Published in final edited form as:

Curr Opin Virol. 2012 April ; 2(2): 176–187. doi:10.1016/j.coviro.2012.02.016.

Henipavirus outbreaks to antivirals: the current status of potential therapeutics

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Abstract

The henipaviruses, Hendra virus and Nipah virus, are classic examples of recently emerged viral zoonoses. In a relatively short time since their discoveries in the mid and late 1990's respectively, a great deal of new information has been accumulated detailing their biology and certain unique characteristics. Their broad species tropism and abilities to cause severe and often fatal respiratory and/or neurologic disease in both animals and humans has sparked considerable interest in developing effective antiviral strategies to prevent or treat henipavirus infection and disease. Here, recent findings on the few most advanced henipavirus countermeasures are summarized and discussed.

Introduction

A previously unknown viral zoonosis now known as Hendra virus (HeV) was discovered in 1994 as the cause of two nearly simultaneous but unrelated incidents of a severe respiratory disease in horses associated with high mortality that occurred at distant locations in Queensland, Australia. Each incident was accompanied by the subsequent transmission of the virus to people and of three individuals in total; two succumbed to infection (reviewed in [1]). Just a few years following these events, a large outbreak of disease in pigs, along with cases of severe febrile encephalitis in people who were in close contact exposure to pigs, occurred in peninsular Malaysia 1998–99. In this instance, a second new virus closely related to HeV was determined to be the etiologic agent and it was later named Nipah virus (NiV) (reviewed in [2]). HeV and NiV are enveloped, single-stranded negative-sense RNA paramyxoviruses and are highly pathogenic in humans with case fatality rates ranging from 40–75%. It has since been demonstrated that their natural reservoirs are primarily several

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Conflict of interest

The author is a United States federal employee and coinventor on patents relating to human monoclonal antibodies against Hendra and Nipah viruses, soluble forms of Hendra and Nipah envelope glycoproteins and vaccines and peptide-based fusion inhibitors targeting henipaviruses; assignees are The United States of America as represented by the Department of Health and Human Services (Washington, DC), and the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (Bethesda, MD).

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different species of large fruit bats commonly called flying foxes, in the *Pteropus* genus [3]. The molecular genetic features, unusually broad host range and exceptionally high virulence observed in a number of mammalian species, including humans, have set HeV and NiV apart from all other well-characterized paramyxoviruses. HeV and NiV have been given Biosafety Level-4 (BSL-4) status and are now categorized as the type species of the new genus Henipavirus within the family *Paramyxoviridae* [1,4].

Since the discovery and recognition of HeV and NiV in the mid to late 1990's a considerable amount of new information has been accumulated detailing their cell biological and molecular features, their prevalence in nature and bat host relationships, and their disease and pathogenic processes in a wide variety of hosts (reviewed in [1,5]). Their broad species tropism and abilities to cause severe and often fatal neurologic and/or respiratory disease in both animals and humans and the lack of available vaccines or therapeutics, has highlighted the potential biothreats they pose from natural outbreaks, laboratory accidents, or their deliberate dispersal. There has been considerable interest in developing effective antiviral strategies to prevent or treat henipavirus infection and disease in people and economically important livestock, along with the animal models to test them, and here the recent progress in these areas are summarized.

Henipavirus spillovers

HeV was first isolated from fatal cases of severe respiratory disease in horses and humans and shown to be distantly related to measles virus and other members of the *Morbillivirus* genus, and provisionally named equine morbillivirus [6]. The outbreak occurred in the Brisbane suburb of Hendra causing fatal respiratory disease in horses and their trainer, and the non-fatal infection of a stable hand. Nearly concurrently, in an unrelated and only retrospectively identified incident near Mackay, Queensland, ~1000 km north of Hendra, another individual experienced a brief meningitic illness after assisting in the necropsies of two horses that were only later confirmed to have succumbed to an infection caused by the same virus that appeared in Hendra. Thirteen months later this individual experienced a recrudescence of fatal meningoencephalitis caused this virus [7,8] which was later re-named Hendra virus after the Brisbane suburb where the initial outbreak occurred. NiV subsequently emerged in peninsular Malaysia in 1998–99, in a large outbreak of respiratory disease in pigs and encephalitis among pig farmers, and also included the infection some abattoir workers in Singapore due to the transport of infected pigs, resulting in 265 cases and 105 deaths in people, and was later shown to be closely related both antigenically and genetically to HeV (reviewed in [1]).

