REVIEW ARTICLE

Hantaviruses

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Since the recognition of the hantavirus pulmonary syndrome (HPS) in the USA in 1993, interest in hantavirus diseases has intensified worldwide. It is clear that hantaviruses have been historically responsible for a variety of human illnesses. Hantaviruses form a separate genus within the *Bunyaviridae* family. There are currently >20 recognised sero/genotypes and many others are under investigation. Each hantavirus type appears to be specific to a different rodent host. Virus phylogeny very closely reflects rodent phylogeny. The different hantavirus types are associated with different types of disease both in terms of target organs and disease severity. Two major diseases are recognised: haemorrhagic fever with renal syndrome (HFRS) and HPS. HFRS is primarily a disease of the old world while HPS is only recognised in the Americas. Over the past few decades the understanding and recognition of hantavirus disease throughout the world have greatly expanded. The number of recognised virus types continues to grow, as does the spectrum of hantavirus disease. There is evidence of hantavirus causing human disease in the British Isles, but at present it remains a largely uncharacterised disease.

Introduction and historical context

Haemorrhagic fever with renal syndrome (HFRS) has been recognised in the medical literature of Europe and Asia under many names [1, 2]. It has been suggested that the illness may have first been recognised as early as 1000 years ago in China [3]. Interest in hantaviruses has been stimulated by a marked increase in the recognition of infection throughout the world. During the Korean War (1950–1953), a form of HFRS, Korean haemorrhagic fever (KHF), was responsible for the hospitalisation of >3000 United Nations soldiers. Typically the disease presented as an acute prostrating febrile illness with renal failure and shock. Haemorrhagic manifestations developed in 30% of those affected. The mortality rate was *c.* 10% [4]. This outbreak attracted world attention. However, it became clear that this was not a new disease. It was recognised that there was close similarity to a long recognised milder disease in Scandinavia and the USSR, nephropathia epidemica (NE). A full description of the typical NE presentation had been published in 1934 [5] and it was postulated that the two might share a common aetiology. Despite much research, the agent of this

disease remained unknown until 1978, when a new virus, Hantaan virus, named after the Hantaan river, was isolated by passage in its rodent host, *Apodemus agrarius* (striped field mouse). Antibody to antigen in lung was demonstrated [6]. The virus was eventually isolated in cell culture in 1981 in a susceptible human cell line, A-549 [7]. It is now clear that many wars other than the Korean war have been complicated by HFRS-type illnesses consistent with a hantavirus aetiology during the past 120 years, including the First and Second World Wars [8].

The outbreak of hantavirus pulmonary syndrome (HPS) in the south-western USA, starting with a cluster of deaths in May 1993 and with a very high fatality rate in the initial outbreak [9], changed the recognised spectrum of hantavirus disease (HVD). The presentation was primarily a febrile illness complicated by the rapid development of respiratory failure; renal and haemorrhagic manifestations were not pronounced. The emergence of HPS further increased world attention and research efforts in the diagnosis, control and treatment of hantavirus infections.

Taxonomic status and genetic organisation

Hantaviruses form a separate genus within the *Bunyaviridae* family. Unlike the other four genera in this family, the hantaviruses are not transmitted *via*

an arthropod vector. Like all members of the *Bunyaviridae* family, the genome is trisegmented. The hantavirus coding strategy is the simplest of the five genera of the *Bunyaviridae*. All three segments encode only one protein in the virus complementary sense. Although minor open reading frames have been noted in both virus sense and virus complementary sense, there is no evidence for protein products [10]. As with other *Bunyaviridae*, each of the three segments has a consensus 3'-terminal nucleotide sequence (AUCAUCAUC), which is complementary to the 5' terminal sequence and is distinct from those of the other four genera [11]. Such complementary sequences are capable of forming pan-handle structures, a consistent feature of the *Bunyaviridae* [12]. The pan-handles probably serve an important role in viral transcription and replication similar to other viruses with this structure, such as vesicular stomatitis virus and influenza virus, whose transcription and replication strategies are better understood. The genome consists of a large, 6530–6550 nucleotides (nt), segment (L) coding for the viral transcriptase, a medium (3613–3707 nt) segment (M) coding for a polyprotein cleaved by co-translational cleavage to form the two viral glycoproteins, and a small (1696–2083 nt) segment (S) coding for the nucleocapsid protein [10]. There is no evidence of an NSs protein which is present in the rest of the *Bunyaviridae* [13]. A short, 37–51 nt, 5' non-coding region (NCR) (virus complementary sense) is present on each of the three segments. A 3' NCR is also present: L segment 38–43 nt, M segment 168–229 nt, and S segment 370–730 nt [13]. The 3' NCR of the S segment is conserved with regard to length and sequence within hantavirus types, suggesting some functional role. However, between types it is very variable in length and sequence except for the terminal pan-handle sequence [14].

