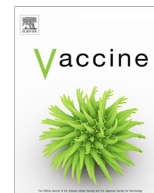
ARTICLE IN PRESS

Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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



A novel approach to a rabies vaccine based on a recombinant single-cycle flavivirus vector

Maryann Giel-Moloney [a,*], Alexander A. Rumyantsev [a], Fred David [b], Monica Figueiredo [b], Brad Feilmeier [b], Teshome Mebatsion [b], Mark Parrington [a], Harry Kleanthous [a], Konstantin V. Pugachev [a]

[a] Sanofi Pasteur, R&D Discovery US, 38 Sidney Street, Cambridge, MA 02139, United States
[b] Merial Bio R&D, 1730 Olympic Drive, Athens, GA 30601, United States

ARTICLE INFO

Article history:
Received 4 May 2017
Received in revised form 26 August 2017
Accepted 26 August 2017
Available online xxxx

Keywords:
RepliVax
Rabies vaccine
Flavivirus vector
Replication defective
Preclinical

ABSTRACT

The RepliVax® vaccine (RV) platform is based on flavivirus genomes that are rationally attenuated by deletion. These single-cycle RV vaccine candidates targeting flavivirus pathogens have been demonstrated to be safe, highly immunogenic, and efficacious in animal models, including non-human primates. Here we show utility of the technology for delivery of a non-flavivirus immunogen by engineering several West Nile-based RV vectors to express full-length rabies virus G protein. The rabies virus G protein gene was incorporated in place of different West Nile structural protein gene deletions. The resulting RV-RabG constructs were demonstrated to replicate to high titers (8 $\log_{10}$ infectious particles/ml) in complementing helper cells. Following infection of normal cells, they provided efficient rabies virus G protein expression, but did not spread to surrounding cells. Expression of rabies virus G protein was stable and maintained through multiple rounds of *in vitro* passaging. A sensitive neurovirulence test in 2–3 day old neonatal mice demonstrated that RV-RabG candidates were completely avirulent indicative of high safety. We evaluated the RV-RabG variants in several animal models (mice, dogs, and pigs) and demonstrated that a single dose elicited high titers of rabies virus-neutralizing antibodies and protected animals from live rabies virus challenge (mice and dogs). Importantly, dogs were protected at both one and two years post-immunization, demonstrating durable protective immunity. The data demonstrates the potential of the RepliVax® technology as a potent vector delivery platform for developing vaccine candidates against non-flavivirus targets.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Rabies is a substantial health issue in many countries [1,2]. The rabies virus is an enveloped, non-segmented negative-sense RNA virus of the Rhabdoviridae family. Its envelope contains glycoprotein G (RabG) which is responsible for cell attachment and membrane fusion, and is a key immunogen targeted for vaccine development utilizing new delivery technologies in nucleic acid, VLPs, and recombinant viral vectors [3–8]. Currently licensed human rabies vaccines are inactivated viral vaccines requiring multiple doses/boosters. The immunoprotection induced by prophylactic vaccination wanes relatively rapidly, which necessitates post-exposure treatment [9]. Licensed animal rabies vaccines also require multiple doses to induce protection. An ideal vaccine could be used prophylactically in both humans and animals, providing reliable, long-lasting protection after a single dose.

The RepliVax® (RV) approach was developed as a replication-defective vaccine platform against flaviviruses, including yellow fever (YF), dengue (DEN), Japanese encephalitis (JE), West Nile (WN), and tick-borne encephalitis (TBE) viruses [10–13]. Flavivirus genomic RNA contains an open reading frame encoding C-prM-E structural proteins (prM, precursor for mature M) and nonstructural proteins NS1–NS5. RV vaccine candidates are single-component pseudo infectious viruses (sPIVs) produced by deleting most of the structural capsid gene, and propagated in helper cells (HC) supplying the C protein *in trans* [10]. RV undergoes a single round of replication, and highly attenuated RV sPIV mimics natural infection, triggering protective immunity. Additionally, a two-component

Abbreviations: CV-WN, ChimeriVax®-West Nile; DEN, dengue; HC, helper cells; LAV, live attenuated vaccine; prM, precursor for mature M protein; RabG, rabies virus glycoprotein G; RV, RepliVax®; RFFIT, Rapid Focus Fluorescent Inhibition Test; rVEE, Venezuelan equine encephalitis virus replicon; sPIV, single-component pseudo infectious virus; tcPIV, two-component pseudo infectious virus; TBE, tick-borne encephalitis; WN, West Nile; YF, yellow fever.

