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The immune response to Nipah virus infection

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Abstract

Nipah virus has recently emerged as a zoonotic agent that is highly pathogenic in humans. Outbreaks have occurred regularly over the last two decades in South and Southeast Asia, where mortality rates reach as high as 100%. The natural reservoir of Nipah virus has been identified as bats from the *Pteropus* family, where infection is largely asymptomatic. Human disease is characterized by both respiratory and encephalitic components, and thus far, no effective vaccine or intervention strategies are available. Little is know about how the immune response of either the reservoir host or incidental hosts responds to infection, and how this immune response is either inadequate or might contribute to disease in the dead-end host. Experimental vaccines strategies have given us some insight into the immunological requirements for protection. This review summarizes our current understanding of the immune response to Nipah virus infection and emphasizes the need for further research.

Introduction

Nipah virus is a member of the genus *Henipavirus* in the family *Paramyxoviridae*. It was recently suggested that Nipah viruses should be classified into two genotypes: genotype M,including virus isolates from Malaysia and Cambodia, and genotype B, including isolates from Bangladesh and India [35]. Nipah virus has a nonsegmented, negative-strand RNA genome of 18,246 or 18,252 nucleotides [27]. The Nipah virus genome encodes six structural proteins: the nucleoprotein N, phosphoprotein P, matrixprotein M, fusion protein F, receptor-binding glycoprotein G, and RNA-dependent RNA polymerase L. Within the P gene, an alternative start codon results in the production of the small protein C. Furthermore, editing of the P mRNA results in the production of proteins V and W through the insertion of one or two nontemplated G residues [17]. The three nonstructural proteins have been shown to antagonize the host innate immune response *in vitro*; proteins V and C have been shown to play an important role in Nipah virus pathogenicity in a hamster model [71].

Nipah virus was first detected during an outbreak of respiratory and neurological disease in pigs and humans in Malaysia in 1998-1999. The virus spread to Singapore and caused 276 human cases of encephalitis, with 106 fatalities [10]. A second outbreak of Nipah virus infection was retrospectively shown to have occurred in India in 2001 [6]. Since 2001, outbreaks of Nipah virus infection have occurred almost yearly in Bangladesh.

Different Nipah virus transmission cycles from the natural reservoir to humans appear to exist. In Malaysia, pigs function as an intermediate and amplifying host. Epidemiological data suggest that in Bangladesh, Nipah virus is transmitted to humans via consumption of date palm juice that is contaminated by bats during collection [36, 45]. Moreover, human-to-human transmission appears to play an important role in the spread of Nipah virus during

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outbreaks in Bangladesh. It is estimated that ~50% of cases in Bangladesh between 2001 and 2007 were the result of human-to-human transmission [37].

The majority of clinical data on Nipah virus infections were gathered during the initial Nipah virus outbreak in Malaysia. The average incubation period for Nipah virus is 10 days, but incubation periods as short as several days up to more than 4 weeks have been observed [8]. Initial symptoms of Nipah virus infection include fever, headache, myalgia, chills and rigors [8, 23]. Approximately a quarter of patients in Malaysia also showed signs of respiratory disease. A large percentage of patients go on to develop neurological signs including drowsiness and confusion. Approximately 19% of patients suffered from neurological deficits lasting at least 4 months after the onset of illness [8, 51]. The mortality rate during the outbreak in Malaysia was ~40% [8], but higher case fatality rates have been reported for Nipah virus outbreaks in Bangladesh. Nipah virus could be isolated from urine, saliva and oropharyngeal secretions up to seven days after onset of disease [12, 23]. Isolation of Nipah virus from cerebrospinal fluid (CSF) was associated with a poor disease outcome [11].