Morphology, physical and chemical properties

By electron microscopy, hantaviruses are roughly spherical with a diameter of 100 nm. A 5-nm bilayered membrane surrounding a granulo-filamentous interior composed of nucleocapsids is easily discerned. The morphology of hantaviruses differs slightly from the other *Bunyaviridae* genera in that an organised grid-like structure can be discerned within the interior [11]. Membrane projections of *c.* 6 nm are composed of the two glycoproteins designated G1 and G2. Like other enveloped viruses, hantaviruses are readily inactivated by heat, detergents, organic solvents and hypochlorite solutions. As with the other *Bunyaviridae* the buoyant density is *c.* 1.18 g/ml in sucrose gradients. The virion consists of >50% protein, 20–30% lipid and 2–7% carbohydrate [15]. Disruption of virions with non-ionic detergent releases nucleocapsids with a buoyant density of 1.18 g/ml.

Virus diversity within the hantavirus genus

Hantaviruses have been recognised in many different rodent populations throughout the world [8, 16]. There are currently >20 recognised sero/genotypes (Table 1) [17–37] and many others are under investigation. The criterion for the definition of type is either a distinct sequence-based phylogenetic position or, for those viruses that have been grown in cell culture, a four-fold difference in neutralisation titre between homologous and heterologous viruses [38]. Each hantavirus is specific to a different rodent or insectivore host. Virus phylogeny very closely reflects rodent phylogeny [36]. This implies that hantaviruses are very ancient infectious agents which have co-evolved with their rodent hosts. Within hantavirus types there may be distinct lineages reflecting geographical differences such as those seen in Hantaan (HTN) and Seoul (SEO) isolates from China [39].

The convention of naming putative new types after place names has become established, as has the use of a two, three, or four letter capitalised abbreviation (Table 1). All known hantaviruses have been isolated from murid rodents (Order: *Rodentia*, family: *Muridae*) except Thotta-palayam (TPM), which was isolated in India from a shrew (Order: *Insectivora*, family: *Suncus*). However, there have been isolated reports of isolation of hantaviruses from birds [40], from two species of bats [41] and from both rabbits and cats [16]. The significance of these non-rodent isolates remains unclear. The region of genome with the most sequence data for phylogeny is a 330-nt region of the M segment (nts 1987–2315). This small sequence has been validated as producing a phylogenetic tree identical to that formed with the whole M segment sequence [36]. When this small sequence is used, HTN, DOB, SEO and THAI form one distinct common lineage, while PUU, PH and SN form a second lineage.

Growth in cell culture

Hantaviruses are routinely cultured in Vero E6 cells where they do not readily cause any cytopathic effect (CPE). Other cell lines that support the growth of hantaviruses include hybridoma cells [42], primary human adult endothelial cells [43], primary human umbilical vein endothelial cells [44], murine macrophage-like continuous cell lines [45], primary rat peritoneal exudate cells and primary rat macrophages and primary human adherent mononuclear cells [46], a large number of human continuous cell lines of lung, kidney, liver and salivary origin and primary human kidney cells [47]. There is no evidence of CPE in any of these cell types. It has been noted that infection of cells with some enveloped viruses, including hantavirus, results in cell fusion under acidic conditions

Table 1. Hantavirus types, their hosts, distribution and disease associations

Geno/serotype	Host	Distribution	Disease	Reference
Andes (AND)	<i>Oligoryzomys longicaudatus</i> (long tail pygmy rice rat)	Argentina	HPS (renal variant)	[17]
Bayou (BAY)	<i>Oryzomys palustris</i> (rice rat)	North America	HPS (renal variant)	[18]
Black Creek Canal (BCC)	<i>Sigmodon hispidus</i> (cotton rat)	North America	HPS (renal variant)	[19]
Cano Delgadito	<i>Sigmodon alstoni</i>	South America	Unknown	[20]
Dobrova (DOB)	<i>Apodemus flavicollis</i> (yellow-necked field mouse)	Balkans	HFRS (severe)	[21]
El Moro Canyon (ELMC)	<i>Reithrodontomys megalotis</i> (American harvest mouse)	North America	Unknown	[22]
Hantaan (HTN)	<i>Apodemus agrarius</i> (field mouse)	Asia	HFRS (severe)	[23]
Isla Vista (ILV)	<i>Microtus californicus</i> (Californian vole)	North America	Unknown	[24]
Khabarovsk (KBR)	<i>Microtus fortis</i> (reed vole)	Asia	Unknown	[25]
Laguna Negra (LN)	<i>Calomys laucha</i> (vesper mouse)	South America	HPS	[26]
Lechiguanas	<i>Oligoryzomys flavescens</i>	South America	HPS	[27]
New York (NY)	<i>Peromyscus leucopus</i> (white-footed mouse)	North America	HPS (prototype)	[28]
Oran	<i>Oligoryzomys longicaudatus</i>	South America	HPS	[27]
Prospect Hill (PH)	<i>Microtus pennsylvanicus</i> (meadow vole)	North America	Non-pathogenic?	[29]
Puumala (PUU)	<i>Clethrionomys glareolus</i> (red bank vole)	Northern and central Europe	HFRS (mild)	[30]
Rio Mamore (RM)	<i>Oligoryzomys microtis</i> (pygmy rice rat)	South America	HPS	[31]
Rio Segundo (RIOS)	<i>Reithrodontomys mexicanus</i> (harvest mouse)	North America	Unknown	[32]
Seoul (SEO)	<i>Rattus norvegicus</i> and <i>R. rattus</i> (rats)	SE Asia and world-wide	HFRS (moderate)	[33]
Sin Nombre (SN)	<i>Peromyscus maniculatus</i> (deer mouse)	North America	HPS (prototype)	[34]
Topografov (TOP)	<i>Lemmus sibiricus</i> (Siberian lemming)	Siberia	? 'Lemming fever'	[35]
Thailand (THAI)	<i>Bandica indica</i> (bandicoot)	Thailand	Unknown	[36]
Thottapalayam (TPM)	<i>Suncus murinus</i> (shrew species)	India	Unknown	[36]
Tula (TUL)	<i>Microtus arvalis</i> and other <i>Microtus</i> spp. (European common vole)	Europe	Unknown	[37]

