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A recombinant rabies vaccine expressing the trimeric form of the glycoprotein confers enhanced immunogenicity and protection in outbred mice

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ABSTRACT

Rabies is a disease characterized by an invariably lethal encephalitis of viral origin that can be controlled by preventive vaccination programs of wildlife, domestic animals and humans in areas with a high risk of exposure. Currently available vaccines are expensive, cumbersome to produce and require intensive immunization and booster schemes to induce and maintain protective immunity. In the present study, we describe the development of candidate recombinant subunit rabies vaccines based on the glycoprotein G of the prototype rabies virus (RABV-G) expressed either as a monomer (RABV-mG) or in its native trimeric configuration (RABV-tG), with or without Matrix-M™ adjuvant. Immunogenicity and protective efficacy of the respective candidate vaccines were tested in outbred NIH Swiss albino mice. The RABV-tG candidate vaccine proved to be superior to the RABV-mG vaccine candidate both in terms of immunogenicity and efficacy. The relatively poor immunogenicity of the RABV-mG vaccine candidate was greatly improved by the addition of the adjuvant. A single, low dose of RABV-tG in combination with Matrix-M™ induced high levels of high avidity neutralizing antibodies and protected all mice against challenge with a lethal dose of RABV. Consequently RABV-tG used in combination with Matrix-M™ is a promising vaccine candidate that overcomes the limitations of currently used vaccines.

1. Introduction

Rabies encephalitis can be caused by any of the members of the lyssavirus genus of the family *Rhabdoviridae* including the prototype rabies virus (RABV). It accounts for more than 55,000 reported deaths per year, mostly among children in the developing world [1]. Productive infection of humans with any of the lyssaviruses will lead to 100% lethal encephalitis. RABV and other lyssaviruses are transmitted via the bite or scratch of an infected animal. Essentially all mammals are susceptible to rabies and can transmit the virus. Human and animal vaccines against RABV are available, allowing effective rabies control, based on extensive vaccination programs of wildlife species and usually mandatory vaccination programs for domestic carnivores. For humans, pre-exposure immunization is recommended for travellers to endemic areas and professionals who may be occupationally exposed to the virus or virus-infected animals. Despite the effectiveness of existing vaccines, they are among the most expensive vaccines with a relatively poor immunogenicity, since protection is conferred after several immunizations with high antigen doses. Most commonly three doses are required to confer protective immunity and booster vaccinations are necessary to ensure long term protection. Therefore, the vaccine is considered expensive and often rather used for post exposure prophylaxis (PEP) than for prevention. Although PEP can protect vaccine recipients from clinical rabies and death, it should be given promptly (within 24 to 48 h post exposure) [2]. However, in endemic areas PEP is often not available when needed and preventive vaccination coverage are low. Therefore more immunogenic and cheaper rabies vaccines are urgently needed.

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In the recent years several approaches have been explored to improve immunogenicity and reduce cost of existing vaccines using novel production and presentation platforms. Of these the development of DNA and recombinant protein vaccines are among the most promising approaches (reviewed in [3]). The glycoprotein G of RABV (RABV-G) assembles in the membrane envelope of the virion in the form of homotrimers, which harbour the major antigenic determinants of the virus [4]. Therefore, most of the recombinant candidate vaccines described today, are based on RABV-G protein produced with several different expression systems [5–9]. Some of these candidates were shown to be immunogenic in mice, but none have so far been registered for human or animal use. In the present paper we describe the enhanced immunogenicity and protective efficacy of a novel recombinant RABV-G vaccine candidate based on the trimeric form of the RABV-G protein (RABV-tG) expressed in HEK293T cells. A similar approach has been shown to be effective in the development of candidate vaccines against other viral infections such as influenza [10]. The RABV-tG based candidate vaccine performed superior to a predominantly monomeric form of RABV-G recombinant protein (RABV-mG) produced in insect cells. Furthermore, we show that the addition of a potent adjuvant to these vaccine preparations increases the immunogenicity of the candidate vaccine, resulting in dose sparing and fewer immunizations required to confer complete protection from rabies in an outbred mouse model. Collectively these data hold promise for the development of novel generations of effective, cheap and safe vaccines against rabies.

2. Materials and methods

2.1. Construction of candidate vaccines

2.1.1. RABV-mG

A 1518 bp DNA containing the RABV-G ectodomain with transmembrane domain and C-terminus (accession number: M13215) was amplified by PCR and cloned into a baculovirus transfer vector. *Spodoptera frugiperda* cells were infected with the recombinant baculovirus and harvested approximately 55 h post infection with a cell viability of approximately 75%. The monomeric protein was purified by extracting the cells using mild detergent conditions, followed by ion-exchange and lentil lectin affinity chromatography.