Autopsies were performed on 32 deceased patients from the Malaysia outbreak [67]. Autopsy showed extensive involvement of the blood vessels in the CNS, lung, heart, and kidney, with the CNS being the most affected tissue. Vasculitis was observed in small arteries, arterioles, capillaries and venules. Syncytia were found in endothelial cells in the CNS in 29% of patients. In the CNS, vasculitis, thrombosis, parenchymal necrosis and the presence of viral inclusions in neurons were the most common observations. Sixty-seven percent of patients showed parenchymal inflammation with infiltration of neutrophils, macrophages, lymphocytes and reactive microglia. In the lung, vasculitis and fibrinoid necrosis was observed in ~60% of patients. Spleens mainly showed depletion of white pulp [67].

Follow-up studies showed that a relapse or late-onset encephalitis occurred in a small number of patients that had recovered from the initial Nipah virus infection. The recurrence of Nipah virus was observed as late as 22 months after initial disease [60, 69]. Although Nipah virus outbreaks in India and Bangladesh also resulted in a combination of respiratory and neurological disease, severe respiratory involvement was much more common during the latter outbreaks, with case-fatality rates of up to 100% [9, 31].

Because of the close genetic relationship between Nipah virus and Hendra virus, the search for the natural reservoir of Nipah virus focused primarily on pteropid bat species (flying foxes), the natural host for Hendra virus in Australia [26]. Evidence for Nipah virus circulation in flying foxes has been detected over a wide geographical range including Malaysia, Bangladesh, India, Papua New Guinea, Cambodia, Indonesia and Thailand [5, 13, 18, 48, 52, 62, 63, 70]. Recently, serological surveys identified the circulation of henipaviruses in fruit bats in Africa, suggesting an extended geographical distribution of henipaviruses [16, 29, 32]. Based on serology, various species of flying foxes have recently been identified to play a role in the circulation and maintenance of Nipah virus: the island flying fox (*Pteropus hypomelanus*), Malayan flying fox. (*Pteropus vampyrus*), the Indian flying fox (*Pteropus giganteus*) and Leylei's flying fox (*Pteropus lylei*) [13, 48, 58]. Moreover, Nipah virus has been isolated from the Malayan flying fox and the island flying fox, confirming the role of flying foxes as a natural reservoir for Nipah virus [13, 46, 58]. Nipah virus is considered to be non-pathogenic in its natural hosts. Experimental infection of Australian grey-headed fruit bats (*Pteropus poliocephalus*) with Nipah virus did not result in clinical disease, but virus replication occurred as indicated by virus excretion, virus isolation from organs, and seroconversion [43]. These findings were confirmed upon

experimental inoculation of Malayan flying foxes and black flying foxes with Hendra virus and Nipah virus, with no disease signs and virus shedding predominantly in urine [28, 43].

This review provides an overview of the current knowledge on the innate and adaptive immune response to Nipah virus infection and discusses the importance of understanding the immune response in the context of vaccine development.

The immune response to Nipah virus infection in the reservoir host-bats

To date, very little is known about how either the reservoir hosts or incidental hosts respond to Nipah virus infection immunologically. Pteropid bats have recently been identified as the natural reservoir of Nipah virus [13, 26, 70]. Presumably, these bats mount a robust immune response to infection, thus controlling viral replication and overt disease. It is likely that Nipah virus is able to modulate the immune response in a way that allows a low-level of viral replication and potential persistence, potentially through evading innate immune responses, as has recently been shown to occur in bat cells [61]. As with many viruses, it is thought that the ability to evade innate immune activation correlates with their pathogenic potential in humans, as evidenced by the multitude of antagonistic strategies employed by virtually all RNA viruses that have been studied to date [4]. This has largely been studied in the context of disease modeling, but little is known how this evasion has come about evolutionarily within the reservoir hosts of these viruses. This ability of Nipah virus to evade innate immune sensing must have evolved in the reservoir bat species, and therefore must be beneficial to the virus, without being overtly deleterious to the host. This evasion likely gives the virus time to establish an active infection, while also modulating the adaptive immune response to allow low-level virus replication. It is possible that the virus is largely eliminated by immune mechanisms but persists at low levels at unknown sites for a period of time. This is supported by the difficulty in isolating virus (or even detecting viral proteins immunohistochemically) from wild-caught or experimentally infected bats [13, 43]. Bats infected with Nipah virus seroconvert and generate measurable titers of neutralizing antibodies, indicative of at least a transient infection, and experimentally infected bats can shed virus intermittently, showing that Nipah virus replicates within these hosts [13, 43]. Currently, other than limited cell culture experiments and the presence of Nipah virus-specific antibodies, little is known about the innate or adaptive immune response to infection in bats. The observation that bats possess neutralizing antibodies (IgG) demonstrates that humoral, as well as $CD4^+$ T cell responses are activated upon infection [28]. Future studies aimed at elucidating the mechanisms through which bats respond to infection without overt pathology might aid in the development of immunomodulatory countermeasures to combat human disease.

