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Electroporation enhances protective immune response of a DNA vaccine against Japanese encephalitis in mice and pigs

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ABSTRACT

Japanese encephalitis virus (JEV) is a pathogenic cause of Japanese Encephalitis (JE), which is a zoonotic disease transmitted by mosquitoes and amplified by pigs. Infection of JEV may lead to severe neurological sequelae, even death in humans and reproductive disorders in pigs. Vaccination is the only way to control JEV infection in humans. For pigs play important role in the JEV transmission cycle, developing a new veterinary vaccine is considered as a useful strategy for cutting off the transmission route of JEV. We have previously reported that DNA vaccine pCAG-JME, expressing prM-E proteins of JEV, is effective in mice through intramuscular injection (IM). However, the poor immunogenicity, due to low expression of immunogen, is the major obstacle for the development of DNA vaccine in large animals. In the present study, therefore, we immunized mice and pigs with pCAG-JME intramuscularly accompanied with electroporation (EP) stimulation, the attractive gene delivery approach. As compared with IM, EP-mediated vaccination markedly increased the expression of immunogen in the injection site and induced a dose- and time-dependent immune response. 100% survival rate was observed in groups vaccinated with doses ranged from 10 to 100μg, indicating that 10μg of DNA with EP for individual was enough for inducing effective protection in mice. Surprisingly, survival rate and end-point titers of anti-JEV antibodies were higher in mice even at lower dose of DNA (5μg) than that in mice inoculated 100μg through IM. Notably, the prM-E antigens also induced high antibody response in pig, while the neutralizing antibody titer achieved 1:320. Our results suggested that EP-mediated DNA immunization might act as an effective strategy against JEV, at least in pig, and that EP has a potential application prospect in DNA vaccination.

1. Introduction

Japanese encephalitis virus (JEV), belonging to the family of *Flaviviridae*, is the etiologic agent of Japanese encephalitis (JE), which is an epidemic mosquito-borne disease mostly prevalent in Southeast Asia and East Asia. In humans, JEV infection may result in severe central nervous system symptoms, including high body temperature, neck rigidity, hemiparesis and convulsions [1]. The survivors are often left with irreversible neurological sequelae. Presumably, the number of annual JE clinical cases is nearly 68,000 globally, with up to 20,400 deaths [2]. However, there is no specific antiviral treatment for JEV available yet, although vaccination might be the most efficient way for controlling the disease. Three types of licensed JE vaccines, namely, mouse brain-derived inactivated vaccine, cell-derived inactivated vaccines, and live attenuated vaccine (SA-14-14-2) [3], are currently used in humans.

JEV, as a zoonotic virus, can also be naturally amplified in pigs which are the only important mammal in the JEV transmission cycle despites high seroprevalence rates in many other mammal species [4]. Pigs could develop viremia for several days. If the female mosquito (*Culex*) sucks the blood of the infected pigs, the mosquito could transmit the virus to a new host, especially pig. Clinical symptoms in pigs are usually self-limited or mild. Notably, JEV can cause abortion, mummified fetuses, stillbirth and infertility in the sows, and orchitis in the boars which lead to significant economic costs in pig industry. More importantly, following the infective mosquito bites, humans might develop subclical or clinical symptoms. Because of large breeding numbers, short feeding period and quick consumption flow, pigs frequently remain naïve and available as the new source of infection, posing as a great threat to humans. Thus, controlling the spread of JEV in pigs should be paid more attention.

However, global demand for JE vaccine is far beyond supplement, even regular human vaccination is not affordable or sustainable in some endemic area currently [5], which makes it more difficult to introduce the JEV vaccine program in raising pig

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industry. In addition, there are also some limitations of inactivated vaccine, including allergic reaction, expensive preparation and fussy operations. The potential risk of virulence reversion of the attenuated vaccine should also be taken into consideration. The exploration on safer, more effective and lower-cost vaccines, therefore, is necessary, while DNA vaccine might be a potential candidate.

DNA vaccines have been developed for decades with attractive advantages, such as inexpensiveness, ease of production, stability for storage and shipping [6]. However, no licensed DNA vaccine is available in humans till now, although two DNA vaccines have been approved for animal infectious diseases at present, including horse vaccine against West Nile virus (WNV) and atlantic salmon vaccine against infectious hematopoietic necrosis virus (IHNV) [7,8]. The main obstruction hampering the success of DNA vaccines in big animal is probably due to the poor immunogenicity compared to the exciting results in mice models. To overcome the obstacle, several technical improvements have been investigated, including gene optimization strategies, novel formulations and immune adjuvants, and effective delivery approaches [8].

