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A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge

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ABSTRACT

The henipaviruses, Hendra virus (HeV) and Nipah virus (NiV), are two deadly zoonotic viruses for which no vaccines or therapeutics have yet been approved for human or livestock use. In 14 outbreaks since 1994 HeV has been responsible for multiple fatalities in horses and humans, with all known human infections resulting from close contact with infected horses. A vaccine that prevents virus shedding in infected horses could interrupt the chain of transmission to humans and therefore prevent HeV disease in both. Here we characterise HeV infection in a ferret model and show that it closely mirrors the disease seen in humans and horses with induction of systemic vasculitis, including involvement of the pulmonary and central nervous systems. This model of HeV infection in the ferret was used to assess the immunogenicity and protective efficacy of a subunit vaccine based on a recombinant soluble version of the HeV attachment glycoprotein G (HeVsG), adjuvanted with CpG. We report that ferrets vaccinated with a 100 μg, 20 μg or 4 μg dose of HeVsG remained free of clinical signs of HeV infection following a challenge with 5000 $TCID_{50}$ of HeV. In addition, and of considerable importance, no evidence of virus or viral genome was detected in any tissues or body fluids in any ferret in the 100 and 20 μg groups, while genome was detected in the nasal washes only of one animal in the 4 μg group. Together, our findings indicate that 100 μg or 20 μg doses of HeVsG vaccine can completely prevent a productive HeV infection in the ferret, suggesting that vaccination to prevent the infection and shedding of HeV is possible.

1. Introduction

Hendra virus (HeV) is a zoonotic virus transmitted from bats to humans via horses. While HeV related disease in bats has not been documented, the virus can cause a severe systemic illness, with severe pathology associated with the respiratory and neurological systems in both horses and humans [1]. Four of the seven human infections recorded so far have been fatal and the disease is usually fatal in horses – in the first recorded outbreak of HeV in 1994 in Brisbane, Queensland, Australia 14 horses died out of a total of 20 infected with HeV [2]. Including the initial outbreak there have been 14 known spillovers of HeV and all except one (in northern New South Wales) occurred in Queensland [3].

HeV is one of only two members of the genus *Henipavirus* in the family *Paramyxoviridae* [4,5]. The henipaviruses are characterised by a large genome and their ability to infect a wide range of animals, including humans. The other member of the genus, Nipah virus (NiV), was first isolated from a disease outbreak that occurred in Malaysia in 1998 in humans and pigs [6]. Out of 265 human cases, 105 were fatal. Since 2001 there have been numerous NiV outbreaks in Bangladesh and two in India [7], the most recent occurrence in early 2011, in Bangladesh [8]. At least two outbreaks have been associated with virus transmission from human-to-human [9–11] with both respiratory and neurological signs observed in humans, and mortality rates ranging from 40% to 75%.

As a result of the potential for henipaviruses to cause significant mortality and morbidity in humans they are classified as Biosafety Level 4 (BSL-4) agents. Further, due to their carriage by wildlife and their relative ease of propagation, the henipaviruses are considered select agents of concern for biodefense by the Centers for Disease Control and Prevention (CDC) and the National Institute of Allergy and Infectious Diseases (NIAID). In spite of this no licensed prophylactic or therapeutic treatments are currently available although several therapeutic modalities are under active investigation.

Like most paramyxoviruses, henipavirus infection of host cells involves two viral glycoproteins [12]. The G glycoprotein is the viral

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attachment protein and exists as a tetramer embedded in the lipid membrane of the virus. Henipavirus G binds to the host cell receptors; ephrin-B2 and ephrin-B3 [13–16], important bi-directional cell–cell signalling molecules that are highly conserved and widely expressed particularly within the nervous and vascular systems [17] across all mammalian species. The second viral glycoprotein is the fusion (F) protein, which upon triggering facilitates the fusion between the viral and cellular membranes.

An immune response to viral surface proteins/glycoproteins is often necessary for resistance to viral infection [18] and is particularly effective in controlling infections with a viraemic phase such as the human paramyxoviruses that cause mumps and measles [19,20]. Similarly, passive protection against NiV infection has recently been demonstrated in a ferret model by transferring a G glycoprotein specific, HeV and NiV cross-reactive, human monoclonal antibody [21] and in a hamster model by transferring G or F glycoprotein specific polyclonal or monoclonal antibody [22–24]. In the hamster model, vaccination with recombinant vaccinia viruses expressing G or F also induced protection against a lethal challenge with NiV [22]. A similar outcome has been demonstrated in pigs vaccinated with a canarypox vaccine carrying G or F [25]. In two different experiments cats vaccinated with a soluble G glycoprotein (sG) based subunit immunogen survived a lethal NiV challenge with no clinical signs [26,27]. Although no clinical disease was observed, in one experiment genome was detected in oral swabs, urine and the brain of several animals, virus was isolated from the brain of one animal [26] and in the other experiment genome was detected in the tissues of two animals at levels that were so low as to be questionable [27]. There is 83.3% identity between the amino acid sequences of the HeV and NiV G glycoproteins [28] and it has been shown that immunisation with sG of either HeV or NiV produces cross neutralising antibodies, with a better cross neutralisation response elicited by HeV soluble G (HeVsG) [27]. HeVsG therefore has potential as a subunit vaccine immunogen for preventing both HeV and NiV infection.