The table includes viruses which have not been fully characterised but which have clear evidence presented indicating that they are unique. A number of other putative types have been reported.

[48–51]. A study to survey the appearance of CPE produced in this way by seven different hantaviruses indicated that CPE induced at low pH varies in appearance with virus type, SEO viruses producing large syncytia and PUU viruses producing small syncytia [52].

Infection in rodents

It appears that the presence of hantavirus in an individual rodent does not confer any survival disadvantage nor any detrimental effect on reproductive fitness. No effect on survival rate or rate of sexual maturation was noted in wild rats in Baltimore, although weight gain was slower [53]. However, rodents may have some histopathological changes associated with infection. Mononuclear infiltrates and oedematous alveolar septae have been demonstrated in both *Peromyscus leucopus* naturally infected with NY [54] and *P. maniculatus* naturally infected with SNV [55]. Some endothelial hyperplasia and evidence of inflammatory changes have been noted in *Clethrionomys glareolus* experimentally infected with PUU [56]. However, other studies have shown no obvious histopathological changes in virus-containing tissues of *C. glareolus* experimentally infected with PUU [57].

During natural and experimental infections, virus can be detected in many tissues including lung, spleen and kidney. Experimental infection of rodents is asymptomatic in most models studied, including intracerebral HTN infection of suckling mice [58], intramuscular PUU infection of *C. glareolus* [57] and infection of *Apodemus* with HTN [6]. However, experimental infection of some newborn rodents results in fatal disease [10, 59].

Once persistently infected, the rodent continues to secrete infectious virus for prolonged periods, perhaps for life, despite the presence of neutralising antibody. The transmission of hantavirus in rodent species occurs between adult animals, and vertical transmission is unimportant both in wild and experimental settings [60]. However, infection has been cleared from an infected laboratory colony of rats by use of caesarian section and seronegative fostering [61]. Seropositivity in juvenile animals is much less common than in adults and the highest antibody prevalence is generally in the oldest animals [62, 63]. Bites and fighting have been implicated in transmission between adult rats [63, 64]. In some rodents such as *Peromyscus* and *Reithrodontomys* spp. a higher prevalence of infection has been reported in male animals [32, 60, 63], but one large study of almost 2000 *P. maniculatus* showed no

significant sex difference in infection rate [65]. There is no sex difference in prevalence of infection in rats [66].

Hantavirus disease: epidemiology, transmission and clinical features

General remarks

World-wide, it has been estimated there are between 60 000 and 100 000 hospitalised cases of hantavirus disease annually, with the bulk of these occurring in China [8]. For most hantavirus infections in humans, asymptomatic or non-specific mild infections probably outnumber the symptomatic, characteristic infections. Transmission among rodents and to humans is generally *via* respiratory secretions, urine and saliva and the aerosol route is felt to be most important [67]. However, bites may also be a route of infection as there is evidence that bites may play a role in transmission among rats [64] and *Peromyscus* [68]. There is a report of human infection after a rodent bite [69]. Generally occupational risk is a dominant factor, with occupations such as animal trappers, forestry workers, farmers and military personnel at highest risk [8, 70]. Spring and summer rises in prevalence of HFRS in Asia are related to greater human contact with rodents during seasonal sowing/planting and harvesting activities [15]. The start of the annual peak incidence of disease in Sweden coincides with the first frost in October when rodents take refuge in barns and other farm buildings [71]. Various epidemiological investigations have implicated heavy farm work, threshing, sleeping on the ground, participation in field military exercises and low socio-economic status [60]. Human epidemics are often a consequence of increases in rodent host population resulting from climatic and environmental changes, changes in agricultural practices that favour human-rodent contact, or natural cyclical variation in rodent populations. Childhood infections are generally uncommon and men are more commonly affected than women, reflecting occupation. Laboratory- and animal facility-acquired hantavirus disease is well recognised throughout the world, arising from contact with naturally infected wild rodents [72], experimentally infected laboratory rodents [73] and from laboratory rodents with unsuspected infections [74, 75]. Human disease caused by hantaviruses present in continuous cell lines has also been reported [42, 76]. There were 33 outbreaks of HFRS from 1976 to 1985 among personnel in laboratory animal facilities in Korea and Japan [8]. Until recently there has been no evidence of person-to-person transmission of any hantavirus and such transmission was thought unlikely [77]. However, recent reports regarding transmission of Andes virus in an outbreak of 18 cases of HPS in southern Argentina have changed this perception [78, 79]. Five of the cases were physicians treating the index or subsequent cases and the others had significant exposure to cases and no rodent contact.