HeV and NiV continue to repeatedly cause spillover events from their natural bat reservoir hosts, with occasional reports of HeV in the first few years following its appearance in 1994 but has now occurred on an annual basis since 2006 and all through the infection of horses in Queensland and New South Wales, Australia, along with a total of 7 human cases of which 4 have been fatal [9,10]. However, in 2011, between the months of June to October, a dramatic increase in the number of HeV spillovers had occurred totaling 18 separate instances of HeV infection among horses in Australia leading to the death or euthanasia of 23 horses as well as 1 dog and the monitoring of more than 60 people for possible HeV

infection [11,12]. Most recently, an early appearance of HeV infection in a horse was reported during the first week of January, 2012, outside of what is generally considered to be the peak period of occurrence (June through August) [13]. In total, there have now been 33 HeV spillover events in Australia since 1994. Likewise, nearly annual outbreaks of NiV infection, primarily in Bangladesh but also including India, have been recorded since 2001 since NiV was first recognized in the Malaysian outbreak in 1998. The most recent appearance of NiV in Bangladesh occurred in January of 2012, with six human fatalities, primarily children [14]. Here, these NiV outbreaks have been associated with significantly higher case fatality rates in people (ranging between 10% to 100%) and several outbreaks have also been linked to the consumption of raw date palm sap contaminated with virus as well as human-to-human transmission of NiV infection [15,16]. In total, there have been 570 reported cases of NiV infection in people with 305 fatalities (reviewed in [17,18]) [14].

Henipavirus host range, cellular tropism and pathogenesis

HeV and NiV have an exceptionally broad species tropism and in addition to bats which do not experience apparent clinical disease, natural and experimental infections with henipaviruses have revealed that they can cause severe and often fatal disease in horses, pigs, cats, dogs, ferrets, hamsters, guinea pigs, monkeys, and humans, spanning 6 mammalian Orders [19–33]. Henipaviruses attach and infect susceptible host cells by the coordinated actions of their attachment (G) and fusion (F) glycoproteins [4]. The F glycoprotein is a type I transmembrane glycoprotein and consists of two disulfide-linked subunits, F₁ and F₂, derived from the proteolytic processing of an F₀ precursor. The F glycoprotein mediates the membrane fusion event between the viral and host cell membranes by a Class I fusion mechanism involving two α -helical heptad repeat domains known as (HR-1 and HR-2) that mediate the formation of a 6-helix bundle structure during or concomitant with membrane merger (recently reviewed in [34]). The attachment G glycoprotein is a type II transmembrane glycoprotein consisting of a stalk and globular head and both the NiV and HeV globular head domain structures have recently been determined, both alone and in complex with the host cell receptor proteins the henipaviruses employ for binding and entry, summarized below [35–38].

The cellular tropism of both HeV and NiV is determined by their utilization of the cellular membrane proteins ephrin-B2 and ephrin-B3 as virus receptors, and binding of virus to cells is mediated by their G glycoproteins (reviewed in [39]). Ephrin-B2 and ephrin-B3 are members of a large family of cell surface expressed ligands that bind to Eph receptor tyrosine kinases mediating important bi-directional cell-cell signaling events within the vascular, nervous and skeletal systems, and ephrin-B2 is found in arteries, arterioles and capillaries whereas ephrin-B3 is more prominent in the nervous system as well as the vasculature (reviewed in [40,41]). Ephrin-B2 and ephrin-B3 are highly conserved proteins across vertebrate species (95% amino acid identity, respectively), including the animal hosts susceptible to HeV and NiV infection such as human, horse, pig, cat, dog, and flying foxes [42]. The recognition of ephrin-B2 and ephrin-B3 as the host cell receptors for henipaviruses helped clarify both their broad species tropisms as well as the systemic distribution of virus in endothelial and smooth muscle cells, and neurons and some epithelial

cells in infected animals and humans, along with the resultant observed pathogenic processes (reviewed in [1,43]).

In susceptible animals and humans, HeV and NiV cause a systemic infection characterized by wide-spread vasculitis and endothelial cell tropism often with the appearance of syncytia, thrombosis, ischemia and necrosis, and parenchymal cell infection and pathology in many major organ systems, and disease is particularly prominent in the lung and brain, but also the heart, lymphoid tissues and kidney (recently reviewed in [43]). Reflecting the major involvement of the both the lung and brain, severe HeV and NiV disease can manifest as an acute severe respiratory syndrome, encephalitis or a combination of both. In addition to acute clinical disease, henipavirus infection in people can take a prolonged course after recovery from an initial infection and then followed by a recrudescence of virus replication in the central nervous system (CNS), a process that was first noted in the second fatal case of HeV. It was also noted that neurological disease could also present later (>10 weeks) following a non-encephalitic, asymptomatic infection or in patients who recovered from acute encephalitis during the initial NiV outbreak in Malaysia, and relapsed-encephalitis has been shown to occur from several months to several years following infection (reviewed in [44]). A recent comparative analysis of the first two fatal human cases of HeV infection (acute respiratory and relapsed encephalitis cases) has also been reported, revealing that HeV is capable of causing either acute or relapsed encephalitis with pathological features similar to those caused by NiV [8]. The mechanisms by which henipaviruses can cause chronic infection and recrudescence within the CNS are unknown, but clearly this is an important area for further investigation.

Animals used for modeling henipavirus infection and pathogenesis

The development of animal modeling platforms of henipavirus infection and pathogenesis has been driven by both a desire to understand the viral pathogenic processes but also to develop the systems and tools needed in the evaluation of potential vaccines and therapeutics to combat infection in humans and livestock. Well-established animal models of HeV and NiV infection and pathogenesis have been reviewed in detail elsewhere, and include the guinea pig, hamster, cat, pig, ferret and African green monkey (AGM) (reviewed in [43,45]) and more recently details from further experimental HeV infection in the horse has been reported [46]. Of these models those that most closely mirror the pathogenic processes of henipavirus infection in people appear to be the hamster, ferret and AGM; whereas the most appropriate models for livestock vaccine development are those relevant species: the pig and horse. Here, a brief summary of the hamster, ferret and AGM animal models, along with the pig and horse models will be included because they have now all been used in the evaluation of henipavirus targeted antivirals and in the testing of the most advanced immunization strategies against the henipaviruses carried out to date.

