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Induction of immune responses and protection in mice against rabies using a self-replicating RNA vaccine encoding rabies virus glycoprotein

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ABSTRACT

A self-replicating RNA vaccine encoding rabies virus glycoprotein gene was developed utilizing sindbis virus RNA replicon. The *in vitro* transcribed RNA (Sin-Rab-G RNA) was transfected in mammalian cells and analysed for self-replication and expression of rabies glycoprotein. To generate immune responses against rabies, mice were immunized with 10 μg of Sin-Rab-G RNA and immune responses developed were compared with mice immunized with rabies DNA vaccine and commercial cell culture vaccine (Rabipur). The self-replicating rabies RNA vaccine generated cellular and humoral IgG responses similar to rabies DNA vaccine. On challenge with rabies virus CVS strain, rabies RNA vaccine conferred protection similar to rabies DNA vaccine. These results demonstrated that replicon-based self-replicating rabies RNA vaccine with 10 μg dose was effective in inducing immune responses and protection similar to rabies DNA vaccine.

1. Introduction

Rabies is one of the major zoonotic diseases of public health importance affecting warm-blooded mammals. It is a neurotropic disease transmissible to man by contact with infected animals (Spencer, 1994). In developing countries, dogs represent the major rabies reservoir causing about 60,000 annual deaths worldwide (Tordo et al., 2006). In developing countries, only 30–40% vaccination is performed in dog that is too low to break the disease transmission cycle. Although several effective rabies vaccines derived from cell culture are available, yet over 60,000 persons worldwide and 30,000–35,000 in developing countries die of rabies each year as these vaccines are unaffordable for animals and poor section of human population (WHO, 2006). The failure to eliminate dog rabies in developing countries stresses the necessity of improving rabies control program by applying new vaccines or new vaccination strategies. Therefore, there is a need to develop safer, cheaper and efficacious vaccine against rabies overcoming the shortcomings of the current vaccines. Several groups, including ours, have experimented with the use of DNA vaccines for protection against rabies in mice (Xiang et al., 1994, 1995; Gupta et al., 2006) and in other animal species (Lodmell et al., 1998; Perrin et al., 1999; Osorio et al., 1999; Gupta et al., 2005a; Patial et al., 2007).

Although robust immune responses can readily be induced using DNA vaccine in small animals such as mice, multiple immunizations of high DNA doses are often required to achieve modest responses in large animal species and in many cases DNA vaccination is hampered by poor efficacy (Calarota et al., 1998; MacGregor et al., 1998; Babiuk et al., 2003). Apart from this some potential risks related to integration of plasmid DNA into host chromosome, tolerance to DNA vector and generation of autoimmune diseases (Mor et al., 1996; MacGregor et al., 1998; Martin et al., 1999; Beger et al., 2002) have limit the progress of DNA vaccines in clinical trials.

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RNA replicon-based self-replicating vaccines are recently being explored as an alternative approach to increase the immunogenicity and biosafety of DNA vaccines. These vaccines are self-amplifying and self-limiting in nature. Their replicon component consists of alphavirus non-structural replicase genes, 5′- and 3′-end genome sequences (required for replication) and a heterologous gene which has been substituted in place of the viral structural gene. Expression of the heterologous gene is achieved by linking to the highly active alphavirus subgenomic promoter (Xiong et al., 1989). These can be delivered as RNA (self-replicating RNA) or replication deficient recombinant virus. Replicon systems have been developed for Sindbis virus (Xiong et al., 1989; Herweijer et al., 1995), Semliki Forest virus (Berglund et al., 1998), Venezualan Equine Encephalitis virus (Lee et al., 2003) and for Kunjin virus (Anraku et al., 2002) and has been found significantly more immunogenic than DNA vaccines with low dose to induce the same level of immune responses as that achieved with the DNA vaccine. Further, expression of immunogenic gene is achieved by RNA, therefore the risks associated with DNA vaccines such as integration with host chromosome can be circumvented.

The present study investigated the potential of replicon-based self-replicating RNA vaccine against rabies in order to improve the biosafety and immunogenicity of rabies DNA vaccine. The rabies-specific humoral and cellular immune responses and the protection against virulent rabies were evaluated in immunized mice and compared with rabies DNA vaccine and commercial cell culture vaccine, Rabipur.

