MINIREVIEW

Henipavirus infection of the central nervous system

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One sentence summary: This mini review provides an overview and discusses current knowledge and gaps on the neuropathobiology of henipavirus infections.

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ABSTRACT

Nipah virus (NiV) and Hendra virus are highly pathogenic zoonotic viruses of the genus *Henipavirus*, family *Paramyxoviridae*. These viruses were first identified as the causative agents of severe respiratory and encephalitic disease in the 1990s across Australia and Southern Asia with mortality rates reaching up to 75%. While outbreaks of Nipah and Hendra virus infections remain rare and sporadic, there is concern that NiV has pandemic potential. Despite increased attention, little is understood about the neuropathogenesis of henipavirus infection. Neuropathogenesis appears to arise from dual mechanisms of vascular disease and direct parenchymal brain infection, but the relative contributions remain unknown while respiratory disease arises from vasculitis and respiratory epithelial cell infection. This review will address NiV basic clinical disease, pathology and pathogenesis with a particular focus on central nervous system (CNS) infection and address the necessity of a model of relapsed CNS infection. Additionally, the innate immune responses to NiV infection *in vitro* and in the CNS are reviewed as it is likely linked to any persistent CNS infection.

Keywords: henipavirus; pathogenesis; acute encephalitis; relapsed/late-onset encephalitis

EPIDEMIOLOGY AND CLINICAL MANIFESTATION OF HENIPAVIRUS INFECTION

Hendra virus (HeV) was the first recognized member of the henipavirus (HNV) genus, originally designated equine morbillivirus (Selvey *et al.* 1995; Murray *et al.* 1995). The initial cases were identified in 1994 in Hendra, Brisbane, Australia affecting 20 horses with severe respiratory infections. During this outbreak, three humans were infected with HeV while caring for sick horses (O'Sullivan *et al.* 1997). These patients experienced flu like illness followed by signs of neurological disease such as headaches and confusion. One case developed meningitis (Wong *et al.* 2009).

Since these initial infections, there have been 83 laboratory confirmed cases and 20 suspected cases in horses along the Queensland coast with an 89% case fatality rate (CFR) and seven human cases with a 57% CFR (Field 2016; Queensland Government 2018). After the first outbreak, fruit bats of the *Pteropus* genus were identified as the natural reservoirs of HeV, and a model was proposed in which horses act as intermediate amplifying hosts after being infected by eating partially eaten fruit or through contact with infected bat urine (Young *et al.* 1996; Halpin *et al.* 2000; Field *et al.* 2012). Several *Pteropus* species are infected in the wild, but *Pteropus alecto* and *Pteropus conspicillatus* appear to be the most relevant reservoirs (Field 2016).

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Figure 1. Epidemiology of HNV infection of humans. *Pteropus* fruit bats represent the primary reservoirs of NiV and HeV. HeV infection on the Queensland coast occurs after horses are exposed to HeV from bats, likely via exposure to urine or partially eaten fruits. HeV then is transmitted to humans in close contact to infected horses such as veterinarians or trainers. A similar pattern is suspected for the NiV-M outbreak in the Philippines. During the Malaysia/Singapore outbreak 1998–99, NiV was transmitted to pigs, likely via partially eaten fruits. From pigs the virus infected workers on pig farms and abattoirs. Pig-to-pig transmission was also observed. NiV-B has spilled over into humans via numerous routes, but perhaps the most notable is the direct infection of humans via the consumption of date palm sap contaminated by bat urine or saliva. There has also been significant human-to-human transmission during NiV-B outbreaks.

Nipah virus (NiV) emerged during a 1998–1999 outbreak in peninsular Malaysia and Singapore (Chua et al. 1999; Chua et al. 2000). The disease was first recognized as a dual outbreak in pigs and pig farmers/abattoir workers. Pigs developed a mild febrile illness with respiratory involvement, while humans developed severe encephalitic illness (Chua et al. 1999; Goh et al. 2000). The incubation period in humans was between 2 days to 2 months, and onset was abrupt with fever, headache, dizziness and vomiting. Later neurological signs included reduced levels of consciousness, areflexia, hypotonia and abnormal doll's eye-reflex (Goh et al. 2000). Nearly a quarter of patients had seizures, and almost all seizures were generalized tonic-clonic (Paton et al. 1999; Goh et al. 2000). In Malaysia, focal neurological signs were common, with the most prominent being segmental myoclonus in 32% of cases (Goh et al. 2000).

The Malaysian NiV outbreak resulted in 265 human cases with a 38% CFR and the culling of over one million pigs (Ang, Lim and Wang 2018). One study estimated that the outbreak resulted in over \$2 billion in damages across Malaysia alone due to the disruption of the pig farming industry (Hosono et al. 2006). Again, *Pteropus* bats were found to be the reservoir of infection, and it is speculated that partially eaten fruits first infected pigs in farms adjacent to the natural rainforest habitat of the *Pteropus* bats (Chua et al. 2002). No other outbreaks have been detected in Malaysia or Singapore. However, there was a suspected NiV outbreak in the Philippines in 2014 associated with sick horses that appears to have been caused by the NiV-Malaysia strain (NiV-M) or a closely related virus (Ching et al. 2015).

Since 2001 there have been nearly yearly outbreaks of NiV infection in Bangladesh and India associated with approximately 300 human cases, person-to-person transmission and a 75% CFR (Luby, Gurley and Hossain 2009; Ang, Lim and Wang

2018). Most of these cases have occurred in Bangladesh or Western Bengal, India, but in 2018 there was an outbreak in Kerala, on the Western coast of India demonstrating the widespread distribution of this virus across the Indian subcontinent (Luby and Gurley 2012; Arunkumar et al. 2018). These outbreaks have been associated with various routes of transmission, including the consumption of bat-contaminated raw date palm sap, human-to-human transmission, exposure to sick cows or pigs and exposure to bat droppings (Hsu et al. 2004; Chadha et al. 2006; Luby et al. 2006; Montgomery et al. 2008). While these outbreaks are smaller, from 1–66 cases, overall mortality is much higher than that observed in Malaysia. Whether differences in mortality are due to differences in virus strains or differences in healthcare availability and quality are debated (strain comparison studies are discussed in another section). Moreover, NiV outbreaks in Bangladesh are usually identified retrospectively (Gurley et al. 2007).

Additionally, NiV-Bangladesh strain (NiV-B) tends to have a different clinical presentation with more frequent and severe respiratory involvement manifesting as cough and respiratory distress in 75% of cases compared to 14% for NiV-M (Chadha et al. 2006; Hossain et al. 2008). However, presentation with neurologic symptoms is still common and similar to that seen in NiV-M cases with fever, altered mental status, headache and vomiting being found in the majority of cases (Hossain et al. 2008). These differences are intriguing, as NiV-M and NiV-B share 91.8% nucleotide homology (Harcourt et al. 2005). The epidemiology and clinical disease of HNV infection is summarized in Figs 1 and 2.

