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Review

Arenaviruses and hantaviruses: From epidemiology and genomics to antivirals

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ABSTRACT

The arenaviruses and hantaviruses are segmented genome RNA viruses that are hosted by rodents. Due to their association with rodents, they are globally widespread and can infect humans via direct or indirect routes of transmission, causing considerable human morbidity and mortality. Nevertheless, despite their obvious and emerging importance as pathogens, there are currently no effective antiviral drugs (except ribavirin which proved effective against Lassa virus) with which to treat humans infected by any of these viruses. The EU-funded VIZIER project (Comparative Structural Genomics of Viral Enzymes Involved in Replication) was instigated with an ultimate view of contributing to the development of antiviral therapies for RNA viruses, including the arenaviruses and bunyaviruses. This review highlights some of the major features of the arenaviruses and hantaviruses that have been investigated during recent years. After describing their classification and epidemiology, we review progress in understanding the genomics as well as the structure and function of replicative enzymes achieved under the VIZIER program and the development of new disease control strategies.

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1. Introduction

Life-threatening RNA viruses emerge regularly, and often in an unpredictable manner. Yet, the very few drugs available against known RNA viruses have sometimes required decades of research for development. At present, very long delays between molecule identification and commercial application are common, and this situation is unlikely to change in the immediate future at least in part owing to the constant upgrade of safety procedures and regulations. Can we generate preparedness for outbreaks of the, as yet, unknown viruses? Here we address these questions for arenaviruses and hantaviruses, two groups of zoonotic RNA viruses that include several major human pathogens, and for which novel viruses have repeatedly been discovered during the last decade. To understand the current situation, we present a comprehensive review covering various aspects of epidemiology, evolutionary relationships, diseases in humans, and the means currently available for preventing and combating human infections. For each group of viruses, the final paragraph deals with objectives and achievements conducted by all partners within the VIZIER European research project.

The VIZIER (VIral enZymes InvolvEd in Replication) (http://www.vizier-europe.org/) project was set up to develop the scientific foundations for countering this challenge to society. VIZIER studied the most conserved viral enzymes (that of the replication machinery, or replicases) that constitute attractive targets for drug-design. The aim of VIZIER was to determine as many replicase crystal structures as possible from a carefully selected list of viruses in order to comprehensively cover the diversity RNA viruses, and generate critical knowledge that could be efficiently utilized to jump-start research on any emerging RNA virus. VIZIER was a multidisciplinary project involving (i) bioinformatics to define functional domains, (ii) viral genomics to increase the number of characterized viral genomes and prepare defined targets, (iii) proteomics to express, purify, and characterize targets, (iv) structural biology to solve their crystal structures, and (v) pre-lead discovery to propose active scaffolds of antiviral molecules.

2. Arenaviruses

2.1. Taxonomy and epidemiology

The eighth edition of the Report of the International Committee for Taxonomy of Viruses (ICTV) indicates that the *Arenaviridae* family contains a single genus *Arenavirus* including 22 viral species (Salvato et al., 2005). However, several new viruses (1 south American virus [Chapare], 4 north American arenaviruses [Skinner Tank, Big Brushy Tank, Tonto Creek, Catarina], and 4 African viruses [Kodoko, Morogoro, Merino Walk, and Lujo viruses]) were discovered very recently and therefore are not yet included in the ICTV list. On the basis of their antigenic properties, the arenaviruses form two groups: the Tacaribe serocomplex (New World) and the Lassa-Lymphocytic choriomeningitis serocomplex (Old World) (Salvato et al., 2005). Nucleocapsid antigens are antigenically similar for most arenaviruses, and quantitative relationships show the basic split between Old World and New World viruses. Individual viruses are immunologically distinct in neutralization assays, which depend on the specificity of epitopes contained in the envelope glycoproteins (Salvato et al., 2005).

Specific rodents are the principal hosts of the arenaviruses, the only exception to date being Tacaribe virus which was isolated from *Artibeus* fruit-eating bats. Each virus species is closely associated with a specific vertebrate species. Thus, the distribution of the host dictates the distribution of the virus. Lymphocytic choriomeningitis virus (LCMV) is the only arenavirus to exhibit a worldwide distribution due to its close association with *Mus musculus*, which has been disseminated on all continents mostly by association with human activities. Other arenaviruses are distributed either in the New World or in Africa. Humans usually become infected through contact with infected rodents, or via inhalation of infectious rodent excreta or secreta. The domestic and peridomestic behavior of rodent reservoir hosts in general is a major contributory factor to viral transmission from rodent to human. However, in most cases, transmission of arenaviruses to humans occurs following recreational or agricultural incursions into environments providing critical habitat for rodent hosts. Additionally, professionals handling infected rodents in the field or laboratory are at increased risk of infection (Sewell, 1995).

Perturbation of the environment due either to human activities (modern farming practices), or natural ecological changes (flooding, storms) may result in behavioral changes of the reservoir hosts. Such changes have been implicated in the emergence of human disease caused by arenaviruses, such as Machupo virus infection cases consecutive to flooding in the El Beni region of Bolivia. Lassa, Junin, Machupo, Guanarito, and Sabia viruses are known to cause severe hemorrhagic fever, in western Africa, Argentina, Bolivia, Venezuela, and Brazil, respectively (Peters et al., 1996). Lassa virus is believed to cause up to 300,000 annual infections with 30% morbidity and 16% mortality (McCormick et al., 1987; McCormick and Fisher-Hoch, 2002). These five viruses are included in the Category A Pathogen List, and considered Biosafety Level 4 (BSL-4) agents in the United States and in Western Europe.

LCMV, the family prototype, was first isolated in 1933 during serial passage in monkeys of human material obtained from a fatal infection in the first documented epidemic of St. Louis encephalitis (Armstrong and Lillie, 1934). LCMV is an agent of acute central nervous system disease (Barton and Hyndman, 2000), congenital malformations (Barton et al., 1993), and has recently been identified in organ-transplantation recipients (CDC, 2005; Fischer et al., 2006; Amman et al., 2007) and immunocompetent patients (Charrel et al., 2006; Emonet et al., 2007). Clusters of fatal infections in organ-transplanted patients have recently been reported

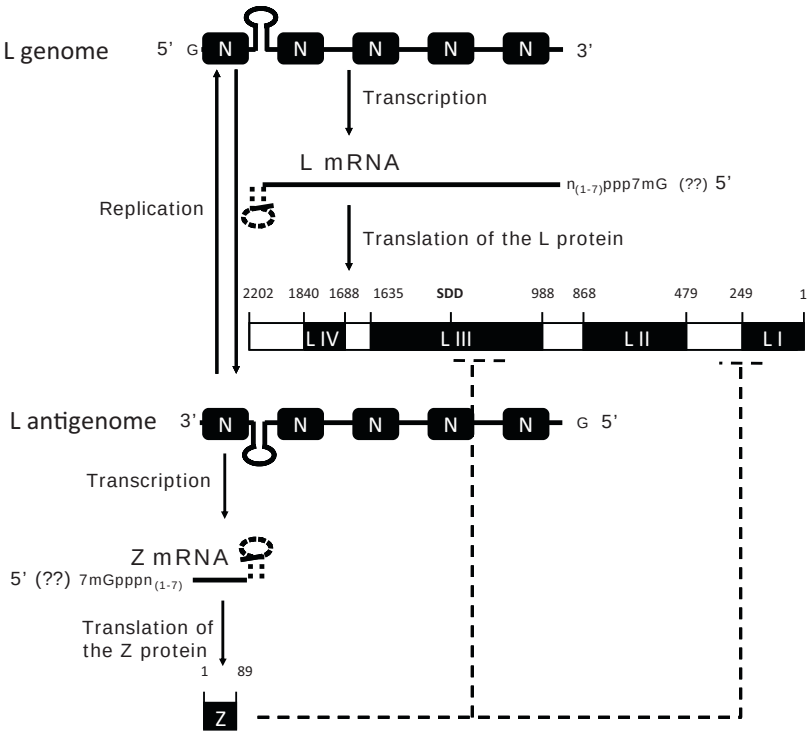

Fig. 1. Schematic representation of the replication and transcription mechanism of the Large L RNA genome of arenaviruses, leading to the translation of the Z and L proteins (adapted from Meyer and Southern, 1993). N is the Nucleoprotein interacting with both genome and antigenome. The L protein is shown with its four conserved domains LI, LII, LIII and LIV, where LIII is carrying the canonical SDD motif. Capping elements (n(1-7)ppp7mG) have been found on the N mRNA and an extra G is found at the 5′ of the genome and antigenome (Garcin and Kolakofsky, 1990). The amino acids positions correspond to the boundaries designed within VIZIER for the Parana virus L protein. The dashed lines illustrate the interaction between Z and L (adapted from Wilda et al., 2008) with the expected interacting regions within the L sequence.

and may constitute a new example of an emerging disease associated with a novel situation related to advances in medicine (Fischer et al., 2006; CDC, 2005, 2006, 2008; Palacios et al., 2008; Amman et al., 2007). Very little is known about the health consequences of human infection by other arenaviruses. Flexal and Tacaribe viruses have caused febrile illness in laboratory workers (Peters et al., 1996). Whitewater Arroyo virus may have been associated with three fatal cases of infection in California (CDC, 2000). Exposure to Pichinde virus has resulted in seroconversion without symptoms (Buchmeier, unpublished results). Tacaribe virus is believed to have caused a single case of a febrile disease which resolved with mild meningitis (J. Casals, unpublished data).

2.2. Genome organization and replication strategy

Arenaviruses possess single-stranded bi-segmented RNA genomes. Each of the two RNA segments encode two non-overlapping reading frames of opposite polarity: the viral RNA-dependent RNA polymerase (L protein) and a zinc-binding matrix protein (Z protein) for the large (L) genomic segment (~7200 nucleotides); the nucleocapsid protein (NP) and the glycoprotein precursor (GPC), secondarily cleaved into the envelope proteins G1 and G2, for the small (S) genomic segment (~3500 nucleotides). The genes on both S and L segments are separated by an intergenic non-coding region with the potential of forming one or more hairpin configurations. The 5′ and 3′ untranslated terminal sequences of each RNA segment possess a relatively conserved reverse complementary sequence spanning 19 nucleotides at each extremity.

The genome organization and ambisense nature of the arenavirus gene transcription strategy result in these viruses displaying characteristics of both (−) and (+) RNA viruses (Fig. 1). For RNA replication, the viral polymerase binds at the 3′ end of the templates, traverses the template RNA from end to end, and synthesizes a full-length complementary RNA. The 5′ ends of the S-derived subgenomic mRNAs extend beyond the ends of the genomic RNA templates (Garcin and Kolakofsky, 1990; Raju et al., 1990; Meyer and Southern, 1993). These non-templated extensions are variable in length (1–7 nucleotides) and terminate with 5′ cap structures. The process of "cap snatching" has been well documented for influenza viruses and bunyaviruses, but a similar mechanism for cap acquisition by arenaviruses has not been confirmed yet (Dias et al., 2009; Jin and Elliott, 1993). The arenavirus subgenomic mRNAs terminate within the intergenic noncoding region, and they are not polyadenylated (Singh et al., 1987; Southern et al., 1987). In many cases, it appears that transcription termination occurs on the distal side of the intergenic stem-loop structure so that the 3′ termini of the mRNA could be stabilized by the formation of terminal hairpin structures (Franze-Fernández et al., 1987; Meyer and Southern, 1993).

