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SUPPLEMENT ARTICLE

Bunyavirales: Scientific Gaps and Prototype Pathogens for a Large and Diverse Group of Zoonotic Viruses

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Research directed at select prototype pathogens is part of the approach put forth by the National Institute of Allergy and Infectious Disease (NIAID) to prepare for future pandemics caused by emerging viruses. We were tasked with identifying suitable prototypes for four virus families of the *Bunyavirales* order (*Phenuiviridae*, *Peribunyaviridae*, *Nairoviridae*, and *Hantaviridae*). This is a challenge due to the breadth and diversity of these viral groups. While there are many differences among the *Bunyavirales*, they generally have complex ecological life cycles, segmented genomes, and cause a range of human clinical outcomes from mild to severe and even death. Here, we delineate potential prototype species that encompass the breadth of clinical outcomes of a given family, have existing reverse genetics tools or animal disease models, and can be amenable to a platform approach to vaccine testing. Suggested prototype pathogens outlined here can serve as a starting point for further discussions.

Keywords. bunyavirus; hantavirus; phlebovirus; peribunyavirus; nairovirus.

The order *Bunyavirales* (whose members are colloquially referred to as "bunyaviruses") includes hundreds of viruses within at least 14 families as of the latest International Committee on the Taxonomy of Viruses (ICTV) release [1], although classification of *Bunyavirales* is constantly being refined as new species are discovered. The majority of bunyaviruses are not known to infect or cause disease in humans, but there are currently 5 families containing viruses responsible for human (or other vertebrate) infections [2]. For the purposes of this review, 4 of these 5 families will be discussed (*Phenuiviridae*, *Peribunyaviridae*, *Nairoviridae*, and *Hantaviridae*) (see Table 1), because the *Arenaviridae* are covered separately. Historically, phenuiviruses, peribunyaviruses, nairoviruses, and hantaviruses were classified as separate genera within the former *Bunyaviridae* family, but reclassification in 2017 promoted them to individual families within the *Bunyavirales* order. They all have trisegmented genomes comprising single-stranded ribonucleic acid (RNA) of negative-sense or ambisense polarity. The large (L) segment encodes the viral RNA polymerase. The medium (M) segment encodes the viral glycoproteins, which are homologs of class II fusion proteins. The small (S) segment encodes the nucleoprotein. Some bunyaviruses also encode nonstructural proteins on the S (NSs) and M (NSm) segments that are responsible for interaction with host cell antiviral machinery. The current phylogeny of the order is based on sequence of conserved motifs within the RNA-dependent RNA polymerase (RdRp) [3].

Identification of prototype pathogens for the *Bunyavirales*, and even within each family, presents many challenges due to the breadth and diversity of viral species. Across the 4 families discussed here, transmission occurs by several different arthropods (mosquitoes, ticks, midges) or rodents (in the case of the *Hantaviridae*). Zoonotic hosts are also wide-ranging and include mammals, reptiles, and birds. For most bunyaviruses, humans are dead-end hosts, with infrequent human-to-human transmission. Clinical disease presents varying degrees of severity from inapparent to febrile illness with flu-like symptoms to acute hemorrhagic fever, encephalitis, and/or arthralgia [2]. Human cases can occur sporadically, seasonally, or regionally, depending upon environmental factors related to host species and vectors for transmission. A major concern is that changes in climate may alter the natural host range of both vector and reservoir species, making emergence in human populations more likely. The segmented genomes of bunyaviruses also make them susceptible to reassortment and thus emergence of new viruses. Due to the historically sporadic and/or geographically constrained nature of bunyaviral disease, there is currently little interest from the pharmaceutical industry in developing vaccines or therapeutics (at least for humans). In this review, the 4 families will be

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Table 1. Select *Bunyavirales* Species Responsible for Human Infections