BASIC VIROLOGY AND PATHOGENESIS

Henipaviruses share the major molecular and genetic characteristics of other paramyxoviruses with a negative sense non-segmented RNA genome (Fig. 3) (Halpin et al. 2004). They encode six genes which produce nine proteins, the nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G) and polymerase (L) (Rota and Lo 2012). Additionally, the P gene contains RNA editing sites which can lead to the production of the proteins V and W as well as an alternate open reading frame encoding the C protein. The functions of these proteins are summarized in Table 1 and are similar to those found in other paramyxoviruses. NiV and HeV G proteins utilize ephrin-B2 and ephrin-B3 as cellular receptors (Bonaparte et al. 2005; Negrete et al. 2005). This correlates strongly with the observed tropism of HeV and NiV to endothelial cells and neurons, which express these ephrin receptors (Pernet, Wang and Lee 2012).

INNATE IMMUNE RESPONSES DURING HNV INFECTION

Interferon type I (IFN- α/β) responses are critical for control of NiV infection as demonstrated by the susceptibility of IFN- α/β receptor (IFNAR) knock out (IFNAR^{-/-}) mice (Dhondt et al. 2013). Additionally, it has been shown that infected primary endothelial and glial cells can produce IFN- β in response to NiV or HeV infection (Lo et al. 2010; Dhondt et al. 2013; Escaffre et al. 2013, 2016). However, neuroblastoma cells failed to mount a successful IFN response, suggesting differences in the regulation of IFN responses between cell types (Dhondt et al. 2013). The ability of HNVs to evade the IFN response is critical for the development of disease and is therefore antagonized by several viral proteins

Figure 2. Pathology and clinical manifestation of HNV infection in humans. This figure highlights the primary features of HNV infection noted in humans. Text in white highlights pathologic findings, while red signifies clinical signs/symptoms. The primary organs affected are the brain and lungs. Infection of the brain is divided into acute and relapsed disease as the pathology and clinical presentations are distinct. Of note, NiV-M tends to present with a more encephalitic disease, while NiV-B tends to present as a primarily respiratory disease. However, aspects of both neurologic and respiratory disease are found during infection with both viruses.

Figure 3. HNV structure and genome. The lipid envelope of HNV virions contains the attachment (G) and fusion (F) glycoproteins. Matrix protein (M) forms an ordered structure beneath the membrane. The RNA genome is coated by a ribonucleoprotein complex primarily composed of nucleocapsid protein (N). Additionally, the RNA-dependent RNA polymerase (L) and phosphoprotein (P) are present. The genome of HNVs encodes six genes and nine proteins. The P gene also encodes V, W, and C proteins. V and W are produced via RNA editing in which one or two guanines are added to mRNAs at an editing site. C is produced by an alternate ribosomal start signal.

Table 1. HNV protein functions.

Function	Function
N	Nucleocapsid encasing viral genome Prevents STAT1 translocation (Sugai et al. 2017)
P	Component of RNA polymerase complex Sequestration of STAT1/2 (Shaw et al. 2004)
V	Block RIG-I/MDA5 signaling (Andrejeva et al. 2004; Sanchez-Aparicio et al. 2018) Inhibits NLRP3 inflammasome activation (Komatsu et al. 2018) Sequestration of STAT1/2 (Shaw et al. 2004)
W	Inhibits IRF3 phosphorylation (Shaw et al. 2005) Sequestration of STAT1/2 (Shaw et al. 2004) Inhibition of inflammatory responses (Satterfield et al. 2015)
C	Enhancement of virion budding (Park et al. 2016) Blocks IRF7 signaling (Yamaguchi et al. 2014)
M	Virion budding (Ciancanelli and Basler 2006; Patch et al. 2007) IFN antagonism (inhibits TRIM6 mediated IKK ϵ ubiquitination) (Bharaj et al. 2016) Reduction of rRNA production (Rawlinson et al. 2018)
F	Fusion protein responsible for viral entry and syncytia formation (Tamin et al. 2002)
G	Attachment glycoprotein binds to ephrin-B2/B3 (Bonaparte et al. 2005; Negrete et al. 2005)
L	Viral RNA-dependent RNA polymerase (Harcourt et al. 2001)

(Table 1). The basic IFN response can be separated into three basic components: (a) recognition of viral RNA by cellular sensors, (b) the signaling pathways activated by these sensors that lead to IFN production and (c) the signaling mediated by binding of IFN to the IFNAR complex leading to the production of interferon stimulated genes (ISGs). HNVs are able to antagonize all of these components. The role of these proteins in HNVs may be uniquely important as HNVs produce far more RNA edited transcripts (V and W) than other paramyxoviruses (Kulkarni et al. 2009). This disrupted response likely plays a central role in the possible persistence of HNVs resulting in relapsed encephalitis. Fortunately, HNV antagonism of the IFN response has been extensively studied.

The HNV V proteins are able to bind and inhibit host dsRNA sensors MDA5 and LGP2 (Andrejeva et al. 2004; Parisien et al. 2009). More recently, NiV V protein was shown to inhibit the ubiquitination of RIG-I by tripartite motif-containing protein 25 (TRIM25), and therefore block downstream activation of MAVS and the induction of IFN (Sanchez-Aparicio et al. 2018).

Signaling pathways induced by the detection of viral RNA ultimately activate IRF3/IRF7 and induce the transcription of IFN- α/β . NiV W and M have been shown to disrupt this pathway. M protein has been shown to interact with and degrade the ubiquitin ligase TRIM6 and therefore block the ubiquitination of IKK ϵ which is necessary for the phosphorylation of IRF3 and STAT1 (Bharaj et al. 2016). Additionally, W was shown to traffic to the nucleus where it inhibited TLR-3 signaling induced IRF3 phosphorylation (Shaw et al. 2005). There has also been a report that C can inhibit TLR7/TLR9 activation via interactions with IKK α which prevent activation of IRF7 (Yamaguchi et al. 2014). This strategy would be particularly important in plasmacytoid dendritic cells (DCs) that are responsible for the majority of IFN production outside of the central nervous system (CNS).

P, V and W all target IFN- β signal transduction and transcription of ISGs via the inhibition of STAT1/STAT2. NiV and HeV V proteins were shown to interact with STAT1 and STAT2 (but only in the presence of STAT1) and lead to their aggregation into high-molecular weight complexes which prevent phosphorylation (Rodriguez, Parisien and Horvath 2002; Rodriguez, Wang and Horvath 2003). This can block both the type I and type II IFN

responses. P and W share the same STAT1 binding domains, but W appears to primarily sequester STAT1 to the nucleus (Shaw et al. 2004). P appears to be less efficient than V or W in inhibiting STAT1 responses (Shaw et al. 2004). Recent reports have also described a role for N in blocking nuclear import of STAT1, which may represent a novel paramyxovirus IFN antagonism strategy (Sugai et al. 2017).