From the perspective of the replication machinery, the L protein (RdRp) must be adapted to synthesize RNA from an RNA template embedded into an RNP as well as from a naked RNA molecule. The L protein carries the SDD motif suggesting it is encodes the RdRp activity. It has been demonstrated that the L protein in association with the Nucleoprotein can replicate and transcribe the Tacaribe genome (Lopez et al., 2001). Two key residues within the Lassa virus domain III were shown to be involved in transcription but not replication (Hass et al., 2008). In the structural model proposed by Hass and collaborators, these residues would be positioned at the intersection of the palm and thumb of the polymerase. The priming reaction is likely to be different according to which RNA template is used. Indeed an original feature of this reaction is a resulting non-templated G molecule at the 5′ end of non-capped genomes and antigenomes (Garcin and Kolakofsky, 1990) whereas there may be a peculiar, though not unique to *Arenaviridae*, "cap snatching" or

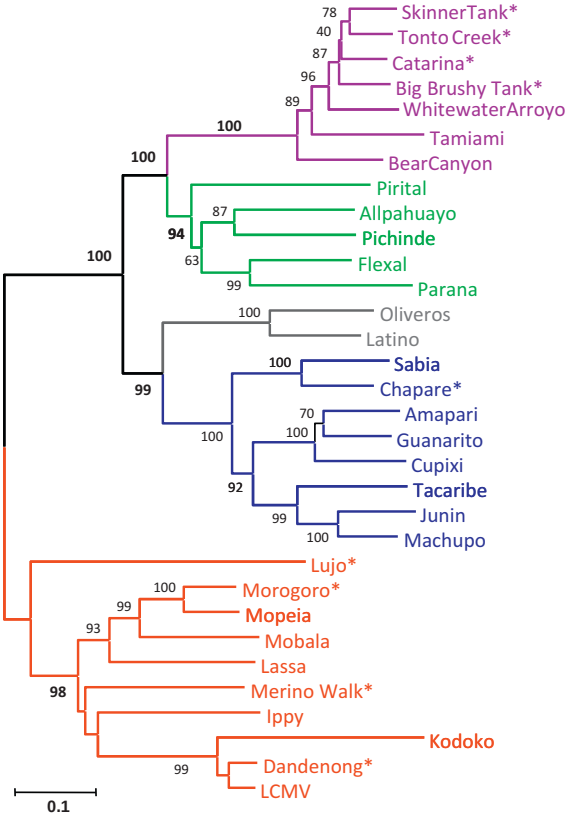

Fig. 2. Phylogeny of arenaviruses based on the analysis of complete amino acid sequences of the nucleoprotein (see Charrel et al., 2008). Phylograms were obtained by a combination of the pairwise distance method, neighbor-joining algorithm for tree reconstruction, and bootstrap analysis using 500 pseudoreplications. Evolutionary lineages are colored: red, Old World arenaviruses; green, clade A new world arenaviruses; blue, clade B new world arenaviruses, grey, clade C new world arenaviruses, purple, recombinant new world arenaviruses. *, newly discovered arenaviruses not included in the ICTV list.

primer/realignment mechanism in the case of mRNA. This latter mechanism has also been reported in influenza virus (Shaw and Lamb, 1984) and bunyavirus (Bouloy et al., 1990).

2.3. Recently discovered arenaviruses

Several arenaviruses have been discovered during the past three years in the Americas and in Africa. Their relative phylogenetic relationships with other members of the *Arenaviridae* are depicted in Fig. 2. Chapare virus was recently isolated from a fatal human case of hemorrhagic fever in the Chapare River region close to Cochabamba in Bolivia (Delgado et al., 2008). To date, there is no evidence for other cases in the same region or elsewhere. There is currently no information concerning its vertebrate host. Morogoro virus was isolated from *Mastomys sp* trapped in Tanzania (Vieth et al., 2007). This virus is most closely related to Mopeia virus regardless of the gene used for analysis (Charrel et al., 2008).

Dandenong virus was identified through a metagenomic approach, and then isolated from tissue specimens collected from a fatal case of infection in a patient who underwent kidney transplantation in Australia from a donor who had just completed a three-month visit to the former Yugoslavia, where he had travelled in rural areas (Palacios et al., 2008). Catarina, Skinner Tank, Tonto Creek and Big Brushy Tank viruses were recently discovered in the southwestern regions of the United States of America from Neotoma rodents (woodrats) (Cajimat et al., 2007, 2008; Milazzo et al., 2008). They appear most closely related to Whitewater Arroyo virus, another North American arenavirus. Kodoko virus was detected, but not isolated, in tissues of *Mus Nannomys minutoides* trapped in Guinea. Partial sequence analysis indicates that Kodoko virus roots LCMV (Lecompte et al., 2007).

Partial sequences of Pinhal virus (*Calomys tener* from Brazil) were obtained from Genbank with minimal information, the reason for which it has not been included in the phylogeny presented in Fig. 2; based on these partial genetic data, Pinhal virus appears to be most closely related to Oliveros virus (Charrel et al., 2008). Most recently, five cases of viral hemorrhagic fever (VHF) were reported to have been caused in South Africa by a novel arenavirus, provisionally named Lujo virus, which is most closely related to the Old World group viruses (Briese et al., 2009). Merino Walk virus (MWV) was isolated from a rodent, *Myotomys unisulcatus*, collected in South Africa, in 1985; MWV has been recently genetically and antigenically characterized and is proposed as a novel species within the Old World arenavirus complex (Palacios et al., 2010).

2.4. Evolutionary relationships among the arenaviruses

The first phylogenetic studies of arenaviruses were based on analysis of partial NP gene sequence in the S RNA segment. There was a strong correlation with their antigenic relationships (Bowen et al., 1996, 1997). Four major phylogenetic lineages were identified: the Old World lineage, the three New World lineages (Fig. 2). The Old World (Lassa-LCM serocomplex) lineage comprises seven viruses: LCM, Lassa, Mopeia, Mobala, Ippy, Morogoro and Kodoko virus. New world arenaviruses were subdivided into three lineages, designated A, B, C. Lineage A includes five South American viruses, Pirital, Pichinde, Flexal, Parana, Allpahuayo. Lineage B includes eight South American viruses, Sabia, Junin, Machupo, Guanarito, Amapari, Tacaribe, Cupixi and Chapare virus. Lineage C comprised two South American viruses: Oliveros and Latino.

Efforts to determine full-length genomes initiated in the early 2000s showed that Whitewater Arroyo virus, indigenous to North America, was a recombinant virus resulting from recombination between ancestors belonging to lineages A and B. Additional viruses indigenous to North America were also found to be recombinants since they possess a common ancestor with Whitewater Arroyo virus (Charrel et al., 2001). Accordingly a fifth lineage, A^{rec}, was described and includes viruses possessing a genome organization resulting from recombination in the S RNA segment: Whitewater Arroyo, Tamiami, Bear Canyon, Catarina, Skinner Tank, Big Brushy Tank, and Tonto Creek (Charrel et al., 2002, 2003; Archer and Rico-Hesse, 2002; Cajimat et al., 2007, 2008; Milazzo et al., 2008). This was the first time recombination had been described in the family *Arenaviridae*.

Since reassortant (inter-segmental recombination) arenaviruses had been generated experimentally (Lukashevich, 1992), sequences were analyzed to investigate whether or not field viruses may be the result of reassortment, but to date there is no evidence to support this possibility (Charrel et al., 2008). With the accumulation of complete genome sequences phylogenetic analyses were progressively updated. The most comprehensive study, based on the comparative analysis of complete sequences of the four genes for the largest set of viruses, was published recently (Charrel et al., 2008). In addition to placing new viruses in the family, the new data support and extend the previous analyses.

2.5. Arenavirus infections of humans

There are a variety of syndromes associated with arenavirus infection. In most cases, they depend on the virus causing the infection. Viral hemorrhagic fever can be caused by Lassa and Lujo viruses in Africa, or by Junin, Machupo, Sabia, Guanarito and Chapare viruses in South America (Peters et al., 1996; Delgado et al., 2008; Briese et al., 2009). It is likely that these viruses can also

cause less severe forms of infection, even non-symptomatic infections, but these aspects are poorly understood, and the relative proportions are unknown.

Infection by LCM virus can result in acute central nervous system disease and congenital malformations (Barton and Hyndman, 2000; Barton et al., 1993); it has recently been described as an important cause of fatal infection in organ transplantation recipients, and immunocompetent patients (CDC, 2005; Fischer et al., 2006; Amman et al., 2007; Charrel et al., 2006; Emonet et al., 2007; Palacios et al., 2008). LCM virus is currently the arenavirus for which the largest amount of data has been accumulated on pathogenesis, seroepidemiology, and array of clinical symptoms. However, its role remains obviously underestimated because of the very limited number of laboratories able to perform diagnosis (Asnis et al., 2010; Barton, 2010; De Ory et al., 2009).

Flexal and Tacaribe viruses have caused febrile illness in laboratory workers (Peters et al., 1996). Whitewater Arroyo virus may have been associated with 3 fatal cases of infection in California (CDC, 2000). Exposure to Pichinde virus has resulted in numerous seroconversions among humans without any noticeable clinical significance (Buchmeier, unpublished results). Tacaribe virus is believed to have caused a single case of a febrile disease with mild CNS symptomatology (J. Casals, unpublished data). Very little is known about the health consequences of infection with the other arenaviruses. The lack of data is mostly due to the absence of investigations in regions where these viruses circulate.

2.6. Vaccines

A live attenuated Junin virus vaccine (Candid#1) was produced in the early 1990s. Its efficacy was proven in a double-blind trial in 15,000 agricultural workers at risk of natural infection. Subsequently, more than 100,000 people were immunized in Argentina. A prospective study showed that Candid #1 vaccine efficacy was greater or equal to 84%, and no serious adverse effects were detected (Maiztegui et al., 1998). This vaccine was licensed in 2006 for use exclusively in Argentina, whereas in the USA Candid #1 remains only as an investigational new drug and studies addressing long-term immunity and safety have not been conducted. The current availability within the USA of a Candid #1 Master Virus Seed is uncertain and re-importation of Candid #1 vaccine from Argentina is likely to meet unsolvable obstacles due to foot-and-mouth-disease virus (FMDV) activity in several geographic regions of Argentina, and potential lack of FDA-compliant documentation (Emonet et al., 2011).

Currently there is no definitive evidence that immunity conferred by Candid#1 vaccine is protective against the other South American arenaviruses causing viral hemorrhagic fevers.

Several attempts to produce a satisfactory Lassa virus vaccine have been made during the past 30 years, some of which show significant promise. The most advanced projects include (i) a replication-competent vaccine based on attenuated recombinant vesicular stomatitis virus vectors expressing the Lassa viral glycoprotein showed that a single intramuscular vaccination elicited a protective immune response in nonhuman primates against a lethal challenge (Geisbert et al., 2005); (ii) ML29, a recombinant Lassa/Mopeia virus vaccine demonstrated protection against Lassa virus challenge in guinea pigs and Rhesus macaques (Lukashevich et al., 2008); (iii) a yellow fever 17D vaccine expressing Lassa virus glycoprotein precursor protected guinea pigs against fatal Lassa fever (Bredenbeek et al., 2006). Recently, a recombinant LCMV/Vesiculovirus vaccine was described that is attenuated, prevents lethal challenge with LCMV in mice, elicits rapid and long-lived cell-mediated immunity against lethal challenge with wild-type LCMV, confers rapid and long-lived cell-mediated protection against overwhelming systemic infection and liver disease, and presents no detectable gain in pathogenicity after propagation in immunodeficient hosts (Bergthaler et al., 2006).

Despite the urgent need for vaccines against arenaviruses and numerous scientific attempts to develop safe, effective and acceptable vaccines, there is currently no WHO-approved vaccine available.

2.7. Therapy of arenavirus infections

2.7.1. Immune plasma

The emergence of Argentine hemorrhagic fever in the 1950s with a mortality rate from 15 to 30% was first combated with immune sera or gamma globulins obtained from convalescent patients. The efficacy of this treatment was established through a double blind placebo-controlled trial organized from 1974 to 1978 that showed that case-fatality rate among cases treated with normal plasma was 16.5% while the rate in those patients treated with immune plasma was 1.1% (Maiztegui et al., 1979). However, alternative forms of treatment/prophylaxy need to be investigated because (i) immune plasma therapy lacks efficacy if given after 8 days of evolution (Enria et al., 1984; Enria and Maiztegui, 1994), (ii) there is a risk of transfusion-associated diseases, (iii) 10% of patients treated with immune plasma develop a late neurological syndrome (Maiztegui et al., 1979; Enria et al., 1985), (iv) the maintenance of adequate stocks of immune plasma is difficult due to the reduced number of cases.

2.7.2. Antiviral drugs

To date, ribavirin (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is the only antiviral approved and commercially available for arenaviruses. It has proved to be efficient in reducing fatality rates if administered at the early stage of infection Lassa fever virus (McCormick et al., 1986). Recently, there has been intense activity centered on molecules with potential antiviral activity against arenaviruses. High-throughput screening of molecules for their antiviral effects is being increasingly performed by both public laboratories and private companies. From 2003 to 2006, six articles reporting testing of molecules with antiviral efficacy on arenaviruses were published (Albiol Matanic and Castilla, 2004; Asper et al., 2004; Bolken et al., 2006; Castilla et al., 2005; Gunther et al., 2004; Uckun et al., 2005). To date, the most promising molecules are those which interfere with virus membrane fusion through the interaction of the G2 fusion subunit with the signal peptide, with an IC_{50} below 10 nM (Larson et al., 2008; York et al., 2008; Lee et al., 2008). N-substituted acridone derivatives showed *in vitro* efficacy against Junin virus with excellent selectivity indices. The inhibitory effect was exerted via blocking virus replication (Sepúlveda et al., 2008).