The evidence from this outbreak suggests that three generations of passage occurred in man. The mode of transmission was most likely respiratory. A review of the epidemiology of hantavirus transmission in the USA in the light of the data from Argentina concluded that there was no evidence of person-to-person spread there [80].

The clinical presentation of hantavirus disease is very variable both in terms of the predominant organ systems affected and in the severity of the illness. This is largely dependent on the type of hantavirus causing the infection. However, there are two well-recognised broad clinical presentations, HFRS and HPS [81]. In addition to the classical presentations of these syndromes (described below), a variety of other symptoms and signs have been reported including central nervous system and gastrointestinal manifestations.

HFRS caused by Hantaan (HTN) and Dobrova (DOB) virus infection

The most severe form of HFRS is caused by HTN in eastern Asia and DOB in former Yugoslavia and other parts of Europe. This severe form manifests clinically with the long recognised acute triad of fever, haemorrhage and renal failure [82]. The classical course of severe HFRS can be considered to have five recognised phases: febrile, hypotensive, oliguric, diuretic and convalescent [83, 84]. The febrile phase usually has an abrupt onset with no prodrome and is accompanied by headache and myalgia and lasts 3–7 days. This is then followed by a hypotensive phase with thrombocytopenia, petechial haemorrhages and proteinuria. Conjunctival injection and acute myopia with or without eye pain is a characteristic feature, although not always present. The hypotensive phase lasts hours or days and if severe haemorrhagic disease occurs, its onset is at this stage. The oliguric phase starts with the return of blood pressure to normal. It lasts for 3–7 days before the urinary output increases. The diuresis phase lasts for up to several weeks with the patient passing several litres of urine per day. Convalescence is usually prolonged, with the patient often not feeling back to normal for many months. The mortality rate is 5–10% [85] with deaths occurring due to shock and multi-organ hypoperfusion during the hypotensive phase or due to uraemia during the oliguric phase. Leucocytosis and haemoconcentration are usually present early in the illness and thrombocytopenia is often present even in the absence of haemorrhagic disease. Concomitant acute pancreatitis has been noted in some reports [86].

HFRS caused by Puumala (PUU) virus infection

PUU causes the mild form of HFRS, usually referred to as NE, which occurs throughout central and northern Europe, Russia and the Balkans [87]. Although the same temporal sequence of phases as in Hantaan virus

infections may be recognised, the phases are less clearly delineated. The disease is milder and the mortality is low, *c.* 0–0.2% of symptomatic cases [88, 89]. Severe haemorrhagic manifestations and shock do not occur, but mild haemorrhagic symptoms such as petechiae are seen in about one-third of patients. As with HTN infection, the distinctive acute myopia with or without eye pain is often present. Conjunctival injection tends to be less marked. Many cases are recognised and managed in the community without admission to hospital.

HFRS caused by Seoul (SEO) virus infection

SEO infections are responsible for the moderate form of HFRS and are mainly recognised in south-east Asia. However, SEO has been noted in rat populations throughout the world, as have cases of human disease [90–92]. Cases tend to occur more in urban areas, reflecting the distribution of the rodent reservoir. The disease tends to have a mortality rate intermediate between HTN and PUU (1–2%). The clinical presentation and course are very similar to HFRS caused by HTN and haemorrhagic manifestations are present in a significant proportion of those affected. SEO virus infections are associated particularly with the presence of hepatitis in a significant proportion of patients [93]. This is generally not present in other hantavirus infections, although there may be biochemical evidence of abnormal liver function as indicated by raised aminotransferase levels.

Hantavirus Pulmonary Syndrome (HPS)

HPS has been recognised in North America since 1993 [94]; >250 cases of HPS have been reported in North and South America [60]. The virus responsible for the initial outbreak in the Four Corners area was named Sin Nombre (SN) and its natural host is *P. maniculatus*, a sigmodontine rodent. It has since become clear that many other sigmodontine rodents in the Americas carry similar related viruses and that at least some are capable of causing HPS (Table 1). HPS has a very different presentation from HFRS in that renal involvement is not marked and haemorrhagic manifestations have not been noted. However, thrombocytopenia, haemoconcentration and leucocytosis are present as in HFRS. The illness generally progresses through three phases: prodromal, cardiopulmonary and convalescent [95–97]. The prodromal phase is generally a short non-specific illness characterised by fever, headache and myalgia, followed by rapid progression to the cardiopulmonary phase with severe respiratory insufficiency caused by non-cardiogenic pulmonary oedema and hypotension. Rhabdomyolysis is common. The case fatality rate is reported to be 50% [96], but was as high as 60% in the first series of cases [98]. Although renal failure is not a feature of HPS caused by SN and New York (NY) viruses, it is emerging that some of the more recently recognised viruses which cause HPS –

including Bayou (BAY), Black Creek Canal (BCC) and Andes (AND) – do have a much higher incidence of renal failure, suggesting that HPS and HFRS are not as clinically distinct as first perceived. For this reason it has been suggested [60] that the disease HPS should be thought of as having two variants: HPS (prototype) caused by SN and NY, and HPS (renal variant) caused by BAY, BCC, and AND viruses (Table 1).