Studies using recombinant NiV lacking V, W and C have provided conflicting results regarding their roles *in vivo*. P knockouts cannot be generated, as P is an essential part of the RNA polymerase complex. Two groups have reported attenuation following C deletion in the hamster model (Yoneda et al. 2010; Mathieu et al. 2012b). One study further demonstrated an increase in inflammatory cytokines and chemokines during C deletion (Mathieu et al. 2012b), while the other also showed a decrease in virulence after V deletion (Yoneda et al. 2010). However, a separate group with a different reverse genetics system and mutation strategy demonstrated that C deletion had no effect on virulence in the ferret model of NiV-M infection, whereas deletion of V resulted in 100% survival, suggesting that V is the major virulence factor (Satterfield et al. 2015, 2016). Interestingly, deletions of W (as well as W and C concurrent deletion) resulted in a protracted disease course with long term neurologic symptoms (Satterfield et al. 2015, 2016). Furthermore, V more strongly inhibited IFN responses, while W appeared to inhibit inflammatory responses via suppression of cytokines/chemokines (Satterfield et al. 2015). These results suggest that V may be necessary for virulent infection due to its impact on IFN inhibition, while antagonists of inflammatory responses, W and C may be dispensable for persistent/relapsed CNS infection. Additionally, V has recently been shown to inhibit the activation of the nucleotide-binding domain and leucine-rich repeat containing protein 3 (NLRP3) inflammasome (Komatsu et al. 2018). This, together with the effects of W deletion, demonstrate that in addition to being IFN antagonists, the P gene products are also potent inhibitors of inflammation.

ACUTE HNV ENCEPHALITIS

The pathology of NiV and HeV infection in humans appears similar (Wong et al. 2002, 2009), but almost all pathological reports

of NiV infection are from the initial Malaysia outbreak. Both viruses are characterized primarily by infection of endothelial cells leading to syncytia formation and small vessel vasculitis throughout the body (Wong et al. 2002, 2009). Systemically, HeV and NiV vasculitis was noted in the lung, heart and kidney, and parenchymal inflammation and necrosis was evident in the lung (Wong et al. 2002, 2009). In the cited studies, the brain appeared to be the most severely affected organ. Acute HNV CNS infection appears to consist of a dual pathogenic mechanism of vasculitis and direct neuronal infection. Endothelial infection and vasculitis can result in platelet activation and thrombi, producing microinfarcts which are likely responsible for the discrete necrotic plaques in the grey and white matter of the brain (Wong et al. 2002). Viral antigen is also detectable in neurons near these vascular lesions, but rarely detected in glial and ependymal cells (Wong et al. 2002). The olfactory nerve has been shown to be a potential route of infection in animal models, but the locations of neuronal lesions throughout the brain near vascular lesions suggest blood-brain barrier breakdown due to vascular inflammation may be a more relevant mechanism of entry for NiV during human infection (Wong et al. 2002; Munster et al. 2012; Escaffre et al. 2018). However, a recent study showed that NiV and HeV replicated efficiently in human olfactory epithelial cells and olfactory sensory neurons, suggesting that this route may in fact be a feasible route of entry during human infection (Borisevich et al. 2017). Alternatively, infected dendritic cells could serve as a 'Trojan horse' and cross the blood-brain barrier (Tiong et al. 2018). Perivascular cuffing and microglial nodules were also noted along with mononuclear infiltrates (Wong et al. 2002). Interestingly, these pathological lesions correlate to magnetic resonance imaging (MRI) studies during acute NiV-M infection which show small, discrete, hyperintense lesions throughout the brain (Paton et al. 1999; Goh et al. 2000). These likely reflect necrotic plaques secondary to microinfarcts.

LATE ONSET AND RELAPSING HNV ENCEPHALITIS

In 1994 a patient with exposure to HeV infected horses experienced a brief episode of aseptic meningitis (O'Sullivan et al. 1997). Thirteen months later, the same patient presented with mood changes, back pain and generalized tonic-clonic seizures with no additional exposures to horses. His condition deteriorated with focal motor seizures with secondarily generalized seizures, low-grade fever, hemiplegia, brainstem signs, loss of consciousness and death 25 days after admission (O'Sullivan et al. 1997). Polymerase chain reaction of cerebrospinal fluid was positive for HeV, with a 500 nucleotide sequence of the M gene identical to virus isolated from previous acute cases (O'Sullivan et al. 1997). MRI revealed high grey matter signal, and anti-HeV immunoglobulin G titers were greatly increased. Taken together, the presentation and laboratory findings suggested a recrudescent of HeV within the brain. Pathological findings revealed discrete foci of necrosis in the grey matter with viral antigen present in neurons and endothelial cells (O'Sullivan et al. 1997). Additionally, rare syncytia were observed in the brain, liver, spleen and lungs, although this finding has not been reported in later cases of HNV relapsing encephalitis (O'Sullivan et al. 1997). No infectious virus was isolated. Further pathologic examination of CNS tissue from this case revealed extensive inflammation of the cortex, perivascular cuffing, glial proliferation, reactive blood vessels and severe neuronal necrosis (Wong et al.

2009). Importantly, no vasculitis, syncytia (as opposed to earlier analysis) or thrombosis were observed. Viral RNA and antigen were detected primarily in neurons, with few glial cells also displaying RNA and antigen (Wong et al. 2009). Additionally, meningeal inflammation was present, and no syncytia were noted in other organs.

Cases of late-onset and relapsed encephalitis were also described after the 1998–99 Malaysian outbreak of NiV infection (Tan et al. 2002). Late-onset Nipah encephalitis was defined as primary onset of encephalitis 10 or more weeks after exposure to NiV accounting with a prevalence of 5% in Malaysia, and a relapse was defined as neurological presentation after recovery from NiV encephalitis with a prevalence of 9% in Malaysia (Abdullah and Tan 2014). Late-onset and relapsed encephalitis appeared similar clinically, pathologically and radiologically, suggesting that they are a continuum of the same pathologic process (Tan et al. 2002). In addition, 18% of 22 patients followed during one study of NiV survivors in Bangladesh reported delayed neurological deficiencies such as oculomotor defects, but NiV-B relapse has been studied and followed much less than NiV-M (Sejvar et al. 2007). The overall mortality for these cases was 18%, but a higher rate of neurologic deficits in survivors (66%) was seen compared to acute NiV encephalitis (Goh et al. 2000). Average time to relapse was 7.6 months after primary encephalitis, and the average time between exposure and late-onset encephalitis was 8.4 months, although one case has been reported 11 years after exposure (Abdullah et al. 2012). There were no significant demographic differences identified between relapsed and non-relapsed populations (Tan et al. 2002).

