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Evaluation of nonspreading Rift Valley fever virus as a vaccine vector using influenza virus hemagglutinin as a model antigen

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ABSTRACT

Virus replicon particles are capable of infection, genome replication and gene expression, but are unable to produce progeny virions, rendering their use inherently safe. By virtue of this unique combination of features, replicon particles hold great promise for vaccine applications. We previously developed replicon particles of Rift Valley fever virus (RVFV) and demonstrated their high efficacy as a RVFV vaccine in the natural target species. We have now investigated the feasibility of using this nonspreading RVFV (NSR) as a vaccine vector using influenza virus hemagglutinin as a model antigen. NSR particles were designed to express either the full-length hemagglutinin of influenza A virus H1N1 (NSR-HA) or the respective soluble ectodomain (NSR-sHA). The efficacies of the two NSR vector vaccines, applied via either the intramuscular or the intranasal route, were evaluated. A single vaccination with NSR-HA protected all mice from a lethal challenge dose, while vaccination with NSR-sHA was not protective. Interestingly, whereas intramuscular vaccination elicited superior systemic immune responses, intranasal vaccination provided optimal clinical protection.

1. Introduction

Vector vaccines based on RNA virus replicons are designed to optimally combine efficacy with safety. These nonspreading viruses lack at least one gene encoding a structural protein, rendering them unable to produce progeny virions and spread beyond the site of initial infection. The viral genome is however able to replicate in the infected cell, thereby providing "danger signals", the recognition of which elicits innate and adaptive immune responses without the need of co-stimulatory adjuvants. Altogether, these properties provide replicon particle-based vaccines a unique status in vaccinology.

The best-studied replicon platforms originate from representatives of the *Togaviridae* and *Flaviviridae* families, which have positive-strand RNA genomes. These replicons have shown promise as vaccines against various pathogens and also as therapeutics against cancer [1–3]. Positive-strand RNA genomes, however, are prone to mutation and recombination events [4–6]. In contrast, genomes of negative-strand RNA viruses are much more stable and recombination events among these viruses are much less common [7,8]. Replicon particles based on the negative-strand RNA virus vesicular stomatitis virus (VSV) have shown promise in various studies. These replicons, however, seem to perform poorly when applied via the intranasal route [9].

Here, we report the establishment of a new vector platform that is based on previously created Rift Valley fever virus (RVFV) replicon particles [10], here referred to as nonspreading Rift Valley fever virus (NSR). RVFV belongs to the *Bunyaviridae* family and comprises a tri-partite negative-strand RNA genome [11]. The large (L) genome segment encodes the viral polymerase. The medium (M) segment encodes the viral surface glycoproteins Gn and Gc, a 78-kDa protein of unknown function [12,13] and a 14-kDa protein named NSm, which was reported to have anti-apoptotic properties [14]. The small (S) segment encodes the nucleocapsid (N) protein and a non-structural protein named NSs. The NSs protein functions as an antagonist of host innate immunity and is considered the major virulence factor [15,16]. NSR particles resemble the

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authentic virus in structure, but lack the NSs gene and are deprived of the M segment, rendering them avirulent and incapable of producing progeny particles.

We previously created NSR particles that express the enhanced green fluorescent protein (eGFP) gene from the NSs locus to facilitate monitoring of infection (NSR-eGFP) [10]. In the present work, the NSs gene was replaced by genes encoding either the full-length hemagglutinin (HA) protein of influenza A virus A/California/04/2009 (H1N1) or the corresponding soluble HA ectodomain, thereby creating NSR-HA and NSR-sHA, respectively. The HA hemagglutinin was selected as the model antigen because it has already been evaluated in previously developed vector-based influenza vaccines allowing us to gain first insights into the relative efficacy of our system [3,17,18].

In the present work, vaccination with NSR-sHA provided no protection when administered via either the intramuscular (IM) or the intranasal (IN) route, whereas a single vaccination with NSR-HA provided solid protection in a mouse model of lethal influenza. Although vaccination with NSR-HA via both routes was highly effective, protection was optimal when the vaccine was delivered via the IN route. Analyses of antibody and T-cell responses demonstrated that the NSR vaccines induce immune responses with polarized Th1 profiles.

2. Materials and methods

2.1. Challenge virus

Swine-origin influenza virus A/Netherlands/602/09 (H1N1) was used as challenge virus in this study [19]. Mouse adaptation was obtained by three passages in mouse lungs [20].

2.2. Preparation of the vaccines

NSR-sHA, NSR-HA and NSR-eGFP vaccine preparations were produced as described previously [21], diluted in phosphate-buffered saline and kept at −80 °C until use.