Syrian golden hamster model

The first successful small animal model of henipavirus infection and resulting pathogenesis was demonstrated using the Syrian golden hamster and a challenge with NiV [24]. NiV infection in the hamster revealed severe pathological lesions in the brain, with animals

succumbing to infection 5 to 9 days after intraperitoneal administration of NiV, 24 hours after the appearance of tremors and limb paralysis [24]. Hamsters inoculated intranasally survived ~5 days longer following challenge and displayed progressive neurological signs along with breathing difficulties. Vascular pathology appeared in a wide-range of organs, including brain and lung, with virus present in endothelial cells. The vascular and parenchyma lesions were consistent with CNS-mediated clinical signs. Another more recent study revealed that hamsters challenged with higher doses of NiV resulted in an acute respiratory distress syndrome (ARDS) whereas lower challenge doses resulted in the development of neurological signs and a more systemic spread of the virus throughout the endothelium [47]. HeV infection of hamsters also causes a similar disease process as seen in acute human cases, including both respiratory and brain pathology, with endothelial infection and vasculitis, and direct parenchymal cell infection notably in the CNS [29]. Similar to NiV infection in hamsters, higher doses of HeV resulted in ARDS and lower doses developed a more neuro-pathogenic syndrome [47].

Ferret model

The possibility of a henipavirus ferret model was first examined using NiV infection. The NiV challenged ferret develops both severe respiratory and neurological disease and systemic vasculitis following oral-nasal challenge with doses as low as 500 TCID₅₀ by 6–10 days post-infection [28,48]. Major clinical disease signs in infected ferrets included combinations of severe depression, serous nasal discharge, cough and shortness of breath, and tremor and hind limb paresis, and pathological findings included vascular fibrinoid necrosis in a wide variety of organs, necrotizing alveolitis, and syncytia of endothelium and alveolar epithelium. Histological and immunohistological analysis revealed severe focal necrotizing alveolitis vasculitis and focal necrosis in a wide-range of tissues, and significant levels of viral antigen in blood vessel walls. NiV antigen was also present within the brain along with infected neurons, and recoverable virus from the brain and multiple other organs was reported. HeV challenged ferrets, also by the oral-nasal route, with doses as low as 50 TCID₅₀ rapidly progressed with severe disease 6 to 9 days following infection with essentially identical outcomes as reported for NiV challenged animals [49] and all together the henipavirus disease processes in the ferret accurately reflects those reported in NiV-infected people. The ferret model has been used in evaluating both antiviral drug and immunization strategies against henipavirus infections, discussed below.

African green monkey model

The first successful nonhuman primate models for both NiV and HeV infection were recently developed using the African green monkey (AGM) [30,33]. Both NiV and HeV can cause a uniformly lethal disease process following low dose virus challenge by intratracheal inoculation within 7–10 days post-infection. There is a rapid spread of the virus within the first 3 to 4 days following challenge to numerous organ systems. Monkeys begin to develop a severe respiratory disease ~7 days post-infection and the progression of henipavirus-mediated lung pathology with congestion and infiltration in the lung fields has been recorded by radiological examination [30,33]. The lungs become enlarged and are particularly affected with high levels of virus replication, congestion, hemorrhage and

polymerized fibrin. Findings included the presence of wide-spread vasculitis with endothelial and smooth muscle cell syncytia with viral antigen, along with viral genome detected in most organs and tissues along with associated pathology. Monkeys infected with either NiV or HeV also exhibit neurological disease signs with the presence of meningeal hemorrhaging and edema, along with vascular and parenchymal lesions in the brain including infection of neurons with the brainstem particularly involved [30,33].

Pig models

Models of henipavirus infection in pigs have also been explored and developed. NiV infection of pigs shows the respiratory system as a primary target of virus replication and pathology, with viral antigen present in the respiratory epithelium (tracheal, bronchial, bronchiolar, and alveolar) with syncytia in small blood and lymphatic vessels [22,23]. Virus was also found in the kidneys and endothelial and smooth muscle cells of small blood vessels [23]. The involvement of the CNS was less common, and meningitis or meningoencephalitis noted more often than encephalitis [23]. Other studies carried out in piglets generally resulted in a mild clinical disease with fever and respiratory signs [25], with virus replication seen in the respiratory system, lymphoid tissues and the CNS. Recoverable virus was obtained from the respiratory, lymphatic and nervous systems, and virus shedding in nasal, pharyngeal and ocular fluids was noted. Experimental HeV infection of pigs also manifests primarily as a respiratory disease in both Landrace piglets and older Gottingen minipigs, with possible CNS involvement observed in minipigs, and similar patterns of virus shedding were observed [31]. HeV appeared to cause a more severe respiratory syndrome in pigs in comparison to NiV. Although henipavirus disease in pigs is often less severe than in other hosts the virus does replicate and disseminate to a variety of organs along with significant levels of virus shedding.