Clinically, relapsed/late-onset encephalitis differs from acute NiV encephalitis. Notably, fever was less common while seizures and focal signs were more common in relapsing encephalitis (Tan et al. 2002). In addition, the NiV-B late-onset neurologic deficits included ophthalmoplegia and cervical dystonia (Sejvar et al. 2007). MRI findings also differ compared to acute cases, with the most common findings being patchy confluent cortical lesions as opposed to small, diffuse and discrete subcortical and white matter lesions found in acute encephalitis (Sarji et al. 2000). Pathologically, relapsed/late-onset NiV cases displayed only CNS involvement (Wong et al. 2002). Importantly, vasculopathy and demyelination were not noted. The hallmarks of relapsed encephalitis were large confluent lesions with extensive viral inclusions both in neurons and the neuropil (Wong et al. 2002). This corresponds with severe neuronal necrosis, gliosis, perivascular cuffing and inflammatory infiltrate. Glial and ependymal cells were also positive for viral antigen in contrast to acute NiV infection (Wong et al. 2002). Overall, the differences in symptoms, radiology and pathology suggest that relapsed and late-onset NiV encephalitis represent a different pathological process than acute NiV encephalitis.

NEUROLOGICAL ASPECTS OF HNV DISEASE IN ANIMAL MODELS

Since HNVs are the only paramyxoviruses classified as biosafety level four pathogens, studying the mechanisms contributing to development of pathogenicity is difficult. However, pathogenesis studies of NiV and HeV have primarily been aided by a variety of animal models of infection (De Wit and Munster 2015). Neurologic disease is apparent in most animals. The naturally amplifying hosts, pigs and horses, are susceptible to experimental infection with NiV and HeV, respectively, and display similar neurological disease secondary to encephalitis as seen

in humans, although the course of disease appears more mild in pigs and does not have prominent encephalitic components (Middleton et al. 2002; Marsh et al. 2011). Interestingly, when lesions were noted in pig brains, glial cells were more prominently infected compared to other models (Weingartl et al. 2005). However, due to their size, cost and special biocontainment facility requirements, pathogenicity studies in pigs and horses are very limited. Cats were characterized as an early model, but again, encephalitic disease was limited (Williamson et al. 1998; Middleton et al. 2002). Guinea pigs have also been evaluated, and within the CNS the meninges and ependyma were affected but not the brain parenchyma (FJ. et al. 2008).

The primary animal models used to study HNV pathogenesis have been Syrian hamsters, ferrets and African green monkeys (De Wit and Munster 2015). The hamster model has been the most widely used small animal model. Hamsters develop both respiratory and encephalitic disease, but the route and dose of infection, as well as age of animal, bias the disease course (Wong et al. 2003; Rockx et al. 2011). A low dose infection tends to lead to encephalitic disease, while higher doses lead to rapid development of respiratory disease (Rockx et al. 2011). Intranasal infection tends to result in neurological disease while intraperitoneal infection tends to result in severe respiratory disease (Wong et al. 2003; Rockx et al. 2011). Additionally, it was recently shown that aerosol infection uniformly leads to both respiratory disease and neurological disease in 40% of infected hamsters (Escaffre et al. 2018). One of the major restrictions of this model is the limited availability of hamster specific reagents. While the ferret model is larger, more expensive and also has limited availability of specific reagents, ferrets develop disease very similar to humans with consistent encephalitic disease occurring (Bossart et al. 2009; Pallister et al. 2009; Leon et al. 2018). African green monkeys are the currently accepted NHP model for NiV and HeV infection, as they very closely resemble clinical disease as observed in human NiV and HeV infection (Geisbert et al. 2010; Rockx et al. 2010). Exposure to intermediate particle aerosol particles (7 μ m) consistent with droplet transmission resulted in respiratory disease and CNS lesion (Hammoud et al. 2018).

In addition to the above-mentioned animal models, limited studies have also been performed in mice. Immunocompetent mouse strains are not susceptible to wildtype NiV infection, although they can develop lethal infection following direct intracerebral infection (Dhondt et al. 2013; Dups et al. 2014). IFNAR^{-/-} mice develop encephalitis and meningitis following intraperitoneal infection highlighting the importance of the type I IFN response in control of HNV infection (Dhondt et al. 2013; Yun et al. 2015). Aged immunocompetent mice infected intracranially with HeV developed a protracted infection confined to the brain parenchyma (largely neurons), and may be a suitable model to study HeV brain parenchymal infection (Dups et al. 2012). However, this finding could not be replicated with NiV infection (Dups et al. 2014). Few studies have addressed the described difference in the neurological disease in patients caused by NiV-M and NiV-B, although one study in the African green monkeys model suggested that NiV-B infection resulted in more severe respiratory pathology, leading to mortality before neurological disease could become apparent (Mire et al. 2016). Of note, respiratory epithelial cells appear more susceptible to NiV-B infection compared to NiV-M infection, possibly contributing to the increased incidence of respiratory disease seen in NiV-B patients (Escaffre et al. 2016). However, other studies using ferrets and hamsters suggest that while NiV-B replicates more quickly and to higher titers, there is no observable difference in disease course or outcome (Baseler et al. 2015; Clayton et al.

2016). Therefore, the role of strain differences on clinical presentation and CFR remains to be determined. Most of the amino acid differences between NiV-M and NiV-B are found in the intergenic regions, but the P protein and accessory V protein have the largest differences among translated sequences at approximately 92% amino acid similarity (Harcourt et al. 2005). Differences in P and V may be due to the necessity of the virus to overcome the immune response in their reservoir hosts, and these differences may also contribute to differences in pathogenicity related to their ability to inhibit human immune responses.

HNV INDUCED PATHOGENESIS OF THE CNS

Until now, only few studies have addressed molecular mechanisms of HNV CNS infection. CNS entry appears to be a later phenomenon during NiV infection. While viral RNA can be detected in the lungs of hamsters hours after infection, it is not present in the CNS until day two after infection (Baseler et al. 2016). CNS damage during acute HNV infection seems to arise from a dual pathogenic mechanism of vasculitis and direct neuronal infection. Therefore, the molecular mechanisms of neuronal infection, evasion of immune responses in the CNS, persistence and relapse remain unknown and a model of persistence and relapse would be invaluable in assessing antiviral and vaccine candidates. *In vivo*, hamster neurons and endothelial cells produce the lymphocytic CXCL10 which may play a pathogenic or protective role in recruiting lymphocytes to sites of infection (Mathieu et al. 2012a). This finding was confirmed in human patient tissues (Mathieu et al. 2012a). Infiltrating lymphocytes are present during encephalitis and may be necessary to clear viral infection and limit disease course, or they may induce neuronal damage and worsen the clinical outcome of disease. The role of individual inflammatory cell populations within the brain during HNV infection remains to be determined.