2.3. Immunogenicity and vaccine efficacy in mice

Six-week-old female BALB/cAnCrl mice (Charles River Laboratories) were housed in five groups of six mice each (Table 1, experiment 1) or five groups of ten mice and one group of five mice (Table 1, experiment 2). The animals were kept in type-III filter top cages under BSL-3 conditions. After acclimatization for 6 days, two groups of mice from experiment 1 received NSR-HA vaccine via either the IM or the IN route, another two groups received NSR-sHA vaccine via either the IM or the IN route and one group received a control NSR-eGFP vaccine via both routes. All vaccines were administered at a dose of 10^7 $TCID_{50}$/mouse. Mice from experiment 1 were euthanized 28 days after vaccination and spleens were collected for analysis of memory T-cell responses (Fig. 1). Mice from experiment 2 were treated according to the same vaccination protocol as those in experiment 1 but included an additional group of 5 animals, which was mock-vaccinated with PBS via both routes.

The mice from experiment 2 were challenged 3 weeks after vaccination by IN administration of 25× LD_{50} (equal to 5.6 $\log_{10}$ $TCID_{50}$) of mouse-adapted influenza virus. Challenged mice were monitored twice per day in the first week and daily during the second week after the challenge infection and survival, body weights and clinical scores were recorded. The following clinical scoring system was used: score 0 = no clinical signs; 1 = rough coat; 2 = rough coat, less reactive, passive during handling; 3 = rough coat, rolled up, laboured breathing, passive during handling; 4 = dead. Mice with clinical score of 3 at two consecutive observations or a body weight loss of more than 20% as compared to the body weight on the day before the challenge were considered to have reached the humane end point and were euthanized by cervical dislocation. Two weeks after challenge, all surviving mice were euthanized. Blood samples were collected on the day before vaccination (day −1) and on the day before the challenge (day 20, Fig. 1).

All vaccines were administered in a volume of 50 μl. IM vaccinations were performed by injection into the thigh muscle using a ∅ 0.30 mm, 12 mm needle. IN vaccinations were performed by equal distribution of the vaccine between both nostrils. All IN vaccinations were preceded by anaesthesia with 7 mg/kg xylazine and 70 mg/kg ketamine, applied intraperitoneally with a 25 gauge, 16 mm needle. Blood samples were collected by puncture of the facial vein.

All animal experiments were conducted in accordance with the Dutch Law on Animal Experiments (Wod, ID Number BWBR0003081) and approved by the Animal Ethics Committee of the Central Veterinary Institute of the Wageningen UR (Permit Number: 2013105).

2.4. ELISA

Sera were tested for the presence of HA-specific IgG and IgA antibodies, as well as for IgG1 and IgG2α isotypes in indirect ELISA. A soluble HA ectodomain derived from influenza virus A/California/04/2009 (H1N1) (residues 17–523), fused to a T4 foldon trimerization domain [22] and a 3×FLAG tag was used as antigen. The HA ectodomain was expressed in HEK293T cells [23] and purified from the cell culture supernatant using anti-FLAG M2 magnetic beads according to the instructions of the manufacturer (Sigma).

Starting at dilution 1:40, two-fold serial dilutions were prepared of each mouse serum and optical densities were determined for all dilutions. To determine the end-point titres, cut-offs were calculated according to the method described by Frey et al. [24] at a level of confidence of 95%, using negative sera collected from the same mice on the day before the vaccination. Ten negative samples per plate were used for the IgG and IgA tests and four negative samples were used for the IgG1 and IgG2α tests.

Detailed description of the ELISA method is provided in the Supplementary data.

2.5. Hemagglutination inhibition assay

Hemagglutination inhibition (HI) assays were performed as described previously [18]. Briefly, the serum samples were heat-inactivated at 56 °C for 30 min and then treated overnight with Vibrio cholera-derived neuraminidase (Roche) at 37 °C. Inactivation of the neuraminidase was achieved by incubation at 56 °C for 30 min after which sera were pre-treated with turkey red blood cells. Starting at 1:12.5, serial two-fold dilutions of the sera were prepared and tested with 4 hemagglutination units (HAU) of inactivated H1N1 virus (A/Neth/602/09).

2.6. Enzyme-linked immunospot (ELISPOT) assay

Spleen lymphocytes were tested in an ELISPOT assay as previously described [21] and the number of interferon gamma secreting cells was determined after stimulation with: 5 μg/ml of soluble HA ectodomain (described in Section 2.4); 5 μg/ml of a peptide derived from the transmembrane region of the HA protein (IYSTVASSL [25]); or 5 μg/ml of a peptide derived from GFP (HYLSTQSAL [26]). Peptides were synthesized by the Genscript Corporation (Piscataway, NJ, USA). Each sample was tested in triplicate, the average numbers of spots were calculated and normalized with the averages of background signals.