* Corresponding author.
E-mail address: Maryann.Giel-Moloney@sanofi.com (M. Giel-Moloney).

http://dx.doi.org/10.1016/j.vaccine.2017.08.055
0264-410X/© 2017 Elsevier Ltd. All rights reserved.

Please cite this article in press as: Giel-Moloney M et al. A novel approach to a rabies vaccine based on a recombinant single-cycle flavivirus vector. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.08.055

pseudo infectious virus (tcPIV) vaccine system was developed, based on two mutually trans-complementing genomes that can be propagated in regular cells [14,15]. Head-to-head comparisons have shown that RV prototypes have similar immunogenicity and efficacy to live attenuated vaccines (LAVs) against flaviviruses in rodents [11], and the antibody response following one dose of RV-TBE sPIV vaccine candidate in monkeys was more durable than three human doses of a licensed inactivated TBE vaccine [13]. The LAV against YF, strain 17D, is considered one of the strongest human immunogens, with a single dose providing lifelong immunity [16]. New vaccines, capable of eliciting similarly durable immunity are needed, warranting the development of flavivirus vectors as gene delivery vehicles. The principle of developing flavivirus gene expression and vaccine vectors has been previously demonstrated with the Kunjin virus replicon [17].

Here we expanded the RV approach to non-flavivirus targets by replacing WN virus structural protein genes with the RabG gene, and evaluated the resulting RV-RabG variants for replication *in vitro*, expression of RabG gene product, genetic stability, attenuation, and immunogenicity in animal models.

2. Materials and methods

2.1. Construction of RV-RabG variants

The nucleotide (nt) sequence of the RabG gene of India95 isolate was human codon-optimized and direct nt sequence repeats of ≥8 nts were removed by silent nt substitutions (DNA2.0, Menlo Park, CA). The native RabG signal sequence was inserted at the N-terminus and a foot-and-mouth disease virus 2a self-cleavage element (FMDV2a) was incorporated at the C-terminus. The cassette was cloned in frame at the C/prM junction in WN vectors with capsid (ΔC, 210nt), prM-E (ΔprM-E, 1935nt) or C-prM-E (ΔC-prM-E, 2145nt) gene deletions. Resulting plasmids were linearized with SwaI and *in vitro*-transcribed using Amplicap Max T7 High Yield message Maker kits (Cellscript). *In vitro*-transcribed RNA was transfected into baby hamster kidney (BHK) HC expressing structural WN C-prM-E proteins required for packaging the RV-RabG replicons as sPIVs to generate infectious RV-RabG particles.

2.2. Cells, viruses, and in vitro characterization

HC for propagation of RV-RabG variants were generated by transfecting BHK cells with Venezuelan equine encephalitis virus replicon (rVEE) expressing WN virus C-prM-E genes and a puromycin N-acetyl-transferase selective marker constructed similarly to rVEE-WN C helper replicon [10,18]. Cells were maintained in Minimum Essential Medium (MEM) alpha medium supplemented with fetal bovine serum (FBS) and 10 μg/ml puromycin. RV-RabG viruses were propagated in the HC and titers determined by immunofocus assay in Vero cells, as described previously [11], using mouse monoclonal antibodies against RabG (MAb 1002; Abcam) and WN NS1 (MAb8152; Millipore). Primary antibodies were detected with goat anti-mouse IgG-HRP (Thermo/Pierce) or goat anti-mouse IgM-HRP (Millipore) conjugate antibodies. Titers were calculated by counting WN NS1- and RabG-stained cells and expressed as focus-forming units (FFU)/ml. Serial passages of RV-RabG variants to assess genetic stability were performed in HC at multiplicity of infection (MOI) 0.01–0.1 and harvesting viruses 3–4 days post-infection. Detection of RabG protein on infected Vero cells was performed 48 h post-infection by fixing cells with 4% paraformaldehyde and probing with RabG MAb1002 and anti-mouse IgG-Alexa488 (Thermo Fisher Scientific).