Mouse primary glial cells are susceptible to NiV-M infection, and viral replication was limited by the type I IFN response (Dhondt et al. 2013). This study also demonstrated that glial cells can produce IFN- β in response to NiV infection. However, most *in vitro* studies of neuronal cells have relied on neuroblastoma cell lines, which are a valuable, easy to work with, and widely available model; however, cancer derived cell lines often have impaired signaling pathways and genetic regulations. Therefore, cautious interpretation of data derived from these lines is necessary. M17 neuroblastoma cell line was highly susceptible to NiV-M infection, but failed to induce an inflammatory or IFN response (Aljofan et al. 2009; Lo et al. 2010). Infection of SK-N-MC cell line with NiV-M was not productive, but did induce apoptosis (Chang et al. 2006, 2007). In contrast, primary human endothelial cells and bronchial/small airway epithelial cells have been better characterized to induce IFN and inflammatory cytokine/chemokine responses (Lo et al. 2010; Satterfield et al. 2015; Escaffre et al. 2016, 2017). Interestingly, recent *in vivo* studies in African green monkeys found an absence of inflammation in the brain with low levels of lymphocytes (Hammoud et al. 2018). This study proposed that NiV encephalitis is not a true neurotropic infection, but rather virus-induced vasculitis. Still, while this may be generally accurate during acute infection, the presence of chronic/relapse encephalitis suggests neuronal infection is a highly relevant aspect of HNV infection. Meanwhile in the ferret model, a recent study showed reduced, but not absent, inflammatory responses in the brain (Leon et al. 2018). It is still unknown if more physiologic human neuronal cells can still respond to NiV infection with IFN and/or chemokine responses. Inflammatory lesions within the brain suggest that

such responses exist, although they could be explained by endothelial cell infection during acute disease. However, during relapsed infection neuronal cells are the primary cell type infected, and would be expected to be the primary source of any inflammatory mediators.

SUBACUTE SCLEROSING PANENCEPHALITIS: ANOTHER PARAMYXOVIRAL RELAPSED ENCEPHALITIS

Relapsed HNV encephalitis has drawn comparison to subacute sclerosing panencephalitis (SSPE) caused by another paramyxovirus, measles virus (MeV). Indeed, several other paramyxoviruses (particularly morbilliviruses) such as canine distemper virus and dolphin morbillivirus have demonstrated a capacity for relapsed or late onset CNS infection (Headley et al. 2009; Van Bresse et al. 2014), but SSPE is by far the most well studied of the paramyxovirus relapsed encephalitides. SSPE is a severe, almost universally fatal, episode of demyelinating encephalitis due to MeV infection of neurons and glia months to years after initial infection (Anlar 2013; Ludlow et al. 2015). In contrast, NiV relapsed encephalitis has only an 18% mortality rate and is not associated with demyelination (Tan et al. 2002).

Initial signs of disease often involve subtle cognitive and behavioral changes (Garg 2008). As the disease progresses, mental function declines and speech deteriorates. Myoclonic jerks, which decrease with late progression of the disease, are a hallmark of SSPE. Ultimately, patients become quadriplegic, vegetative and often suffer hypothalamic failure leading to respiratory failure. MRI studies reveal non-specific changes to the white matter and cerebral edema (Praveen-kumar et al. 2007). The hallmark pathologic finding in SSPE is neuronal and glial (oligodendrocytes and astrocytes) viral inclusion bodies consisting of protein or ribonucleoprotein complexes. Findings during the early stages of disease include widespread inflammation, neuronal degeneration, demyelination, perivascular cuffing and gliosis. In later stages of infection, inflammation is less pronounced, but other findings are more severe. Additionally, neurofibrillary tangles are prominent (Garg 2008; Anlar 2013). Indeed, the clinical presentation is distinct from that seen in NiV relapse, being more generalized while NiV is more focal. Additionally, while the two diseases share intraneuronal inclusions and inflammation, NiV relapsed encephalitis is not demyelinating. Clearly, there are major differences in the pathophysiology between these two relapsed infections, but they may still share mechanisms of immune evasion, persistence and/or reactivation.

SSPE occurs despite host immune responses against MeV. Additionally, polymorphisms within the genes encoding interleukin IL-2, IL-4, IL-12, MxA and TLR-3 have been implicated in increased susceptibility to SSPE (Reuter and Schneider-Schaulies 2010). Neurons appear to fail to induce a type I IFN response or induce MHC I expression when infected with MeV (Dhib-Jalbut et al. 1999; Fang, Song and Dhib-Jalbut 2001; Fang et al. 2002). Together, these studies suggest abnormal immune responses may result in MeV persistence within the CNS. No studies have assessed IFN responses in NiV infected neurons or assessed genetic polymorphisms associated with relapse.

Expression of the structural genes M, F and H are reduced in SSPE, and hypermutations in the coding sequences of these genes, especially M and F, are often found and are thought to

contribute to increased RNA replication (Cattaneo et al. 1988; Patterson et al. 2001; Watanabe et al. 2018). Furthermore, recombinant MeV expressing M from an SSPE isolate becomes neurovirulent, suggesting that these mutations play a functional role in the development of SSPE (Patterson et al. 2001). Recent research has suggested hyperfusogenic F proteins also seem to be important for neurovirulence along with mutations in the P gene which reduce RNA editing and expression of V (Watanabe et al. 2015; Millar et al. 2016). To date no host factors involved with persistence or relapse of NiV infection have been identified. Unpublished reports claim that no mutations have been identified in viral RNA recovered from relapsed cases (Wong 2010). However, recent reports of ferret infection with recombinant NiV deficient in P gene products suggest that alterations of P gene expression may play a role in persistent neurologic infection (Satterfield et al. 2015, 2016).

CONCLUSIONS AND OUTLOOK

While NiV has recently received more attention and calls for increased research, the basic mechanisms of neuropathogenesis remain relatively unknown. In particular, almost no studies have attempted to address the mechanisms of NiV persistence or relapse within the CNS. Several other paramyxoviruses produce similar persistence and CNS relapse, and it is possible that this family of viruses (or at the very least the morbilliviruses and henipaviruses) have shared mechanisms that enable this process.

In order to develop a clearer understanding of HNV encephalitis, the development of a model of relapsed encephalitis is greatly needed. Due to the sporadic nature of HNV outbreaks and the fact that most cases occur in Bangladesh where autopsies are not commonly performed due to religious reasons, access to human samples is extremely limited if not impossible. Therefore, animal models which accurately recapitulate human infection are critical to study HNV pathogenesis. Currently, no consistent model of relapse exists, but the aged mouse model of HeV and NiV W deletion mutants in ferrets develop protracted neurological disease, which may be suitable models to study certain aspects of infection. The development of an *in vivo* model could be aided by the use of relevant *in vitro* models. To date, no published work has studied NiV infection in primary human neuronal tissue. One possible model could be the use of human neural stem cell derived neuron/astrocyte co-cultures, which have recently been used to study other encephalitic viruses (McGrath et al. 2017; Dawes et al. 2018). Such *in vitro* and *in vivo* systems will also be critical for the development of vaccines and antiviral therapeutics. Antivirals against NiV should be able to cross the blood-brain-barrier and reach therapeutic concentrations in the brain while not being directly neurotoxic. Another particular concern with antiviral development will be whether treatments can prevent persistence and relapse, and if treatment is effective during relapse.

