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Hantaviruses include serious human pathogens that are maintained in nature in persistently infected rodents and that can also persistently infect cultured mammalian cells, causing little or no cytopathology. The mechanisms of hantavirus persistence are only beginning to be explored. Recent data point to subtle changes in the viral genome that might result in the differential regulation of replication and lead to persistence.

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Hantaviruses comprise a genus in the family Bunyaviridae. They are spherical, enveloped viruses, with a genome consisting of three segments of single-stranded, negative-sense RNA. Hantaviruses are found worldwide and are known to cause two serious and often fatal human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). With the exception of the hantaviruses, all Bunyaviridae are maintained in arthropods and are amplified in animal or plant hosts that are often killed by the infecting virus. By contrast, hantaviruses are maintained by cyclical transmission between persistently infected rodents, with incidental infection of humans. Transmission occurs primarily by inhalation of aerosols from infected rodents' urine, feces or saliva (reviewed in Ref. 1). More than 20 hantaviruses have been identified, approximately half of which are known to cause HFRS or HPS (Table 1). Although all hantaviruses are believed to infect their natural reservoir persistently, many have not been studied in detail, and it is therefore possible that some infection characteristics remain to be discovered. Here, we will review the evidence for, and properties of, hantavirus persistence in nature and will speculate on the possible mechanisms of this persistence.

Hantaviruses in nature

Hantaviruses are the archetypes of microorganisms that have adapted well to their environment. This adaptation allows hantaviruses to coexist with one of nature's most abundant vertebrates – rodents – without the need for a complex host–vector transmission cycle. In general, distinct hantaviruses are associated with a single rodent species of the *Murinae*, *Arvicolinae* or *Sigmodontinae* subfamilies (Table 1). Genetically related rodents carry genetically similar hantaviruses, a fact illustrated by phylogenetic studies in which hantaviral gene or protein sequences were examined (Fig. 1). Comparing mitochondrial gene sequences of hantaviral hosts further corroborates this relationship, as nearly identical phylogenetic trees can be constructed by using host mitochondria or viral gene sequences[2]. These data suggest that hantaviruses have coevolved with their rodent hosts, probably over thousands of years. Whether hantaviruses were always able to infect rodents persistently or whether persistence characteristics were selected during the course of this coevolution is unknown.

It is generally believed that hantavirus infection in rodents is lifelong and has no deleterious effects. However, the evidence for this comes from studies that used only a few hantavirus–rodent pairs and that did not always include measurement of virus shedding or pathological examinations. Therefore, although it is likely that hantaviruses can be transmitted for extended periods by infected adult rodents, and that there is little consequence to the health of the reservoir, such generalizations await further research.

Hantavirus infection of natural rodent reservoirs

Hantaan virus

Hantaan virus, the prototype of the Bunyaviridae, is an etiologic agent of severe HFRS and is carried by field mice of the genus *Apodemus*. Experimental infection of wild-caught, sero-negative *Apodemus* with Hantaan virus resulted in a transient viremia seven to 12 days after infection, which was followed by persistent viral shedding in the rodents' urine for at least one year[3] (Table 2). Infectious virus was also recovered from saliva and feces for approximately one month after infection. Viral antigen was present in the lungs throughout the course of the one-year study and in the kidneys and parotid glands for approximately two months and six months, respectively. Although horizontal transmission of virus between caged *Apodemus* was readily observed, vertical transmission from pregnant mice to offspring was not[3].

A study of wild-caught *Apodemus* in eastern Russia suggested temporal changes in the carrier status of individual rodents[4]. Viral antigen could be detected in the lungs of a proportion of mice at all times but, at certain times, was also found in other organs and tissues, suggesting increased viral replication. This increase occurred in the spring and summer months