Family	Genus	Clinically Significant Members	Human Clinical Disease	Range	Vectors	Biosafety Level	Reverse Genetics System	Prototype
Phenuiviridae	*Phlebovirus*	Rift Valley fever virus (RVFV)	fever, hemorrhagic fever, ocular disease, neurologic disease, possible vertical transmission	Africa	Mosquitos	BSL-3/ Select Agent	Yes	x
…	*Phlebovirus*	Punta Toro virus (PTV)	fever, possible arthralgia	South America	Sandflies	BSL-2	No	x
…	*Phlebovirus*	Toscana virus (TOSV)	fever, possible neurological disease	Mediterranean	Sandflies	BSL-2	Yes	x
…	*Bandavirus*	Sever fever with thrombocytopenia syndrome virus (SFTSV)	fever, thrombocytopenia	East Asia	Ticks	BSL-3	Yes	x
…	*Bandavirus*	Heartland virus (HRTV)	fever, thrombocytopenia	United States	Ticks	BSL-3	Yes	…
Peribunyaviridae	*Orthobunyavirus*	Bunyamwera virus (BUNV)	fever	Africa	Mosquitos	BSL-2	Yes	…
…	*Orthobunyavirus*	La Crosse virus (LACV)	fever, neurological disease in children	United States	Mosquitos	BSL-2	Yes	x
…	*Orthobunyavirus*	Oropouche virus (OROV)	fever, possible neurological disease	South America	Mosquitos; midges	BSL-2	Yes	x
…	*Orthobunyavirus*	Cache Valley virus (CVV)	febrile illness; possible neurological disease; congenital disease in animals	North America	Mosquitos	BSL-3	Yes	x
…	*Orthobunyavirus*	Schmallenberg virus (SBV)	no known illness in humans; congenital disease in animals	Europe	Midges	BSL-3	Yes	…
Nairoviridae	*Orthonairovirus*	Crimean Congo Hemorrhagic Fever virus (CCHFV)	febrile illness, severe hemorrhagic fever	Africa, Asia, Europe, Middle East	Ticks	BSL-4 / Select Agent	Yes	x
…	*Orthonairovirus*	Nairobi Sheep Disease virus (NSDV)	febrile illness, nausea, vomiting	Africa	Ticks	BSL-3	No	…
…	*Orthonairovirus*	Dugbe virus (DUGV)	febrile illness	Africa	Ticks	BSL-3	No	…
…	*Orthonairovirus*	Hazara virus (HAZV)	no known disease in humans	Pakistan	Ticks	BSL-2	Yes	x
Hantaviridae	*Orthohantavirus*	Andes virus (ANDV)	Hantavirus cardiopulmonary syndrome	South America	Rats/mice	BSL-3/ ABSL-4	Minigenome only	x
…	*Orthohantavirus*	Sin Nombre virus (SNV)	Hantavirus cardiopulmonary syndrome	North America	Deer mice	BSL-3/ ABSL-4	No	x
…	*Orthohantavirus*	Hantaan virus (HNTV)	hemorrhagic fever with renal syndrome	Asia	Field mice	BSL-3/ ABSL-4	Minigenome only	x
…	*Orthohantavirus*	Seoul virus (SEOV)	hemorrhagic fever with renal syndrome	Asia	Rats	BSL-3/ ABSL-4	No	…
…	*Orthohantavirus*	Puumala virus (PUUV)	hemorrhagic fever with renal syndrome	Europe	Voles	BSL-3/ ABSL-4	No	…

Abbreviations: ABSL, animal biosafety level; ANDV, Andes virus; BSL, biosafety level; BUNV, Bunyamwera virus; CCHFV, Crimean-Congo hemorrhagic fever; CVV, Cache Valley virus; DUGV, Dugbe virus; HAZV, Hazara virus; HRTV, Heartland virus; HTNV, Hantaan virus; LACV, La Crosse virus; NHP, nonhuman primate; NSDV, Nairobi sheep disease virus; OROV, Oropouche virus; PTV, Punta Toro virus; PUUV, Puumala virus; RVFV, Rift Valley fever virus; SBV, Schmallenberg virus; SEOV, Seoul virus; SFTSV, severe fever with thrombocytopenia syndrome virus; SNV, Sin Nombre virus; TOSV, Toscana virus.

examined individually, with a summary of the basic gaps, models of disease, landscape of vaccine and monoclonal antibody (mAb) countermeasures, and proposed prototype pathogens. We conclude with a discussion of future directions and thoughts on using the prototype pathogen approach for pandemic preparedness for emerging bunyaviruses.

PHENUIVIRIDAE

Pathogenic Species

Although the *Phenuiviridae* family contains at least 20 genera as of the most recent ICTV listing, 2 of them (*Phlebovirus* and *Bandavirus*) are responsible for the majority of vertebrate infections (Table 1). Most of the former are transmitted by

phlebotomine sand flies (hence the name phlebovirus), whereas the latter are transmitted by ticks. However, *Rift Valley fever phlebovirus* (RVFV) is an exception in that it is a phlebovirus spread primarily by mosquitos. RVFV is a priority pathogen and arguably the most well studied bunyavirus for several reasons. It was first identified in 1932 and can result in severe hemorrhagic fever in humans, thus it was historically placed in a category with *Ebolavirus*. RVFV has caused several notable human outbreaks in South Africa (1950), Egypt (1976), and Saudi Arabia (2000–2001) [4]. During the Cold War, it was part of the biological weapons cache of several countries due to its potential crippling effect on animals and the agricultural industry [5, 6]. Other notable phleboviruses include *Punta Toro phlebovirus* (PTV), found in South America, which causes primarily febrile disease with arthralgia. Sand fly fever, caused by several viruses including Toscana virus (TOSV), Sicilian virus (SFSV), and Naples virus (SFNV), is a febrile disease found in the Mediterranean region and can occasionally progress to encephalitis.

As an example of the danger of emerging bunyaviruses, severe fever with thrombocytopenia syndrome virus ([SFTSV] also known as *Dabie bandavirus* or *Huaiyangshan banyangvirus*) was first identified in 2008 and causes highly pathogenic disease in humans in the east Asian countries of China, Korea, and Japan [7]. The vector for SFTSV is the Asian long-horned tick (*Haemaphysalis longicornis*), which has now been found in the United States [8]. A closely related virus, Heartland virus (HRTV), has recently caused several cases of human disease in the southern and central United States [9].