Several aspects of HNV acute and relapsed or late-onset encephalitis represent important avenues to understand the pathogenesis of NiV and HeV. If HNVs persist, what cell types are the reservoirs for persistence? Persistently infected cell populations remain unknown in SSPE/MeV infection, and no studies have assessed HNV persistence. The identification of these populations is critical for the development of *in vitro* models of persistence. Secondly, how are HNVs able to subvert the immune response and lead to relapse? W deletions leading to prolonged neurologic disease and the finding of mutations in P in SSPE suggest that modulation of the IFN antagonists is important for

chronic CNS infection (Satterfield et al. 2015; Millar et al. 2016). Future studies on the role of P, V, W and C in the CNS is required. What are the factors leading to relapse and what patients are at highest risk? These questions require urgent attention, but as SSPE has demonstrated, these diseases can be incredibly difficult to study.

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REFERENCES

- Abdullah S, Chang L-Y, Rahmat K et al. Late-onset Nipah virus encephalitis 11 years after the initial outbreak: a case report. *Neurol Asia* 2012;**17**:71–74.
- Abdullah S, Tan CT, Henipavirus Encephalitis, *Handb Clin Neurol* 2014;**123**:663–70.
- Aljofan M, Saubern S, Meyer AG et al. Characteristics of Nipah virus and Hendra virus replication in different cell lines and their suitability for antiviral screening. *Virus Res* 2009;**142**:92–9.
- Andrejeva J, Childs KS, Young DF et al. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN- β promoter. *Proc Natl Acad Sci USA* 2004;**101**:17264–9.
- Ang BSP, Lim TCC, Wang L. Nipah virus infection. *J Clin Microbiol* 2018;**56**. doi:10.1128/JCM.01875-17.
- Anlar B, Subacute Sclerosing Panencephalitis and Chronic Viral Encephalitis, *Handb Clin Neurol* 2013;**112**:1183–89.
- Arun Kumar G, Chandni R, Mourya DT et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. *J Infect Dis* 2018. doi:10.1093/infdis/jiy612.
- Baseler L, de Wit E, Scott DP et al. Syrian hamsters (*Mesocricetus auratus*) oronasally inoculated with a Nipah virus isolate from Bangladesh or Malaysia develop similar respiratory tract lesions. *Vet Pathol* 2015;**52**:38–45.
- Baseler L, Scott DP, Saturday G et al. Identifying early target cells of Nipah virus infection in syrian hamsters. *PLoS Negl Trop Dis* 2016;**10**:1–18.
- Bharaj P, Wang YE, Dawes BE et al. The matrix protein of Nipah virus targets the E3-Ubiquitin ligase TRIM6 to inhibit the IKK ϵ Kinase-Mediated Type-I IFN antiviral response. *PLoS Pathog* 2016;**12**:1–27.
- Bonaparte MI, Dimitrov AS, Bossart KN et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci USA* 2005;**102**:10652–7.
- Borisevich V, Ozdener MH, Malik B et al. Hendra and Nipah virus infection in cultured human olfactory epithelial cells. *mSphere* 2017;**2**. doi:10.1128/mSphere.00252-17.
- Bossart KN, Zhu Z, Middleton D et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS Pathog* 2009;**5**:e1000642.
- Cattaneo R, Schmid A, Billeter MA et al. Multiple viral mutations rather than host factors cause defective measles virus gene expression in a subacute sclerosing panencephalitis cell line. *J Virol* 1988;**62**:1388–97.
- Chadha MS, Comer JA, Lowe L et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis* 2006;**12**:235–40.
- Chang L-Y, Ali AM, Hassan S et al. Human neuronal cell protein responses to Nipah virus infection. *Virol J* 2007;**4**:54.
- Chang L-Y, Ali ARM, Hassan SS et al. Nipah virus RNA synthesis in cultured pig and human cells. *J Med Virol* 2006;**78**:1105–12.
- Ching PKG, de los Reyes VC, Sucaldito MN et al. Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis* 2015;**21**:328–31.
- Chua KB, Bellini WJ, Rota PA et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science* (80) 2000;**288**:1432–5.
- Chua KB, Goh KJ, Wong KT et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* 1999;**354**:1257–9.
- Chua KB, Koh CL, Hooi PS et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 2002;**4**:145–51.
- Ciancanelli MJ, Basler CF. Mutation of YMYL in the Nipah virus matrix protein abrogates budding and alters subcellular localization. *J Virol* 2006;**80**:12070–8.
- Clayton BA, Middleton D, Arkinstall R et al. The nature of exposure drives transmission of Nipah viruses from Malaysia and Bangladesh in ferrets. *PLoS Negl Trop Dis* 2016;**10**. doi:10.1371/journal.pntd.0004775.
- Dawes BE, Gao J, Atkins C et al. Human neural stem cell-derived neuron/astrocyte co-cultures respond to La Crosse virus infection with proinflammatory cytokines and chemokines. *J Neuroinflammation* 2018;**5**:1–15.
- De Wit E, Munster VJ. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *J Pathol* 2015;**235**:196–205.
- Dhib-Jalbut S, Xia J, Rangaviggula H et al. Failure of measles virus to activate nuclear factor-kappa B in neuronal cells: implications on the immune response to viral infections in the central nervous system. *J Immunol* 1999;**162**:4024–9.
- Dhondt KP, Mathieu C, Chalons M et al. Type I interferon signaling protects mice from lethal henipavirus infection. *J Infect Dis* 2013;**207**:142–51.
- Dups J, Middleton D, Long F et al. Subclinical infection without encephalitis in mice following intranasal exposure to Nipah virus-Malaysia and Nipah virus-Bangladesh. *Virol J* 2014;**11**:1–5.
- Dups J, Middleton D, Yamada M et al. A new model for Hendra virus encephalitis in the mouse. *PLoS One* 2012;**7**:1–14.
- Escaffre O, Borisevich V, Carmical JR et al. Henipavirus pathogenesis in human respiratory epithelial cells. *J Virol* 2013;**87**:3284–94.
- Escaffre O, Borisevich V, Vergara LA et al. Characterization of Nipah virus infection in a model of human airway epithelial cells cultured at an air-liquid interface. *J Gen Virol* 2016;**97**:1077–86.
- Escaffre O, Hill T, Ikegami T et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. *J Infect Dis* 2018;**218**:1602–10.
- Escaffre O, Saito TB, Juelich TL et al. Contribution of human lung parenchyma and leukocyte influx to oxidative stress and immune system-mediated pathology following Nipah virus infection. *J Virol* 2017;**91**:1–15.
- F.J. T-V, W.J. S, P.E. R et al. Histopathologic and immunohistochemical characterization of Nipah virus infection in the guinea pig. *Vet Pathol* 2008;**45**:576–85.