The *innate* immune response to Nipah virus infection in humans and animal models of disease

The first line of defense against invading microbes is the innate immune response. Innate immunity generally refers to the ability of most nucleated cells to sense the invasion of a microbe by the engagement of pathogen-associated molecular patterns (PAMPs) with host-encoded pattern recognition receptors (PRRs). This interaction directs the activation of transcription factors that control the expression of several antiviral proteins, as well as type I and type III interferons (IFNs), which activate additional antiviral responses via the Jak/STAT pathway [53]. Nipah virus genomic RNA is recognized by cellular cytoplasmic RNA helicases to activate IFN responses [25]. Upon recognition, there is a balance between the cell's ability to activate the innate immune response to defend itself, and the virus' ability to antagonize it. *In vitro*, endothelial cells (an important cell type targeted *in vivo*) infected with Nipah virus produce IFNβ as well as innate chemokines and cytokines, including IP-10

and IL-6, respectively [34]. IP-10 is a chemokine that attracts activated T lymphocytes, whereas IL-6 is a cytokine that stimulates acute-phase proteins and acts as an inflammatory molecule [19, 38]. Not surprisingly, these chemokines have the ability to functionally recruit T cells, which has been demonstrated using *in vitro* migration assays [34]. This likely contributes to the extensive vasculitis reported in virtually all histopathology studies. *In vivo*, little is know about the activation of the innate immune system. The expression of innate immune genes has been documented as a response to Nipah virus infection in animal models of disease, including the upregulation of IP-10 and IL-6 [40, 49].

The ability of RNA viruses that cause disease in humans to antagonize the innate immune response seems to be ubiquitous [22]. Nipah virus has been extensively characterized *in vitro* for its ability to subvert innate immunity by several mechanisms. The P gene of Nipah virus is transcriptionally edited to produce not only the phosphoprotein but also two alternate V and W proteins, and an alternate ORF expresses a C protein [33]. These proteins possess multiple capabilities to inhibit IFN production, as well as downstream signaling. In addition, the expression of these antagonistic genes is temporally regulated during infection [54]. The V protein has been shown to interact with the cytoplasmic RNA helicases to prevent downstream signaling and activation of the IFNb promoter [1]. The W protein inhibits IFNb promoter activation by disrupting the transcription factor IRF3 in the nucleus [57]. Not only do these proteins inhibit IFNb production, but along with the V protein, downstream signaling via IFNb is also antagonized. Both STAT1 and STAT2, which are required for IFNb signaling, are antagonized by Nipah virus P gene products [14, 50, 56].

Employing a reverse genetics approach, the antagonistic function of the P, V, W and C proteinshas been assessed *in vivo* using the hamster model of pathogenesis. Hamsters inoculated with recombinant virus lacking the ability to produce either the C or the V protein display no pathology, and the viral genome is almost undetectable in these animals, indicating that the antagonistic properties for these proteins are responsible for pathogenesis [71]. Additional evidence that the innate immune response can alter the pathogenic process comes from the observation that synthetic RNA (poly I:C), which strongly activates IFN production, is effective in limiting disease and increasing survival of Nipah virus–infected hamsters [21]. Whether the effects are due to the inhibition of viral replication solely through the innate interfering properties of IFN or whether the resulting cytokine/chemokine response augments the adaptive immune response is currently unknown.