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Table 1. Hantaviruses

Virus	Original source	Source location	Disease
***Murinae* subfamily-associated viruses**			
Hantaan	*Apodemus agrarius*	Korea	HFRS
Seoul	*Rattus norvegicus, Rattus rattus*	Korea	HFRS
Dobrava-Belgrade	*Apodemus flavicollis*	Slovenia	HFRS
Thai-749	*Bandicota indica*	Thailand	Unknown
***Arvicolinae* subfamily-associated viruses**			
Puumala	*Clethrionomys glareolus*	Finland	HFRS
Prospect Hill	*Microtus pennsylvanicus*	Maryland	Unknown
Tula	*Microtus arvalis*	Russia	Unknown
Khabarovsk	*Microtus fortis*	Russia	Unknown
Topografov	*Lemmus sibiricus*	Siberia	Unknown
Isla Vista	*Microtus californicus*	California	Unknown
***Sigmodontinae* subfamily-associated viruses**			
Sin Nombre	*Peromyscus maniculatus*	New Mexico	HPS
New York	*Peromyscus leucopus*	New York	HPS
Black Creek Canal	*Sigmodon hispidus*	Florida	HPS
Bayou	*Oryzomys palustris*	Louisiana	HPS
Caño Delgadito	*Sigmodon alstoni*	Venezuela	Unknown
Rio Mamore	*Oligoryzomys microtis*	Bolivia	Unknown
Laguna Negra	*Calomys laucha*	Paraguay	HPS
Muleshoe	*Sigmodon hispidus*	Texas	Unknown
El Moro Canyon	*Reithrodontomys megalotis*	California	Unknown
Rio Segundo	*Reithrodontomys mexicanus*	Costa Rica	Unknown
Andes	*Oligoryzomys longicaudatus*	Argentina	HPS
Insectivore-associated virus			
Thottapalayam	*Suncus murinus*	India	Unknown
Other hantavirus-rodent pairs			
Monongahela	*Peromyscus maniculatus*	Unknown	Unknown
Blue River	*Peromyscus leucopus*	Unknown	Unknown
Oran	*Oligoryzomys longicaudatus*	Unknown	Unknown
Lechiguanas	*Oligoryzomys flavescens*	Unknown	Unknown
Bermejo	*Oligoryzomys chacoensis*	Unknown	Unknown
Maciel	*Bolomys obscurus*	Unknown	Unknown
Pergamino	*Akadon azarae*	Unknown	Unknown

Abbreviations: HFRS, hemorrhagic fever with renal syndrome; HPS, hantavirus pulmonary syndrome.

when rodents are most active, and was suggested to be influenced by seasonal physiological changes[4].

Seoul virus

The rodent reservoirs of Seoul virus, which causes a moderately severe form of HFRS, are common urban brown or black rats of the genus *Rattus*. As rats were distributed worldwide over the past few centuries (primarily as passengers on cargo ships), Seoul virus became equally widely distributed. Although persistent infection of rats with Seoul virus is generally accepted, few studies have documented lifelong infection. In nature, adult rats (six months and older) show a significantly higher sero-prevalence rate for Seoul virus than do younger rats[5–8]. One reason for the lower rate in younger rats is that there is no vertical transmission of Seoul virus, and newborn rats are protected from infection by the presence of maternal antibodies[9–11]. In one study, however, it was shown that ~50% of newborn rats with circulating maternal antibodies could be infected if given a large intraperitoneal (i.p.) dose of Seoul virus[12]. The rest of the newborn rats were apparently protected by maternal antibodies, but when challenged again as adults, they were susceptible to infection[12]. Although the authors of this study reported that these rats became persistently infected, no data were provided regarding the duration of infection.

In the absence of maternal antibodies, lethal infection of suckling mice or rats has been demonstrated by intracerebral (i.c.) or i.p. infection with Hantaan or Seoul viruses[10,13–17]. In nature, it is likely that infection of newborn rodents only occurs by aerosol exposure or contact with a recently infected dam; the significance of these findings is therefore unclear. It is possible that rat pups born to hantavirus-naive dams could suffer lethal infection, but as yet, there are no supporting data for this.

In addition to maternal-antibody-mediated protection, age-dependent infection of wild rats with Seoul virus has been linked to aggressive behavior of sexually mature animals. This is reflected in the correlation between the number of wounds on an adult rat and increased sero-prevalence of Seoul virus[18]. These data were interpreted to indicate that mechanical transmission might be a common means of viral spread. An alternative interpretation is that urine aerosols are generated during aggressive interactions, leading to increased aerosol exposure to virus. In laboratory studies, rats and mice were found to be more sensitive to hantavirus infection by intramuscular (i.m.) injection than by aerosol exposure[19]. However, because a urine aerosol probably contains much greater amounts of infectious virus than saliva imparted by bite, these laboratory findings do not offer much support for mechanical transmission being the primary means of infection in nature.

Not only is the manner by which rats become infected with Seoul virus unclear, so are the characteristics of viral persistence. As was found for Hantaan virus infection of *Apodemus*, experimentally inoculated and

wild-caught Seoul-virus-infected rats excrete virus in urine; however, virus shedding is not consistently observed[20]. Although both naturally and experimentally infected rats have viral antigen in many of their tissues and organs, with lungs generally having the highest antigen concentration[13,20], the duration of Seoul virus persistence is poorly defined. In separate studies, newborn rats were found to have viral antigen in their organs for at least 25 weeks[13] and adult rats for at least 75 days[20] after infection (Table 2). This age-dependent difference in Seoul virus persistence in rats has also been observed in two other studies. In one study, i.p. inoculation of one-day-old rats resulted in viral persistence for at least 184 days, whereas seven-week-old rats appeared to cure the infection after 50 days[21]. In the other study, using a different strain of Seoul virus, inoculation of newborn rats resulted in viral antigen persisting in tissues and organs for at least six weeks, whereas inoculation of three-week-old rats did not result in any detectable viral antigen[22]. In the first study, a cell-culture-adapted strain of Seoul virus was used; it is therefore possible that the results do not mimic a natural infection. In the second study, however, the authors corroborated their findings by inoculating three-week-old rats with the original lung suspension from which the Seoul virus had been isolated (i.e. virus that had never been passed through cell cultures). Although they were able to infect rats with this inoculum (as demonstrated by the elicited antibody responses), they could not recover infectious virus from the lungs, suggesting that persistent infections were not established[22]. These data argue against cell-culture adaptation as a cause of the reduced ability of Seoul virus to infect rats persistently. This is contradicted by another study[23], in which Seoul virus was isolated from a single wild rat by passage in cell culture or by sequential inoculation of newborn rats. Only the rat-passaged isolate was able to infect three-week-old rats and cause virus shedding[23]. Hence, it is likely that cell-culture adaptation does influence the ability of Seoul virus to replicate in rats. Further studies, using non-adapted strains of Seoul virus, are required to determine conclusively if rats maintain lifelong persistent infections with Seoul virus or whether they are able to cure infection. In nature, this question might not be significant as viral persistence for a few months would probably encompass the life span of the host.