T-705 (6-fluoro-3-hydroxy-2-pyrazinecarboxamide), a substituted pyrazine derivative offered significant protection against Pichinde virus-infected hamsters when administrated orally after virus challenge (Gowen et al., 2007, 2008). An essential step in antiviral development is an appropriate animal model to document efficacy of molecules against arenaviruses; several models have been proposed and are summarized by Gowen and Holbrook (2008). Recently, MHC class I knock-out mice were shown to be susceptible to Lassa virus and thus could constitute a good animal model for Lassa virus infection (Flatz et al., 2010, PMID 20360949). AG129 IFN-α/β and -γ receptor-deficient mice were also successfully used to validate the antiviral of MY-24 against Tacaribe virus (Gowen et al., 2010).

2.8. The VIZIER quest for new antiviral drugs against arenaviruses

For arenaviruses, domain design was performed from the little genomics data available at the outset of the project. A total

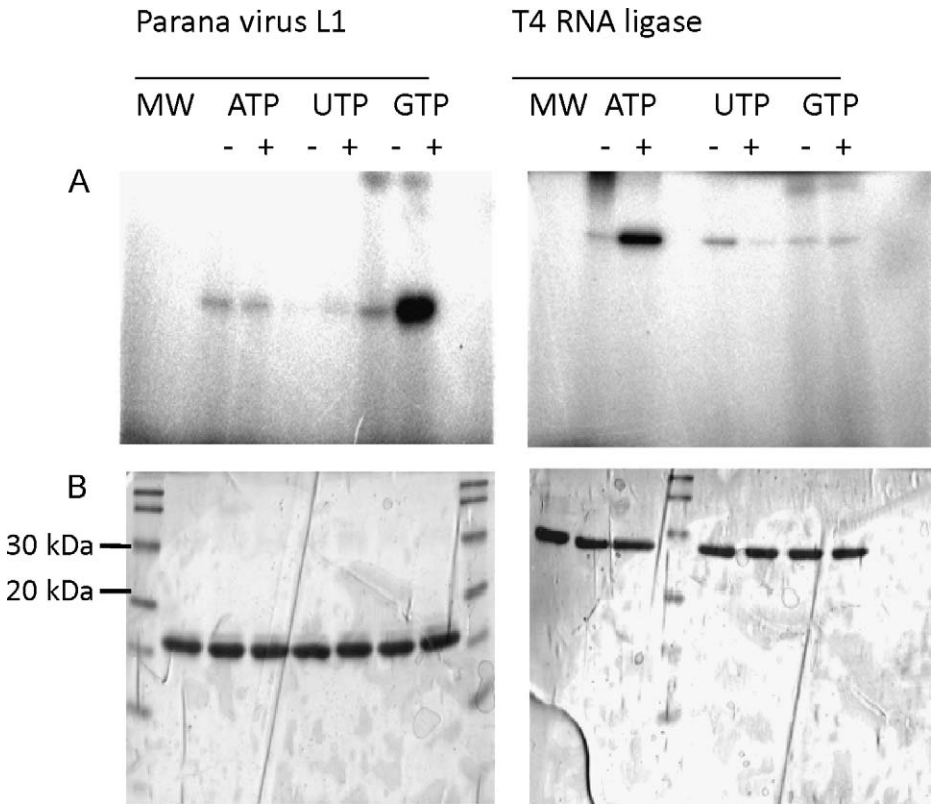

Fig. 3. NTP binding assay of Parana virus L1 domain (New England Biolabs). The experimental procedure was as described previously (Egloff et al., 2002). Briefly, 2 μg of protein was incubated in 10 μl Tris 10 mM NaCl 50 mM, pH 7.5, with either 0 or 50 μM (1 μCi) of [α-32P]ATP, [α-32P]UTP or [α-32P]GTP. The mixture was then UV-irradiated 3 min using a UV lamp (40 W, λ = 254 nm) at a 12 mm distance. Samples were then boiled before SDS PAGE electrophoresis. Wet and dried gels were analyzed by Coomassie blue staining (Panel B) or using a FujiImager to visualize radiolabelled products (Panel A), respectively. The T4 RNA ligase was used as a positive control for specific ATP binding.

of six full-length L RNA segments were sequenced and available from public databases at the outset of VIZIER. Efforts during VIZIER project led to the complete sequence characterization for a total of 15 additional arenaviruses listed in the ICTV catalogue, but also for new arenaviruses not yet listed in the ICTV, such as Morogoro virus (Charrel et al., 2008). So, VIZIER achievement in the field of genomics has to be considered as a major contribution not only to the project objectives, but also for the scientific community.

2.8.1. The search for a soluble sub-domain of the arenavirus L protein

No rational antiviral strategy has been followed to target specifically the replication machinery, probably because of our current meager knowledge of the L protein structure and function. The bottleneck for structural and functional studies is an appropriate production yield of pure recombinant L protein. To date, functional studies related to the L protein have been derived from replicon studies (Hass et al., 2008), or a minigenome transfected into infected cells (Lee et al., 2000). The arenavirus L protein plays an essential role in genome transcription and replication (Lopez et al., 2001). It consists of 2220 amino-acid residues. Because of its size, expression in heterologous expression systems remains problematic. Recently, the design of smaller subdomains has been successfully implemented to improve expression yields compatible with the requirements to obtain biochemical and structural data. For example, a random cloning approach based on deletion library screening selected a soluble, functional and crystallizable fragment of the influenza PB2 subunit (Guilligay et al., 2008).

Efficient domain design can also be achieved by combining bio-informatic and experimental data, as exemplified by the strategy used to produce crystallizable fragments of a pestivirus RdRp (Choi et al., 2004). Both random and rational strategies were available in the VIZIER pipeline (Coutard et al., 2008; Manolaridis et al., 2009; Gorbalenya et al., 2010). Extensive sequence analysis based on homologous proteins from similar viruses or different strains of Lassa virus led to the prediction of four domains encompassing residues 1–250, 493–887, 1007–1653, and 1757–1909, which were termed regions LI to LIV, respectively (see Fig. 1) (Vieth et al., 2004). A biological function was predicted exclusively for region LIII as the putative RNA-dependent RNA Polymerase, RdRp. For Tacaribe virus, both domains LI and LIII were recently shown to be involved in the interaction with the Z protein (Wilda et al., 2008). Taken together, these data led to the rational design of 4 domains for protein expression, the latter being shown in Fig. 1 for the Parana virus L protein.

Lassa virus domains LI and LIV were cloned into the pIVEX2.4d vector and successfully over-expressed using cell-free translation. The data indicated that these domains can fold independently. Crystals of LI domain from Lassa virus were obtained but exhibited only poor diffraction. Several alternative expression constructs for this domain, with different domain boundaries, were prepared in order to improve the quality of the crystals. These design efforts did not only rely on theoretical predictions (secondary structure, homology to corresponding domains of other arenaviruses), but also on limited proteolysis of larger fragments and subsequent analysis of the resistant stable domain cores. Experimental findings showed that significant improvement of the crystals could be achieved.

The domains LI to LIV were cloned for 14 other viruses (Parana, LCM, Ippy, Mopeia, Guanarito, Pirital, Allpahuayo, Bear Canyon, Cupixi, Amapari, Machupo, Sabia, Latino, Oliveros) to bring sequence diversity and improve thus the success rate of expression, crystallization, or crystal diffraction (Coutard and Canard, 2010).

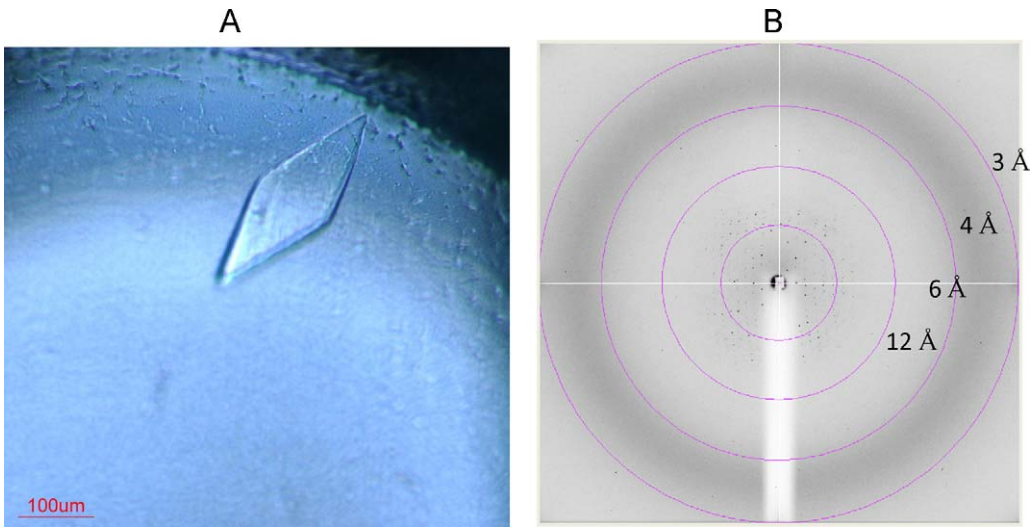

Fig. 4. Crystallization and crystal diffraction of the Parana virus L1 Domain. Panel A: Crystals of Parana virus L1 Domain were obtained from a two step screening procedure. Initial crystals hits were grown from commercial screens using the vapor diffusion technique in $(NH_4)_2PO_4$ 500 mM, Imidazole 100 mM pH 8.0. Crystals were then refined in a secondary screening procedure by adding two parts of the described crystallization solution to one part of screening solutions (as described in Jabafi et al., 2007). The best crystals were obtained in a solution made of 2 part of $(NH_4)_2PO_4$ 500 mM, Imidazole 100 mM pH 8.0 and one part of NH_4Cl 1.75 M. Panel B: Diffraction pattern of the obtained crystals. 3, 4, 6 and 12 Å resolution limits are indicated by purple circles.

The expression procedure followed a previously described protocol (Berrow et al., 2006) for expression in E. coli. Only 3 constructs were expressed in the soluble fraction. All of them correspond to an LI domain. When purified, only the domain of Parana virus was stable in a non-denaturing buffer (CHES 10 mM, NaCl 50 mM, pH 9). Surprisingly, the protein mass observed on SDS-Page stained with Coomassie blue was 5 kDa lower than expected (23 kDa instead of 28 kDa). The Mass Spectrometry analysis confirmed that about 50 amino acids were missing at the C-terminal end of the protein, probably resulting from proteolytic cleavage. Although the protein did not exhibit the expected mass, wide range functional and crystallization screenings were launched. In parallel, a smaller construct (1–196 aa) for Parana virus L was re-cloned as well as for 13 other arenavirus LI domains.

2.8.2. Preliminary functional data for the N-terminal end of the Parana virus L protein

The structure prediction of the Parana virus LI domain using PHYRE (Kelley and Sternberg, 2009) suggested that it could be a nucleotide binding domain. A nucleotide binding screening was assayed for the Parana LI domain, and the results are shown in Fig. 3. The Parana LI domain was found to bind GTP specifically, under experimental conditions requiring UV-cross-linking. In comparison, none of the tested conditions provided any significant binding of ATP or UTP These results represented an interesting starting point to elucidate progressively the LI function since (i) the 3′ end of the genome and antigenome is a G, (ii) an extra G was shown to be added at their 5′ end (Garcin and Kolakofsky, 1990), and (iii) mRNAs are capped following a probable cap-snatching pathway indicating that at least a G or ^{7m}GTP binding site may exist in the L protein (Fig. 1). The involvement of the LI domain in these three stages of viral replication/transcription was thus investigated.

2.8.3. Crystallization of the Parana virus LI protein domain

Both 1–196 aa and 1–249 aa constructs were used for crystallization trials. No improvement was observed with the shorter construct, compared to the degraded protein resulting from the long one. Nevertheless, despite many crystallogenesis efforts, X-ray diffraction could not be improved below a 6 Å resolution (Fig. 4). However, the strategy based on homologue screening with the 13 other arenavirus LI domains led to the crystallization of the cognate LCM virus LI domain, for which the crystal structure was determined at a 2.7 Å resolution (Morin et al., 2010).