RVFV and SFTSV represent the spectrum of concern over emerging bunyaviruses. The former is a well studied priority pathogen, whereas the latter is newly emerging and highly pathogenic, underscoring the importance of continued research to understand not only existing bunyaviruses, but also the ecological drivers of emergence of previously unknown species.

Major Gaps in the Field

It is not well understood why phenuiviruses cause such disparate disease outcomes in people, although some progress has been made in understanding the pathogenesis of encephalitis and vertical transmission using animal models [10–12]. Despite this, many unanswered questions remain as to what causes differential outcomes in people. Animal studies suggest that exposure route and dose may play an important role [13–15], as well as host genetic factors such as polymorphisms in innate immune genes [16].

Like all other bunyaviruses, the *Phenuiviridae* use their glycoprotein (Gn/Gc) for attaching to receptor(s) and attachment factor(s) on the surface of host cells. However, attachment factors have been identified for only a few phenuiviruses [17], and the details of cell entry remain largely unknown. In a recent study, the conserved host protein low-density lipoprotein (LDL)-related protein 1 (Lrp1) was implicated in the entry of RVFV into host cells from different species [18], providing a new avenue for understanding virus-host interactions. Receptors and attachment factors for bandaviruses such as SFTSV are currently unknown.

Existing Models of Disease

A limitation in the study of phenuiviruses, which is also true for the other 3 bunyavirus families below, remains the paucity of rodent disease models (Table 2). With a few exceptions (most notably RVFV), most bunyaviruses do not cause disease in immunocompetent adult rodents. Some of the viruses demonstrate age-dependent lethality and cause disease only in neonatal animals; whereas others require intracranial inoculation or the use of immunodeficient (interferon-α/β receptor [IFNAR] knockout [KO], AG129, etc) animals [19–22]. In the case of PTV, lethality in adult mice and hamsters is virus strain-dependent and attributable to the ability of the viral NSs to inhibit the interferon response [23].

For phenuiviruses that have been tested in nonhuman primate (NHP) species such as macaques, the resulting disease is typically mild (transient viremia, modest changes in blood chemistry, mild/moderate fever) unless the virus is administered intranasally (IN) or by aerosol inhalation [13, 14, 24]. Advancements in continuous radiotelemetry monitoring allow measurement of subtle changes in body temperature, electrocardiography, electroencephalography, and intracranial pressure in animals that survive with mild illness [25–30]. These physiological changes, even in mild disease, can be used to statistically evaluate the efficacy of vaccines and therapeutics.

Current Countermeasure Landscape

Of all the *Phenuiviridae*, the vaccine field is most advanced for RVFV [31]. Several vaccine candidates were developed in the 1970s and 1980s and have undergone clinical trials in humans, including formalin-inactivated preparations (TSI-GSD-200) and the attenuated MP-12 strain [32–35]. Numerous preclinical approaches (deoxyribonucleic acid [DNA], virus-like particle [VLP], rationally attenuated, vectored) have been tested in animals and proposed as veterinary or human vaccines, with the majority showing promising efficacy as prophylactic vaccines. As with many emerging viruses, there has not been significant pharmaceutical interest in further clinical testing or manufacturing of human RVFV vaccines. Most recently, the public/private partnership known as the Coalition for Epidemic Preparedness Initiative (CEPI) is supporting the preclinical development of 2 different single-dose live-attenuated RVFV vaccines (DDVax and RVFV-4S) for use in humans [36, 37]. In addition, a vectored vaccine using replication-deficient chimpanzee adenovirus (ChAd-Ox1 RVF) expressing the RVFV glycoproteins is currently in phase 1 clinical studies; this vaccine has shown safety and efficacy in pregnant livestock [38, 39]. With respect to antibody therapeutic approaches,