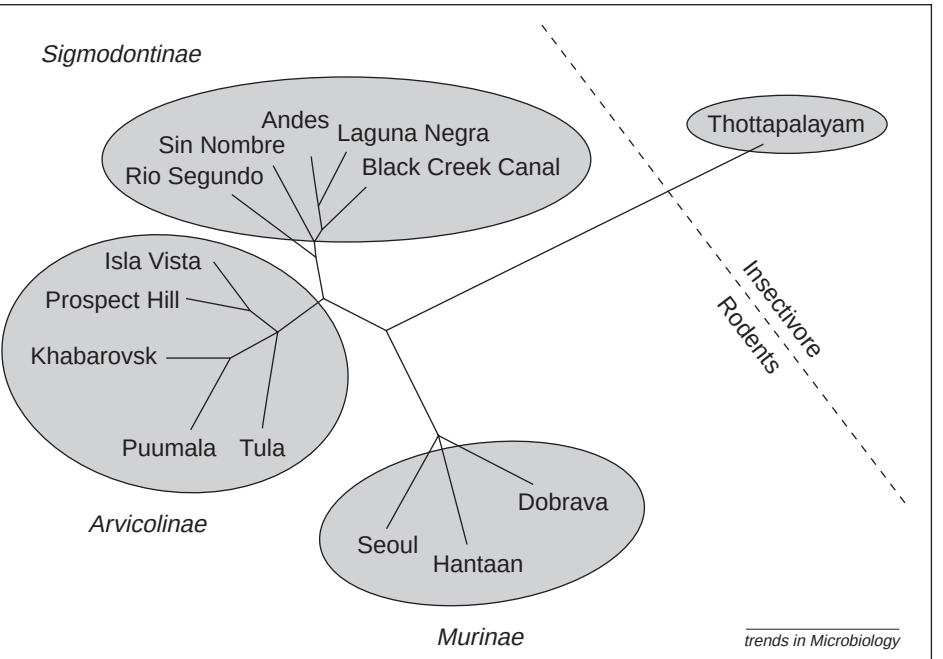

Fig. 1. Phylogenetic relationships of hantaviruses based on alignment of amino acid sequences of the nucleocapsid protein (N). Hantaviruses and their rodent reservoirs included in the analysis are as described in Table 1. The subfamilies of hantaviral rodent hosts (*Murinae*, *Arvicolinae* and *Sigmodontinae*) and the insectivore host are designated by the shaded regions. Accession numbers for S segment sequences (from which the amino acid sequences for N were derived) are: Andes virus, AF004660; Black Creek Canal virus, L39949; Dobrava virus, L41916; Hantaan virus, M14626; Isla Vista virus, U19302; Khabarovsk virus, U35255; Laguna Negra virus, AF005727; Prospect Hill virus, X55128; Puumala virus, X61035; Rio Segundo virus, U18100; Seoul virus, M34881; Sin Nombre virus, L33683; Tula virus, Z48235. The sequence for the *Thottapalayam* S segment is not published but is available upon request. Amino acid sequences were aligned using the Clustal W Multiple Sequence Alignment Program Version 1.7. Phylogenic relationships were displayed by using TreeView tree drawing software.

Puumala virus

Puumala virus, which causes HFRS with relatively low mortality, is found throughout Europe and western Russia. The principal rodent reservoirs for Puumala virus are bank voles of the genus *Clethrionomys*. As demonstrated for Seoul virus in rats, the prevalence rate for antibodies against Puumala virus in wild-caught *Clethrionomys* correlates with increased weight (age), suggesting that horizontal infection could be important[24]. Puumala virus antigens were detected in many organs and tissues in the natural rodent reservoir, including lungs, liver, spleen, kidneys and salivary glands, and also in urine[25,26]. Interestingly, viral antigen was more readily detected in brown fat than in lungs of overwintering voles, suggesting that brown fat might play a role in maintaining the persistent infection in the winter months[25]. As also described for Seoul virus, cell-culture-adapted Puumala virus has a reduced ability to infect the natural host[27].