2.8.4. Structural and functional study of LCM virus LI domain

The LCMV LI Domain consists of 4 β-strands surrounded by seven α-Helices. This organization was found to be related to the recently discovered N-terminal domain of influenza PA protein (Dias et al., 2009). Together with the conserved amino acid motif PD. . .(D/E)XK, the domain was proposed to be a type II endonuclease. This function was confirmed using enzymatic assays. Following mutational analysis and a cell-based replicon system, endonuclease knock-out was associated to a transcription-null phenotype, suggesting that this domain is involved in functional RNA production, probably in the cap snatching process. Sequence analysis suggested that the endonuclease was conserved among *Arenaviridae* and *Bunyaviridae*. This finding was concomitantly confirmed by the crystal structure determination of the La Crosse virus endonuclease (Reguera et al., 2010). Moreover, the structure determination of both LCMV and La Crosse virus endonucleases showed that they are very closely related to that of influenza virus. From an antiviral design point-of-view, it means that the ongoing efforts to design influenza endonuclease inhibitors may directly benefit to the neglected *Arenaviridae* and *Bunyaviridae* virus family. Indeed, 2,4-dioxo-4-phenylbutanoic acid, a potent inhibitor of the influenza endonuclease, is also active on La Crosse virus endonuclease and has been crystallized in its active site (Reguera et al., 2010).

2.8.5. Lassa virus Z protein

Arenaviral Z proteins are small zinc-binding proteins of 90–103 amino-acid residues. For LCM virus and Lassa virus, the Z proteins were shown to be the driving force for the budding of virus from infected cells (Strecker et al., 2003, 2006). The expression of Lassa virus Z protein alone in Vero cells is sufficient for the production and release of lipid-enveloped virus-like particles (Strecker et al., 2003). N-terminal myristoylation of the Lassa virus Z protein has been demonstrated to be essential for the interaction of the protein with host-cell membranes (Strecker et al., 2006). The arenaviral Z proteins have also been reported to interact with several host-cell proteins including the promyelotic leukemia protein (Borden et al., 1998a), the nuclear fraction of the ribosomal protein P0 (Borden et al., 1998b), and eIF4E (Campbell Dwyer et al., 2000).

For some arenaviruses, the Z protein has been reported to be involved in regulation of transcription and RNA replication (Strecker et al., 2003). Recently, the Z protein of Tacaribe virus has been shown to inhibit the polymerase activity of the L protein (see below; Wilda et al., 2008). The core of the Z protein contains a RING domain of about 60 residues, which is able to bind two zinc ions (Salvato and Shimomaye, 1989). The so-called late domains (PTAP and PPPY) at the C-terminus of the proteins are also required for the formation and release of virus-like particles (Strecker et al., 2003).

Within the VIZIER project, the Lassa Z protein was expressed with an N-terminal His tag in E. coli and purified by Ni-NTA chromatography. The findings suggest that the addition of metal ions to the growth medium affected the solubility and the oligomerization state of the protein. Z protein from non-metal-supplemented cultures consisted of a mixture ranging from monomers to polymers. In contrast to the observations reported by Garcıá et al. (2006), we were able to produce a stable and soluble, metal ion-free form of the Lassa virus Z protein. The addition of Zn^{2+} or Co^{2+} resulted in a narrower oligomer-distribution with a maximum corresponding to 12- to 14-mers (according to gel filtration and Dynamic Light Scattering). The metal content was determined by Atomic Absorption Spectroscopy (AAS) and by Proton-Induced X-ray Emission-spectroscopy (PIXE). The maximum metal-ion content was determined as two and three Zn^{2+} per protein, by AAS and PIXE, respectively. The nature and the location of a possible third metal-binding site are currently unknown. Studies to characterize the binding sites of ^{57}Fe-substituted Lassa virus Z protein by Mößbauer spectroscopy are in progress. Homogeneous protein fractions (according to Dynamic Light Scattering) yielded small crystals, which are currently being improved.

3. Hantaviruses

3.1. Taxonomy and epidemiology

Hantaviruses form a unique genus (*Hantavirus*) within the *Bunyaviridae* family. Twenty-three species are currently recognized by the ICTV but many recently discovered novel hantaviruses remain to be included. On the other hand, proposed new stringent species definition criteria may lead to a reduction in the number of species (Maes et al., 2009a,b). Hantaviruses differ from other bunyaviruses in one important ecological aspect: they are not transmitted by arthropod vectors. In common with the arenaviruses, their natural hosts are mainly rodents and the hantaviruses also have a strict association with specific natural hosts. Thus, by analogy with the arenaviruses their distribution is determined by that of their vertebrate host (Schmaljohn and Hjelle, 1997). They are dispersed and infect hosts via aerosolized rodent excreta and in the case of their natural hosts they produce an asymptomatic persistent infection. In contrast, infected humans may develop hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe, or hantavirus cardiopulmonary syndrome (HCPS) in the Americas.

Hantaan virus (HTNV) and Seoul virus (SEOV) are prominent examples of hantaviruses that cause HFRS in Asia. Approximately 150,000 HFRS cases are estimated to occur worldwide annually, more than 90% being reported from Asia where the most severe cases with fatality rates reaching 15% are recorded (Kariwa et al., 2007). The most common European hantavirus is Puumala virus which causes a mild form of HFRS often called *nephropathia epidemica.* Severe HFRS is also seen in Europe and is caused by Dobrava-Belgrade virus (DOBV) (Krüger et al., 2001; Klempa et al., 2008). Sin Nombre virus (SNV) from North America and Andes virus (ANDV) from South America are the most important representatives of viruses causing HCPS. Thus far, ANDV is the only known hantavirus that displays human-to-human transmission (Padula et al., 1998; Martinez et al., 2005; Ferres et al., 2007).

3.2. Genome structure and replication strategy

Hantaviruses are lipid-enveloped, spherical viruses of about 80–110 nm in diameter. They have a genome organization typical of the *Bunyaviridae* family, consisting of three negative-stranded RNA segments: a small (S) segment (~1700–2100 nucleotides) encoding the viral nucleocapsid (N) protein, a medium (M) segment (~3700 nucleotides) encoding the envelope glycoproteins G1 and G2 (co-translationally cleaved from a glycoprotein precursor), and a large (L) segment (~6500 nucleotides) encoding the viral RNA-dependent RNA polymerase (L protein). The L protein acts as a replicase, transcriptase, endonuclease and possibly, RNA helicase. The 5′ and 3′-terminal sequences of all three genome segments are genus-specific, highly conserved and display reverse complementarity to each other, thus being capable of forming panhandle structures. They are thought to play a role in viral transcription and in the proposed prime-and-realign mechanism of replication (Plyusnin et al., 1996; Maes et al., 2004; Khaiboullina et al., 2005).

3.3. Recently discovered hantaviruses

Over the past three years hantavirus research has passed two important milestones. The first was the discovery of hitherto unknown endemic hantaviruses in Africa and the second was the discovery of a group of novel, phylogenetically very distinct hantaviruses in non-rodent hosts.

Although several studies in African populations have shown the presence of antibodies that cross-react with Eurasian hantaviruses, no indigenous hantaviruses in African rodents were known until 2006 when the first genetic evidence for the presence of hantaviruses in Africa was reported. The first African hantavirus was found in the African wood mouse (*Hylomyscus simus*) trapped in a forest habitat in Guinea, West Africa, and named Sangassou virus (SANGV) after the village where the animal had been trapped (Klempa et al., 2006). A second African hantavirus was found in Guinea soon after and its discovery was even more surprising than the first one, thus initiating the search for hantaviruses in non-rodent hosts. Very divergent hantavirus sequences were found in Therese's shrew (*Crocidura theresae*). It was named Tanganya virus (TGNV), and is only distantly related to other hantaviruses, reflecting the fact that it was found in a shrew instead of a rodent (Klempa et al., 2007). Although HFRS and HCPS are not recognized diseases in Guinea, or Africa in general, the pathogenic potential of SANGV, TGNV, and probably other, yet undiscovered African hantaviruses should not be underestimated. Hantavirus-associated disease may be confused with other severe diseases or may be unrecognized because of limited health-care conditions.

Interestingly, Thottapalayam virus (TPMV) found in 1971 in Asian house shrew (*Suncus murinus*) was for decades considered to be the single exception of a hantavirus with a non-rodent reservoir. However, shortly after the discovery of the shrew-associated TGNV several other unique shrew-borne isolates of hantavirus were reported. These include the detection of Camp Ripley virus in the northern short-tailed shrew (*Blarina brevicauda*) (Arai et al., 2007), Ash River virus in the masked shrew (*Sorex cinereus*), Jemez Springs virus in the dusky shrew (*Sorex monticolus*) in the United States (Arai et al., 2008a), Cao Bang virus (CBNV) in the Chinese mole shrew (*Anourosorex squamipes*) in Vietnam (Song et al., 2007a), Seewis virus in the Eurasian common shrew (*Sorex araneus*) in Switzerland (Song et al., 2007b), and Imjin virus (MJNV) in the Ussuri white-toothed shrew (Crocidura lasiura) in Korea (Song et al., 2009). Most recently, the spectrum of hantavirus hosts was further extended to moles when Asama virus was found in the Japanese shrew mole (*Urotrichus talpoides*) trapped in Japan (Arai et al., 2008b) and Oxbow virus in the American shrew mole (*Neurotrichus gibbsii*) trapped in the United States (Kang et al., 2009).

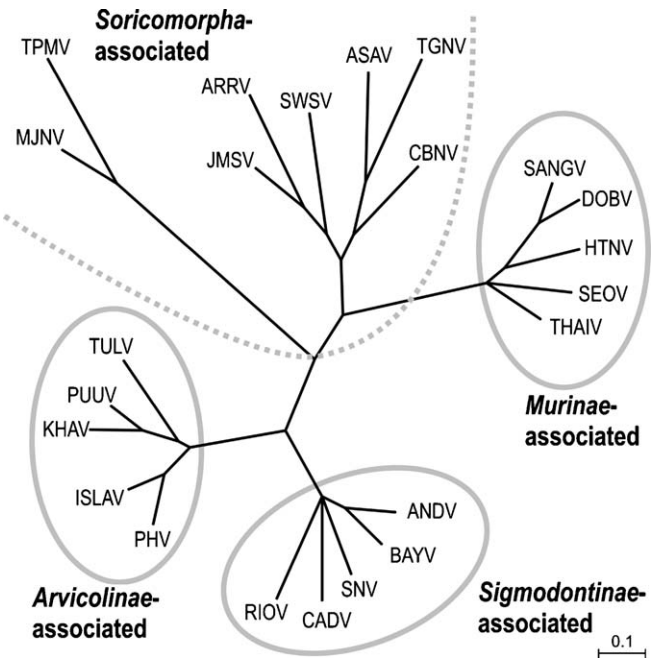

Fig. 5. Maximum likelihood phylogenetic tree of hantavirus representatives illustrating relationships between the three major groups of rodent-borne hantaviruses (indicated by grey ellipsoids) and novel shrew- and mole-associated hantaviruses (indicated by interrupted grey curve) based on partial N protein amino acid sequences (147 aa, position 217–365). The tree was computed with the TREE-PUZZLE package by using the JTT evolutionary model. The scale bar indicates an evolutionary distance of 0.1 substitutions per position in the sequence. SANGV, Sangassou virus; HTNV, Hantaan virus; SEOV, Seoul virus; DOBV, Dobrava-Belgrade virus; THAIV, Thailand hantavirus; PUUV, Puumala virus; TULV, Tula virus; KHAV, Khabarovsk virus; ISLAV, Isla Vista virus; PHV, Prospect Hill virus; SNV, Sin Nombre virus, ANDV, Andes virus; BAYV, Bayou virus; RIOV, Rio Segundo virus; CADV, Cano Delgadito virus; TPMV, Thottapalayam virus; MJNV, Imjin virus; TGNV, Tanganya virus; CBNV, Cao Bang virus; ARRV, Ash River virus; JMSV, Jemez Springs virus; SWSV, Seewis virus; ASAV, Asama virus.

3.4. Evolutionary relationships among the hantaviruses

Hantavirus species are strongly associated with one (or a few closely related) specific rodent species as their natural reservoir hosts, mainly rodents but, as recently reported, also insectivores (order *Soricomorpha*; shrews and moles). This close association is mirrored also in their phylogeny; rodent-associated hantaviruses form three major evolutionary clades corresponding to the three subfamilies of their rodent hosts. HTNV, SEOV, DOBV, as well as the recently discovered African SANGV are examples of *Murinae*-associated hantaviruses, PUUV and Tula virus (TULV) belong to the *Arvicolinae*-associated hantaviruses while SNV and ANDV are members of the group called *Sigmodontinae*-associated hantaviruses. Interestingly, the *Soricomorpha*-associated viruses do not seem to be monophyletic. At least with the currently available limited sequence data, there seem to be at least two main clusters of shrew- and mole-borne viruses but without a clear association to their hosts (Fig. 5).