Table 2. Animal Models of Bunyavirus Disease

Virus	Mouse	Rat	Hamster	Ferret	NHP
Phenuiviridae	…	…	…	…	…
RVFV	Lethal hepatic disease by all routes and ages	Hepatic or neurologic disease, depending on strain and inoculation route. Vertical transmission in pregnant rats.	Lethal hepatic disease	Neurologic disease, depending on inoculation route	Mild febrile disease or lethal neurologic disease depending on exposure route and NHP species
PTV	Lethal in newborn mice; virus strain-dependent lethality in adult animals	N/A	Virus strain-dependent lethality in adult animals	N/A	N/A
TOSV	Lethal in adult mice after IC infection with a neuroadapted strain	N/A	N/A	N/A	N/A
SFTSV	Lethal in immunodeficient mice	Lethal in newborn rats; nonlethal infection in adult rats	Lethal in STAT2 KO hamsters	Lethal disease in aged animals	Mild disease
HRTV	Lethal in immunodeficient mice	Lethal in newborn rats; nonlethal infection in adult rats	Lethal in STAT2 KO hamsters	N/A	Mild disease
Peribunyaviridae	…	…	…	…	…
LACV	Lethal in newborn mice; lethal by IN or IC infection in adult mice	N/A	N/A	N/A	Mild disease in rhesus macaques
OROV	Lethal in newborn mice; lethal by IC infection of adult mice	N/A	Neurologic disease after IP or IC inoculation of adult animals	N/A	N/A
CVV	Lethal in adult immunodeficient mice; vertical transmission in pregnant mice	N/A	N/A	N/A	N/A
SBV	Lethal in adult immunodeficient mice	N/A	N/A	N/A	N/A
Nairoviridae	…	…	…	…	…
CCHFV	Lethal disease in immunodeficient, immunosuppressed, humanized adult mice	N/A	STAT2 KO	N/A	Mild disease; severe disease with Hoti strain
HAZV	Lethal disease in immunodeficient adult mice	N/A	N/A	N/A	N/A
DUGV	Lethal in newborn mice; lethal by IN or IC infection in adult mice	N/A	N/A	N/A	N/A
Hantaviridae	…	…	…	…	…
ANDV	Asymptomatic transient infection of deer mice	N/A	Lethal in adult animals	N/A	N/A
SNV	Asymptomatic persistent infection of deer mice	N/A	Mild sublethal disease in immunocompetent hamsters; lethal disease in immunosuppressed hamsters; hamster-adapted strain causes more severe disease in adults	…	Lethal in rhesus macaques after respiratory exposure (deer-mice passaged virus only)
HTNV	Lethal in newborn mice; some adult mouse strains susceptible to disease when injected IP; lethal in immunodeficient adult mice	N/A	Persistent asymptomatic infection	Persistent asymptomatic infection	Asymptomatic disease in marmosets
SEOV	Lethal in immunodeficient adult mice	Persistent asymptomatic infection	Persistent asymptomatic infection	…	…
PUUV	No disease in adult mice	N/A	Persistent asymptomatic infection	Persistent asymptomatic infection	Mild clinical disease in cynomolgus macaques

Abbreviations: ANDV, Andes virus; DUGV, Dugbe virus; HAZV, Hazara virus; HRTV, Heartland virus; IC, intracerebral; IN, intranasal; IP, intraperitoneal; KO, knockout; LACV, La Crosse virus; N/A, no information available or not tested; NHP, nonhuman primate; PTV, Punta Toro virus; PUUV, Puumala virus; RVFV, Rift Valley fever virus; SEOV, Seoul virus; SFTSV, severe fever with thrombocytopenia syndrome virus; SNV, Sin Nombre virus; TOSV, Toscana virus.

several studies identified highly potent neutralizing antibodies against RVFV that are promising as effective therapeutics at low concentrations [40, 41].

For SFTSV, vaccine efforts have proceeded quickly since its discovery due to the severity of disease that it causes. Several preclinical vaccines have been tested in animals, including live-attenuated viruses and various vectored (vesicular stomatitis virus [VSV], vaccinia [VAC]), DNA, and subunit approaches [42], but none have yet been tested in human clinical trials. Antibody-based therapeutics, including camel-produced nanobodies, have demonstrated protection in mouse models [43, 44].

Challenges

Biosafety and biosecurity issues are significant when working with phenuiviruses such as RVFV, SFTSV, or HRTV, all of which require biosafety level (BSL)-3 containment [45]. Furthermore, RVFV is classified as a Select Agent because of its severe disease and potential for use in bioterrorism and/or biowarfare. In contrast, PTV and TOSV can be used at BSL-2, and the Adames strain (PTV-A) causes acute lethal disease in immunocompetent adult hamsters and mice [23], making it an attractive prototype for the higher-containment phenuiviruses. However, a limitation of PTV is that, unlike the other phenuiviruses listed in Table 1, there is no reverse genetics system available for genetic manipulation [46].

Proposed Prototypes

We propose 4 prototypes for the *Phenuiviridae*. RVFV makes sense because it is a priority pathogen and has been historically studied, it has advanced animal models covering different disease outcomes, advanced vaccine candidates that have been tested in animals and humans, as well as significantly advanced mAb development. It is also arguably one of the most pathogenic of the phleboviruses that causes a range of human clinical outcomes. For these reasons, a vaccine platform that is effective against RVFV will have high likelihood to also be effective against emerging novel phleboviruses. *PTV* or TOSV may serve as alternative prototypes for the Phlebotomus fever antigenic group, because they can be worked with under BSL-2 conditions. Toscana virus has an available reverse genetics system, whereas the PTV-A strain causes disease in immunocompetent animals. We propose SFTSV as the prototype bandavirus, because it is newly emerged, highly pathogenic, and induces a unique clinical outcome compared to most other members of the family. Therefore, it provides a good representation of potential emerging bandaviruses.