A laboratory investigation of Puumala virus infection of colonized *Clethrionomys* demonstrated that, following i.m. inoculation, weanling voles developed asymptomatic, persistent infections, that virus could be recovered from the lungs for at least 270 days and that virus was excreted in urine for at least 130 days after infection[26]. Horizontal transmission to cage

Table 2. Hantavirus persistence in experimentally infected adult rodent reservoirs

Virus	Rodent	Time after infection that virus, antigen or RNA was detected				Refs
		Organ				
		Blood	Saliva/salivary gland	Urine/kidney	Lung/spleen	
Hantaan	*Apodemus agrarius*	12 d	35 d	360 d	14 d	3[a]
Seoul	*Rattus norvegicus*	0 d[b]	17 wk[c]	5 wk[d]	75 d[e], 17 wk[c]	13[d], 20[e], 21[b], 40[c]
Puumala	*Clethrionomys glareolus*	14 d	300 d	240 d	360 d	28[f]
Black Creek Canal	*Sigmodon hispidus*	150 d	150 d	150 d	150 d	37[g]

[a]Sero-negative *Apodemus* were inoculated by the intramuscular (i.m.) route with $10^{8.2} \times ID_{50}$ (ID_{50} is the dose of virus required to infect 50% of all animals) of the Lee strain of Hantaan virus, which had previously been passed three times in *Apodemus*. Infectious virus was measured by inoculation of samples into naive *Apodemus*. The duration of the study was 360 days.
[b]Seven-week-old Wistar rats were inoculated by the intraperitoneal (i.p.) route with 6.6×10^3 focus-forming units of the KI-83-262 strain of Seoul virus, which had previously been passed in Vero E6 cell cultures. Reverse transcriptase (RT)-PCR was used to measure the concentration of viral DNA in the sera and clots of samples collected from three–180 days after inoculation.
[c]Rowett rats, which were immunologically normal but were heterozygous for T-cell deficiency (rnu/+), were inoculated by the i.p. route with $>10^3 \times LD_{50}$ (LD_{50} is the dose of virus required to kill 50% of all newborn rats) of the B1 strain of Seoul virus. The virus was previously passed in Vero E6 cells. Infectious virus was measured by inoculating tissue cultures with suspensions of tissues or organs followed by immunofluorescent antibody detection of viral antigen. The duration of the study was 17 weeks.
[d]Six-week-old Fischer rats were inoculated by the i.p. route with the B1 strain of Seoul virus. The titer of the inoculum was 1×10^4 focus-forming units ml^{-1}. The amount of inoculum was not specified. Virus was isolated from tissues or organ explants to Vero E6 cells. The duration of the study was six weeks.
[e]Wistar rats were infected with Seoul virus, strain 80/39. Lung, kidney, liver, spleen, parotid gland and lacrimal gland were assayed by immunofluorescent antibody staining at 30 days after infection and in lung and liver at 75 days after infection.
[f]'Red-mice' were inoculated via the i.m. route with $100 \times ID_{50}$ of the Kazan strain of Puumala virus. The duration of the study was 390 days. Viral antigen was measured by a direct immunoenzyme method.
[g]Six-week-old cotton rats were infected by the subcutaneous route with $1000 \times TCID_{50}$ ($TCID_{50}$ is the amount of inoculum required to infect 50% of cells in culture) of Black Creek Canal virus. Virus-complementary RNA was detected by RT-PCR. The duration of the study was 150 days.

mates was also demonstrated, with the highest transmission occurring at times when virus shedding was most prevalent in saliva. It was suggested that virus maintenance in endemic foci might be related to the grooming behavior of the voles[26]. The distribution of viral antigen in tissues and organs was found to be the same for voles infected by i.m. inoculation or by aerosol exposure to virus from cage mates[26]. In the same study, suckling *Clethrionomys* infected with Puumala virus by i.c. inoculation also developed asymptomatic infections, with widespread tissue distribution of viral antigen that persisted throughout the course of the study[26]. As is also the case for Hantaan and Seoul viruses, vertical transmission of Puumala virus was not observed[28].

In contrast to these findings in laboratory-bred voles, a study of trapped *Clethrionomys* revealed that only approximately one-quarter of sero-positive animals had detectable viral antigen or RNA in their lungs[29]. Additionally, no virus was recovered from the antigen-negative, sero-positive animals, whereas infectious virus could be isolated from some of the antigen-positive voles. These findings were interpreted to indicate that, in nature, lifelong Puumala virus persistence probably does not occur in *Clethrionomys*. However, an earlier study, by Bogdanova *et al.*[28] of wild-caught and laboratory-colonized *Clethrionomys* revealed the presence of viral antigen and the shedding of infectious virus for at least one year after infection with Puumala virus (Table 2). A possible explanation given for this discrepancy was that because antibodies to the hantavirus were shown to undergo a 'wavelike fluctuation', an asynchronous oscillation in antibody and antigen titers in different organs could be characteristic of a persistent infection[28]. However, Bogdanova *et al.* cautioned that the absence of antigen and virus in lungs of animals with high levels of circulating antibodies might not prove the absence of infectious virus in the rodent. Further studies to resolve these disparate results are needed to clarify the issue of Puumala virus maintenance in nature.