Human disease caused by other hantaviruses

Many hantaviruses such as TUL, ELMC, ILV and KBR have no clear disease association at present (Table 1). This may be because human exposure to certain hantaviruses may be very infrequent. Some may be truly non-pathogenic for man. Prospect Hill (PH) virus has been recognised in North America since the early 1980s; however, no human disease has been noted despite the finding of seropositive individuals in groups such as American mammologists [99]. The lemming population is famously prone to large fluctuations following an approximate 40-year cycle. It has long been recognised in years of peak lemming population density that a distinctive illness ‘Lemming fever’ is present in people living in the same regions. The recent discovery of a new hantavirus, Topografov (TOP), in the Siberian lemming suggests that the historic disease may be hantavirus-mediated [100], and may account for an outbreak of disease in German troops plagued by lemmings in 1942 (a ‘lemming year’) in Lapland [90]. However, there are no current data linking TOP with human disease. It is likely that many more hantaviruses will continue to emerge and that associated human disease will be recognised.

Pathogenesis

Hantaviruses appear to have adapted to their natural hosts in a process of co-evolution producing chronic persistent infection with no disease. Transmission of these indolent viruses across a species barrier results in human disease. There are many parallels for this increased pathogenicity after the crossing of a species barrier, including herpes simiae transmission from monkeys of the genus *Macaca* to man [101], arena virus transmission from rodents to man [102] and morbilli virus transmission from dogs to large cats [103]. Man and animals other than the natural reservoir are ‘dead end’ or incidental hosts and are unimportant in the transmission and evolution of hantaviruses.

Virus type correlates closely with disease severity. Generally, hantaviruses originating from murid rodents of the subfamily *Sigmodontinae* are associated with HPS, those originating from the subfamily *Murinae* are associated with severe and moderate HFRS, while those originating from the subfamily *Arvicolinae* are associated with NE (Fig. 1). However, within any one type there is a wide spectrum of disease and

Fig. 1. Illustration of the parallel phylogeny between hantaviruses and their rodent hosts. The positions and lengths of lines do not imply precise phylogenetic relationships. Abbreviations are as given in Table 1.

asymptomatic cases are probably common with all hantavirus types. The pathogenesis of hantavirus disease remains incompletely understood [104]. The factors governing the localisation of virus and pathology in specific tissues and the full repertoire of cell types infected are not known. It has been demonstrated that pathogenic hantaviruses enter cells *via* beta-3 integrins, which are present on the surfaces of endothelial cells, macrophages and platelets. Beta-3 integrins are important in the regulation of vascular permeability and platelet function and the interaction of hantaviruses with these molecules may be central in hantavirus pathogenesis [105]. Specific antibodies are present early in the disease and are often detectable at presentation, and there is a suggestion that increased level of antibody correlates with more severe disease, suggesting an immunopathological mechanism [83, 106]. Neutralising antibodies have been shown to be directed to the envelope glycoproteins and humoral response alone is sufficient for protection [13]. T-Lymphocyte responses to HTN nucleocapsid protein have been demonstrated in man [107].

Vascular dysfunction appears to be the principal abnormality in HFRS and HPS [108]. Although hantaviruses have been shown to replicate in cultured human endothelial cells and are present in endothelial cells in HFRS [83] and HPS [109], there is considerable evidence that immune mechanisms rather than direct viral cytopathology are responsible for this abnormality [110]. Increased vascular resistance has

been noted in the acute phase of HFRS, but it is not clear whether this is mediated by glomerular or post-glomerular capillaries. Roles for the renin-angiotensin system, atrial natriuretic hormone and the vasoconstrictor endothelin have been suggested as mediators of altered vascular tone [111]. The origin of the thrombocytopenia is not understood. Unlike arena virus infections this does not appear to be on the basis of megakaryocyte infection. Nor is it consumptive in origin, as thrombocytopenia occurs in the absence of haemorrhagic manifestations or disseminated intravascular coagulation. The main histopathological findings in fatal cases of HFRS are haemorrhagic necrosis of the renal medulla with widespread tubular degeneration. In HPS, the main histopathological changes consist of interstitial pneumonitis with congestion, oedema and mononuclear cell infiltration and areas of hyaline membrane formation with an intact respiratory epithelium [109]. The oedema fluid has the characteristics of a transudate rather than an exudate.