PERIBUNYAVIRIDAE

Pathogenic Species

The *Peribunyaviridae* family contains at least 7 different genera [1]. *Orthobunyavirus* contains the majority of viral species responsible for human infections, with those in the California, Bunyamwera, and Simbu serotypes representing the most medically important (Table 1) [47]. These viruses are found worldwide and are primarily transmitted by mosquitoes and other arthropods [2]. *La Crosse orthobunyavirus* (LACV) causes a significant burden of pediatric encephalitis in the United States. *Bunyamwera orthobunyavirus* (BUNV), on the other hand, is restricted to Africa and causes acute febrile disease. *Cache Valley orthobunyavirus* (CVV), also within the Bunyamwera serogroup, is found in the North America and causes fetal infection of ruminant animals such as sheep, but its pathogenicity in humans is understudied. Viruses within the Simbu serogroup, such as *Oropouche orthobunyavirus* (OROV) in South America and *Schmallenberg orthobunyavirus* (SBV) in Europe, are transmitted by *Culicoides* midges and some mosquito species. OROV causes a dengue-like febrile disease in humans, with occasional neurological complications, whereas SBV causes stillbirths and congenital malformations in domestic livestock, including cattle, sheep, and goats. The human disease manifestations caused by orthobunyaviruses range from mild to acute or recurring febrile illness, neurological disease, and arthralgia [48, 49]. A subset of orthobunyaviruses, such as CVV and SBV, cause congenital infections in animals, with undocumented but potential occurrence in humans.

Major Gaps in the Field

It is not understood why orthobunyaviruses generally have restricted host and geographical ranges or why infection results in different, strain-specific, clinical outcomes in human and veterinary hosts. Little is known about the molecular processes involved in attachment and invasion of host cells, but recently the LDL-receptor family protein Lrp1 has been implicated as a host factor mediating efficient cellular infection by OROV [50]. The recent cryoelectron microscopy (CryoEM) structures of the L protein (RdRp) from LACV provide the most complete understanding of RNA polymerase function for any bunyavirus [51].

Existing Models of Disease

The largest limitation on the study of orthobunyaviruses in vivo is that they do not cause disease in immunocompetent laboratory animals (Table 2), which hampers the preclinical development of vaccines and therapeutics. LACV, OROV, and BUN demonstrate age- and route-dependent lethality in wild-type mice [52–54]. Both LACV and OROV are lethal when injected intracranially (IC) or administered IN in adult mice or when injected intraperitoneally into newborn mice. Most of these viruses will cause disease in immunodeficient adult animals (eg, IFNAR KO mice) [55–58]. LACV infection of nonhuman primates such as rhesus monkey results in viremia with subclinical infection [53]. LACV infects several different human cell lines and primary neurons in vitro, as well as brain organoids [59, 60].

Current Countermeasure Landscape

To our knowledge, there have not been any vaccines for orthobunyaviruses tested in humans, and a very limited number of preclinical vaccine candidates have been evaluated in mice and other animals. A chimeric LACV/Jamestown Canyon virus (JCV) virus is attenuated when inoculated IC into mice and provides protection from lethal challenge with either parental virus [61]. The same vaccine also protects monkeys from viremia in a nonlethal monkey model [61]. The DNA-based LACV vaccines demonstrated efficacy in an immunodeficient mouse model [62, 63]. For OROV, a VSV-OROV chimera elicits a protective immune response against wild-type OROV challenge in immunocompetent mice [64]. In comparison, several different clinical and preclinical veterinary vaccine approaches have been developed for SBV due to its rapid spread and threat to the European livestock industry. Inactivated whole-virus vaccines prevent viremia in sheep and cattle and have been quickly approved for use in livestock in the United Kingdom [65]. Presentation of SBV Gc glycoprotein in a scaffolding construct provides protection in immunodeficient mice and in cattle [66]. This approach could serve as a template for testing of other orthobunyaviruses. A subunit vaccine consisting of the N protein from SBV also induced antibodies and reduced viremia in mice [67]. A gap in the orthobunyavirus field is the absence of therapeutic neutralizing antibodies for use in pre-exposure or post-exposure circumstances.

Challenges

Many of the orthobunyaviruses cause neurological disease and are tropic for the brain. Targeting vaccine-induced immune responses to prevent neuroinvasion, as well as getting mAb and other therapeutics across the blood brain barrier, is arguably the premier challenge presented by this group of viruses.

Proposed Prototypes

We propose 3 prototypes for the *Peribunyaviridae*. LACV (California serogroup) is a clinically significant orthobunyavirus, and compared to the other members of the family, several studies have been published on LACV and the neurological disease it induces in in animals [60, 68, 69]. Although animal models are challenging due to lack of disease in wild-type mice, advantages of LACV are that it requires only BSL-2 containment and reverse genetics systems are in place [70]. Second, we propose CVV (Bunyamwera serogroup) as a prototype for vertically transmitted orthobunyaviruses, and in utero transmission models in mice are suitable for testing vaccines and mAb therapeutics [56]. CVV requires BSL-3 containment but is not a Select Agent. Reverse genetics systems exist for CVV [71], and the foundational vaccine work done with SBV provides a solid starting point for similar work with CVV. Finally, OROV is a good prototype of the Simbu serogroup. It has an existing reverse genetics system and requires BSL-2 containment.

