Antiviral Research 101 (2014) 26–29

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Short Communication

Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters

Michael K. Lo [a,*,1], Brian H. Bird [a,1], Anasuya Chattopadhyay [c,1], Clifton P. Drew [b], Brock E. Martin [a], Joann D. Coleman [a], John K. Rose [c], Stuart T. Nichol [a], Christina F. Spiropoulou [a,*]

[a] Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States
[b] Infectious Disease Pathology Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States
[c] Department of Pathology, Yale University School of Medicine, New Haven, CT, United States

ARTICLE INFO

Article history:
Received 10 September 2013
Revised 18 October 2013
Accepted 22 October 2013
Available online 30 October 2013

Keywords:
Henipavirus
Nipah
Vaccine
VSV
Single-dose
Hamster

ABSTRACT

Nipah virus (NiV) continues to cause outbreaks of fatal human encephalitis due to spillover from its bat reservoir. We determined that a single dose of replication-defective vesicular stomatitis virus (VSV)-based vaccine vectors expressing either the NiV fusion (F) or attachment (G) glycoproteins protected hamsters from over 1000 times LD_{50} NiV challenge. This highly effective single-dose protection coupled with an enhanced safety profile makes these candidates ideal for potential use in livestock and humans.
Published by Elsevier B.V.

Nipah virus (NiV) is a highly pathogenic paramyxovirus responsible for causing fatal human encephalitis with high case fatality rates from 40% to 75% (Lo and Rota, 2008). Since its initial outbreak in Malaysia from 1998 to 1999, NiV has caused smaller sporadic outbreaks of fatal encephalitis in Bangladesh on a near-annual basis (Luby and Gurley, 2012). A soluble subunit glycoprotein vaccine approved for animal use against the closely-related Hendra virus requiring a two-dose prime-boost regimen has shown protection against NiV in several animal models (Bossart et al., 2012; Broder et al., 2013; McEachern et al., 2008; Mungall et al., 2006; Pallister et al., 2013). Previous work demonstrated that a single dose of replication-defective single-cycle recombinant vesicular stomatitis viruses (VSV-ΔG) expressing either the NiV fusion (F) (VSV-ΔG-NiVF) or attachment (G) (VSV-ΔG-NiVG) glycoproteins induced neutralizing antibodies in mice against VSV-ΔG-particles pseudotyped with NiV F and G glycoproteins (VSV-ΔG-eGFP-NEUT) (Chattopadhyay and Rose, 2011). In order to evaluate the protective efficacy of the VSV-ΔG-NiVF and VSV-ΔG-NiVG vaccines against lethal NiV challenge in an animal model that mimics NiV disease, we tested these vaccines in the Syrian golden hamster (DeBuysscher et al., 2013; Guillaume et al., 2004; Rockx et al., 2011; Wong et al., 2003). We obtained approval for animal experiments from the Centers for Disease Control and Prevention (CDC) Institutional Animal Care and Use Committee (IACUC). All animal work was performed by certified staff in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved biosafety level 2(BSL-2) (vaccination phase) or BSL-4 (challenge phase) facilities at CDC.

We produced stocks of the single-cycle viruses VSV-ΔG-NiVG, VSV-ΔG-NiVF, VSV-ΔG-eGFP pseudotyped with VSV G glycoprotein, and also the VSV-ΔG-eGFP-NEUT pseudotyped with NiV F and G as previously described (Chattopadhyay and Rose, 2011). For vaccination 6-week old female Syrian golden hamsters (*Mesocricetus auratus*, Charles River Laboratories, Wilmington, VA) were anesthetized (isoflurane) and inoculated intramuscularly in the right quadriceps with 1×10^6 infectious particles of either VSV-ΔG-NiVG (10 animals), VSV-ΔG-NiVF (10 animals), or VSV-ΔG-eGFP (10 animals). At 28 days post-vaccination, ~100 μl of blood was collected for determination of serum neutralizing antibody titers (SNT) as previously described (Chattopadhyay and Rose, 2011). The vaccinated hamsters, along with 3 additional unvaccinated hamsters (to serve as unvaccinated controls) were transferred into the BSL-4 lab and were given 3 days to adjust to their new surroundings. On day 32 post-vaccination (challenge day 0), all hamsters were inoculated via the intraperitoneal route with a

* Corresponding authors. Address: Centers for Disease Control and Prevention, Viral Special Pathogens Branch, 1600 Clifton Road, Mailstop G-14, Atlanta, GA 30333, United States. Tel.: +1 4046390327.
E-mail addresses: mko2@cdc.gov (M.K. Lo), ccs8@cdc.gov (C.F. Spiropoulou).
[1] M.K.L., B.H.B., and A.C. contributed equally to this work.