Previous studies have revealed that ferrets are a very successful model for NiV infection, closely mirroring the characteristics of the infection in humans [21,29]. NiV infected ferrets exhibit severe respiratory and neurological disease as well as generalised vasculitis. Here, we have evaluated HeV infection of ferrets and extend the use of this new animal model to assess the protective efficacy of HeVsG as a vaccine immunogen against lethal HeV challenge. We show that, like NiV, the manifestation of HeV infection and pathogenesis in ferrets is similar to that seen in humans exhibiting both respiratory and neurological disease. Further, in this model system, the three HeVsG vaccine doses tested prevented clinical disease after a lethal HeV challenge, and following the two higher doses of immunogen there was no detectable evidence of HeV infection.

2. Materials and methods

2.1. Animals, accommodation, handling, and biosafety

Eight male ferrets aged 12–18 months were used for the HeV model development study and eight were used for the HeVsG vaccination study. The animal husbandry methods and experimental design were endorsed by the CSIRO Australian Animal Health Laboratory's Animal Ethics Committee. Animals were housed in a single room at BSL-4 in pairs in cages that incorporated two "squeeze" compartments for administration of chemical restraint, given a complete premium dry food and provided with water *ad libitum*. Room temperature was maintained at 22 °C with 15 air changes per hour; and humidity varied between 40% and 60%. Before any manipulation, animals were immobilized with a mixture of ketamine HCl (3 mg/kg; Ketamil; Ilium, Smithfield, Australia) and medetomidine (30 μg/kg; Domitor; Novartis, Pendle Hill, Australia) by intramuscular injection. For reversal, atimepazole (Antisedan; Novartis) was given intramuscularly at 50% of the dose used for medetomidine. At least one week prior to virus challenge single stage transmitters fitted with an internal loop antenna and coated with an inert two-pot epoxy resin (Sirtrack, Havelock North, New Zealand) were implanted subcutaneously in the flank of the ferrets for the purpose of real-time continuous monitoring of body temperature. Staff wore fully encapsulated suits with breathing apparatus while in the animal room. Serology, virus isolations, and the initial stages of RNA extraction were carried out at BSL-4.

2.2. Animal infections

Ferrets were exposed to a low passage isolate of HeV (Redlands 2008) by the oronasal route. For the HeV infection study, 2 ferrets per group were exposed to 50 $TCID_{50}$ (ferrets 1-50, 2-50), 500 $TCID_{50}$ (3-500, 4-500), 5000 $TCID_{50}$ (5-5000, 6-5000) or 50,000 $TCID_{50}$ (7-50,000, 8-50,000) and for the sG vaccination experiment ferrets were exposed to 5000 $TCID_{50}$ at day 41 of the experiment i.e. 21 days post the booster vaccination.

General clinical observations were documented daily prior to as well as after challenge. Animals were weighed while under sedation at the time of vaccination and challenge and at days 6, 8, 10, and 21 post-challenge (pc). Rectal temperature was also determined at sedation by using digital thermometers to augment data derived remotely from the implanted temperature transponders. Ferrets were euthanized when reaching a previously determined endpoint or 21 days pc. The humane endpoint was defined as rapidly progressive clinical illness of up to 2 days duration including fever and depression, possibly accompanied by increased respiratory rate or posterior paresis or ataxia. In susceptible animals, this typically occurs within the first 10 days after viral challenge. In preliminary studies, these signs were found to correlate with the requirement to euthanize ferrets on subsequent days on humane grounds; thus, they have been utilized as surrogates for lethality.

2.3. Vaccine immunogen preparation

A human codon optimized HeV soluble glycoprotein G (sG) construct was used to produce recombinant HeVsG. The construct was generated by cloning the entire ectodomain coding regions of HeV G linked to an IgK leader sequence and S-peptide tag into pcDNA CMV+hygro. The expression plasmid pcDNA-CMV+hygro was generated by insertion of the CMV promoter element from plasmid phCMV-1(Gelantis, San Diego, CA) into pCDNA3.1(hygro) (Invitrogen, Carlsbad, CA). A stable HeVsG secreting cell line was generated by transfecting plasmid pcDNA-CMV+hygro-HeVsG into human 293F cells and selection using hygromycin B followed by limiting dilution cloning, generating the cell line HeVsG#4-2 293F. HeVsG was prepared by growing HeVsG#4-2 293F cells in shaker cultures using serum-free medium- 293 SFM II (Invitrogen) and purified by S-protein agarose affinity chromatography followed by preparative gel filtration chromatography with a Hiload 16/60, Superdex 200 column. CpG oligodeoxynucleotide (ODN) 2007 (TCGTCGTTGTCGTTTTGTCGTT) containing a fully phosphorothioate backbone was purchased from Invivogen (San Diego, CA, USA) and Alhydrogel™ was purchased from Accurate Chemical & Scientific Corporation (Westbury, NY, USA). Although the CpG component of the adjuvant is species specific, in the absence of any information on ferret specific CpG sequences the same CpG component was used for the ferret vaccine as was used for cats [26]. Vaccine doses containing fixed amount of CpG ODN 2007 and varying amounts of HeVsG and aluminium ion (at a weight ratio of 1:25) were formulated as follows: 100 μg dose: 100 μg HeVsG, 2.5 mg aluminium ion and 150 μg of CpG ODN 2007; 20 μg dose: 20 μg