Other etiological agents

Since the 1993 discovery of HPS in the southwestern United States (reviewed in Ref. 30), numerous hantaviruses have been detected in New World rodents in North and South America. The etiologic agent of most North American HPS cases is Sin Nombre virus, which is carried by deer mice of the genus *Peromyscus*. As with the Old World hantavirus–rodent pairs, wild-caught deer mice were found to have Sin Nombre viral antigens in many tissues and organs, with lungs having the greatest amount of antigen and viral nucleic acid[31,32]. The presence of viral antigen in the kidneys was most common in deer mice that did not have antibodies to Sin Nombre virus, suggesting that virus shedding is greatest in the early stages of infection[32]. Also analogous to findings with the Old World hantaviruses, a higher sero-prevalence rate for Sin Nombre virus was observed in old deer mice than in young deer mice, with males infected more often than females[33–35]. Although these data are consistent

with horizontal transmission by aggressive interactions and biting, they could also reflect the larger home range of adult males compared with adult females and the resulting increased risk of aerosol exposure[33].

The consequences of Sin Nombre virus infection of *Peromyscus* have not been examined in detail, but are probably negligible. Two studies have reported that hantavirus-infected, wild-caught *Peromyscus* show evidence of lung edema and it has been suggested that acute infection might cause a brief pathology[32,36]. Additional, well controlled studies, including laboratory studies, are needed to explore more fully the impact of Sin Nombre virus infection on the natural reservoir.

Another etiologic agent of HPS, Black Creek Canal virus, is carried by cotton rats of the genus *Sigmodon*. A laboratory study of this virus in its natural reservoir demonstrated that subcutaneous infection of six-week-old animals resulted in persistent infections and virus shedding for at least 150 days[37]. Infectious virus was also isolated from many tissues and organs, including lung, liver, spleen, kidney, brain and blood, throughout the course of the study.

Possible mechanisms of hantavirus persistence

Host-related changes

Viral persistence ultimately depends on the ability of the infecting virus to maintain its genetic information while simultaneously avoiding the host immune system. Generally, this involves non-lytic infection of one or more cell types in the host or, alternatively, replication in a site that is not readily accessed by the host immune system (i.e. replication in a 'privileged site'; reviewed in Ref. 38). This mechanism probably does not apply to hantaviruses, which replicate in many tissues and organs that are readily accessed by the host immune system, resulting in vigorous humoral responses[3,13,21,39]. Little is known about the hosts' cell-mediated immune responses during hantaviral persistence. Laboratory studies have shown that, in the absence of a competent T-cell response, nude rats succumb rapidly to Seoul virus infection, suggesting that cell-mediated immunity plays an important role in controlling infection[40]. In laboratory mice, cytotoxic T cells were found to crossreact with distantly related hantaviruses better than neutralizing antibodies did; cell-mediated immunity, therefore, could also be important for broadening the hosts' immune response[41].

Another possible mechanism of persistence is infection of immunocompromised individuals or those with incompetent immune systems, such as infection *in utero* or infection of neonates. As already described, vertical transmission of hantaviruses is not believed to occur, and newborns are generally protected from infection by maternal antibodies. There could be differences among hantavirus–rodent pairs in their age-related susceptibility to infection, but current data indicate that adult animals with competent immune systems can be persistently infected and that these infections are apathogenic for most or all hantaviruses.

Persistence can also result from viral replication in immune cells such as monocytes, macrophages or T cells, which interfere with or actively suppress immunity. Most viruses that cause persistent infections infect lymphocytes and/or monocytes[38], and hantavirus replication in lymphocytes and peripheral blood macrophages has been well documented. The effects of viral replication in immune cells on host immunity have not been studied in detail for hantaviruses.

Viral alterations

In addition to host-related mechanisms, changes in the virus that result in downregulation of replication or antigenic variation can also lead to persistence by minimizing host cell destruction. These changes could occur at many levels in the virus life cycle including transcription, translation, encapsidation, packaging or release from the cell. Support for the importance of these mechanisms is provided by studies of hantaviruses in their rodent reservoirs[3,4,11,21,26,28,37]. The infections were usually characterized by a transient viremia that peaked approximately seven to 14 days post-infection, followed by a prolonged period when virus was rarely detected in blood. In some tissue samples, infectious virus or viral antigen also peaked during the first three to four weeks of infection, and afterwards varied in relative amounts or disappeared and reappeared multiple times. These data suggest that persistence is established after the first three to four weeks of infection, and that some stage of the viral life cycle is downregulated around that time, and perhaps at cyclic intervals thereafter. In one study that measured levels of anti-genomic RNA as an indication of active viral replication, the concentration of RNA varied between tissues and, in some tissues, varied over time, suggesting that intermittent bursts of virus replication occur during persistence[37]. Whether the downregulation is a result of changes in the virus, the host immune system, or both, has not been determined. Recent studies from our laboratory have examined this aspect of hantavirus persistence by looking at alterations in the virus genome that might influence persistence in the absence of a host immune response.