The obvious similarities of hantavirus and rodent phylogenies have been the basis for the hantavirus-rodent co-evolution and co-speciation concept (Plyusnin et al., 1996). However, recent phylogenetic analyses and evolutionary rate calculations as well as recent findings of shrew- and mole-associated hantaviruses suggest that this concept will have to be re-evaluated (Ramsden et al., 2008, 2009). Moreover, high diversity and wide geographic distribution of the recently discovered hantaviruses together with the fact that insectivores and mice are very divergent in evolutionary terms make it reasonable to predict that other groups of mammals will be found to carry yet undiscovered hantaviruses in the near future (Henttonen et al., 2008; Klempa, 2009).

3.5. Hantavirus infections of humans

Both hantavirus diseases, HFRS and HCPS, are acute febrile infections. The initial symptoms are very similar and include abrupt onset of high fever, malaise, myalgia, back and abdominal pain, and other flu-like symptoms. HFRS is mainly characterized by renal failure and hemorrhages, varying from small petechiae to severe internal bleeding and the disseminated intravascular coagulation syndrome. On the other hand, pneumonia and cardiovascular dysfunction are characteristic for HCPS.

Common factors of hantavirus infections include increased permeability of the microvascular endothelium and thrombocytopenia. The complex pathogenesis of HFRS and HCPS is currently assumed to be a multifactoral process which includes T-cell mediated endothelial damage, immune effectors, and β_3-integrin dysfunction-mediated increase of vascular permeability (Krüger et al., 2001; Maes et al., 2004; Khaiboullina et al., 2005; Gavrilovskaya et al., 2008).

3.6. Vaccines

Different rodent brain and cell culture-derived inactivated vaccines are used locally in South Korea and China but the methods of their preparation are considered unacceptable for human vaccination in most western countries (Piyasirisilp et al., 1999). Hantavax®, a formalin-inactivated suckling mouse brain marketed in Korea demonstrated capacity to elicit neutralizing antibodies in only about half of the recipients (Cho et al., 2002; Sohn et al., 2001). Field trials have not been conducted, and a case–control study was inconclusive because of insufficient statistical power (Park et al., 2004). In China, HTNV- and SEOV-inactivated vaccines have been produced in cell cultures, and elicited neutralizing antibody responses in about 50% and 80% of recipients receiving three doses, respectively. An inactivated, bivalent vaccine (HTNV and SEOV), was also developed and tested in a total of 1100 persons in China: more than 90% of vaccines developed neutralizing antibodies to both HTNV and SEOV after 3 doses (Dong et al., 2005).

A variety of techniques, including recombinant proteins, virus-like particles and chimeric viruses as well as DNA vaccines have been investigated and found protective in animal trials (reviewed in Maes et al., 2009b). In the most recent approach, adenovirus vectors expressing hantavirus proteins protected hamsters against lethal challenge with ANDV (Safronetz et al., 2009).

To avoid some of the problems associated with cell culture or rodent brain-derived vaccines, two molecular vaccines for HFRS have been tested in humans. The first was a recombinant vaccinia virus (VACV)-vectored vaccine expressing the M and the S segments of HTNV (Woo et al., 2005). Low rate of neutralizing antibodies to HTNV were observed, and this vaccine has not been pursued. Recently, plasmid DNA delivered by gene gun has been developed and tested in animals (Hooper et al., 1999; Spik et al., 2008).

3.7. Therapy of hantavirus infections

3.7.1. Immune plasma

At present, there have been no published reports of controlled clinical trials of immunotherapy for HFRS or HCPS (Jonsson et al., 2008).

3.7.2. Antiviral drugs

Currently, no approved antiviral drug is available for specific treatment of the hantavirus diseases. The only antiviral drug which has been shown to have *in vitro* activity and to some extent also in

vivo activity against some hantaviruses is ribavirin (Severson et al., 2003). The efficacy of ribavirin therapy given to HTNV-infected suckling mice showed that the ribavirin-treated mice had a higher survival rate than the placebo control group (Huggins et al., 1986). Ribavirin was tested for efficacy in HFRS patients in China and shown to have a statistically significant beneficial effect if initiated early in the disease course (Huggins et al., 1991). More recently, results of a clinical study using intravenous ribavirin A in a total of 38 individuals enrolled between 1987 and 2005 were supportive of an efficacy demonstrated by a decreased occurrence of oliguria and decreased severity of renal insufficiency (Rusnak et al., 2009).

On the other hand, the results of trials in patients suffering from HCPS yielded disappointing results (Chapman et al., 1999; Mertz et al., 2004). Two double-blind, placebo-controlled efficacy trials have been performed in persons with HCPS in the cardiopulmonary phase (Chapman et al., 1999; Mertz et al., 2004). The majority of the patients were in the cardiopulmonary stage when they enrolled, and treatment with ribavirin had no clinical benefit, suggesting that its efficacy may depend on the phase of infection and the severity of disease when treatment is initiated and calling attention to the need for early intervention. The major problem is that progression to respiratory failure, shock and death typically occurs within hours of presentation in the cardiopulmonary phase, leaving little time for the study intervention to have an effect.

Recently, a diverse series of 3-substituted 1,2,4-triazole-beta-ribosides were prepared and one compound with antiviral activity was identified, 1-beta-D-ribofuranosyl-3-ethynyl-[1,2,4] triazole (ETAR). It showed promising antiviral activity against HTNV and ANDV. ETAR did not increase mutation frequency of the HTNV genome, which suggests it has a different mechanism of action from ribavirin. Mechanism and metabolism studies identified its activity as being primarily due to inosine monophosphate dehydrogenase inhibition with reduction of GTP pools, which was combined with residual complementary activity possibly affecting the L protein. Although ETAR protected suckling mice from infection with HTNV only to a degree comparable to the efficacy of ribavirin it is a promising scaffold for antiviral drug development (Chung et al., 2008).

Recently, other antiviral strategies have been evaluated such as the use of cyclic peptides which bind $\alpha_v\beta_3$ integrin as a virus receptor and thereby blocked SNV and HTNV infection of Vero E6 cells (Larson et al., 2005; Hall et al., 2007) or multivalent cyclic peptides presented on nanoparticles which specifically prevented SNV infection *in vitro* (Hall et al., 2008).

3.8. The VIZIER quest for antiviral drugs against hantaviruses

The main achievements of the VIZIER Program in the field of hantaviruses were obtained in the virus discovery and genomics part of the project. Identification of Sangassou virus as the first African hantavirus (Klempa et al., 2007) dramatically extended the dogmatic view about geographic range, evolution, and epidemiology of hantaviruses. Tanganya virus (Klempa et al., 2007) as the second African hantavirus was, moreover, the second shrew-borne hantavirus ever found (after TPMV, known for decades but considered exceptional) and initiated the recent boom of novel shrew- and mole-borne hantaviruses. Moreover, the novel genetic lineage of Dobrava virus causing severe HFRS cases in southern European Russia, and the Dobrava strain causing HFRS outbreaks in central European Russia were identified and genetically characterized (Klempa et al., 2008).

Unfortunately, the final goal of the VIZIER project, obtaining crystal structures, was not reached for hantavirus L protein domains. Nevertheless, intensive efforts were made in the field of hantavirus L protein domain predictions, expression, and solubility problems. Altogether, 85 expression constructs were prepared involving 20 different domain predictions/constructs for seven different hantavirus strains. The main effort was focused on the LI domain of still unknown function and the polymerase domain by itself. Different domain prediction approaches were used. First of all, tools developed within the consortium were used, such as Viralis and VaZyMoLo. Altogether, twelve soluble proteins of five different viruses were prepared, including two constructs of the LI domain, three constructs of the LII domain, and one construct of the N protein.

4. Conclusions

Although many studies are under way to discover and further investigate antivirals active against pathogenic arenaviruses and hantaviruses, there will be a significant delay before drugs may be available for patient treatment in hospital settings (approach #1). Therefore, the option to test licensed drugs for their efficacy against arenaviral and hantaviral infections should be considered and encouraged actively (approach #2). While on the face of it, this approach may appear unorthodox, it could provide the indisputable advantage of drastically reducing the delay between drug discovery and possible use as a treatment for patients. These two approaches should not be considered competitive, but complementary. They should be combined to bring active molecules against highly pathogenic viruses onto the market.In the case of both arenavirus and hantavirus protein constructs, we observed that the same constructs prepared using different viruses showed different expression levels for unknown reasons. Therefore the option of increasing the number of targets, on a purely empirical basis, may be efficient. Accordingly, using biodiversity, specifically point mutations selected in strains circulating in nature to overcome solubility, crystallization and diffraction problems showed to be an efficient strategy. Practical evidence demonstrated that increasing the number of viruses or strains, including a large diversity of clinical strains with minimal genomics heterogeneity, may be a successful option to obtain diffracting crystals. Usage of the wide spectrum of naturally occurring variants of the same viral protein to overcome solubility problems is therefore a very important lesson that can be taken from the VIZIER effort on hantaviruses. Although the obtained soluble proteins did not reach the crystallization stage during the VIZIER project, they provide a solid basis for further development in the near future.

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References

Albiol Matanic, V.C., Castilla, V., 2004. Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus. Int. J. Antimicrob. Agents 23, 382–389.

Amman, B.R., Pavlin, B.I., Albariño, C.G., Comer, J.A., Erickson, B.R., Oliver, J.B., Sealy, T.K., Vincent, M.J., Nichol, S.T., Paddock, C.D., Tumpey, A.J., Wagoner, K.D., Glauer, R.D., Smith, K.A., Winpisinger, K.A., Parsely, M.S., Wyrick, P., Hannafin, C.H., Bandy, U., Zaki, S., Rollin, P.E., Ksiazek, T.G., 2007. Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. Emerg. Infect. Dis. 13, 719–725.

Arai, S., Song, J.W., Sumibcay, L., Bennett, S.N., Nerurkar, V.R., Parmenter, C., Cook, J.A., Yates, T.L., Yanagihara, R., 2007. Hantavirus in northern short-tailed shrew, United States. Emerg. Infect. Dis. 13, 1420–1423.

Arai, S., Bennett, S.N., Sumibcay, L., Cook, J.A., Song, J.W., Hope, A., Parmenter, C., Nerurkar, V.R., Yates, T.L., Yanagihara, R., 2008a. Phylogenetically distinct hantaviruses in the masked shrew (textitSorex cinereus) and dusky shrew (Sorex monticolus) in the United States. Am. J. Trop. Med. Hyg. 78, 348–351.

Arai, S., Ohdachi, S.D., Asakawa, M., Kang, H.J., Mocz, G., Arikawa, J., Okabe, N., Yanagihara, R., 2008b. Molecular phylogeny of a newfound hantavirus in the Japanese shrew mole (Urotrichus talpoides). Proc. Natl. Acad. Sci. U. S. A. 105, 16296–16301.

Archer, A.M., Rico-Hesse, R., 2002. High genetic divergence and recombination in Arenaviruses from the Americas. Virology 304, 274–281.

Armstrong, C., Lillie, R.D., 1934. Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St Louis encephalitis epidemic. Public Health Rep. 49, 1019–1027.

Asnis, D.S., Muana, O., Kim do, G., Garcia, M., Rollin, P.E., Slavinski, S., 2010. Lymphocytic choriomeningitis virus meningitis, New York, NY, USA, 2009. Emerg. Infect. Dis. 16, 328–330.

Asper, M., Sternsdorf, T., Hass, M., Drosten, C., Rhode, A., Schmitz, H., Gunther, S., 2004. Inhibition of different Lassa virus strains by alpha and gamma interferons and comparison with a less pathogenic arenavirus. J. Virol. 78, 3162–3169.

Barton, L.L., 2010. Human infection with lymphocytic choriomeningitis virus. Emerg. Infect. Dis. 16, 1046–1047.

Barton, L.L., Budd, S.C., Morfitt, W.S., Peters, C.J., Ksiazek, T.G., Schindler, R.F., Yoshino, M.T., 1993. Congenital lymphocytic choriomeningitis virus infection in twins. Pediatr. Infect. Dis. J. 12, 942–946.

Barton, L.L., Hyndman, N.J., 2000. Lymphocytic choriomeningitis virus: reemerging central nervous system pathogen. Pediatrics 105, E35.