Propagation of RV-RabG variants in BHK HC to generate viral stocks for animal studies was performed as previously described [10,18–20]. HC were infected at MOI 0.1 and overlaid with FBS (2%)-containing medium, at 24–48 h post-infection medium was removed, cells were washed with PBS, and overlaid with non-FBS-containing medium. Supernatants were harvested 72–96 h post-infection, concentrated (Centricon Plus-70 centrifugal filter unit, Amicon), supplemented with sorbitol (10% final concentration) and stored at −80 °C. Control YF/WN (ChimeriVax-WN [CV-WN]) [21,22] and YF 17D (YF-VAX, Sanofi Pasteur) LAV viruses were grown in Vero cells.

2.3. Animal studies

All animal procedures were performed under approved IACUC protocols (Sanofi Pasteur or Merial) in accordance with National Institutes of Health requirements for humane treatment of laboratory animals.

2.4. Mouse neurovirulence tests

Litters of neonatal Institute of Cancer Research (ICR) mice (Taconic) aged 2–3 days were infected intracerebrally (IC) with sPIVs, tcPIVs (formulated with the ΔC deletion-containing RV-WN vaccine candidate [10,11] as the trans-complementing component), or LAV controls (9–10 animals/group) at indicated doses in 10 μl volume using Gastight microsyringes with microdispensers (Hamilton). Animals were monitored for 21 days after inoculation. Pups appearing ill or exhibiting signs of paralysis were euthanized.

Pathology in formalin-fixed brain tissue was examined after euthanasia at indicated time points. Brain samples were sectioned coronally from rostral anterior to caudal posterior and analyzed by hematoxylin and eosin stain (Mass Histology). The presence of infectious virus in the brain was assessed; two-to-three animals were sacrificed on days 3, 7 and 11 post-inoculation, brains were collected, homogenized in phosphate-buffered solution (PBS) or MEM and the resulting 10% suspensions clarified and titrated on Vero cells.

2.5. Mouse immunogenicity and efficacy study

Non-Swiss albino (NSA) mice aged ≥6 weeks (Georgia Clinical Research Center Animal Resources) were immunized intraperitoneally (IP) once (day 0) or twice (days 0 and 14) with RV-RabG variants administered as sPIVs or tcPIV formulations (5×10^6 FFU/dose; 20 animals/group). In each group, mice were randomized for serology testing (day 20) or challenge (day 21) (10 mice each). The negative control groups (RV-WN ΔC empty vector) were inoculated once or twice, bled for serology tests and challenged as above. The positive control was a commercially available inactivated rabies vaccine diluted 1:10, administered IP at days 7 and 14, the recommended schedule for optimal response.

Mice for serological testing were terminally bled. Levels of rabies virus-neutralizing antibodies in the sera were quantified using a Rapid Focus Fluorescent Inhibition Test (RFFIT) (Atlanta Health Associates, Inc., Cumming, GA). Pooled sera were also titrated by $PRNT_{50}$ assay against chimera YF/WN virus to determine WN-specific neutralizing antibody titers [21].

The challenge rabies virus (New York Strain 1 42.90) was diluted to a target concentration of 25 MLD_{50} in 0.03 ml, mice were challenged IC (back titration-calculated dose was 16LD_{50}), and observed daily for 15 days. Rabies-related deaths were recorded 6–15 days post-challenge. Animals exhibiting signs of rabies (paresis, paralysis and/or convulsions) were euthanized and categorized as rabies deaths.

2.6. Canine immunogenicity and efficacy study

Eighteen 4-month-old beagles (Covance Laboratories) were randomized to one of four treatment groups (n = 6). On day 0, dogs

Please cite this article in press as: Giel-Moloney M et al. A novel approach to a rabies vaccine based on a recombinant single-cycle flavivirus vector. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.08.055

Download English Version:

https://daneshyari.com/en/article/8486572

Download Persian Version:

https://daneshyari.com/article/8486572

Daneshyari.com