantaviruses in their rodent hosts have been discovered in recent years, there are a number of intriguing questions that remain to be addressed:

- Do hantaviral NSs inhibit type I IFN responses in rodents and therefore contribute to viral persistence?
- Is DC and macrophage activity (e.g., antigen presentation, cytokine production, and T cell activation) suppressed by hantavirus infection in rodent reservoirs?
- Does suppression of excessive proinflammatory cytokine responses (e.g., TNF-α) prevent disease in rodents at the cost of causing viral persistence?
- Is the activity of hantavirus-specific CD8+ T cells suppressed during infection to mediate viral persistence in rodents?
- How is regulatory T cell activity induced by hantaviruses in reservoir hosts?
- What is the mechanism of regulatory T cell–mediated hantaviral persistence (i.e., suppression of proinflammatory and/or CD8+ T cell activity)?
- Do host genetic factors, in addition to MHC alleles, contribute to the susceptibility of rodents to hantaviruses?
- What is the role of non-immune mediators, including MMP-9 [61] and receptor use for cellular entry [75,76], in hantaviral dissemination and persistence in rodents?

We propose that comparing immune responses in rodents to those in humans may provide insight into ways to prevent pathology in humans. Although advances are being made in the development of a hantavirus vaccine, there currently is no FDA-approved vaccine or drug for prevention or treatment of hantaviral disease [77,78]. Elevated regulatory T cell responses in rodents contribute to hantavirus persistence, possibly by suppressing proinflammatory responses (i.e., production of TNF-α) [42,48]. Regulatory T cell responses during hantavirus infection have not been well characterized in humans, but may be downregulated [32] and contribute to symptoms of HFRS and HCPS. Targeted manipulation of regulatory T cell responses by adoptive transfer of regulatory T cells, administration of anti-TNFα therapy, or treatment with glucocorticoids may control the “cytokine storm” that is initiated when hantaviruses infect humans and cause severe immunopathology. Understanding the mechanisms mediating viral persistence in the absence of disease in reservoir hosts may contribute to advances in the treatment of HFRS and HCPS in humans.

**References**

1. Klein SL, Calisher CH (2007) Emergence and persistence of hantaviruses. Curr Top Microbiol Immunol 317: 217–252.
2. Plyusnin A, Morzunov SP (2001) Virus evolution and genetic diversity of hantaviruses and their rodent hosts. Curr Top Microbiol Immunol 256: 47–75.
3. Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, et al. (1995) Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol 146: 552–579.
4. Khaboullina SF, St Jor SC (2002) Hantavirus immunology. Viral Immunol 15: 609–625.
5. Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Kisank TG, et al. (1993) Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science 262: 914–917.
6. Meyer BJ, Schmaljohn CS (2000) Persistent hantavirus infections: characteristics
and mechanisms. Trends Microbiol 8: 61–67.
7. Glass GE, Cháls JE, Korch GW, Leude JW (1980) Association of intraspecific wounding with hantaviral infection in wild rats (Rattus norvegicus). Epidemiol Infect 101: 459–472.

10. Hinson ER, Fujiaki M, Yoshimura K, Arikawa J, Takashima I, et al. (1996) Urine-associated hantavirus transmission among male Norway rats. Arch Virology 140: 363–374.

12. Klein SL, Maron AL, Scott AL, Kernet G, Glass GE (2002) Neonatal sex steroids affect responses to Seoul virus infection in male but not female Norway rats. Brain Behav Immun 16: 736–746.

13. Lee HW, Lee PW, Baek LJ, Song CK, Seong IW (1988) Association of intraspecific wounding with hantavirus strains in eastern and western Pennsylvania. Am J Trop Med Hyg 75: 1127–1134.

14. Yanagihara R, Amyx HL, Gajdusek DC (1985) Hantaan virus infection in the deer mouse (Peromyscus maniculatus) model: Sites of replication and differences in immune responses and viral shedding following Seoul virus infection in Norway rats. Am J Trop Med Hyg 70: 310–317.

15. Easterbrook JD, Klein SL (2000) Seoul virus enhances regulatory and reduces proinflammatory responses in male Norway rats. J Med Virol 60: 1308–1316.

16. Raftery MJ, Kraus AA, Ulrich R, Kruger DH, Schonrich G (2002) Hantavirus infection of dendritic cells. J Virol 76: 10724–10733.

17. Blakker G, Delhaye S, Habjan M, Blair CD, Sanchez-Vega JR, Yee J, et al. (2007) La Crosse bunyavirus nonstructural protein NSs serves to suppress the T1 interferon system of mammalian hosts. J Virol 81: 4991–4999.

18. Jaskelainen KM, Puma E, Mimura Y, Erimo N, Kurokawa K, Arakawa K, Yoshimura K, Kuroiwa H, et al. (2007) Tula and Puuma hantavirus ORFs are functional and the products inhibit activation of the interferon-α promoter. J Med Virol 79: 1540–1550.

19. Takashima I, Amiya H, Gajdusek DC (1985) Experimental infection with Puuma virus, the etiologic agent of nephropathia epidemica, in bank voles (Clethrionomys glareolus). J Virol 53: 34–38.

20. Lee HW, Lee PW, Baek LJ, Song CK, Seong IW (1981) Intraspecific transmission of Hantaan virus, etiologic agent of Korean Hemorrhagic fever with renal syndrome. Am J Trop Med Hyg 30: 1106–1112.