0166-3542/$ - see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.antiviral.2013.10.012

previously described uniformly lethal challenge dose (10^5 $TCID_{50}$/hamster, >1000 times LD_{50}) of NiV Malaysia strain passaged three times on Vero E6 cells (Chua et al., 2000; DeBuysscher et al., 2013; Harcourt et al., 2000; Rockx et al., 2011). Animals were examined and scored daily for two weeks post-challenge for signs of clinical illness, neurologic disease, respiratory distress, and weight loss (weight evaluation for 3 vaccinated groups began on day 3 post-challenge). Animals showing significant weight loss (>25% of initial weight on challenge day 0) alongside any neurological or respiratory signs were humanely euthanized. Animals without clinical illness after 14 days post-infection (p.i.) continued to be monitored daily but were only weighed in 2–5 day intervals until day 32 p.i. in which all surviving animals were humanely euthanized. At time of euthanasia, ~3 ml of blood was collected by cardiac puncture for SNT determination. Necropsies were performed to collect lung, spleen, kidney, and brain tissues. Tissues were either inactivated in MAGMAX RNA lysis buffer (Life Technologies, Carlsbad, CA) for subsequent RNA extraction and real-time RT-PCR as previously described (Lo et al., 2012), or fixed in 10% formalin for histopathology and immunohistochemistry (IHC) analysis as previously described (Wong et al., 2003).

On day 6 post-challenge, all unvaccinated control hamsters either died or were euthanized due to the development of neurologic signs and respiratory distress. Similarly, 5 out of 10 (50%) backbone control VSV-ΔG-eGFP-vaccinated hamsters either died or were euthanized due to the onset of illness. By day 9 post-challenge, all VSV-ΔG-eGFP-vaccinated hamsters had either died or were euthanized (Fig. 1A). In contrast, hamsters vaccinated with VSV-ΔG-NiVG or VSV-ΔG-NiVF did not develop any clinical illness nor weight loss throughout the course of infection, and were euthanized on day 32 p.i. (day 64 post-vaccination) (Fig. 1A and B). SNTs against NiV were defined as the reciprocal of the highest serum dilution at which duplicate wells of each serum sample showed complete neutralization of 50 infectious particles of VSV-ΔG-eGFP-NEUT. Prior to NiV challenge, hamsters vaccinated with either VSV-ΔG-NiVG or VSV-ΔG-NiVF developed respective SNTs of 640 and 160, while hamsters vaccinated with VSV-ΔG-eGFP did not develop any detectable SNT (Fig. 2A). Following NiV challenge, both unvaccinated control and VSV-ΔG-eGFP-vaccinated groups developed low levels of neutralizing antibodies (Fig. 2B), but even at serum dilutions of 1:20 could not completely neutralize 50 particles of VSV-ΔG-eGFP-NEUT. The lack of an anamnestic immune response in the VSV-ΔG-NiVG or VSV-ΔG-NiVF-vaccinated groups following NiV challenge possibly indicates sterilizing immunity, as their respective post-challenge SNTs at the time of euthanasia remained similar to pre-challenge levels with comparatively lower percentages of neutralization at higher serum dilutions of 1:2560, 1:5120 and 1:10240 (Fig. 2A and B). Histopathology and IHC results in unvaccinated and VSV-ΔG-eGFP-vaccinated hamsters were similar to those observed in a recent study of NiV pathogenesis in the hamster model (DeBuysscher et al., 2013), indicating bronchointerstitial pneumonia with vasculitis associated with virus replication (Fig. 3). In the unvaccinated and eGFP control-vaccinated groups we detected both viral RNA and viral antigen in all tissues sampled from both groups except for the brain, in which we detected viral RNA but not viral antigen (Table 1, Fig. 3, data not shown). In contrast, we did not observe any pathology and could neither detect the presence of viral RNA nor antigen in any tissues collected from VSV-ΔG-NiVG or VSV-ΔG-NiVF-vaccinated groups (Table 1, data not shown).