Horse model

Natural HeV infection in horses is often associated with severe disease, and experimental infections are essentially uniformly fatal (reviewed in [43,45] [46]). The incubation period in naturally infected horses is ~8 to 11 days and animals initially present as anorexic and depressed with general uneasiness and ataxia, along with the development of fever with sweating. Respiration becomes rapid, shallow and labored with pulmonary edema and congestion with nasal discharge being a common terminal feature from 1 to 3 days following the onset of clinical signs. In severe cases of naturally acquired HeV infection the airways of the animals are often filled with a blood-tinged frothy exudate. There is hemorrhage, thrombosis of capillaries, necrosis and syncytial cells in the endothelium of pulmonary vessels. Viral antigen is present in endothelial cells in a wide variety of organs and virus can be recovered from a number of internal organs, including the lung, and from saliva and urine. Neurologic disease is also present but less frequent and has been noted in both terminally ill horses and in those that recovered from respiratory infection [50,51]. Experimental infection of horses with NiV has not been carried out although the brain and spinal cord of one naturally infected horse was examined and infection confirmed by immunohistochemical staining of viral antigen and revealing non-suppurative meningitis [22].

Antiviral strategies

Ribavirin, a well-known ribonucleoside analog with broad antiviral activity was used during the initial NiV outbreak in Malaysia in an open-label study of some 140 patients with encephalitis, and the morbidity and mortality in this treated group was significantly reduced in comparison to some 54 individuals that were untreated because of a lack of drug availability or refusal of treatment [52]. In light of the possible therapeutic benefit of ribavirin in treating NiV infection, and its availability for use in humans, it has been further evaluated *in vivo* in experimental henipavirus infections. Additionally, chloroquine, an anti-malarial drug first demonstrated to block the proteolytic processing of HeV-F [53], was later shown to inhibit henipavirus infection *in vitro* [54] and it has also been tested *in vivo*.

The hamster model of henipavirus infection was used in the evaluation of both ribavirin and chloroquine both individually and in combination treatment strategies (Table 1). Ribavirin only delayed NiV disease and death in challenged subjects and had no therapeutic benefit against HeV infection in hamsters [55,56]. In addition, ribavirin therapy also only delayed typical HeV disease and clinical outcome by 1 or 2 days in AGMs [33]. In contrast, the study by Georges-Courbot et al., also compared ribavirin treatment to poly(I:C) treatment [56] (Table 1). Various forms of poly(I:C), long known as strong inducers of IFN- α and - β production, have been extensively explored as potential antiviral therapies for over 40 years. In particular, polyIC₁₂U [57] has been shown to be very specific in triggering the Toll-like receptor (TLR)3 pathway, along with a reduced *in vivo* toxicity (reviewed in [58]). PolyIC₁₂U could block NiV replication in cell culture and it was tested *in vivo* against NiV infection in the hamster model [56]. Continuous administration of polyIC₁₂U for 10 days beginning at the time of NiV challenge did result in prevention of lethal disease in 5 of 6 subjects. This result suggests that further studies using TLR3 agonists such as PolyIC₁₂U as immunotherapeutic agents, perhaps in combination with other antiviral strategies, should be explored.

By comparison, chloroquine treatment, either alone or in combination with ribavirin, had no therapeutic benefit in ferrets challenged with NiV or hamsters challenged with either NiV or HeV [48,55,56] (Table 1). However, ribavirin is still considered as a possible treatment for henipavirus infection, but its impact on disease progression is questionable as two HeV infected patients in 2008 showed no discernible benefit after treatment with ribavirin [10] and combined treatment with chloroquine and ribavirin proved ineffective in one HeV-infected person in 2009 [59].

Heptad peptide based fusion inhibitors corresponding to either the HR-1 and HR-2 domains of a paramyxovirus F glycoprotein are particularly effective at inhibiting virus infection *in vitro* when present prior to triggering the F-mediated membrane fusion process (reviewed in [60]). HR-2 peptides have been used more often and act by blocking the formation of the 6-helix bundle structure in F by binding in the grooves of the HR-1 trimeric core preventing the viral F HR-2 domains from binding. The first henipavirus-specific peptide fusion inhibitor was a 36 amino acid HR-2 based F glycoprotein sequence (NiV-FC2) [61] analogous to the approved HIV-1 specific therapeutic peptide enfuvirtide (Fuzeon). More recent studies have determined that an HR-2 peptide derived from human parainfluenza

virus type-3 (hPIV3) F proved more potent at inhibiting HeV mediated fusion [62], and these observations were followed up with testing of a sequence-optimized and cholesterol tagged hPIV3-based HR2-derived peptide in the hamster model of NiV infection [63] (Table 1). The cholesterol-tagged antiviral peptide could also penetrate the CNS and exhibit some effective therapeutic activity against NiV infection in the hamster model and this approach could be particularly effective in treating the encephalitic manifestations of henipavirus infection. Additional *in vivo* efficacy testing of peptide fusion inhibitors as henipavirus therapeutics merits further investigation.