The hantavirus genome consists of three single-strand, negative-sense RNAs, termed small (S), medium (M) and large (L) RNAs, which encode the viral nucleocapsid, envelope glycoproteins and polymerase, respectively[42]. Changes in any of these three gene segments could influence the course of infection and contribute towards persistence by downregulating one or more aspects of viral replication. In particular, changes in the terminal conserved regions of the genes could have drastic effects on viral replication. Like other Bunyaviridae, the 3′ and 5′ termini of the L, M and S segments of hantaviruses have ~20 complementary nucleotides that are believed to have a panhandle structure (Fig. 2). Although not yet defined, it is possible that both the terminal nucleotide sequence and the panhandle structure are important signals for correct transcription initiation of the viral RNAs and the viral-complementary RNAs. To investigate this

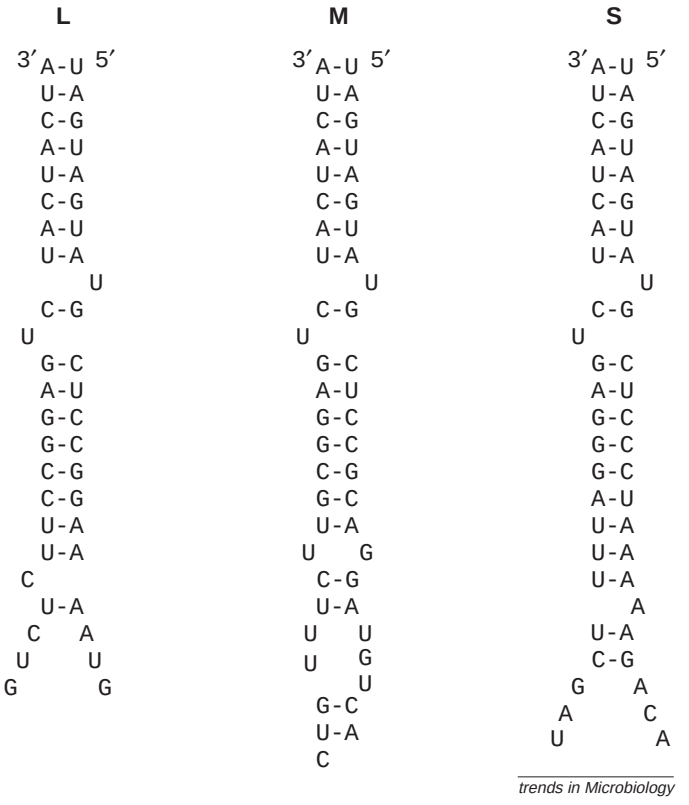

Fig. 2. The 3′ and 5′ terminal nucleotides of the L, M and S genomic segments of Seoul virus and their predicted base pairing to form panhandle structures[43–45].

possibility, changes in the termini and other regions of the S, M and L RNAs that arose during establishment and maintenance of persistence were characterized from two independently derived Seoul virus infections of cultured mammalian cells, one lasting 46 days and the other lasting 139 days[43]. The cultured cells mimicked the early stages of rodent infection in that they had an acute, non-cytolytic stage with peak virus titers one to two weeks after infection. At the end of the acute stage and at intervals during persistence, viral replication and the titer of released virus fluctuated cyclically and appeared to be downregulated. Typical defective interfering particles, with large genome deletions, were not produced; however, an accumulation of populations of S, M and L RNAs with short deletions at both ends, primarily within the terminal 20 nucleotides, was observed. The length and concentration of the L RNA deletions were found to vary during persistence and correlate with reduced levels of viral replication. We postulate that these terminally deleted RNAs have a causal role in downregulating replication and viral gene expression[43]. A model has been proposed in which terminal nucleotide deletions arise via the nuclease activity of the viral polymerase. According to this model, nucleotide deletions extending beyond the panhandle region would severely curtail replication. Further studies are needed to corroborate these findings and to determine if similar genome changes occur in persistently infected rodents.

Questions for future research

- How long do hantaviruses persist in their natural rodent reservoirs?
- Which host immune mechanisms contribute to viral persistence?
- Does the age of the rodent at the time of infection influence the duration of viral persistence?
- Do terminal nucleotide deletions of the L, M or S RNAs occur in hantavirus-infected rodents, and do such deletions downregulate viral replication?