NAIROVIRIDAE

Pathogenic Species

Within *Nairoviridae*, the *Orthonairovirus* genus contains the major species responsible for human infections, and the most relevant species are listed in Table 1. These include *Crimean-Congo Hemorrhagic Fever orthonairovirus* (CCHFV), *Dugbe orthonairovirus* (DUGV), *Hazara orthonairovirus* (HAZV), and *Nairobi Sheep Disease orthonairovirus* (NSDV), from which the group name originated. Orthonairoviruses are primarily transmitted by ticks.

CCHFV is a priority pathogen and the most clinically relevant nairovirus for humans. It causes outbreaks in many countries in Africa, Asia, Middle East, and most recently in parts of Europe, making it the most geographically widespread tick-borne virus [72]. Although human outbreaks are sporadic, case fatality can be up to 30% [73]. CCHFV circulates enzootically between ixodid ticks and mammals such as livestock; however, viremia in these animals is transient, and the animals do not show signs of disease. Humans are dead-end hosts and the only known species to develop disease as a result of CCHFV infection. Humans can become infected from a tick bite or from handling blood from a viremic, asymptomatic mammal. Human symptoms begin as a general fever, headache, and body aches that may progress to the hemorrhagic form characterized by rash and bleeding from mucosal sites [2].

The other orthonairoviruses generally do not cause significant human disease burden. DUGV is apathogenic in livestock animals and causes nonlethal febrile illness in people [74, 75]. NSDV causes severe disease in sheep and the role in human infections is less clear. HAZV, isolated from ticks, does not cause disease in humans or animals and does not experimentally infect cattle or sheep [76, 77].

Major Gaps in the Field

Proteinaceous cellular receptor(s) or attachment factors for nairoviruses have not been identified, and therefore viral attachment and internalization in target cells is unclear. The host cell protein nucleolin has been implicated as a host factor promoting CCHFV infection [78], but further mechanistic studies are warranted. The mechanisms of binding and fusion by CCHFV Gn and Gc are not known, but Gc contains a predicted fusion loop and anti-Gc mAbs can neutralize CCHF [79]. Further study on the attachment factor(s) and receptor(s) mediating nairovirus binding and entry to cells from ticks, animals, and people will be crucial to understanding tropism and spread.

Existing Models of Disease

The mouse models for orthonairoviruses require the use of immunodeficient animals (Table 2). Most of the focus on nairovirus animal model development has been on CCHFV due to its

clinical significance as a priority pathogen. Several avenues have been used to develop suitable animal models for CCHFV, including Type I-IFN deficient animals (A129), STAT-1 KO mice, or transient use of anti-IFN-α receptor antibodies [80–83]. In these models, CCHFV replicates to high levels in the liver and the animals rapidly succumb to disease. Liver tropism in mouse models mimics the human infection situation. The IFNAR KO mice, in contrast, develop severe disease and subsequently recover, which provides a model for convalescence [84]. Another recent approach has been to adapt CCHFV to mice, resulting in a strain that causes lethal hepatic infection and inflammation in wild-type BL/6 mice [85]. STAT2-KO hamsters [86] are susceptible to lethal disease and are notable due to development of rash and coagulation abnormalities. Infection of most NHP species with CCHFV results in mild, if any, disease. However, use of the more virulent Hoti strain, isolated from a fatal human patient, causes disease in cynomolgus macaques [87].

HAZV has been used in IFNAR KO mice as a surrogate for CCHFV, and it induces a hepatotropic lethal disease in these animals [88]. However, HAZV and CCHFV elicit vastly different inflammatory responses in polarized cells in vitro [89]. DUGV only causes disease in very young mice, adults infected IC, or A129 mice [90, 91].

Current Countermeasure Landscape

The focus of vaccine and mAb development has naturally been on CCHFV due to its status as a priority pathogen. Early versions of inactivated CCHFV vaccine preparations (known as the Russian/Bulgarian vaccine) are currently used in humans in Eastern Europe and parts of the former Soviet Union. Concerns about the origin of this vaccine from mouse brain passaging, as well as the need for several booster shots, make it less appealing. A number of additional preclinical CCHFV vaccine approaches have been tested in mice [92]. Deoxyribonucleic acid vaccines, modified vaccinia virus Ankara (MVA)-vectored, and VLP-based vaccines have shown immunogenicity and at least some protective efficacy in mouse models, whereas adenovirus-vectored and protein-based vaccines have not [92–95]. Veterinary vaccines are a potential avenue because curtailing CCHFV circulation in animals will limit spread to humans via ticks, and veterinary vaccines present lower hurdles for vaccine approval.

Neutralizing antibodies from survivors show promise in preventative and therapeutic settings in IFNAR KO mice [79]. In addition, nonneutralizing antibodies targeting the gp38 protein provide protection in adult IFNAR KO mice using a complement-dependent mechanism [96].

Challenges

Although animal models are well developed for CCHFV, work with it requires BSL-4 containment and CCHFV is also a Select Agent, making it challenging to work with and limited to relatively few facilities. NSDV and DUGV are BSL-3, non-Select Agents, whereas HAZV is BSL-2 [45].