Passive immunization strategies

Early passive immunization studies carried out in hamster NiV-challenge model provided the proof-of-concept findings that immunotherapy against henipavirus infection by targeting the viral envelope glycoproteins was possible. Several studies carried out in hamsters demonstrated that protective passive immunotherapy with both NiV G and F-specific polyclonal antisera, as well as with various mouse monoclonal antibodies (mAbs) specific for the henipavirus G or F glycoproteins, could be achieved [29,64,65] and together these studies revealed a major role of viral glycoprotein specific antibody in protection from severe henipavirus-mediated disease (reviewed in [66]). However, the administration of antibody therapy systemically and following virus infection would be the likely scenario encountered in treating a potentially infected person, and a more potent mAb would likely be needed and one that was either humanized or was a human mAb.

Using recombinant antibody methods, henipavirus-neutralizing human mAbs specific for the G glycoprotein were previously isolated [67]. These mAbs were derived from a large naïve human phage-display antibody library and isolated as Fabs using a recombinant HeV soluble G glycoprotein (HeV-sG) as the panning antigen. One particular antibody, m102, exhibited strong cross-reactive neutralization activity against both HeV and NiV, and it was later affinity matured and individual clones re-isolated. One of the selected clones, m102.4, had improved affinity for HeV-sG and was converted to IgG1 format and produced in a CHO-K1 cell line. The m102.4 mAb had exceptionally potent neutralizing activity against NiV and HeV with 50% inhibitory concentrations below 0.04 and 0.6 µg/ml, respectively [68] and its epitope maps to the ephrin receptor binding site (Xu, Broder and Nikolov, unpublished). Additional testing of m102.4 confirmed its neutralization activity against several isolates; NiV-Malaysia, HeV-1994, HeV-Redlands, NiV-Bangladesh [28].

The first efficacy study of human mAb m102.4 was conducted in the ferret model of NiV infection [28] (Table 1). In a pre- and post-exposure NiV challenge study in the ferret, groups of three animals each were given a single 50 mg dose (~25mg/kg) at 24 hrs before (pre-) or 10 hrs following (post-) challenge. The mAb m102.4 was administered by intravenous infusion with control ferrets given PBS, and the animals were challenged oronasally with a 5,000 TCID₅₀ of NiV (10-fold the minimal infectious dose –50%). Control ferrets became febrile with clinical illness by day 6, with severe disease by day 8 and were euthanized. By day 8 one animal in the pre-group and all in the post-group were febrile with variable levels of depression, but by day 10 fevers began to abate. At day 13 post-infection, two of three ferrets in the pre-group experienced disease progression and were euthanized,

however, all other ferrets (3/3 in the post-group and 1/3 pre-group) were well and free of clinical signs and remained so until the study end point. At study completion, the control animals revealed severe systemic pathology while the two pre-group treated animals that had experienced a delayed disease course had a reduced frequency and size of hemorrhagic lesions in the pulmonary parenchyma, suggesting that disease progression in these subjects was slowed. There were no pathological abnormalities found in any of the surviving ferrets which included 3/3 animals that received a single dose of m102.4 10 hrs after lethal challenge [28]. This study was the first human mAb therapy successfully evaluated *in vivo* for prevention of lethal henipavirus infection.

In order to meet the requirements for future approved use of m102.4 in people, additional *in vivo* studies in a second animal model would be needed. To advance towards the goal of potential approved human use in the future, m102.4 was recently examined in the nonhuman primate, AGM, henipavirus challenge model [69] (Table 1). Here again, the study design for passive immunotherapy in the AGM reflected a potential real life scenario requiring mAb as a post-exposure treatment. Fourteen monkeys were challenged intratracheally with HeV and 12 animals were infused twice with a 100 mg dose (~20mg/kg) of m102.4 beginning at 10 hr, 24 hr or 72 hr p.i. with the second infusion ~48 hrs later. A variety of assays confirmed all subjects became infected following challenge, however all 12 animals that received m102.4 survived infection; whereas the untreated control subjects succumbed to severe systemic disease by day 8. Animals in a 72 hr treatment group (therapy initiated 3 days following challenge) exhibited neurological signs but all these animals recovered by day 16. The mAb m102.4 also revealed an excellent distribution half-time (~1 day) and elimination half-time (~11 days) in the AGM. There was no evidence of HeV-specific pathology in any of the m102.4-treated animals, whereas the control subjects revealed high levels of viral genome along with recoverable virus in a wide-range of tissues. No infectious HeV could be recovered from any tissues from any m102.4-treated subjects. All mAb treated survivor tissue architecture appeared normal and all tissues examined were negative for HeV antigen, whereas control subject tissues showed significant HeV antigen staining (Figure 1). This study revealed that mAb m102.4 prevented wide-spread HeV dissemination in virus challenged subjects, and was the first successful post-exposure *in vivo* therapy against HeV and the first in a nonhuman primate.