2. Materials and methods

2.1. Cell, virus and vaccines

Baby Hamster Kidney (BHK-21) cell line was used for *in vitro* transfection. Cell line was procured from National Centre for Cell Science (NCCS), India and grown at 37 °C under 5% CO_2 in Dulbecco's modified minimum essential medium (DMEM, Hyclone) supplemented with 10% foetal bovine serum (FBS, Hyclone), 50 μg/ml gentamicin and 25 mM HEPES (Sigma).

The mice brain adapted rabies challenge virus standard (CVS) strain was used for virulent rabies challenge. The purified BPL-inactivated rabies Pasteur virus-11 (PV-11) was used in ELISA as coating antigen.

The rabies DNA vaccine encoding glycoprotein gene of rabies virus CVS strain used in this study was previously reported by our group (Patial et al., 2007). Purified chick embryo derived cell culture vaccine (PCECV), Rabipur (Chiron vaccines, Behring) was used as control cell culture rabies vaccine.

2.2. In vitro transcription of self-replicating RNA (Sin-Rab-G) encoding rabies glycoprotein

The DNA fragment containing full length glycoprotein gene from CVS strain of rabies virus was subcloned from a recombinant plasmid and ligated into pSin (Invitrogen) plasmid vector at XbaI and StuI sites. The glycoprotein gene insert and ORF in recombinant plasmid pSin-Rab-G was confirmed by restriction digestion and PCR. The Sin-Rab-G RNA was prepared by *in vitro* transcription from recombinant plasmid pSin-Rab-G using SP6 RNA polymerase. The pSin-Rab-G plasmid was linearized downstream of 3′-UTR using the restriction enzyme NotI and used as template for transcription using mMESSAGE mMACHINE Kit (Ambion) following manufacturer's instructions. The transcribed RNAs were capped during transcription reaction using 4 mM of cap analogue (Ambion). The transcribed RNA contained 5′-UTR, non-structural genes (nSP1-4), 26S subgenomic promoter, rabies glycoprotein gene and 3′-UTR (Fig. 1). The quality of the RNA transcripts was analyzed by denaturing agarose gel electrophoresis. The quantity was adjusted to 0.1 mg/ml and stored in −80 °C until used for transfection or immunization.

2.3. Expression analysis of Sin-Rab-G RNA in mammalian cells

The Sin-Rab-G RNA was transfected into BHK-21 cells using Transmessenger transfection reagent (Ambion)

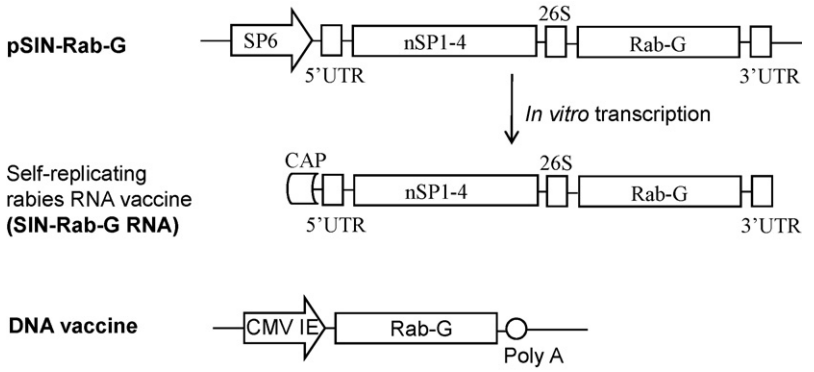

Fig. 1. Schematic representation of replicon-based RNA vaccine and rabies DNA vaccine. The transcriptional control units and gene expression sequences are shown: SP6, SP6 RNA polymerase promoter; 5′-UTR, 5′-untranslated region; nSP1-4, the non-structural proteins of Sindibis virus; 26S, the subgenomic promoter of Sindibis virus; 3′-UTR, 3′-untranslated region; PolyA, polyadenylation signal; Rab-G, rabies glycoprotein gene; CMV IE, cytomegalovirus immediate early promoter/enhancer.