Bergthaler, A., Gerber, N.U., Merkler, D., Horvath, E., de la Torre, J.C., Pinschewer, D.D., 2006. Envelope exchange for the generation of live-attenuated arenavirus vaccines. PLoS Pathog. 2, e51.

Berrow, N.S., Büssow, K., Coutard, B., Diprose, J., Ekberg, M., Folkers, G.E., Levy, N., Lieu, V., Owens, R.J., Peleg, Y., Pinaglia, C., Quevillon-Cheruel, S., Salim, L., Scheich, C., Vincentelli, R., Busso, D., 2006. Recombinant protein expression and solubility screening in Escherichia coli: a comparative study. Acta Crystallogr. D: Biol. Crystallogr. 62, 1218–1226.

Bolken, T.C., Laquerre, S., Zhang, Y., Bailey, T.R., Pevear, D.C., Kickner, S.S., Sperzel, L.E., Jones, K.F., Warren, T.K., Lund, S., Kirkwood-Watts, D.L., King, D.S., Shurtleff, A.C., Guttieri, M.C., Deng, Y., Bleam, M., Hruby, D.E., 2006. Identification and characterization of potent small molecule inhibitor of haemorrhagic fever New World arenaviruses. Antiviral Res. 69, 86–97.

Borden, K.L.B., Campbell Dwyer, E.J., Salvato, M.S., 1998a. An arenavirus RING (zinc-binding) protein binds the oncoprotein promyelocte leukemia protein (PML) and relocates PML nuclear bodies to the cytoplasm. J. Virol. 72, 758–766.

Borden, K.L.B., Campbell Dwyer, E.J., Carlile, G.W., Djavani, M., Salvato, M.S., 1998b. Two RING finger proteins, the oncoprotein PML and the arenavirus Z protein, colocalize with the nuclear fraction of the ribosomal P protein. J. Virol. 72, 3819–3826.

Bouloy, M., Pardigon, N., Vialat, P., Gerbaud, S., Girard, M., 1990. Characterization of the 5′ and 3′ ends of viral messenger RNAs isolated from BHK21 cells infected with Germiston virus (Bunyavirus). Virology 175, 50–58.

Bowen, M.D., Peters, C.J., Nichol, S.T., 1996. The phylogeny of New World (Tacaribe complex) arenaviruses. Virology 219, 285–290.

Bowen, M.D., Peters, C.J., Nichol, S.T., 1997. Phylogenetic analysis of the Arenaviridae: patterns of virus evolution and evidence for cospeciation between arenaviruses and their rodent hosts. Mol. Phylogenet. Evol. 8, 301–316.

Bredenbeek, P.J., Molenkamp, R., Spaan, W.J., Deubel, V., Marianneau, P., Salvato, M.S., Moshkoff, D., Zapata, J., Tikhonov, I., Patterson, J., Carrion, R., Ticer, A., Brasky, K., Lukashevich, I.S., 2006. A recombinant Yellow Fever 17D vaccine expressing Lassa virus glycoproteins. Virology 345, 299–304.

Briese, T., Paweska, J.T., McMullan, L.K., Hutchison, S.K., Street, C., Palacios, G., Khristova, M.L., Weyer, J., Swanepoel, R., Egholm, M., Nichol, S.T., Lipkin, W.I., 2009. Genetic detection and characterization of Lujo virus, a new haemorrhagic fever-associated arenavirus from southern Africa. PLoS Pathog. 5, e1000455.

Cajimat, M.N., Milazzo, M.L., Borchert, J.N., Abbott, K.D., Bradley, R.D., Fulhorst, C.F., 2008. Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the Mexican woodrat (Neotoma mexicana). Virus Res. 133, 211–217.

Cajimat, M.N., Milazzo, M.L., Bradley, R.D., Fulhorst, C.F., 2007. Catarina virus, an arenaviral species principally associated with Neotoma micropus (southern plains woodrat) in Texas. Am. J. Trop. Med. Hyg. 77, 732–736.

Campbell Dwyer, E.J., Lai, H., MacDonald, R.C., Salvato, M.S., Borden, K.L.B., 2000. The lymphocytic choriomeningitis virus RING protein Z associates with eukaryotic initiation factor 4E and selectively represses translation in a RING-dependent manner. J. Virol. 74, 3293–3300.

Castilla, V., Larzabal, M., Sgalippa, N.A., Wachsman, M.B., Coto, C.E., 2005. Antiviral mode of action of a synthetic brassinosteroid against Junin virus replication. Antiviral Res. 68, 88–95.

Centers for Disease Control and Prevention, 2000. Fatal illnesses associated with a New World arenavirus – California, 1999–2000. MMWR Morb. Mortal. Wkly. Rep. 49, 709–711.

Centers for Disease Control and Prevention, 2005. Lymphocytic choriomeningitis virus infection in organ transplant recipients – Massachusetts, Rhode Island, 2005. MMWR Morb. Mortal. Wkly. Rep. 54, 537–539.

Centers for Disease Control and Prevention, 2006. Survey of lymphocytic choriomeningitis virus diagnosis and testing – Connecticut, 2005. MMWR Morb. Mortal. Wkly. Rep. 55, 398–399.

Centers for Disease Control and Prevention, 2008. Brief report: lymphocytic choriomeningitis virus transmitted through solid organ transplantation – Massachusetts, 2008. MMWR Morb. Mortal. Wkly. Rep. 57, 799–801.

Chapman, L.E., Mertz, G.J., Peters, C.J., Jolson, H.M., Khan, A.S., Ksiazek, T.G., Koster, F.T., Baum, K.F., Rollin, P.E., Pavia, A.T., Holman, R.C., Chris-tenson, J.C., Rubin, P.J., Behrman, R.E., Bell, L.J., Simpson, G.L., Sadek, R.F., 1999. Intravenous ribavirin for hantavirus pulmonary syndrome: safety and tolerance during 1 year of open-label experience. Ribavirin Study Group. Antivir. Ther. 4, 211–219.

Charrel, R.N., de Lamballerie, X., Fulhorst, C.F., 2001. The Whitewater Arroyo virus: natural evidence for genetic recombination among Tacaribe serocomplex viruses (family *Arenaviridae*). Virology 283, 161–166.

Charrel, R.N., Feldmann, H., Fulhorst, C.F., Khelifa, R., de Chesse, R., de Lamballerie, X., 2002. Phylogeny of New World arenaviruses based on the complete coding sequences of the small genomic segment identified an evolutionary lineage produced by intrasegmental recombination. Biochem. Biophys. Res. Commun. 296, 1118–1124.

Charrel, R.N., Lemasson, J.J., Garbutt, M., Khelifa, R., De Micco, P., Feldmann, H., de Lamballerie, X., 2003. New insights into the evolutionary relationships between arenaviruses provided by comparative analysis of small and large segment sequences. Virology 317, 191–196.

Charrel, R.N., Retornaz, K., Emonet, S., Noel, G., Chaumoitre, K., Minodier, P., Girard, N., Garnier, J.M., de Lamballerie, X., 2006. Acquired hydrocephalus caused by a variant lymphocytic choriomeningitis virus. Arch. Intern. Med. 166, 2044–2046.

Charrel, R.N., de Lamballerie, X., Emonet, S., 2008. Phylogeny of the genus *Arenavirus*. Curr. Opin. Microbiol. 11, 362–368.

Cho, H.W., Howard, C.R., Lee, H.W., 2002. Review of an inactivated vaccine against hantaviruses. Intervirology 45 (4–6), 328–333.

Choi, K.H., Groarke, J.M., Young, D.C., Kuhn, R.J., Smith, J.L., Pevear, D.C., Rossmann, M.G., 2004. The structure of the RNA-dependent RNA polymerase from bovine viral diarrhea virus establishes the role of GTP in de novo initiation. Proc. Natl. Acad. Sci. U. S. A. 101, 4425–4430.

Chung, D.H., Kumarapperuma, S.C., Sun, Y., Li, Q., Chu, Y.K., Arterburn, J.B., Parker, W.B., Smith, J., Spik, K., Ramanathan, H.N., Schmaljohn, C.S., Jonsson, C.B., 2008. Synthesis of 1-beta-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole and its in vitro and in vivo efficacy against Hantavirus. Antiviral Res. 79, 19–27.

Coutard, B., Canard, B., 2010. The VIZIER project: overview; expectations; and achievements. Antiviral Res. 87 (2), 85–94.

Coutard, B., Gorbalenya, A.E., Snijder, E.J., Leontovich, A.M., Poupon, A., De Lamballerie, X., Charrel, R., Gould, E.A., Gunther, S., Norder, H., Klempa, B., Bourhy, H., Rohayem, J., L'hermite, E., Nordlund, P., Stuart, D.I., Owens, R.J., Grimes, J.M., Tucker, P.A., Bolognesi, M., Mattevi, A., Coll, M., Jones, T.A., Aqvist, J., Unge, T., Hilgenfeld, R., Bricogne, G., Neyts, J., La Colla, P., Puerstinger, G., Gonzalez, J.P., Leroy, E., Cambillau, C., Romette, J.L., Canard, B., 2008. The VIZIER project: preparedness against pathogenic RNA viruses. Antiviral Res. 78, 37–46.

De Ory, F., Gegúndez, M.I., Fedele, C.G., Sánchez-Seco, M.P., 2009. Toscana virus, West Nile virus and lymphochoriomeningitis virus as causing agents of aseptic meningitis in Spain. Med. Clin. (Barc). 132, 587–590.

Delgado, S., Erickson, B.R., Agudo, R., Blair, P.J., Vallejo, E., Albariño, C.G., Vargas, J., Comer, J.A., Rollin, P.E., Ksiazek, T.G., Olson, J.G., Nichol, S.T., 2008. Chapare virus, a newly discovered arenavirus isolated from a fatal haemorrhagic fever case in Bolivia. PLoS Pathog. 4, e1000047.

Dias, A., Bouvier, D., Crépin, T., McCarthy, A.A., Hart, D.J., Baudin, F., Cusack, S., Ruigrok, R.W., 2009. The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. Nature 458, 914–918.

Dong, G.M., Han, L., An, Q., Liu, W.X., Kong, Y., Yang, L.H., 2005. Immunization effect of purified bivalent vaccine to haemorrhagic fever with renal syndrome manufactured from primary cultured hamster kidney cells. Chin. Med. J. (Engl.) 118 (9), 766–768.

Egloff, M.P., Benarroch, D., Selisko, B., Romette, J.L., Canard, B., 2002. An RNA cap (nucleoside-2′-O-)-methyltransferase in the flavivirus RNA polymerase NS5: crystal structure and functional characterization. EMBO J. 21 (11), 2757–2768.

Emonet, S., Retornaz, K., Gonzalez, J.P., de Lamballerie, X., Charrel, R.N., 2007. Mouse-to-human transmission of variant lymphocytic choriomeningitis virus. Emerg. Infect. Dis. 13, 472–475.

Emonet, S.F., Seregin, A.V., Yun, N.E., Poussard, A.L., Walker, A.G., de la Torre, J.C., Paessler, S., 2011. Rescue from cloned cDNAs and in vivo characterization of recombinant pathogenic Romero and live-attenuated Candid #1 strains of Junin virus, the causative agent of Argentine hemorrhagic fever disease. J. Virol. 85 (4), 1473–1483.

Enria, D.A., de Damilano, A.J., Briggiler, A.M., Ambrosio, A.M., Fernández, N.J., Feuillade, M.R., Maiztegui, J.I., 1985. Late neurologic syndrome in patients with Argentinian hemorrhagic fever treated with immune plasma. Medicina (B Aires) 45, 615–620.

Enria, D.A., Briggiler, A.M., Fernandez, N.J., et al., 1984. Dose of neutralising antibodies for Argentine haemorrhagic fever. Lancet 2, 255–256.

Enria, D.A., Maiztegui, J.I., 1994. Antiviral treatment of Argentine hemorrhagic fever. Antiviral Res. 23 (1), 23–31.

Ferres, M., Vial, P., Marco, C., Yanez, L., Godoy, P., Castillo, C., Hjelle, B., Delgado, I., Lee, S.J., Mertz, G.J., 2007. Andes Virus Household Contacts Study Group. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile. J. Infect. Dis. 195, 1563–1571.