Active immunization strategies

The hamster henipavirus challenge model was the first to be used in an evaluation of an active immunization strategy against NiV. The highly attenuated vaccinia virus strain, NYVAC, was employed, and NiV F and G glycoprotein gene encoding recombinant viruses, both individually and in combination, were used to immunize animals and the study revealed that complete protection from NiV-mediated disease was achievable [64]. Evidence of NiV replication and an anamnestic antibody response in challenged animals was noted, but nevertheless this was an important proof-of-concept study indicating that eliciting an immune response to the viral envelope glycoproteins would be important in protection.

Livestock vaccine development for henipaviruses has also been an area of research because of the association of pigs as an amplifying host for NiV and because all HeV outbreaks in

Australia have occurred in horses. The first vaccine that was tested in this regard was a recombinant canarypox-based vaccine candidate for possible use in pigs [70] (Table 1). The NiV F and G glycoprotein genes were employed to generate recombinant canarypox virus (ALVAC) vaccine vectors and these were used to immunize 4-week-old pigs twice within 2 weeks. Each ALVAC vector was tested alone and in combination, and piglets were challenged intranasally with 2.5×10^5 plaque-forming units (PFU) of NiV. Essentially, the goal of vaccination of pigs is aimed at both preventing disease but also to prevent virus shedding to reduce the risk of virus transmission to people if in contact with infected animals. This study demonstrated protection from NiV-mediated disease in all vaccinated pigs by either ALVAC vector alone or in combination. The individual ALVAC-F and ALVAC-G vaccinated animals had only low levels of detectable virus shedding by nucleic acid analysis but no infectious virus could be recovered, however the combined ALVAC-F/G immunization appeared superior and neither infectious virus nor NiV RNA could be detected in sampled tissues from vaccinated animals. NiV neutralizing antibody was elicited by both vectors, with ALVAC-G yielding the strongest response, and the combined ALVAC-F/G vector vaccination appeared to be only marginally better than ALVAC-G alone. Together, the data suggested that an ALVAC-G or combination vector formulation could serve as a protective vaccine against NiV disease in pigs, but the combination of vectors may be needed to assure the prevention of virus shedding in these animals [70].

A subunit vaccine approach against the henipaviruses has also been extensively examined because of the inherent safety of such an approach. Soluble and secreted, oligomeric forms of the G glycoprotein (sG) from both HeV and NiV have been developed [71] and HeV-sG is now in commercial development as a livestock vaccine. The HeV-sG glycoprotein subunit vaccine (Figure 2) is a secreted version of the molecule in which the transmembrane and cytoplasmic tail domains have been deleted from the coding sequence. HeV-sG is produced in mammalian cell culture systems and is properly N-linked glycosylated [35]. HeV-sG retains many native characteristics including its oligomerization into dimers and tetramers and ability to bind ephrin receptors [72]. HeV-sG elicits potent cross-reactive neutralizing (HeV and NiV) antibody responses in a variety of animals including mice, rabbits, cats, ferrets, monkeys and horses.

Immunization and antibody binding studies have indicated that HeV-sG appears to present more cross-reactive epitopes (anti-NiV G antibodies) as compared to sG from NiV [73]. Studies using the HeV-sG subunit immunogen were first carried out in the cat model and a side-by-side comparison was made using a similarly designed and expressed NiV-sG. The cat is acutely susceptible to severe and fatal NiV and HeV disease but virus can be detected in brain only rarely and no evidence of encephalitis has been reported (reviewed in [5,43]). Both HeV-sG and NiV-sG, formulated in CSIRO triple adjuvant (Montanide, Quil A, and DEAE-dextran) and given as 3 doses of 100 μ g at three week intervals, could elicit a completely protective immune response against a lethal subcutaneous NiV challenge in the cat (NiV-Malaysia isolate) [26] (Table 1). This first study suggested that a single vaccine (HeV-sG) could be effective against both HeV and NiV. A follow-up study in the cat model explored the level of protection of pre-challenge virus-neutralizing antibody titers along with a high dose oronasal challenge, the likely natural route of zoonotic exposure [74]. Here, only 2 doses of either 50 μ g, 25 μ g or 5 μ g of HeV-sG formulated with CpG and Allhydrogel™

were given at day 0 and 21. Virus neutralizing antibody titers present at day 35 ranged from 1:32 (low vaccine dose) to 1:512 (high vaccine dose). Animals were challenged on day 42 and all vaccinated animals were completely protected from a high dose oronasal challenge of NiV (50,000TCID₅₀ of NiV-Malaysia).

More recently, a HeV-sG immunization study was reported using the ferret model with a HeV challenge [49]. Here, ferrets were immunized twice (day 0 and day 20) with either a 100 µg, 20 µg or 4 µg dose of HeV-sG formulated in CpG and Allhydrogel™ (similar to the cat study). HeV-sG elicited a significant neutralizing antibody response that correlated to the dose of HeV-sG used, and titers ranged from 1:8192 in the 100 µg group, to 1:1024 and 1:2048 in the 20 µg, and 1:64 and 1:128 for the 4 µg. Animals were challenged oronasally with a 5,000 TCID₅₀ dose (10-fold the minimal infectious dose 50%) of a low passage isolate of HeV (Redlands 2008 isolate), and all vaccinated ferrets remained completely free of any signs of fever or clinical disease from HeV infection, whereas the control ferrets developed fever, depression, and tremors, and at necropsy extensive gross pathological lesions were noted. Post-mortem examination of HeV-sG vaccinated ferrets at the end of the study revealed all animals to be gross pathologically and histopathologically normal (except one of four animals in the low dose vaccine group). There was no evidence of virus or viral genome in any tissues or body fluids in any animals in the 100 µg and 20 µg vaccine groups; and only a low level of genome was detected in the nasal washes from only one of four animals in the 4 µg vaccine group. No infectious virus was recovered from any vaccinated ferrets and together, these findings confirmed that subunit vaccination strategy against henipaviruses to prevent disease and virus shedding appeared possible.