The *adaptive* immune response to Nipah virus infection in humans and animal models of disease

Currently, almost no work has been done to investigate whether the adaptive immune response is simply inefficient at dealing with Nipah virus infection or whether the immune response is itself in some way immunopathogenic, that is, a robust, ineffective immune response exacerbating the pathogenic process. Sera from infected patients contain measurable IgM antibodies as early as four days after exposure, and the presence of IgG in patients following infection indicates that both B-cell and CD4+ T-cell responses are elicited in response to virus infection [47].

Presumably, disease is at least partially a consequence of a insufficient adaptive immune response, although immune cell infiltration observed in patients who have succumbed to disease indicates that immune recruitment, leading to acute vasculitis, might contribute to disease [30, 67]. This question has been difficult to address due to the lack of animal models of disease for which there are ample reagents to study the immune response. Currently, the most successful animal models that at least partially mimic disease seen in humans are the Syrian hamster, ferret, and cat models [2, 42, 68]. While these models are useful in the

initial study and development of vaccines, they are less useful for the study of mechanisms of disease and protection afforded by vaccination, due to their outbred nature and lack of immune-related reagents. Recently, a non-human primate model of Nipah virus disease has been described that seems to closely recapitulate human disease, but this model is cumbersome for use in mechanistic studies for obvious reasons [20]. Unfortunately, a laboratory mouse model has yet to be described for Nipah virus infection, never mind disease. Although it is possible that immunocompromised mouse models, such as aged, interferon-alpha receptor (IFNAR) knockout, or signal transducer and activator of transcription-1 (STAT1) knockout mice would develop disease, these models are of limited use in both innate and adaptive immunology studies.

Although almost nothing is known about natural immunity in naïve humans and animal models, some insight into the requirements of the adaptive immune response in combating infection has been gained by vaccine and immunotherapeutic studies that have been performed with wide success in animal models, as summarized in Table 1. Several vaccine platforms have been enlisted, including subunit, viral vector, virus-like particle (VLP), and DNA vaccines, although not all platforms have been tested in challenge models. All of these vaccines have been designed with the goal of eliciting neutralizing antibody responses by consisting of, or encoding, either the G or F (or both) surface glycoproteins. The efficacy of these vaccines, most if not all of which induce measurable neutralizing antibodies, suggests that an efficient adaptive immune response against Nipah virus infection benefits from, if not requires, the development of a robust neutralizing antibody response. In addition, prophylactic, as well as post-exposure, administration of hyperimmune sera or neutralizing monoclonal antibodies has demonstrated efficacy in challenge models. Passive antibody transfer of pooled sera from hamsters vaccinated with a vaccinia virus (VV) expressing G, given one hour and again at 24 hours post-challenge, protected hamsters from Nipah virus challenge [24]. Using the ferret model, m102.4, a human monoclonal antibody, given ten hours after Nipah virus challenge was completely protective [2]. This antibody has also been successful in protecting non-human primates from Hendra virus-induced disease when given post-exposure [3].

While neutralizing antibodies are highly protective when elicited/administered before or shortly after infection, it is unknown what role, if any, the cellular adaptive immune response plays in either protection by vaccination or natural immunity. Currently, no vaccine strategies have been used to address this, partially due to the lack of syngeneic cell lines to measure CTL activity, or antibodies to measure $CD8^+$ (or $CD4^+$) CTL responses by flow cytometry or to measure cytokines in these animal models. To investigate the contribution of the cellular response, one approach might be to develop and measure the efficacy of vaccines that are comprised of, or encode, either non-structural Nipah virus proteins or parts of structural proteins that do not contain epitopes that elicit neutralizing antibodies.

Immunopathology is another possible result of infection. Interestingly, one way in which the immune response may exacerbate disease is by disseminating virus. Recently, it has been shown that viable Nipah virus can be carried by lymphocytes and monocytes without productively infecting or perhaps even entering these cells, only to be released at sites distant from the initial infection [39]. This may be a way in which the immune system actively participates in the dissemination of virus and may explain the widespread viral distribution observed despite little (or no) viremia detectable in disease models. Recently, it was reported that porcine monocytes, natural killer cells, and $CD6^+/CD8^+$ T cells support Nipah virus replication, which may facilitate dissemination of the virus [59].