The role of cytokine mediators in hantavirus disease has been investigated. An increased expression of tumour necrosis factor- α (TNF- α), transforming growth factor- β and platelet-derived growth factor are seen at the sites of the maximal inflammatory infiltration of the kidneys of patients with NE [112]. Venous plasma of HFRS patients has been shown to have elevated TNF- α , soluble TNF receptors, interleukin (IL)-6 and IL-10 [104]. TNF- α may mediate fever, chills, myalgia and hypotension [113], all of which are seen in hantavirus

disease. The presence of IL-6 and IL-10 is expected, as TNF- α induces the expression of these cytokines. In HPS, T cells act on heavily infected lung endothelial cells and it is suspected that γ -interferon and TNF mediate the increase in endothelial permeability that leads to severe pulmonary oedema [114]. The role of immune complexes has been suggested particularly as a mediator of the renal disease [111, 115], as kidney biopsies in HFRS reveal deposits of IgG, IgM and C3. IgE may be implicated in the pathogenesis of NE, as elevated plasma-soluble CD23 and Puumala virus-specific serum IgE have been documented during the acute phase of illness [116].

Host factors play an important role in determining severity of disease [110]. HLA haplotypes HLA B8 and DRBI*0301 have been shown to predict more severe disease in PUU infections [117, 118]. A genetic predisposition to high-level production of TNF- α via the TNF2 allele is significantly more frequent in hospitalised NE cases than in healthy controls [119].

The lack of animal models has been considered to be a significant obstacle in the development of an understanding of the pathogenesis of hantavirus disease [10]. A possible animal model for HPS is NY infection of *P. leucopus* [54]. In this model, lung pathology is significantly different from HPS in that no hyaline membrane formation occurs and there is no evidence of severe respiratory dysfunction. However, most of the histopathological changes noted are similar to those seen in human HPS cases. A promising model for HFRS is provided by cynomolgus macaques (*Macaca fascicularis*) infected with PUU via the tracheal route [120]. Infected animals developed an illness clinically, immunologically and histopathologically similar to NE; however, the renal disease produced was very mild.

Diagnosis

Serology

As with most virus infections, various diagnostic methods have been applied to hantavirus infections. Serology is the main diagnostic tool. Historically, indirect immunofluorescence (IIF) with native virus grown in E6 cells has been the most widely used serological test [6]. It remains a sensitive, group-specific assay and can be used to detect both IgM and IgG. Enzyme immunoassays (EIA) with both native and recombinant antigens have also been developed in a variety of assay formats including μ capture for very sensitive detection of IgM, facilitating earlier diagnosis [121]. With IgG assays based on the N protein, sera have been shown to fall into two groups of reactivity, either SEO-HTN-DOB-reactive or PUU-SN-reactive [122]. Such assays have been shown to be useful for high volume serological testing for large sero-prevalence surveys [123]. As hantaviruses haemagglutinate certain erythrocytes, haemagglutination inhibition

(HAI) can be used. The complement fixation test (CFT) has also been used. HAI and CFT offer no real advantages over IIF and EIA. The use of a strip immunoblot assay shows promise as a rapid antibody test for SNV [124] and could be applied to other hantaviruses. The assay format as developed is a strip immunoblot bearing four immobilised antigens of SNV and a recombinant N protein of SEO. The SNV antigens include a full-length recombinant-expressed nucleocapsid, a recombinant-expressed G1 protein and synthetic peptides derived from N and G1. Most diagnostic laboratories are already familiar with such assays for HIV and HCV diagnosis. The assay is rapid, robust, sensitive and specific and is potentially usable as a typing assay if the appropriate recombinant antigens are used. The plaque reduction neutralisation test (PRNT) is considered to be the gold standard serological test and, because it is type specific, it can be used to discriminate between infections caused by different hantaviruses. Although normally considered not to produce CPE, hantaviruses can be adapted by long-term serial passage in Vero-E6 cells with repeated selection of the largest plaques. Such plaque-adapted viruses are available from the American Type Culture Collection (ATCC). The plaques form after 14 days in monolayers of E6 cells and this can be used as the basis of a neutralisation test.

Virus detection

Antigen detection in neutrophils and peripheral blood mononuclear cells with polyclonal and monoclonal antibodies has been reported to be useful early in HFRS [125] and can be applied successfully to post-mortem tissues from fatal cases [109]. Isolation of hantaviruses from clinical specimens is often very difficult and, ideally, is best accomplished by first passing through laboratory rodents. Even when successful, primary isolation takes several weeks, as two or three passages may be necessary and therefore this approach is not useful diagnostically. Reverse transcriptase PCR with genus-specific or type-specific primers has been shown to be useful [58]. This was especially illustrated by the speedy investigation into the HPS outbreak in the south-western USA [94]. This was probably the first time that PCR played a prominent role in the acute investigation of a major outbreak of a new infectious agent. Although this investigation was a testament to the power of the molecular approach to viral diagnosis it should be remembered that it was the traditional approach with EIA and IIF that narrowed the search to the *Hantavirus* genus.