In addition to the cat and ferret HeV-sG vaccination studies, HeV-sG vaccination of nonhuman primates (AGMs) followed by intratracheal NiV challenge [75] or intratracheal HeV challenge (Geisbert and Broder, unpublished) has also been carried out and here again, complete protection from henipavirus-induced disease was achievable, and no recoverable virus or evidence of virus shedding has been noted (Table 1), whereas control monkeys developed severe systemic and fatal henipavirus disease. Preclinical development of HeV-sG in vaccine formulations that could potentially be suitable for use in humans are in progress.

Because of the remarkable success in HeV-sG vaccine-mediated protection in multiple animal challenge models the potential application of HeV-sG as a safe and effective equine vaccine has now been taken on as a strategy to both prevent infection in horses and also to ameliorate the risk of HeV transmission to people. HeV-sG has been licensed by multinational animal health company and is commercial development as an equine vaccine for use in Australia. Preliminary horse HeV-sG vaccination and HeV challenge studies have recently been carried in Australia at the BSL-4 facilities of the Animal Health Laboratories (AAHL) in Geelong, Australia. These efforts have been collaborative research program between the Uniformed Services University and Henry M. Jackson Foundation, the AAHL along with a corporate partner. Findings of these preliminary studies were reported at Australian Veterinary Association, Annual Conference in Adelaide, in May 2011 [76]. The HeV-sG was used to immunize horses (a 2 dose regime with a 3 week interval) and both a high and a low dose of HeV-sG antigen was examined, achieving pre-challenge HeV

neutralizing titers between 1:128 to 1:2048. Following a high dose oronasal challenge with 2×10^6 TCID₅₀ of HeV, all vaccinated horses remained clinically disease-free, and there was no evidence of virus replication or virus shedding in any of the immunized horses. It is hoped that the vaccine may be available sometime in 2013.

Conclusions

The henipaviruses are the first and only examples of zoonotic paramyxoviruses that can infect and cause lethal disease across a broad range of vertebrate species including humans. Because of the potential access of these agents from a variety of bat reservoirs, their associated high morbidity and mortality and the risk they pose from natural outbreaks, laboratory accidents or deliberate dispersal, the development of effective countermeasures has been of some importance. Much of the research conducted over the past decade has focused on the virus binding and entry processes and functions of the viral envelope glycoproteins in engaging host cell receptors and the membrane fusion process. These efforts have led to the development and testing of potential antiviral therapeutics (fusion inhibitors) and vaccine candidates (active and passive immunization) with the commonality of targeting the early stages of the virus infection process.

Presently, a cross-reactive human mAb (m102.4), capable of neutralizing all known isolates of HeV and NiV, has been shown to be exceptionally efficacious as a post-exposure therapy in protecting both ferrets and nonhuman primates from lethal henipavirus disease, even under conditions of high dose virus challenge and by oronasal or intratracheal routes of virus inoculation. Recently, during the 2010 HeV spillover occurrence in Queensland, Australia, there were two individuals considered to be at high risk of HeV infection [77]. The m102.4 mAb was requested by Australian health authorities and administered to two individuals as a compassionate use therapeutic option even though no human safety testing has been carried out and it was not recommended for use in humans. There were no adverse reactions resulting from infusion of the mAb, and both individuals remain well. The m102.4 mAb is presently in pre-clinical development stages in both the United States and Australia.

As a vaccination strategy for preventing henipavirus infection and disease a recombinant subunit vaccine, HeV-sG, has shown remarkable efficacy and is cross-protective against HeV and NiV. HeV-sG has been demonstrated to provide protection against henipavirus challenge in cats, ferrets, monkeys and horses. Recent experiments have used only two immunizing doses that can elicit a cross-reactive neutralizing antibody response capable of protecting animals from high dose challenge, with no evidence of an anamnestic humoral response following virus challenge and no evidence of virus shedding from immunized and challenged subjects. The HeV-sG as a subunit vaccine is now in commercial development as a livestock vaccine and currently under testing in Australia as an equine vaccine. Further, recent preliminary data suggests that the HeV-sG subunit vaccine could clearly be developed for potential human use. Because HeV-sG immunization can elicit a cross-protective antibody response against both HeV and NiV and in the absence of any approved and effective alternative therapeutic options, this subunit vaccine could be beneficial for administration to veterinarians and staff workers working with horses in Australia, biologists

engaged in virus surveillance studies in bat populations, laboratory workers performing experiments with HeV or NiV, or people at risk of NiV infection in Bangladesh.