Fischer, S.A., Graham, M.B., Kuehnert, M.J., Kotton, C.N., Srinivasan, A., Marty, F.M., Comer, J.A., Guarner, J., Paddock, C.D., DeMeo, D.L., Shieh, W.J., Erickson, B.R., Bandy, U., DeMaria Jr., A., Davis, J.P., Delmonico, F.L., Pavlin, B., Likos, A., Vincent, M.J., Sealy, T.K., Goldsmith, C.S., Jernigan, D.B., Rollin, P.E., Packard, M.M., Patel, M., Rowland, C., Helfand, R.F., Nichol, S.T., Fishman, J.A., Ksiazek, T., Zaki, S.R., 2006. LCMV in Transplant Recipients Investigation Team. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N. Engl. J. Med. 354, 2235–2249.

Flatz, L., Rieger, T., Merkler, D., Bergthaler, A., Regen, T., Schedensack, M., Bestmann, L., Verschoor, A., Kreutzfeldt, M., Brück, W., Hanisch, U.K., Günther, S., Pinschewer, D.D., 2010. T cell-dependence of Lassa fever pathogenesis. PLoS Pathog. 6 (3), e1000836.

Franze-Fernández, M.T., Zetina, C., Iapalucci, S., Lucero, M.A., Bouissou, C., López, R., Rey, O., Daheli, M., Cohen, G.N., Zakin, M.M., 1987. Molecular structure and early events in the replication of Tacaribe arenavirus S RNA. Virus Res. 7, 309–324.

Garcıá, C.C., Djavani, M., Topisirovic, I., Borden, K.L.B., Salvato, M.S., Damonte, E.B., 2006. Arenavirus Z protein as an antiviral target: virus inactivation and protein oligomerization by zinc finger-reactive compounds. J. Gen. Virol. 87, 1217–1228.

Garcin, D., Kolakofsky, D., 1990. A novel mechanism for the initiation of Tacaribe arenavirus genome replication. J. Virol. 64, 6196–6203.

Gavrilovskaya, I.N., Gorbunova, E.E., Mackow, N.A., Mackow, E.R., 2008. Hantaviruses direct endothelial cell permeability by sensitizing cells to the vascular permeability factor VEGF, while angiopoietin 1 and sphingosine 1-phosphate inhibit hantavirus-directed permeability. J. Virol. 82, 5797–5806.

Geisbert, T.W., Jones, S., Fritz, E.A., Shurtleff, A.C., Geisbert, J.B., Liebscher, R., Grolla, A., Ströher, U., Fernando, L., Daddario, K.M., Guttieri, M.C., Mothé, B.R., Larsen, T., Hensley, L.E., Jahrling, P.B., Feldmann, H., 2005. Development of a new vaccine for the prevention of Lassa fever. PLoS Med. 2, 183.

Gorbalenya, A.E., Lieutaud, P., Harris, M.R., Coutard, B., Canard, B., Kleywegt, G.J., Kravchenko, A.A., Samborskiy, D.V., Sidorov, I.A., Leontovich, A.M., Jones, T.A., 2010. Practical application of bioinformatics by the multidisciplinary VIZIER consortium. Antiviral Res. 87 (2), 95–110.

Gowen, B.B., Wong, M.H., Larson, D., Ye, W., Jung, K.H., Sefing, E.J., Skirpstunas, R., Smee, D.F., Morrey, J.D., Schneller, S.W., 2010. Development of a new tacaribe arenavirus infection model and its use to explore antiviral activity of a novel aristeromycin analog. PLoS One 5 (9), pii: e12760.

Gowen, B.B., Smee, D.F., Wong, M.H., Hall, J.O., Jung, K.H., Bailey, K.W., Stevens, J.R., Furuta, Y., Morrey, J.D., 2008. Treatment of late stage disease in a model of arenaviral hemorrhagic fever: T-705 efficacy and reduced toxicity suggests an alternative to ribavirin. PLoS One 3, e3725.

Gowen, B.B., Holbrook, M.R., 2008. Animal models of highly pathogenic RNA viral infections: hemorrhagic fever viruses. Antiviral Res. 78, 79–90.

Gowen, B.B., Wong, M.H., Jung, K.H., Sanders, A.B., Mendenhall, M., Bailey, K.W., Furuta, Y., Sidwell, R.W., 2007. In vitro and in vivo activities of T-705 against arenavirus and bunyavirus infections. Antimicrob. Agents Chemother. 51, 3168–3176.

Guilligay, D., Tarendeau, F., Resa-Infante, P., Coloma, R., Crepin, T., Sehr, P., Lewis, J., Ruigrok, R.W., Ortin, J., Hart, D.J., Cusack, S., 2008. The structural basis for cap binding by influenza virus polymerase subunit PB2. Nat. Struct. Mol. Biol. 15, 500–506.

Gunther, S., Asper, M., Roser, C., Luna, L.K., Drosten, C., Becker-Ziaja, B., Borowski, P., Chen, H.M., Hosmane, R.S., 2004. Application of real-time PCR for testing antiviral compounds against Lassa virus, SARS coronavirus and Ebola virus in vitro. Antiviral Res. 63, 209–215.

Hall, P.R., Hjelle, B., Brown, D.C., Ye, C., Bondu-Hawkins, V., Kilpatrick, K.A., Larson, R.S., 2008. Multivalent presentation of antihantavirus peptides on nanoparticles enhances infection blockade. Antimicrob. Agents Chemother. 52, 2079–2088.

Hall, P.R., Malone, L., Sillerud, L.O., Ye, C., Hjelle, B.L., Larson, R.S., 2007. Characterization and NMR solution structure of a novel cyclic pentapeptide inhibitor of pathogenic hantaviruses. Chem. Biol. Drug Des. 69, 180–190.

Hass, M., Lelke, M., Busch, C., Becker-Ziaja, B., Günther, S., 2008. Mutational evidence for a structural model of the Lassa virus RNA polymerase domain and identification of two residues, Gly1394 and Asp1395, that are critical for transcription but not replication of the genome. J. Virol. 82, 10207–10217.

Henttonen, H., Buchy, P., Suputtamongkol, Y., Jittapalapong, S., Herbreteau, V., Laakkonen, J., Chaval, Y., Galan, M., Dobigny, G., Charbonnel, N., Michaux, J., Cosson, J.F., Morand, S., Hugot, J.P., 2008. Recent discoveries of new hantaviruses widen their range and question their origins. Ann. N. Y. Acad. Sci. 1149, 84–89.

Hooper, J.W., Kamrud, K.I., Elgh, F., Custer, D., Schmaljohn, C.S., 1999. DNA vaccination with hantavirus M segment elicits neutralizing antibodies and protects against Seoul virus infection. Virology 255 (2), 269–278.

Huggins, J.W., Hsiang, C.M., Cosgriff, T.M., Guang, M.Y., Smith, J.I., Wu, Z.O., LeDuc, J.W., Zheng, Z.M., Meegan, J.M., Wang, Q.N., et al., 1991. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of haemorrhagic fever with renal syndrome. J. Infect. Dis. 164, 1119–1127.

Huggins, J.W., Kim, G.R., Brand, O.M., McKee Jr., K.T., 1986. Ribavirin therapy for Hantaan virus infection in suckling mice. J. Infect. Dis. 153, 489–497.

Jabafi, I., Selisko, B., Coutard, B., De Palma, A.M., Neyts, J., Egloff, M.P., Grisel, S., Dalle, K., Campanacci, V., Spinelli, S., Cambillau, C., Canard, B., Gruez, A., 2007. Improved crystallization of the coxsackievirus B3 RNA-dependent RNA polymerase. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 63 (Pt 6), 495–498.

Jin, H., Elliott, R.M., 1993. Characterization of Bunyamwera virus S RNA that is transcribed and replicated by the L protein expressed from recombinant vaccinia virus. J. Virol. 67, 1396–1404.

Jonsson, C.B., Hooper, J., Mertz, G., 2008. Treatment of hantavirus pulmonary syndrome. Antiviral Res. 78, 162–169.

Kang, H.J., Bennett, S.N., Dizney, L., Sumibcay, L., Arai, S., Ruedas, L.A., Song, J.W., Yanagihara, R., 2009. Host switch during evolution of a genetically distinct hantavirus in the American shrew mole (Neurotrichus gibbsii). Virology 388, 8–14.

Kariwa, H., Yoshimatsu, K., Arikawa, J., 2007. Hantavirus infection in East Asia. Comp. Immunol. Microbiol. Infect. Dis. 30, 341–356.

Kelley, L.A., Sternberg, M.J., 2009. Protein structure prediction on the Web: a case study using the Phyre server. Nat. Protoc. 4, 363–371.

Khaiboullina, S.F., Morzunov, S.P., St Jeor, S.C., 2005. Hantaviruses: molecular biology, evolution and pathogenesis. Curr. Mol. Med. 5, 773–790.

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Denys, C., Koivogui, L., ter Meulen, J., Krüger, D.H., 2006. Hantavirus in African wood mouse, Guinea. Emerg. Infect. Dis. 12, 838–840.

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., Barrière, P., Koivogui, L., ter Meulen, J., Krüger, D.H., 2007. Novel hantavirus sequences in Shrew, Guinea. Emerg. Infect. Dis. 13, 520–522.

Klempa, B., Tkachenko, E.A., Dzagurova, T.K., Yunicheva, Y.V., Morozov, V.G., Okulova, N.M., Slyusareva, G.P., Smirnov, A., Kruger, D.H., 2008. Haemorrhagic fever with renal syndrome caused by 2 lineages of Dobrava hantavirus, Russia. Emerg. Infect. Dis. 14, 617–625.

Klempa, B., 2009. Hantaviruses and climate change. Clin. Microbiol. 15, 518–523.

Krüger, D.H., Ulrich, R., Lundkvist, A., 2001. Hantavirus infections and their prevention. Microbes Infect. 3, 1129–1144.

Larson, R.A., Dai, D., Hosack, V.T., Tan, Y., Bolken, T.C., Hruby, D.E., Amberg, S.M., 2008. Identification of a broad-spectrum arenavirus entry inhibitor. J. Virol. 82, 10768–10775.

Larson, R.S., Brown, D.C., Ye, C., Hjelle, B., 2005. Peptide antagonists that inhibit Sin Nombre virus and Hantaan virus entry through the β_3-integrin receptor. J. Virol. 79, 7319–7326.

Lecompte, E., ter Meulen, J., Emonet, S., Daffis, S., Charrel, R.N., 2007. Genetic identification of Kodoko virus, a novel arenavirus of the African pigmy mouse (Mus Nannomys minutoides) in West Africa. Virology 364, 178–183.

Lee, K.J., Novella, I.S., Teng, M.N., Oldstone, M.B., de La Torre, J.C., 2000. NP and L proteins of lymphocytic choriomeningitis virus (LCMV) are sufficient for efficient transcription and replication of LCMV genomic RNA analogs. J. Virol. 74, 3470–3477.

Lee, A.M., Rojek, J.M., Spiropoulou, C.F., Gundersen, A.T., Jin, W., Shaginian, A., York, J., Nunberg, J.H., Boger, D.L., Oldstone, M.B., Kunz, S., 2008. Unique small molecule entry inhibitors of hemorrhagic fever arenaviruses. J. Biol. Chem. 283, 18734–18742.

Lopez, N., Jácamo, R., Franze-Fernández, M.T., 2001. Transcription and RNA replication of tacaribe virus genome and antigenome analogs require N and L proteins: Z protein is an inhibitor of these processes. J. Virol. 75, 12241–12251.

Lukashevich, I.S., 1992. Generation of reassortants between African arenaviruses. Virology 188, 600–605.

Lukashevich, I.S., Carrion Jr., R., Salvato, M.S., Mansfield, K., Brasky, K., Zapata, J., Cairo, C., Goicochea, M., Hoosien, G.E., Ticer, A., Bryant, J., Davis, H., Hammamieh, R., Mayda, M., Jett, M., Patterson, J., 2008. Safety, immunogenicity, and efficacy of the ML29 reassortant vaccine for Lassa fever in small non-human primates. Vaccine 26, 5246–5254.

Maes, P., Clement, J., Gavrilovskaya, I., Van Ranst, M., 2004. Hantaviruses: immunology, treatment, and prevention. Viral Immunol. 17, 481–497.

Maes, P., Clement, J., Van Ranst, M., 2009a. Recent approaches in hantavirus vaccine development. Expert Rev. Vaccines 8, 67–76.

Maes, P., Klempa, B., Clement, J., Matthijnssens, J., Gajdusek, D.C., Krüger, D.H., Van Ranst, M., 2009b. A proposal for new criteria for the classification of hantaviruses, based on S and M segment protein sequences. Infect. Genet. Evol. [Epub ahead of print].

Maiztegui, J.I., Fernandez, N.J., de Damilano, A.J., 1979. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. Lancet 2, 1216–1217.