Future perspectives

Almost 15 years have passed since the first documented Nipah virus outbreak in humans, and Nipah virus has subsequently caused outbreaks in Bangladesh on an almost yearly basis. The continuous threat to human and animal health posed by Nipah viruses requires a multi-disciplinary approach focused on preventive and therapeutic intervention strategies. Here, we have highlighted the current knowledge of the immune response to Nipah virus infection.

The difference in pathogenicity of Nipah virus infection in the natural reservoir, flying foxes, and dead-end hosts such as humans, indicates that the natural reservoir has the ability to control the detrimental effects of a Nipah virus infection immunologically. The correlates of this natural immunity are not known. It is likely that a rapid and robust innate immune response, coupled with and augmenting a vigorous adaptive immune response, correlates with a positive outcome. The last decade has seen an increase in interest in studying host-reservoir immunology in relation to their pathogens. For Nipah virus, this has so far not resulted in a conclusive insight in the host-pathogen interaction in the natural host. The availability of tools such as host-specific cell lines and high-throughput sequencing will prove essential in the generation of host-specific reagents and the capability to study host-pathogen interactions.

From the other end of the transmission cycle, the dead-end host, a more comprehensive understanding of the correlates of protection is much needed to design effective prophylactic and therapeutic countermeasures against Nipah virus infection. Several vaccine and antibody treatment experiments have so far yielded promising results in their ability to prevent morbidity and/or mortality in a variety of animal models. As a result of these studies, it is apparent that neutralizing antibodies are beneficial, if not required, for protection from Nipah virus infection. Careful study of the exact mechanism of protection in these studies might also lead to a better understanding of the disease process. Identifying aspects of the immune response that are absent or counter-effective during human Nipah virus infection, may lead to the development of targeted intervention strategies to adapt the immune response in Nipah virus–infected individuals, leading to increased survival rates.

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Table 1

Summary of Nipah virus vaccine strategies

Vaccine platform	Species	Vaccine schedule	Nipah virus challenge dose (route)	Efficacy	Reference
VLPs comprised of G, F and M proteins	Balb/c mice	1′ (15 days) 2′ (29 days) 3′	NA	Neutralizing ab measurable after 2nd boost	**[64]**
Venezuelan equine encephalitis virus expressing G or F proteins	C3H/He mice	1′ (5 weeks) 2′ (18 weeks) 3′	NA	Neutralizing ab measurable after prime	**[15]**
Recombinant vesicular stomatitis virus (VSV) expressing G or F proteins	Balb/c mice	Single immunization	NA	Neutralizing ab measurable after vaccination (28d)	**[7]**
Soluble Hendra G protein	Cats	1′ (21 days) 2′	5×10^4 $TCID_{50}$ (oronasal)	Complete protection (6/6). Non-sterile immunity	**[41]**
Soluble Nipah or Hendra G protein	Cats	1′ (2 weeks) 2′ (2 weeks) 3′	5×10^2 $TCID_{50}$ (subcutaneous)	Complete protection (4/4). Non-sterile immunity	**[44]**
DNA vaccination (pCAGGS G or F proteins)	Balb/c mice	1′ (4 weeks) 2′	NA	Neutralizing ab measurable after boost	**[65]**
Recombinant canarypox virus expressing G or F proteins	Pigs	1′ (2 weeks) 2′	2.5×10^5 PFU (intranasal)	Reduction in virus and complete inhibition of viral shedding	**[66]**
Vaccinia virus (VV) expressing G and/or F proteins	Syrian hamster	1′ (4 weeks) 2′	1×10^3 PFU (intraperitoneal)	Complete protection in all groups (passive ab also protects)	**[24]**
1′ = prime, 2′= boost, 3′ = second boost					