Conclusions and future prospects

Hantaviruses cause persistent infections in rodents and in cell culture that are marked by a short acute stage, during which the levels of infectious virus are high. Subsequently, there is a prolonged chronic stage when the infection is productive but the virus is usually present at much lower levels, which can vary cyclically. Viremia in rodents is transient, probably reflecting clearance by circulating neutralizing antibodies. The factors contributing to hantavirus persistence in rodents are not yet clearly defined. Laboratory investigations have used many different experimental conditions and strains of viruses (Table 2), and it is therefore not surprising that varying results have been obtained. The data available suggest that hantaviruses avoid detection and clearance by the host immune system by modulating viral replication (and presumably viral protein expression), so that infection is non-lytic and infectious virus is released from cells in low amounts at cyclical intervals. For any given hantavirus, this delicate balance occurs primarily between that hantavirus and the rodent species with which it has coevolved. Infections in other mammals are cleared. The only known exceptions are humans, in which infection is acute. Although small deletions at the termini of the genomic RNAs could indeed have a role in regulating replication, it is likely that other factors are involved. Many aspects of hantaviral persistence and the host immune response during persistence remain to be examined.

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References

1 Lee, H. and van der Groen, G. (1989) Hemorrhagic fever with renal syndrome. *Prog. Med. Virol.* 36, 62–102
2 Nichol, S.T. (1999) Genetic analysis of hantaviruses and their host relationships. In *Emergence and Control of Rodent–borne Viral Diseases* (Saluzzo, J.F. and Dodet, B., eds), pp. 99–109, Elsevier
3 Lee, H. *et al.* (1981) Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent *Apodemus agrarius*. *Am. J. Trop. Med. Hyg.* 30, 1106–1112
4 Slonova, R. *et al.* (1990) Persistence of the pathogen for hemorrhagic fever with renal syndrome in the body of a natural carrier and its relationship to epizootic and epidemic processes. *Voprosy Virusologii* 35, 250–253
5 Childs, J.E. *et al.* (1985) Geographical distribution and age related prevalence of antibody to Hantaan-like virus in rat populations of Baltimore, Maryland, USA. *Am. J. Trop. Med. Hyg.* 34, 385–387