Maiztegui, J.I., McKee Jr., K.T., Barrera Oro, J.G., Harrison, L.H., Gibbs, P.H., Feuillade, M.R., Enria, D.A., Briggiler, A.M., Levis, S.C., Ambrosio, A.M., Halsey, N.A., Peters, C.J., 1998. Protective efficacy of a live attenuated vaccine against Argentine haemorrhagic fever. AHF Study Group. J. Infect. Dis. 177, 277–283.

Manolaridis, I., Wojdyla, J.A., Panjikar, S., Snijder, E.J., Gorbalenya, A.E., Berglind, H., Nordlund, P., Coutard, B., Tucker, P.A., 2009. Structure of the C-terminal domain of nsp4 from feline coronavirus. Acta Crystallogr. D: Biol. Crystallogr. 65 (Pt 8), 839–846.

Martinez, V.P., Bellomo, C., San Juan, J., Pinna, D., Forlenza, R., Elder, M., Padula, P.J., 2005. Person-to-person transmission of Andes virus. Emerg. Infect. Dis. 11, 1848–1853.

McCormick, J.B., Fisher-Hoch, S.P., 2002. Lassa fever. Curr. Top. Med. Microbiol. Immunol. 262, 75–109.

McCormick, J.B., Webb, P.A., Krebs, J.W., Johnson, K.M., Smith, E.S., 1987. A prospective study of the epidemiology and ecology of Lassa fever. J. Infect. Dis. 155, 437–442.

McCormick, J.B., King, I.J., Webb, P.A., Scribner, C.L., Craven, R.B., Johnson, K.M., Elliott, L.H., Belmont-Williams, R., 1986. Lassa fever. Effective therapy with ribavirin. N. Engl. J. Med. 314, 20–26.

Mertz, G.J., Miedzinski, L., Goade, D., Pavia, A.T., Hjelle, B., Hansbarger, C.O., Levy, H., Koster, F.T., Baum, K., Lindemulder, A., Wang, W., Riser, L., Fernandez, H., Whitley, R.J., Collaborative Antiviral Study Group, 2004. Placebo-controlled, double-blind trial of intravenous ribavirin for the treatment of hantavirus cardiopulmonary syndrome in North America. Clin. Infect. Dis. 39 (9), 1307–1313.

Meyer, B.J., Southern, P.J., 1993. Concurrent sequence analysis of 5′ and 3′ RNA termini by intramolecular circularization reveals 5′ nontemplated bases and 3′ terminal heterogeneity for lymphocytic choriomeningitis virus mRNAs. J. Virol. 67, 2621–2627.

Milazzo, M.L., Cajimat, M.N., Haynie, M.L., Abbott, K.D., Bradley, R.D., Fulhorst, C.F., 2008. Diversity among tacaribe serocomplex viruses (family Arenaviridae) nat-

urally associated with the white-throated woodrat (Neotoma albigula) in the southwestern United States. Vector Borne Zoonotic Dis. 8, 523–540.
Morin, B., Coutard, B., Lelke, M., Ferron, F., Kerber, R., Jamal, S., Frangeul, A., Baronti, C., Charrel, R., de Lamballerie, X., Vonrhein, C., Lescar, J., Bricogne, G., Günther, S., Canard, B., 2010. The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription. PLoS Pathog. 6 (9), pii: e1001038.
Padula, P.J., Edelstein, A., Miguel, S.D., López, N.M., Rossi, C.M., Rabinovich, R.D., 1998. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology 241, 323–330.
Palacios, G., Druce, J., Du, L., Tran, T., Birch, C., Briese, T., Conlan, S., Quan, P.L., Hui, J., Marshall, J., Simons, J.F., Egholm, M., Paddock, C.D., Shieh, W.J., Goldsmith, C.S., Zaki, S.R., Catton, M., Lipkin, W.I., 2008. A new arenavirus in a cluster of fatal transplant-associated diseases. N. Engl. J. Med. 358, 991–998.
Palacios, G., Savji, N., Hui, J., Travassos da Rosa, A., Popov, V., Briese, T., Tesh, R., Lipkin, W.I., 2010. Genomic and phylogenetic characterization of Merino Walk virus, a novel arenavirus isolated in South Africa. J. Gen. Virol. 91, 1315–1324.
Park, K., Kim, C.S., Moon, K.T., 2004. Protective effectiveness of hantavirus vaccine. Emerg. Infect. Dis. 10 (12), 2218–2220.
Peters, C.J., Buchmeier, M., Rollin, P.E., Ksiazek, T.G., 1996. Arenaviruses. In: Fields, B.N., Knipe, D.M., Howley, P.M., Chanock, R.M., Melnick, J.L., Monath, T.P., Roizman, R., Straus, S.E. (Eds.), Fields Virology. Lippincott-Raven Publishers.
Piyasirisilp, S., Schmeckpeper, B.J., Chandanayingyong, D., Hemachudha, T., Griffin, D.E., 1999. Association of HLA and T-cell receptor gene polymorphisms with Semple rabies vaccine-induced autoimmune encephalomyelitis. Ann. Neurol. 45 (5), 595–600.
Plyusnin, A., Vapalahti, O., Vaheri, A., 1996. Hantaviruses: genome structure, expression and evolution. J. Gen. Virol. 77 (Pt 11), 2677–2687.
Raju, R., Raju, L., Hacker, D., Garcin, D., Compans, R., Kolakofsky, D., 1990. Nontemplated bases at the 5′ ends of Tacaribe virus mRNAs. Virology 174, 53–59.
Ramsden, C., Holmes, E.C., Charleston, M.A., 2009. Hantavirus evolution in relation to its rodent and insectivore hosts: no evidence for co-divergence. Mol. Biol. Evol. 26, 143–153.
Ramsden, C., Melo, F.L., Figueiredo, L.M., Holmes, E.C., Zanotto, P.M., 2008. VGDN Consortium. High rates of molecular evolution in hantaviruses. Mol. Biol. Evol. 25, 1488–1492.
Reguera, J., Weber, F., Cusack, S., 2010. Bunyaviridae RNA polymerases (L-protein) have an N-terminal, influenza-like endonuclease domain, essential for viral cap-dependent transcription. PLoS Pathog. 6 (9), pii: e1001101.
Rusnak, J.M., Byrne, W.R., Chung, K.N., Gibbs, P.H., Kim, T.T., Boudreau, E.F., Cosgriff, T., Pittman, P., Kim, K.Y., Erlichman, M.S., Rezvani, D.F., Huggins, J.W., 2009. Experience with intravenous ribavirin in the treatment of hemorrhagic fever with renal syndrome in Korea. Antiviral Res. 81, 68–76.
Safronetz, D., Hegde, N., Ebihara, H., Denton, M., Kobinger, G.P., St Jeor, S., Feldmann, H., Johnson, D.C., 2009. Adenovirus vectors expressing hantavirus proteins protect hamsters against lethal challenge with Andes virus. J. Virol. [Epub ahead of print].
Salvato, M.S., Clegg, J.C.S., Buchmeier, M.J., Charrel, R.N., Gonzales, J.P., Lukashevich, I.S., Peters, C.J., Rico-Hesse, R., Romanowski, V., 2005. Family Arenaviridae. In: Van Regenmortel, M.H.V., Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball (Eds.), Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses, L.A. Academic Press.
Salvato, M.S., Shimomaye, E.M., 1989. The completed sequence of lymphocytic choriomeningitis virus reveals a unique RNA structure and a gene for a zinc finger protein. Virology 173, 1–10.
Schmaljohn, C., Hjelle, B., 1997. Hantaviruses: a global disease problem. Emerg. Infect. Dis. 3, 95–104.
Sepúlveda, C.S., Fascio, M.L., Mazzucco, M.B., Palacios, M.L., Pellón, R.F., García, C.C., d'Accorso, N.B., Damonte, E.B., 2008. Synthesis and evaluation of N-substituted acridones as antiviral agents against haemorrhagic fever viruses. Antivir. Chem. Chemother. 19, 41–47.
Severson, W.E., Schmaljohn, C.S., Javadian, A., Jonsson, C.B., 2003. Ribavirin causes error catastrophe during Hantaan virus replication. J. Virol. 77, 481–488.
Sewell, D.L., 1995. Laboratory-associated infections and biosafety. Clin. Microbiol. Rev. 8, 389–405.
Shaw, M.W., Lamb, R.A., 1984. A specific sub-set of host-cell mRNAs prime influenza virus mRNA synthesis. Virus Res. 1, 455–467.
Singh, M.K., Fuller-Pace, F.V., Buchmeier, M.J., Southern, P.J., 1987. Analysis of the genomic L RNA segment from lymphocytic choriomeningitis virus. Virology 161, 448–456.
Sohn, Y.M., Rho, H.O., Park, M.S., Kim, J.S., Summers, P.L., 2001. Primary humoral immune responses to formalin inactivated hemorrhagic fever with renal syndrome vaccine (Hantavax): consideration of active immunization in South Korea. Yonsei Med. J. 42 (3), 278–284.
Song, J., Kang, H.J., Song, K.J., Truong, T.T., Bennett, S.N., Arai, S., Truong, N.U., Yanagihara, R., 2007a. Newfound hantavirus in Chinese mole shrew, Vietnam. Emerg. Infect. Dis. 13, 1784–1787.
Song, J.W., Gu, S.H., Bennett, S.N., Arai, S., Puorger, M., Hilbe, M., Yanagihara, R., 2007b. Seewis virus, a genetically distinct hantavirus in the Eurasian common shrew (Sorex araneus). Virol. J. 4, 114.
Song, J.W., Kang, H.J., Gu, S.H., Moon, S.S., Bennett, S.N., Song, K.J., Baek, L.J., Kim, H.C., O'Guinn, M.L., Chong, S.T., Klein, T.A., Yanagihara, R., 2009. Characterization of Imjin virus, a newly isolated hantavirus from the Ussuri white-toothed shrew (Crocidura lasiura). J. Virol. 83, 6184–6191.
Southern, P.J., Singh, M.K., Riviere, Y., Jacoby, D.R., Buchmeier, M.J., Oldstone, M.B., 1987. Molecular characterization of the genomic S RNA segment from lymphocytic choriomeningitis virus. Virology 157, 145–155.
Spik, K.W., Badger, C., Mathiessen, I., Tjelle, T., Hooper, J.W., Schmaljohn, C., 2008. Mixing of M segment DNA vaccines to Hantaan virus and Puumala virus reduces their immunogenicity in hamsters. Vaccine 26 (40), 5177–5181.
Strecker, T., Eichler, R., ter Meulen, J., Weissenhorn, W., Klenk, H.D., Garten, W., Lenz, O., 2003. Lassa virus Z protein is a matrix protein sufficient for the release of virus-like particles. J. Virol. 77, 10700–10705.
Strecker, T., Maisa, A., Daffis, S., Eichler, R., Lenz, O., Garten, W., 2006. The role of myristoylation in the membrane association of the Lassa virus matrix protein Z. Virol. J. 3, 93.
Uckun, F.M., Venkatachalam, T.K., Erbeck, D., Chen, C.L., Petkevich, A.S., Vassilev, A., 2005. Zidampidine, an aryl phosphate derivative of AZT: in vivo pharmacokinetics, metabolism, toxicity, and anti-viral efficacy against haemorrhagic fever caused by Lassa virus. Bioorg. Med. Chem. 13, 3279–3288.
Vieth, S., Torda, A.E., Asper, M., Schmitz, H., Günther, S., 2004. Sequence analysis of L RNA of Lassa virus. Virology 318, 153–168.
Vieth, S., Drosten, C., Lenz, O., Vincent, M., Omilabu, S., Hass, M., Becker-Ziaja, B., ter Meulen, J., Nichol, S.T., Schmitz, H., Günther, S., 2007. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. Trans. R. Soc. Trop. Med. Hyg. 101, 1253–1264.
York, J., Dai, D., Amberg, S.M., Nunberg, J.H., 2008. pH-induced activation of arenavirus membrane fusion is antagonized by small-molecule inhibitors. J. Virol..
Wilda, M., Lopez, I., Casabona, J.C., Franze-Fernandez, M.T., 2008. Mapping of the Tacaribe arenavirus Z-protein binding sites on the L protein identified both amino acids within the putative polymerase domain and a region at the N terminus of L that are critically involved in binding. J. Virol. 82, 11454–11460.
Woo, G.J., Chun, E.Y., Kim, K.H., Kim, W., 2005. Analysis of immune responses against nucleocapsid protein of the Hantaan virus elicited by virus infection or DNA vaccination. J. Microbiol. 43 (6), 537–545.