Proposed Prototypes

Proposed prototypes for nairoviruses are CCHFV, because it has been the main focus of vaccine and therapeutic efforts to date due to its high pathogenicity and widespread geographic range. However, the less pathogenic HAZV, which can be handled at BSL-2, has an available reverse genetics system [97], and is lethal in certain mouse models, represents a prototype for the opposite spectrum of pathogenic range among orthonairoviruses.

HANTAVIRIDAE

Pathogenic Species

Unlike the 3 families discussed above, members of the *Hantaviridae* are transmitted by small mammals rather than arthropods. Rodents (mice, rats, voles) are the primary hosts of pathogenic *Orthohantavirus* species that infect and cause disease in humans (Table 1). Each rodent host species is persistently (but asymptomatically) infected, shedding virus in their saliva, urine, and feces and transmitting it horizontally through the rodent population. The virus is aerosolized and can be inhaled by unsuspecting humans [98]. The target organs of hantaviruses are the lung and kidneys, with endothelial cells being a primary cellular target. Thus, disease outcomes in humans typically take 2 primary forms: hemorrhagic fever with renal syndrome (HFRS) manifested by the Old World hantaviruses and hantavirus cardiopulmonary syndrome (HCPS) caused by the New World viruses [2]. As the names indicate, the kidney is the major target in HFRS, whereas the lung is the primary target organ in HCPS.

Hantaan orthohantavirus (HNTV) is the namesake of the *Hantaviridae* and, along with *Seoul orthohantavirus* (SEOV), causes Korean hemorrhagic fever, a form of HFRS in Asia. The natural reservoir of HNTV is the striped field mouse (*Apodemus agrarius*) [2]. SEOV is transmitted by rats (*Rattus norvegicus* and *Rattus rattus*), with human infection occurring by rodent body fluids (blood, saliva, urine), exposure to aerosolized rat excrement, or bites from infected rats. *Puumala orthohantavirus* (PUUV), originally discovered in Finland, causes HFRS in Northern Europe and Russia. The host species of PUUV is the bank vole (*Myodes glareolus*), and humans are infected by inhaling dust from vole droppings. *Dobrava-Belgrade orthohantavirus* (DOBV) was first isolated from a yellow-necked mouse (*Apodemus flavicollis*) in southeastern Slovenia and causes HFRS in throughout Europe. There are 4 different genotypes, each with its own natural reservoir, but Dobrava is the most virulent, causing a case fatality rate (CFR) of ~10% [99].

Sin Nombre orthohantavirus (SNV) was first identified in 1993 as the causative agent of an outbreak of HCPS in the Four Corners region of the United States, and since that time, it has been found wherever its host species (*Peromyscus maniculatus*) is located, including most of the populated areas of North America [2]. The case fatality rate for SNV was initially reported as ~67%, but it is now reported to be ~35% due to increased testing and surveillance. Numerous additional hantavirus strains have been associated with HCPS in North, Central, and South America. Of these, *Andes orthohantavirus* virus (ANDV) is the only one with documented human-to-human transmission [100], although this is still in question. ANDV is most commonly found in the pygmy rat (*Oligoryzomys longicaudatus*) or long-haired grass mouse (*Abrothrix longipilis*). The case fatality rate for HCPS due to ANDV infection of humans is ~25%–37%.

Major Gaps in the Field

The most severe limitation in the field of hantavirus research is the lack of reverse genetics systems, with only 2 minigenome systems available for ANDV and HTNV and no full-length clone systems [46]. The role and mechanism of action of the viral structural proteins, as well as the interaction between the envelope glycoproteins (Gn and Gc) and the host cell surface receptors, have been relatively well studied compared to other *Bunyavirales* [101]. Integrins are the main receptors in vitro, although complement decay-accelerating factor and globular heads of complement C1q receptor (gC1qR) can also mediate attachment of both Old World and New World hantaviruses in cultured cells. Most recently, protocadherin-1 (PCDH1) was demonstrated to mediate entry of the New World hantaviruses (ANDV, SNV) but not Old World viruses such as HTNV or SEOV [102]. The importance of PCDH1 was confirmed in vivo using KO hamsters, which were resistant to disease and death caused by ANDV. Upon entry, hantavirus infection proceeds through several possible routes, including clathrin-dependent endocytosis, clathrin-independent receptor-mediated endocytosis, and micropinocytosis [101].

Existing Models of Disease

Because rodents are natural hosts of hantaviruses, the usefulness of laboratory mice as disease models is limited. Most laboratory strains of mice generally remain asymptomatic (and often remain persistently infected) after infection with hantaviruses. In many cases, infection of newborn animals is required to induce lethal disease [103, 104]. SNV, HTNV, PUUV, and SEOV all cause asymptomatic and possibly persistent infection in Syrian Golden hamsters making them the most attractive rodent model for most hantaviruses [104, 105]. ANDV is the only hantavirus to cause lethal disease in immunocompetent hamsters [106]. Further models have been developed for SNV, including the use of chemically immunosuppressed animals or hamster-adapted virus strains, both of which develop more systemic infection with clinical disease [107–109].