Treatment and prevention

Specific antiviral drug treatment

In-vitro and animal studies suggest that replication of hantaviruses is inhibited by ribavirin [126] and α -

interferon [127]. Ribavirin is often used in the treatment of hantavirus disease in the Peoples Republic of China and clinical trials there have shown that ribavirin therapy can significantly reduce the mortality rate in HFRS if given in the first 5 days after onset [128]. However, a trial of intravenous ribavirin in HPS was inconclusive, but further studies on the use of ribavirin in HPS are under way [96] or being planned [97]. The use of α -interferon has been shown to have no effect on mortality and to have minimal influence on clinical course in HFRS in the Peoples Republic of China [129]. The use of tragacanthin polysaccharides has been suggested as a potential therapeutic approach to hantavirus disease, as these compounds have been shown to have antiviral activity against other bunyavirus infections in mice [130].

General management

The mainstay of treatment in all serious hantavirus disease is general supportive therapy. Maintenance of intravascular volume and cardiac output with inotropic support if necessary and the management of fluid and electrolyte balance are the main elements of this approach [97]. The use of acute peritoneal dialysis and haemodialysis can be life-saving. In HPS, treatment is similarly based on close intensive care monitoring, and cardiovascular support with inotropic and vasopressor drugs. Blood gas monitoring and the use of mechanical ventilation and oxygen are most important aspects [96]. If disseminated intravascular coagulation occurs, heparin and platelet infusions are indicated. Extracorporeal membrane oxygenation (ECMO) has been found useful as a rescue therapy in patients with severe HPS [131].

Vaccines

A variety of vaccines has been developed by use of both killed virus and recombinant DNA technology. Formalin-inactivated vaccines (SEO and HTN) have been shown to produce neutralising antibody [132] and an SEO-derived vaccine protected baby mice from challenge with both SEO and HTN [133]. Such vaccines have been shown to be safe and immunogenic in man [134]. Formalin-inactivated HTN vaccines used in trials in Korea have demonstrated a 75% neutralising antibody response rate; however, the response was short-lived [135]. Recombinant vaccines have also been developed. Approaches utilising baculovirus- and vaccinia-expressed viral glycoproteins have been shown to protect against challenge in animal models [13]. There is some evidence that baculovirus-expressed N protein can also induce protection, which is mediated by cytotoxic T lymphocytes and is potentially cross-reactive against other hantavirus types [13]. A baculovirus vaccine expressing both M and S segment products of Hantaan virus is currently being evaluated in human clinical trials, as is a vaccinia-based vaccine

[136]. Development work on DNA vaccines directed at HPS and HFRS is ongoing [136, 137].

Rodent control

Potentially the most effective means of control of hantavirus disease is by limiting contact with rodents and their excrement. Monitoring of hantavirus prevalence in rodent populations may give some warning of expected increases in numbers of human cases [68, 138]. Attention to environmental factors, such as the increase in precipitation associated with the 1992–1993 El Nino, which may indirectly increase the risk for Sin Nombre virus exposure, may be of value in disease prevention [139]. The application of simple rodent-proofing measures to dwellings has been shown to eliminate or substantially reduce exposure to *P. maniculatus* [140]. Similarly working practices and conditions in agriculture, forestry and military activities should be modified where possible to reduce human rodent exposure. General precautions for residents living in affected areas have been produced [98] and deal with the elimination of rodents inside the home, prevention of rodents from entering the home and reduction in rodent food and shelter near homes. Guidance for hikers and campers has also been produced [97].

In laboratory animal facilities all laboratory work involving the propagation of hantaviruses in cell culture or animals should be conducted in biosafety level III conditions. Generally high standards of animal husbandry and adherence to safety protocols must be used when dealing with experimental animals. Even in work with animals not known to be infected with hantavirus, protocols should minimise potential contact with secretions.

Hantaviruses in the British Isles

Hantaviruses in Great Britain (GB)

Sero-epidemiological studies (1985–1989) in GB have revealed a hantavirus seroprevalence rate of 5–10% in serum specimens from suspected cases of leptospirosis, rickettsial and arboviral infection [1]. These patients were generally from rural areas or had a job involving rodent contact (e.g. sewage and agricultural workers). Further studies on occupational groups revealed considerable evidence of past exposure to hantaviruses. Seropositivity rates were 12.5% of 122 nature conservancy workers in Scotland, 4.3% of 96 sewage and water workers, 21.5% of 130 farmers and 5.1% of 90 water sport enthusiasts [1]. The seroreactivity was considered to be PUU type-specific. Tests on sera received in the Public Health Laboratory in Taunton, Somerset, revealed 29 patients with an acute illness in whom IgG antibodies to hantavirus were detected by IIF. Only 4 of the 29 were positive for IgM [141–144]. The clinical picture that emerges from these descrip-

tions is of a drawn-out influenza-like illness. The most severe cases had a sore throat and swelling of the neck, face and upper limbs and an associated macular rash. Cervical and inguinal lymphadenopathy were prominent in 41% of cases. Abnormal liver function tests were present in 62% and clinical hepatomegaly was present in 24%. Most of the patients had occupations (farmers and sewage workers) or hobbies that brought them into potential contact with rodents. None of the patients had acute renal failure, although proteinuria or microscopic haematuria was detected in a few patients. This atypical constellation of features is significantly different from that described for hantavirus disease in the world literature, especially the absence of significant renal disease and the presence of lymphadenopathy and arthralgia in many patients. Although the reports state that sera were tested against HTN, PUU and SEO antigens it is not made clear to which antigen, if any, the predominant seroreactivity was directed. The PRNT was not done on any of these sera.