21. Lee PW, Yamaichi R, Gibbs CJ Jr, Gajdusek DC (1986) Pathogenesis of experimental Hantaan virus infection in laboratory rats. Arch Virol 88: 37–56.

22. Tanishita O, Takahashi Y, Okuno Y, Tamura M, Asada H, et al. (1998) Persistent infection of rats with Sin Nombre hantavirus with renal syndrome virus and their antibody responses. J Gen Virol 79: 1540–1550.

23. Yanagita R, Amiya H, Gajdusek DC (1985) Experimental infection with Puuma virus, the etiologic agent of nephropathia epidemica, in bank voles (Clethrionomys glareolus). J Virol 53: 34–38.

24. Henttonen H, et al. (2006) Maternal antibodies to hantaviruses are found in cord serum. Virology 350: 614–619.

25. Chen L, Yang W (1999) Abnormalities of T cell immunoregulation in hemorrhagic fever with renal syndrome. J Infect Dis 165: 1016–1019.

26. Mok HC, Zuckerman BA, Caffrey MJ, Jany D, Zanetti M, Rice PL, et al. (1988) Role of maternal antibody in protection from hemorrhagic fever with renal syndrome virus infection in rats. Arch Virol 103: 253–265.

27. Khaiboullina SF, Rizvanov AA, Deyde VM, St Michael G, Ensslin FA. (2005) Interferon regulatory factor 1 (IRF-1) and interferon regulatory factor 5 (IRF-5) were highly expressed and up-regulated in osteosarcoma cell lines. J Virol 79: 1192–1199.
Norrby rats following infection with Seoul virus. J Med Virol 74: 180–190.

61. Easterbrook JD, Klein SL (2008) Corticosteroids modulate Seoul virus infection, regulatory T cell responses, and MMP-9 expression in male, but not female, Norrby rats. J Gen Virol 89: 2723–2730.

62. Klingström J, Lindgren T, Ahlin C (2008) Sex-dependent cytokine responses in cynomolgus monkeys exposed to hantavirus infection. Clin Vaccine Immunol 15: 855–867.

63. Ahlin C, Linderholm P, Juto B, Stegmyr B, Setrgren B (1994) Prevalence of serum IgG antibodies to Puunlla virus (hemorrhagic fever with renal syndrome) in Northern Sweden. Epidemio Infect 113: 129–136.

64. Vapalahti O, Mustonen J, Lundkvist A, Tarnvik A (1996) Human leukocyte antigen-B8-DR3 is a more important risk factor for severe Puumala hantavirus infection than the anti-beta2 integrin antibody inhibition by serum from Norrby virus in the deer mouse model. J Gen Virol 78: 493–505.

65. Hooper JW, Ferro AM, Wahl-Jensen V (2008) Immune serum produced by DNA vaccination protects hamsters against lethal respiratory challenge with Andes virus. J Virol 82: 1332–1338.

66. Dunst R, Mettang T, Kuhlmann U (1998) Severe hemorrhagic fever with renal syndrome caused by Puumala virus infection in Norwegian Norway rats following infection with Seoul virus. Arch Virol 31: 201–221.

67. Tarnvik A, Hynninen M, Kolho E, Kallio-Kokko H, Pettila V (2006) Corticosteroids combined with continuous veno-venous hemodiafiltration for treatment of hantavirus pulmonary syndrome caused by Puunlla virus infection. Eur J Clin Microbiol Infect Dis 25: 261–266.

68. Linderholm M, Björner I, Juto P, Roos G, Sandstrom T, et al. (1995) Local host response in the lower respiratory tract in nephropathia epidemica. Scand J Infect Dis 27: 97–101.

69. Markotic A, Gagro A, Dasic G, Sabioncello A, Krakauer T (2005) Chemokine production predominates in human monocytes infected with Tula virus. Viral Immunol 20: 206–213.

70. Lahetki J, Hyvonen M, Pietilä K, Vapalahti O, et al. (2008) T cell memory generation in puunlla hantavirus infection occurs after the acute phase and is associated with boosting of EBV-specific CD8+ memory T cells. J Immunol 179: 329–334.

71. Carter LM, Kekalainen E, Makela S, Alahuhta I, Ennis FA, et al. (2007) Human CD8+ T cell memory generation in puunlla hantavirus infection occurs after the acute phase and is associated with boosting of EBV-specific CD8+ memory T cells. J Immunol 179: 329–334.

72. Imaizumi T, Katakami Y, Ishihara M, et al. (2005) Double-stranded RNA induces the synthesis of retinoic acid-inducible gene-I in vascular endothelial cells. Endothelium 12: 133–137.

73. Markusova A, Krnovskyova IA (2001) Cellular receptors and hantavirus pathogenesis. Curr Top Microbiol Immunol 256: 91–115.

74. Vaheri A, et al. (1996) Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: An immunohistochemical study. Clin Immunol Immunopathol 78: 47–55.

75. Anderson AO, Krakauer H (1994) Serum levels of alpha and gamma interferons in hemorrhagic fever with renal syndrome. J Infect Dis 173: 38–43.

76. Tatemon M, Matsuura J, Helin H, Pasternack A, Valeri A, et al. (1996) Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: An immunohistochemical study. Clin Immunol Immunopathol 78: 47–55.