For larger animals, ferrets and most monkey species are also asymptomatic [105, 110]. PUUV causes mild, nonlethal disease in cynomolgus macaques [111, 112]. For SNV, lethal disease is observed in rhesus macaques infected with a virus strain that was passaged in deer mice [113]. Due to the limitations in animal models, infection of 3-dimensional tissue models of lung microvascular endothelial cells with ANDV provides an in vitro model of HPS/HCPS [114].

Current Countermeasure Landscape

Although there are currently no US Food and Drug Administration-approved hantavirus vaccines for humans in the United States or Europe, inactivated virus vaccines for HTNV and SEOV (Hantavax) are in clinical usage in Korea and China; this vaccine requires 3 doses to obtain at least 2 years of immunity [115]. The DNA-based vaccines for HTNV/PUUV and ANDV are in phase 2 trials, and numerous other approaches (subunit, VLP, and adenovirus or VSV vectors) have been used in various preclinical studies [116]. Neutralizing mAbs provide protection in animal models such as hamsters or neonatal mice [116, 117]. Human polyclonal antibodies generated by DNA vaccination of transchromosomal bovines provide protection against ANDV, SNV, HNTV, and PUUV in hamsters [118, 119]. Even though laboratory mice are generally not good disease models of hantavirus infections, they can, under certain circumstances, be used for evaluation of vaccine-induced immunogenicity and protection from infection upon challenge.

Challenges

There are several major challenges in working with hantaviruses: lack of full-length clone reverse genetics systems, no good animal models that recapitulate human disease, and biosafety restrictions on animal experiments. Although in vitro studies with most hantaviruses can be performed at BSL-3, animal experiments are restricted to BSL-4 [45] due to the fact that rodents are natural reservoirs and the primary natural human exposure route is via aerosol from rodent excreta.

Proposed Prototypes

Because New World and Old World hantaviruses cause different clinical disease outcomes, we propose HTNV as the prototype for Old World hantaviruses causing HFRS, because this species has been the most widely studied and vaccine candidates are quite advanced. SNV is the proposed prototype for HCPS caused by New World hantaviruses, because it has been fairly well studied and is relevant as an emerging hantavirus. ANDV is included as a second New World prototype because of its severe disease presentation and person-to-person transmission.

CONCLUSIONS

Although there are many differences among the *Bunyavirales*, several common themes tie them all together: complex ecological life cycles that involve movement between vertebrates and invertebrates, segmented genomes (allowing reassortment), and a propensity to cause a range of human clinical outcomes from mild to severe and even death. When presented with the charge to identify appropriate prototype pathogens for the *Bunyavirales* [120], the approach that our focus group took was to identify prototypes that encompass the breadth of clinical outcomes of a given family, have existing reverse genetics tools or animal disease models, and can be amenable to a platform approach to vaccine testing. Although we are certain that arguments can be made for inclusion or exclusion of other members, we believe this is a starting point for further discussions.

A large historical body of work has overcome many of the inherent imitations in bunyavirus animal models, and the breadth of vaccine approaches is outstanding. Furthermore, advances in molecular virology provide foundational knowledge on replication, transcription, and cellular infection. Significant gaps remain, however, particularly in understanding host factors and receptors that determine host range and pathogenesis. In addition, further structural analysis of bunyavirus Gn and Gc proteins is essential to understanding neutralizing antibody and vaccine-mediated protection.

When thinking about rapid deployment of preventative vaccines or therapeutic antibodies for emerging bunyaviruses, several lessons learned from the coronavirus disease 2019 (COVID19) pandemic can inform considerations for prototype pathogen approach. Although live-attenuated vaccines have historically worked well in animal models, in the event of a novel outbreak, they would be too slow to develop and may prove to be a difficult hurdle to educate the public regarding safety. An alternative would be to leverage existing vaccine platforms (eg, lipid nanoparticle mRNA, adenovirus-vectored, or VSV-vectored vaccines) that have now undergone rigorous safety testing. In this instance, established vaccine platforms could be tested in animals using glycoproteins or other antigens from prototype viruses to demonstrate feasibility, and then these findings can be extended quickly to novel emerging viruses by swapping in glycoprotein domains. In addition, self-assembling nanoparticle technology has shown considerable promise for vaccine development against other viral diseases [121], especially because it may allow the use of antigens from several different species within a single vaccine. The use of therapeutic mAb approaches may prove formidable, because lack of cross-reactivity would mean that a single mAb is unlikely to work for more than 1 species. More detailed information on Gn/Gc structure and function of prototype viruses, including conformational epitopes, will inform mAb design, which would in turn form the basis for the rapid development of broadly reactive mAbs against a novel virus.

In summary, identifying suitable prototypes for the 4 virus families discussed is difficult due to the breadth and diversity of the groups. However, use of a platform approach and prototype viruses to develop new vaccines and mAb therapeutics will go a long way towards preparedness for the emergence of a known or novel bunyavirus. Furthermore, development of a Target Product Profile for each prototype virus may be a worthwhile next step. We thank the focus group panelists for their thoughtful contributions to our overall discussion. This document serves as a starting point for considering the prototype pathogen approach to addressing responses to future emerging viral threats.

Notes

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