- Fang YY, Song ZM, Dhib-Jalbut S. Mechanism of measles virus failure to activate NF-kappaB in neuronal cells. *J Neurovirol* 2001;7:25–34.
- Fang YY, Song ZM, Wu T et al. Defective NF- κ B activation in virus-infected neuronal cells is restored by genetic complementation. *J Neurovirol* 2002;8:459–63.
- Field H, Cramer G, Kung NY-H et al. Ecological aspects of hendra virus. *Curr Top Microbiol Immunol* 2012;359:11–23.
- Field HE. Hendra virus ecology and transmission. *Curr Opin Virol* 2016;16:120–5.
- Garg RK. Subacute sclerosing panencephalitis. *J Neurol* 2008;255:1861–71.
- Geisbert TW, Daddario-DiCaprio KM, Hickey AC et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One* 2010;5:e10690.
- Goh KJ, Tan CT, Chew NK et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000;342:1229–35.
- Gurley ES, Montgomery JM, Hossain MJ et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerg Infect Dis* 2007;13:1031–7.
- Halpin K, Bankamp B, Harcourt BH et al. Nipah virus conforms to the rule of six in a minigenome replication assay. *J Gen Virol* 2004;85:701–7.
- Halpin K, Young PL, Field HE et al. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol* 2000;81:1927–32.
- Hammoud DA, Lentz MR, Lara A et al. Aerosol exposure to intermediate size Nipah virus particles induces neurological disease in African green monkeys. *PLoS Negl Trop Dis* 2018;12:e0006978.
- Harcourt BH, Lowe L, Tamin A et al. Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis* 2005;11:1594–7.
- Harcourt BH, Tamin A, Halpin K et al. Molecular characterization of the polymerase gene and genomic termini of Nipah virus. *Virology* 2001;287:192–201.
- Headley SA, Amude AM, Alfieri AF et al. Molecular detection of canine distemper virus and the immunohistochemical characterization of the neurologic lesions in naturally occurring old dog encephalitis. *J Vet Diagn Invest* 2009;21:588–97.
- Hosono H, Kono H, Ito S et al. Economic Impact of Nipah virus infection outbreak in Malaysia. *International Symposia on Veterinary Epidemiology and Economics proceedings* 2006;11:324.
- Hossain MJ, Gurley ES, Montgomery JM et al. Clinical presentation of Nipah virus infection in Bangladesh. *Clin Infect Dis* 2008;46:977–84.
- Hsu VP, Hossain MJ, Parashar UD et al. Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis* 2004;10:2082–7.
- Komatsu T, Tanaka Y, Kitagawa Y et al. Sendai virus V protein inhibits the secretion of Interleukin-1 β by preventing NLRP3 inflammasome assembly. *J Virol* 2018. doi:10.1128/JVI.00842-18.
- Kulkarni S, Volchkova V, Basler CF et al. Nipah virus edits its p gene at high frequency to express the V and W proteins. *J Virol* 2009;83:3982–7.
- Leon AJ, Borisevich V, Boroumand N et al. Host gene expression profiles in ferrets infected with genetically distinct henipavirus strains. *PLoS Negl Trop Dis* 2018;12:e0006343.
- Lo MK, Miller D, Aljofan M et al. Characterization of the antiviral and inflammatory responses against Nipah virus in endothelial cells and neurons. *Virology* 2010;404:78–88.
- Luby SP, Gurley ES, Hossain MJ. Transmission of human infection with Nipah virus. *Clin Infect Dis* 2009;49:1743–8.
- Luby SP, Gurley ES. Epidemiology of henipavirus disease in humans. *Curr Top Microbiol Immunol* 2012;359:25–40.
- Luby SP, Rahman M, Hossain MJ et al. Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis* 2006;12:1888–94.
- Ludlow M, McQuaid S, Milner D et al. Pathological consequences of systemic measles virus infection. *J Pathol* 2015;235:253–65.
- Marsh GA, Haining J, Hancock TJ et al. Experimental infection of horses with Hendra virus/Australia/horse/2008/Redlands. *Emerg Infect Dis* 2011;17:2232–8.
- Mathieu C, Guillaume V, Sabine A et al. Lethal Nipah virus infection induces rapid overexpression of cxcl10. *PLoS One* 2012a;7. doi:10.1371/journal.pone.0032157.
- Mathieu C, Guillaume V, Volchkova VA et al. Nonstructural Nipah virus C protein regulates both the early host proinflammatory response and viral virulence. *J Virol* 2012b;86:10766–75.
- McGrath EL, Rossi SL, Gao J et al. Differential responses of human fetal brain neural stem cells to Zika virus infection. *Stem Cell Reports* 2017;8:715–27.
- Middleton DJ, Westbury HA, Morrissey CJ et al. Experimental Nipah virus infection in pigs and cats. *J Comp Pathol* 2002;126:124–36.
- Millar EL, Rennick LJ, Weissbrich B et al. The phosphoprotein genes of measles viruses from subacute sclerosing panencephalitis cases encode functional as well as non-functional proteins and display reduced editing. *Virus Res* 2016;211:29–37.
- Mire CE, Satterfield BA, Geisbert JB et al. Pathogenic differences between Nipah Virus Bangladesh and Malaysia Strains in primates: implications for antibody therapy. *Sci Rep* 2016;6:1–16.
- Montgomery JM, Hossain MJ, Gurley E et al. Risk factors for Nipah virus encephalitis in Bangladesh. *Emerg Infect Dis* 2008;14:1526–32.
- Munster VJ, Prescott JB, Bushmaker T et al. Rapid Nipah virus entry into the central nervous system of hamsters via the olfactory route. *Sci Rep* 2012;2:1–8.
- Murray K, Selleck P, Hooper P et al. A morbillivirus that caused fatal disease in horses and humans. *Science* 1995;268:94–7.
- Negrete OA, Levroney EL, Aguilar HC et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* 2005;436:401–5.
- O'Sullivan JD, Allworth AM, Paterson DL et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* 1997;349:93–5.
- Pallister J, Middleton D, Cramer G et al. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. *J Virol* 2009;83:11979–82.
- Parisien J-P, Bamming D, Komuro A et al. A shared interface mediates paramyxovirus interference with antiviral RNA helicases MDA5 and LGP2. *J Virol* 2009;83:7252–60.
- Park A, Yun T, Vigant F et al. Nipah Virus C Protein Recruits Tsg101 to Promote the Efficient Release of Virus in an ESCRT-Dependent Pathway. *PLoS Pathog* 2016;12:1–22.
- Patch JR, Cramer G, Wang L-F et al. Quantitative analysis of Nipah virus proteins released as virus-like particles reveals central role for the matrix protein. *Virol J* 2007;4:1.
- Paton NI, Leo YS, Zaki SR et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* 1999;354:1253–6.
- Patterson JB, Cornu TI, Redwine J et al. Evidence that the hypermutated M protein of a subacute sclerosing panencephalitis measles virus actively contributes to the chronic progressive CNS disease. *Virology* 2001;291:215–25.
- Pernet O, Wang YE, Lee B. Henipavirus receptor usage and tropism. *Curr Top Microbiol Immunol* 2012;359:59–78.