Some isolated case histories have been reported from elsewhere in GB in which acute renal failure was a feature. A 10-year-old boy living in a caravan next to a rat-infested scrapyards in Nottingham presented with acute renal failure requiring dialysis [145]. He had a 6-day history of vomiting, diarrhoea, abdominal pain, microscopic haematuria and had loin tenderness. Renal biopsy revealed an acute interstitial nephritis. Testing for IgM was positive by IIF, but again it is not clear in this case if there was a predominant type-specific reactivity. A case of hantavirus disease has been reported in a 21-year-old man from Glasgow, Scotland [146]. The clinical episode was characterised by acute renal failure, fever, conjunctivitis, macular rash, sore throat and, reminiscent of the more recent Somerset cases, there was marked cervical lymphadenopathy.

Sero-surveys of rodents in GB have revealed some evidence of hantavirus infection. A survey in Somerset found that hantavirus antibody was present in 4 of 100 *Rattus norvegicus*, 1 of 102 'mice' (it is not stated whether these were *Mus* or *Apodemus*) and none of 76 *C. glareolus* [144]. The type specificity of these seropositive animal sera was not reported. A sero-survey of *R. norvegicus* trapped on farms in England and Wales used EIA and IIF methodologies with HTN, PUU and SEO antigens [147]. This study revealed a seroprevalence of 4% in 127 rats. Of the five seropositive rats identified, one was reactive to SEO and four were reactive to HTN. To date there are no hantavirus isolates or genetic sequences originating from wild rodents in the UK. A sero-survey of cats in GB using EIA and IIF testing with HTN 76–118 antigens suggested that antibody to hantavirus was widespread among cats [148]. Antibody was demonstrated in 15% of 41 randomly investigated

domestic cats, many of which were known to be healthy; 23% of 81 chronically ill cats and 8% of 85 feral cats were seropositive.

Hantaviruses in Northern Ireland

Serological evidence of hantavirus in the human population in Northern Ireland has been sought in farmers and in cases of leptospirosis [149, 150]. It was noted in these studies that 4 (1.25%) of 320 farmers and 10 (24%) patients with current leptospiral infection were seropositive to SEO virus. A larger sero-epidemiological study was performed to assess the frequency of hantavirus seropositivity in a group of 627 Northern Irish patients presenting with symptoms suggestive of HFRS and 100 healthy controls [151]. Immunofluorescence screening of IgG to nine different hantavirus antigens revealed a seropositivity of 2.1% (15 of 727) with an almost exclusive reaction to SEO antigens.

The species diversity in the rodent population in Ireland is uniquely limited compared with the rest of Europe and even with the rest of the British Isles. The prevalent hantavirus of northern Europe is PUU and the reservoir for this virus is the bank vole (*C. glareolus*), which has been absent from Northern Ireland since the start of the last ice age 30 000 years ago. Its range in Ireland is limited to a small localised population in the extreme south-east (Cork and Kerry), where it was introduced from England in the middle of the last century [152]. Therefore, there is no host for PUU in Northern Ireland. A number of other rodent species such as *Micromys minutus* (harvest mouse), *Apodemus flavicollis* (yellow-necked mouse), *Arvicola terrestris* (water vole) and *Microtus agrestis* (field vole), that are found in GB are absent from Northern Ireland [153]. There are only three rodent species in Northern Ireland, *Mus domesticus* (house mouse), *Apodemus sylvaticus* (wood mouse) and *R. norvegicus* (brown rat) [153]. A survey of serological evidence of rodent hantavirus in Northern Ireland demonstrated antibodies to hantavirus in 11 (21.6%) of 51 *R. norvegicus*, 1 (3.2%) of 31 *Apodemus sylvaticus* and 17 (28.8%) of 59 *M. domesticus* [62].

Conclusion

Over the past few decades the understanding and recognition of hantavirus disease throughout the world has greatly expanded. The number of virus types recognised continues to grow, as does the spectrum of hantavirus disease. Although there is evidence that hantavirus causes human disease in the British Isles, both the viruses responsible and the diseases caused remain largely uncharacterised. Because the presentation of hantavirus in the British Isles is so variable and ill-defined at present, it is difficult to be dogmatic about indications for serological testing. It is reason-

able to consider serological testing in any patient who has a febrile illness and suspected rodent contact. Patients with negative tests for leptospirosis are a clearly defined group in whom hantavirus should be considered.

We are grateful for the support of the Royal Hospitals for a Clinical Research Fellowship for C.M. and to Mrs Dorothy Wyatt for critically reviewing the manuscript.

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