6 Childs, J.E. *et al.* (1987) Epizootiology of hantavirus infections in Baltimore: isolation of a virus from Norway rats and characteristics of infected rat populations. *Am. J. Epidemiol.* 126, 55–68
7 Childs, J.E. *et al.* (1987) Prospective seroepidemiology of hantaviruses and population dynamics of small mammal communities of Baltimore, Maryland. *Am. J. Trop. Med. Hyg.* 37, 648–662
8 Arikawa, J. *et al.* (1986) Epidemiological studies of hemorrhagic fever with renal syndrome (HFRS) related virus infection among urban rats in Hokkaido, Japan. *Arch. Virol.* 88, 231–240
9 Morita, C. *et al.* (1993) Inability of a strain of Seoul virus to transmit itself vertically in rats. *Jpn. J. Med. Sci. Biol.* 46, 215–219
10 Zhang, X. *et al.* (1988) Role of maternal antibody in protection from hemorrhagic fever with renal syndrome virus infection in rats. *Arch. Virol.* 103, 253–265
11 Lee, H. *et al.* (1985) Study of horizontal and vertical transmission of Hantaan and Seoul virus in *Apodemus agrarius* and in rats. *J. Kor. Soc. Virol.* 15, 55–63
12 Dohmae, K. and Nishimune, Y. (1995) Protection against hantavirus infection by dam's immunity transferred vertically to neonates. *Arch. Virol.* 140, 165–172
13 Tanishita, O. *et al.* (1986) Persistent infection of rats with haemorrhagic fever with renal syndrome virus and their antibody responses. *J. Gen. Virol.* 67, 2819–2824
14 Zhang, F. (1984) Seroepidemiological investigation on hemorrhagic fever with renal syndrome III. Carrier state and familial aggregation. *Chin. J. Epidemiol.* 5, 5–8
15 McKee, K.T., Jr *et al.* (1985) Hantaan virus infection in suckling mice: virologic and pathologic correlates. *J. Med. Virol.* 17, 107–117
16 Kim, G.R. and McKee, K.T., Jr (1985) Pathogenesis of Hantaan virus infection in suckling mice: clinical, virologic, and serologic observations. *Am. J. Trop. Med. Hyg.* 34, 388–395
17 Zhang, X. *et al.* (1989) Comparison of virulence between two strains of *Rattus* serotype hemorrhagic fever with renal syndrome HFRS virus in newborn rats. *Microbiol. Immunol.* 33, 195–206
18 Glass, G.E. *et al.* (1988) Association of intraspecific wounding with hantaviral infection in wild rats (*Rattus norvegicus*). *Epidemiol. Infect.* 101, 459–472
19 Nuzum, E. *et al.* (1988) Aerosol transmission of Hantaan and related viruses to laboratory rats. *Am. J. Trop. Med. Hyg.* 38, 636–640
20 Lee, H. *et al.* (1982) Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever from wild urban rats. *J. Infect. Dis.* 146, 638–644
21 Kariwa, H. *et al.* (1996) Modes of Seoul virus infections: persistency in newborn rats and transiency in adult rats. *Arch. Virol.* 141, 2327–2338
22 Morita, C. *et al.* (1985) Age-dependent transmission of hemorrhagic fever with renal syndrome (HFRS) virus in rats. *Arch. Virol.* 85, 145–149
23 Morita, C. *et al.* (1994) Different transmissibility of two isolates of Seoul virus from the same wild brown rat (*Rattus norvegicus*). *J. Vet. Med. Sci.* 56, 549–550
24 Niklasson, B. *et al.* (1995) Temporal dynamics of Puumala virus antibody prevalence in voles and of nephropathia epidemica incidence in humans. *Am. J. Trop. Med. Hyg.* 53, 134–140
25 Gavrilovskaya, I. *et al.* (1983) Features of circulation of hemorrhagic fever with renal syndrome (HFRS) virus among small mammals in the European USSR *Arch. Virol.* 75, 313–316
26 Yanagihara, R. *et al.* (1985) Experimental infection with Puumala virus, the etiologic agent of nephropathia epidemica, in bank voles (*Clethrionomys glareolus*). *J. Virol.* 55, 34–38
27 Lundkvist, A. *et al.* (1997) Cell culture adaptation of Puumala hantavirus changes the infectivity for its natural reservoir, *Clethrionomys glareolus*, and leads to accumulation of mutants with altered genomic RNA S segment. *J. Virol.* 71, 9515–9523
28 Bogdanova, S. *et al.* (1987) Persistent infection caused by hemorrhagic fever with renal syndrome virus in bank voles (*Clethrionomys glareolus*)-the natural host of the virus. *Mikrobiol. Zhur.* 49, 99–106
29 Alexeyev, O.A. *et al.* (1998) A minority of seropositive wild bank voles (*Clethrionomys glareolus*) show evidence of current Puumala virus infection. *Epidemiol. Infect.* 121, 419–425
30 Schmaljohn, C. and Hjelle, B. (1997) Hantaviruses: a global disease problem. *Emerg. Infect. Dis.* 3, 95–104
31 Green, W. *et al.* (1998) Tissue distribution of hantavirus antigen in naturally infected humans and deer mice. *J. Infect. Dis.* 177, 1696–1700
32 Netski, D. *et al.* (1999) Sin Nombre virus pathogenesis in *Peromyscus maniculatus*. *J. Virol.* 73, 585–591
33 Otteson, E.W. *et al.* (1996) Occurrence of hantavirus within the rodent population of northeastern California and Nevada. *Am. J. Trop. Med. Hyg.* 54, 127–133
34 Mills, J.N. *et al.* (1997) Patterns of association with host and habitat: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the southwestern United States. *Am. J. Trop. Med. Hyg.* 56, 273–284
35 Calisher, C.H. *et al.* (1999) Natural history of Sin Nombre virus in western Colorado. *Emerg. Infect. Dis.* 5, 126–134
36 Lyubsky, S. *et al.* (1996) Histopathology of *Peromyscus leucopus* naturally infected with pathogenic NY-1 hantaviruses: pathologic markers of HPS viral infection in mice. *Lab. Invest.* 74, 627–633
37 Hutchinson, K.L. *et al.* (1998) Pathogenesis of a North American hantavirus, Black Creek Canal virus, in experimentally infected *Sigmodon hispidus*. *Am. J. Trop. Med. Hyg.* 59, 58–65
38 Oldstone, M. (1991) Molecular anatomy of viral persistence. *J. Virol.* 65, 6381–6386
39 Arikawa, J. *et al.* (1994) Epizootiological studies of hantavirus infection among urban rats in Hokkaido, Japan: evidences for the persistent infection from the sero-epizootiological surveys and antigenic characterizations of hantavirus isolates. *J. Vet. Med. Sci.* 56, 27–32
40 Dohmae, K. *et al.* (1994) Experimental transmission of hantavirus infection in laboratory rats. *J. Infect. Dis.* 170, 1589–1592
41 Asada, H. *et al.* (1989) Cross-reactive immunity among different serotypes of virus causing haemorrhagic fever with renal syndrome. *J. Gen. Virol.* 70, 819–825
42 Schmaljohn, C.S. (1995) The molecular biology of hantaviruses. In *The Bunyaviridae* (Elliott, R.M., ed.), pp. 63–90, Plenum Press
43 Meyer, B.J. and Schmaljohn, C. (2000) The accumulation of terminally deleted RNAs may play a role in hantavirus persistence. *J. Virol.* 74, 1321–1331
44 Arikawa, J. *et al.* (1990) Coding properties of the S and the M genome segments of Sapporo rat virus: comparison to other causative agents of hemorrhagic fever with renal syndrome. *Virology* 176, 114–125
45 Antic, D. *et al.* (1991) Nucleotide sequence and coding capacity of the large (L) genomic RNA segment of Seoul 80-39 virus, a member of the *Hantavirus* genus. *Virus Res.* 19, 59–66

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