2.1.2. RABV-tG

A human codon-optimized sequence encoding the RABV-G ectodomain (amino acids [aa] 20 to 455) of RABV strain Pasteur (Accession number: M13215) was synthesized (Base Clear, Leiden, The Netherlands) and cloned into a derivative of expression plasmid pS1-Ig [11] for expression in HEK293T cells. The G gene was preceded by a sequence encoding an N-terminal CD5 signal peptide and followed by sequences encoding a 7-aa long linker (LIGGGGI), an artificial GCN4-based trimerization domain (GCN4-pII) and a Strep tag for affinity purification (IBA GmbH) as described recently [10]. The strep-tagged RABV-tG was expressed and purified following a protocol described before [10]. In short, HEK293T cells were transfected with the RABV-tG expression plasmid using polyethyleneimine. After 6 h of incubation, the transfection medium was replaced by 293 SFM II expression medium (Invitrogen) supplemented with sodium bicarbonate (3.7 g/l), glucose (2.0 g/l), Primatone RL-UF (3.0 g/l), penicillin (100 units/ml), streptomycin (100 μg/ml), glutaMAX (Gibco), and 1.5% dimethyl sulfoxide (DMSO). Tissue culture supernatants were harvested 5 to 6 days post transfection, and RABV-tG protein was purified from the culture medium using Strep-Tactin affinity chromatography (IBA GmbH). RABV-tG protein expression and purification were confirmed by Western blotting using a Strep-Tactin-horseradish peroxidase conjugate (IBA GmbH) (data not shown) and SDS-PAGE analysis. Protein concentrations were measured using the NanoDrop ND-1000 spectrophotometer (Nanodrop technologies, Wilmington).

2.2. SDS PAGE gel and Western blotting

Samples were separated on 10% SDS-PAGE gel in a Mini-PROTEAN gel electrophoresis system (Bio-Rad). Gels were transferred to a 0.22 μm nitrocellulose membrane (Li-COR) at 250 mA overnight (Mini-PROTEAN; Bio-Rad). Blots were saturated with 5% nonfat dried milk in PBS and washed three times with PBS containing 0.1% Tween-20. Blots were subsequently incubated with monoclonal antibodies recognizing a RABV-G linear (clone 2-22-C5) or conformational (clone 1-11-D6) epitope [12] or mouse IgG1 (isotype control) and stained with rabbit anti-mouse IRDye 800 (Li-COR).

2.3. Immunization and challenge of mice

Groups of ten 3-week old outbred NIH Swiss-albino mice (Harlan Laboratories, Boxmeer, The Netherlands) were immunized with different doses of the monomeric or trimeric vaccine candidates with or without Matrix-M™ (Table 1). The doses used in this experiment were standardised based on the commercial RABV vaccine. Four weeks later (D28) mice were boosted with the same vaccine formulations, except for Groups no. 7 and no. 14 which received a single adjuvanted dose. One group of mice was immunized twice with a commercially available human RABV vaccine (Sanofi Pasteur, Lyon, France) using 1/10th of a human dose per mouse (containing approx. 1 μg of G protein; data not shown). Other control groups received either PBS alone or Matrix-M™ alone (Table 1). Immunizations were given intra-muscularly in 100 μl volumes. All mice were challenged intracranially with 10 μl volumes containing 10^4 $TCID_{50}$ of CVS-11 three weeks after booster (D49). Challenge dose was chosen based on preliminary experiments in our lab that showed this dose led to 100% mortality of infected mice. Intracranial inoculation was chosen based on the generally accepted NIH potency test for rabies vaccines [13]. All mice that survived challenge were euthanized at day 28 post challenge (D77). Animals were kept in isolator cages after challenge, in a 12 h day–night cycle and were given water and food ad libitum. All animal experiments were carried out in accordance with institutional guidelines for animal welfare and were approved by the animal ethics committee of the Erasmus Medical Centre in compliance with Dutch law.

2.4. Serology

Blood was collected from mice by oblique superficial cut of the tail on day 0, 28, 49 and 77. Serum was separated after centrifugation at 3000 rpm for 10 min, heat-inactivated at 56 °C for 30 min and stored at −20 °C until use. High-binding ELISA plates (Costar, Zwijndrecht, The Netherlands) were coated with RABV antigen (whole virus-BPL inactivated) overnight at 4 °C, washed thoroughly with PBS containing 0.05% Tween-20 (PBS-T) to remove unbound antigen and blocked with ELISA buffer (0. 5% skimmed milk, 3% NaCl in PBS) for 1 h at 37 °C. Mouse serum was diluted 1:100 in ELISA buffer and applied to the plates for 1 h at 37 °C. After thorough washing with PBS-T the secondary antibody (protein-A HRPO; Zymed, Bleiswijk, The Netherlands) was applied for 1 h at 37 °C. Plates were developed with tetramethylbenzidine and absorption was measured at 450 nm using a 620 nm reference filter. Avidity ELISA was performed identical to the antibody ELISA with the exception that antibodies were eluted with 6 M urea for 5 min before addition of secondary antibody. Virus neutralizing antibodies (VNA)