In summary, we have demonstrated that a single dose of these single-cycle vaccine candidates expressing either NiV G or F conferred complete protection from lethal NiV challenge in the Syrian golden hamster animal model. This is in marked contrast with all other previously evaluated viral vaccine vectors expressing NiV G or F that required multi-dose prime-boost regimens (Guillaume

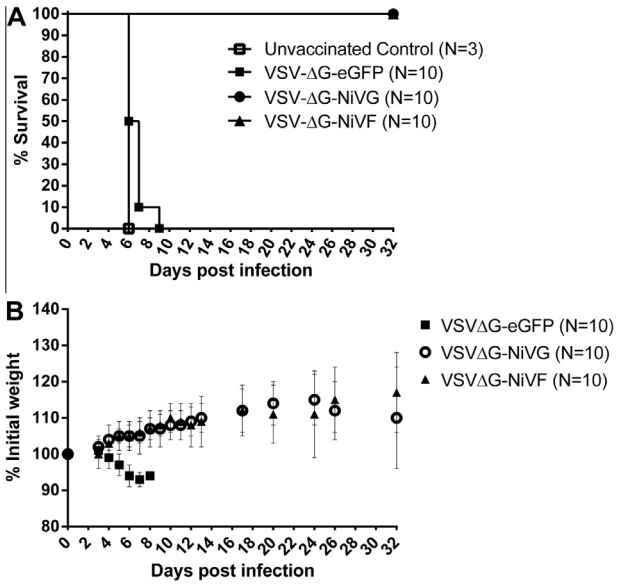

Fig. 1. A single-dose vaccination of hamsters with single-cycle replication-deficient VSV viral vectors (VSV-ΔG) expressing either NiV G or NiV F confers complete protection from lethal NiV challenge. (A) Survival curves of unvaccinated hamsters and hamsters vaccinated with VSV-ΔG-NiVG, VSV-ΔG-NiVF, or VSV-ΔG-eGFP, and challenged 32 days later with virulent NiV. (B) Weight curves of vaccinated hamsters challenged with lethal dose of NiV. Weight changes are expressed as the mean percentage changes for NiV challenged animals relative to their weights at day zero.

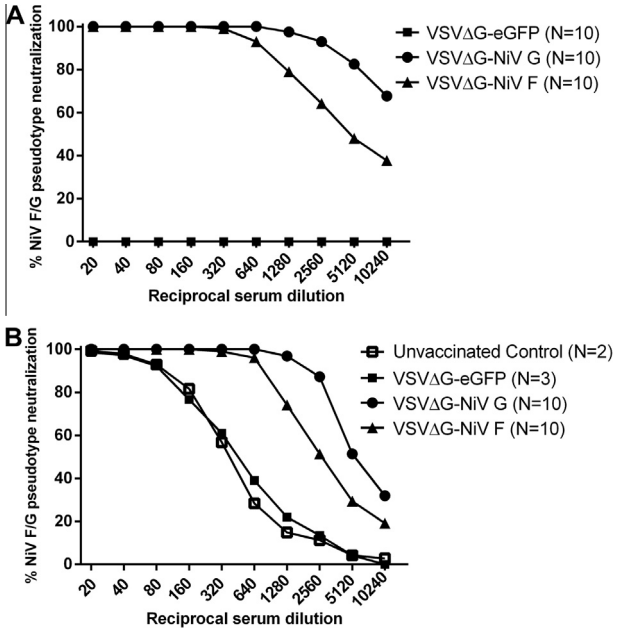

Fig. 2. A single-dose vaccination of hamsters with either VSV-ΔG-NiVG or VSV-ΔG-NiVF induces protective serum neutralizing antibody titers (SNT). Percent neutralization curves of pooled serum from each group of hamsters at (A) 28 days post vaccination pre-NiV challenge, and (B) 64 days post-vaccination, 32 days post-NiV challenge for VSV-ΔG-NiVG or VSV-ΔG-NiVF-vaccinated hamsters, 6 days post-challenge for unvaccinated hamsters, and at 6, 7, and 9 days post-challenge for VSV-ΔG-eGFP-vaccinated hamsters. SNT for each group was determined as the highest reciprocal serum dilution in which 50 particles of VSV-ΔG-eGFP virus pseudotyped with NiV F and G glycoproteins were completely (100%) neutralized.

et al., 2004; Weingartl et al., 2006; Yoneda et al., 2013) with the exception of an adenovirus-associated virus (AAV) vector which required an extremely high dose of vaccine (6×10^{11} genome particles) for protection (Ploquin et al., 2013). Furthermore, when