- Praveen-kumar S, Sinha S, Taly AB et al. Electroencephalographic and imaging profile in a subacute sclerosing panencephalitis (SSPE) cohort: a correlative study. *Clin Neurophysiol* 2007;**118**:1947–54.
- Queensland Government. Summary of Hendra virus incidents in horses. 2018. <https://www.business.qld.gov.au/industries/service-industries-professionals/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary>.
- Rawlinson SM, Zhao T, Rozario AM et al. Viral regulation of host cell biology by hijacking of the nucleolar DNA-damage response. *Nat Commun* 2018;**9**:3057.
- Reuter D, Schneider-Schaulies J. Measles virus infection of the CNS: human disease, animal models, and approaches to therapy. *Med Microbiol Immunol* 2010;**199**:261–71.
- Rockx B, Bossart KN, Feldmann F et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. *J Virol* 2010;**84**:9831–9.
- Rockx B, Brining D, Kramer J et al. Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *J Virol* 2011;**85**:7658–71.
- Rodriguez JJ, Parisien J, Horvath CM. Nipah virus evasion of STAT 1,2 transcription factors. *J Virol* 2002;**76**:11476–83.
- Rodriguez JJ, Wang L-F, Horvath CM. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. *J Virol* 2003;**77**:11842–5.
- Rota PA, Lo MK. Molecular virology of the henipaviruses. *Curr Top Microbiol Immunol* 2012;**359**:41–58.
- Sanchez-Aparicio MT, Feinman LJ, Garcia-Sastre A et al. Paramyxovirus V proteins interact with the RIG-I/TRIM25 regulatory complex and inhibit RIG-I signaling. *J Virol* 2018;**92**. doi:10.1128/JVI.01960-17.
- Sarji SA, Abdullah BJJ, Goh KJ et al. MR imaging features of Nipah encephalitis. *Am J Roentgenol* 2000;**175**:437–42.
- Satterfield BA, Cross RW, Fenton KA et al. Nipah virus C and W proteins contribute to respiratory disease in ferrets. *J Virol* 2016;**90**:6326–43.
- Satterfield BA, Cross RW, Fenton KA et al. The immunomodulating V and W proteins of Nipah virus determine disease course. *Nat Commun* 2015;**6**:1–15.
- Sejvar JJ, Hossain J, Sana SK et al. Long-term neurological and functional outcome in Nipah virus infection. *Ann Neurol* 2007;**62**:235–42.
- Selvey LA, Wells RM, McCormack JG et al. Infection of humans and horses by a newly described morbillivirus. *Med J Aust* 1995;**162**:642–5.
- Shaw ML, Cardenas WB, Zamarin D et al. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol* 2005;**79**:6078–88.
- Shaw ML, Garcia-Sastre A, Palese P et al. Nipah virus V and W proteins have a common STAT1-binding domain yet inhibit STAT1 activation from the cytoplasmic and nuclear compartments, respectively. *J Virol* 2004;**78**:5633–41.
- Sugai A, Sato H, Takayama I et al. Nipah and Hendra virus nucleoproteins inhibit nuclear accumulation of signal transducer and activator of transcription 1 (STAT1) and STAT2 by interfering with their complex formation. *J Virol* 2017;**91**. doi:10.1128/JVI.01136-17.
- Tamin A, Harcourt BH, Ksiazek TG et al. Functional properties of the fusion and attachment glycoproteins of Nipah virus. *Virology* 2002;**296**:190–200.
- Tan CT, Goh KJ, Wong KT et al. Relapsed and late-onset Nipah encephalitis. *Ann Neurol* 2002;**51**:703–8.
- Tiong V, Shu M-H, Wong WF et al. Nipah virus infection of immature dendritic cells increases its transendothelial migration across human brain microvascular endothelial cells. *Front Microbiol* 2018;**9**:2747.
- Van Bresse M-F, Duignan P, Banyard A et al. Cetacean morbillivirus: current knowledge and future directions. *Viruses* 2014;**6**:5145–81.
- Watanabe S, Ohno S, Shirogane Y et al. Measles virus mutants possessing the fusion protein with enhanced fusion activity spread effectively in neuronal cells, but not in other cells, without causing strong cytopathology. *J Virol* 2015;**89**:2710–7.
- Watanabe S, Shirogane Y, Sato Y et al. New insights into measles virus brain infections. *Trends Microbiol* 2018. doi:10.1016/j.tim.2018.08.010.
- Weingartl H, Czub S, Copps J et al. Invasion of the central nervous system in a porcine host by Nipah virus. *J Virol* 2005;**79**:7528–34.
- Williamson MM, Hooper PT, Selleck PW et al. Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. *Aust Vet J* 1998;**76**:813–8.
- Wong KT, Grosjean I, Brisson C et al. A golden hamster model for human acute Nipah virus infection. *Am J Pathol* 2003;**163**:2127–37.
- Wong KT, Robertson T, Ong BB et al. Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathol Appl Neurobiol* 2009;**35**:296–305.
- Wong KT, Shieh W-J, Kumar S et al. Nipah virus infection. *Am J Pathol* 2002;**161**:2153–67.
- Wong KT. Nipah and Hendra viruses: recent advances in pathogenesis. 2010;**5**:129–31.
- Yamaguchi M, Kitagawa Y, Zhou M et al. An anti-interferon activity shared by paramyxovirus C proteins: inhibition of toll-like receptor 7/9-dependent alpha interferon induction. *FEBS Lett* 2014;**588**:28–34.
- Yoneda M, Guillaume V, Sato H et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One* 2010;**5**:1–8.
- Young PL, Halpin K, Selleck PW et al. Serologic evidence for the presence in Pteropus bats of a paramyxovirus related to equine morbillivirus. *Emerg Infect Dis* 1996;**2**:239–40.
- Yun T, Park A, Hill TE et al. Efficient reverse genetics reveals genetic determinants of budding and fusogenic differences between Nipah and Hendra viruses and enables real-time monitoring of viral spread in small animal models of henipavirus infection. *J Virol* 2015;**89**:1242–53.