Acknowledgments

The views expressed in the manuscript are solely those of the author, and they do not represent official views or opinions of the Department of Defense or The Uniformed Services University of the Health Sciences. A special thanks is given to Kai Xu of the Structural Biology Program, Memorial Sloan-Kettering Cancer Center, New York for developing the structural model of HeV-sG dimer. Supported in part by the Department of Health and Human Services, National Institutes of Health, grants AI054715 and AI077995.

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Paper of particular interest, published within the period of review.

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Highlights

- Hendra virus and Nipah virus advanced antiviral therapies are reviewed.
- Recent animal models of henipavirus disease used to test antivirals and vaccines are discussed.
- A potential human-use passive immunotherapeutic is reviewed.
- Potential livestock Hendra and Nipah virus vaccines are discussed.

Figure 1.

Absence of HeV antigen in the lung of m102.4 treated African green monkeys. Localization of HeV antigen by immunohistochemical stain in the middle lung, (a) monkey in the m102.4 treatment group beginning at 72 hr, and (b) a control animal (viral antigen is red staining). Sections were stained with a NiV nucleoprotein (N) specific rabbit polyclonal antibody and images were obtained at an original magnification of at 20X. These images were originally published in Bossart et al, [69] and reformatted with permission.

Figure 2.

Hendra virus soluble G glycoprotein subunit vaccine and details of the ephrin binding site on the G glycoprotein globular head in comparison to the m102.4 binding site. The HeV-sG glycoprotein subunit vaccine consists of the entire predicted ectodomain (amino acids 76–604). (a) HeV-sG shown as the dimer with one monomer colored red and the other is blue. The secondary structure elements of the two globular head domains are derived from the crystal structure of the HeV G head domain, which also revealed that all five predicted N-linked glycosylation sites (N306, N378, N417, N481 and N529) were occupied by carbohydrate moieties (gray spheres) [35]. N378 is not modeled in the figure due to weak electron density. The G glycoprotein head domain folds as a six-blade β -propeller. The structure of the entire HeV G stalk domain (residues 71–173) has not been determined, but here the stalk regions (residues 77–136) of each monomer are modeled [78] and are not a continuous helix. The HeV-sG stalk residues 98–135 appear equivalent to the HN glycoprotein stalk helix domain of the recently reported NDV structure [79]. Here, the position of HeV sG head dimer and stalks are oriented based on the alignment to the NDV structure. The ephrin receptor binding face of the red monomer is facing out and that of the blue monomer is facing left.

Table 1

Antiviral and advanced immunization strategies trialed in animal models by henipavirus challenge.

Strategy	Viral antigen target or immunogen	Challenge model
Antiviral therapy		
Ribavirin		Golden Hamster ^a (NiV and HeV) African Green Monkey ^b (HeV)
Chloroquine		Ferret ^c (NiV), Hamster ^a (NiV and HeV)
polyIC ₁₂ U		Golden Hamster ^d (NiV)
Heptad peptide		Golden Hamster ^e (NiV)
Passive immunization		
Human monoclonal antibody	Nipah / Hendra G glycoprotein	Ferret ^f (NiV) African green monkey ^g (HeV)
Active immunization		
Recombinant canarypox virus	Nipah F and/or G glycoprotein	Pig ^h (NiV)
Subunit	Hendra soluble G glycoprotein	Cat ⁱ (NiV), Ferret ^j (HeV, NiV), African Green Monkey ^k (HeV, NiV) Horse ^l (HeV)

^a Ribavirin and chloroquine alone or in combination are evaluated in the henipavirus hamster model [55,56]

^b Ribavirin was evaluated in an African green monkey model of Hendra virus infection [33].

^c Chloroquine was evaluated in the Nipah virus ferret model [48].

^d Administration of polyIC₁₂U was evaluated in the Nipah virus hamster model [56].

^e A cholesterol-tagged peptide fusion inhibitor was evaluated in the Nipah virus hamster model [63].

^f A cross-reactive neutralizing human mAb against henipavirus G glycoprotein (m102.4) was evaluated as both a pre- and post-exposure therapy in the Nipah virus ferret model [28] and Hendra virus ferret model (Pallister and Broder, unpublished).

^g Human mAb (m102.4) was evaluated by post-exposure application in African green monkeys by intravenous infusion following Hendra virus challenge [69] and Nipah virus challenge (Geisbert and Broder, unpublished).

^h Nipah F and/or G encoding recombinant canarypox viruses are used to immunize pigs followed by intranasal Nipah virus challenge [70].

ⁱ Hendra virus sG is used to immunize cats followed by subcutaneous Nipah virus challenge [26]; Hendra virus sG is used to immunize cats followed by oronasal Nipah virus challenge [74].

^j Hendra virus sG is used to immunize ferrets followed by oronasal Hendra virus challenge [49]; and NiV challenge (Pallister and Broder, unpublished).

^k Hendra virus sG is used to immunize African green monkeys followed by intratracheal Nipah virus challenge [75] and Hendra virus challenge (Geisbert and Broder, unpublished).

^l Hendra virus sG is used to immunize horses followed by oronasal Hendra virus challenge [76] (Middleton and Broder, unpublished).