Attractively, electroporation (EP), as a method to deliver macromolecule drugs into cells by transient electric pulses, has generated considerable attention. EP has been introduced in many non-human investigations, human clinical trials of DNA vaccines and gene therapy products in recent years [9–12]. The expression level of antigens, cytokines or enzymes were markedly increased with a relatively lower dose of immunogens in big animals such as dogs, pigs and non-human primates due to efficient uptake of DNA by cells. Previously we have demonstrated that a DNA vaccine pCAG-JME expressing the premembrane (prM) and envelope (E) proteins of JEV could induce effective protection in mice through intramuscular injection [13]. The present study aimed to further evaluate whether the pCAG-JME-induced immune response could be enhanced by the EP gene transfer strategy in mice and pigs. As expected, high antibody response and effective protection were observed in mice even with low dose of the DNA. Excitingly, the prM-E antigen also induced high antibody response in pigs. Our results indicated that pCAG-JME might serve as a promising pig JE vaccine, while EP, as an effective delivery method, might have a potential application prospect in DNA vaccination.

2. Material and methods

2.1. Cells, virus, vaccine and animals

Vero cells were regularly maintained at 37 °C with 5% CO_2 in minimum essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). C6/36 *Aedes albopictus* cells were regularly maintained at 28 °C with 5% CO_2 in RPMI 1640 supplemented with 10% FBS.

The Beijing-1 strain of JEV was propagated in C6/36 cells and Vero cells respectively. Titers of virus and neutralizing antibody (NAb) were determined by plaque assay on Vero cells.

The DNA vaccine pCAG-JME, expressing the prM-E gene, was constructed as previously described [14].

6–8 week-old female inbred BALB/c mice were purchased from the Vital River Laboratories (Beijing, China). All mice were maintained in specific-pathogen-free environments. The 21–42 day-old, 8–12 kg-weight female Landrace piglets were provided by China Animal Husbandry Group (Beijing, China). All pigs were maintained in barrier environments. All the animal experiments were approved by the animal ethics committees of Capital Medical University, Beijing, China.

2.2. DNA immunization and EP in vivo

For murine immunization, 50 μl of 0.9% sodium chloride containing different doses of DNA was inoculated into the quadriceps muscle of the hind limb. After injection, two silver electrodes (6 mm apart) were immediately inserted into same site and six electric pulse stimulations (36 V, 10 ms) were given by Teresa Gene Delivery Device (Shanghai Teresa Healthcare Sci-Tech, China). Mice were immunized three times at three-week intervals. The groups and plasmid doses were detailed in Table 1. Mice immunized with pCAGGSP7 plasmid served as negative controls (EP-control). Three weeks after the final immunization, the mice were challenged with 50 times LD50 of JEV, and the mortality were observed for 21 days.

In Landrace, 1 mg of pCAG-JME in 1 ml of 0.9% normal saline was administered into the thigh (alternating left and right) four times at two-week intervals. The *in vivo* EP parameters were: six electric pulse stimulations, 50 ms pulse length and 60 V/mm distance between the electrodes. Prior to vaccination, the absence of JEV-specific antibody was confirmed in all pigs.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Concentrations of JEV antibodies were measured in sera collected from the mice and pigs at different time-points using enzyme-linked immunosorbent assay (ELISA) as previously described [15].

2.4. Plaque reduction neutralization test (PRNT)

To determine the NAb titers in sera obtained from immunized mice and pigs, sera were diluted two-fold starting at 1:10 in MEM containing 2% FBS. After heating at 56 °C for 30 min to inactivate complement, 500 μl of serial dilutions were added with an equal volume of JEV solution containing about 1000 PFU, and incubated at 37 °C for one hour. Mixtures of samples (200 μl) were then added to monolayer Vero cell in a 24-well plate and each well contained about 200 PFU of the virus. After one-hour incubation, plates were washed and overlaid with MEM containing 5% FBS and 1.35% methylcellulose. Following five days of incubation at 37 °C with 5% CO_2, plates were stained with crystal violet and plaques were counted. The NAb titer was calculated as the reciprocal of the maximum dilution of serum that yielded a 50% plaque reduction (PRNT 50) when compared to that of the virus controls.

2.5. Immunohistochemical analysis (IHC)

To detect the antigen expression *in situ*, paraffin-embedded sections prepared from the murine quadriceps muscles at three weeks after final immunization were subjected to IHC. Briefly, after removing paraffin, blocking endogenous peroxidase, retrieving antigens and blocking with 1% of bovine serum albumin (BSA), the specimens were incubated with murine polyclonal antibody against JEV and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG in order. Finally, the slides were developed with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. Slides were examined under a light microscope.

2.6. Statistical analysis

All data were analyzed with the SPSS software (version 17.0). Data were considered to be significant difference when P value was less than 0.05.

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