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Electroporation improves the immune response induced by a DNA vaccine against pseudorabies virus glycoprotein B in pigs

Vincent Le Moigne [a], Roland Cariolet [b], Véronique Béven [a], André Keranflec'h [b], André Jestin [a], Daniel Dory [a,*]

[a] ANSES, Ploufragan/Plouzané Laboratory, Viral Genetics and Biosafety Unit, 22440 Ploufragan, France
[b] ANSES, Ploufragan/Plouzané Laboratory, Pathogen-free Pig Breeding and Testing Facility, 22440 Ploufragan, France

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ABSTRACT

This study was performed to determine whether electroporation can be used to enhance the efficacy of a DNA vaccine against pseudorabies virus (PrV) in pigs. Immune responses to PrV were measured in pigs following a single intramuscular injection of plasmids encoding PrV glycoprotein B, with or without electroporation. Plasmid injection coupled with electroporation increased production of specific antibodies against PrV and peripheral blood mononuclear cells proliferated in response to stimulation with PrV glycoproteins. These results show that electroporation can improve the performance of a DNA vaccine against PrV in pigs. However, additional work is required to maximise the effectiveness of the vaccination protocol.

DNA vaccination is described as a potential alternative to conventional vaccination against viral diseases. For example, pigs can be protected against experimental swine alphaherpesvirus pseudorabies virus (PrV) infections after the injection of plasmids encoding the PrV glycoproteins B, C and/or D (Dory et al., 2005b; Dufour et al., 2000; Gerdts et al., 1999; van Rooij et al., 2000). PrV causes Aujeszky's disease, a serious illness characterised by nervous disorders, respiratory distress, severe weight loss, high morbidity and mortality, leading to severe economic losses in the pig industry (Mettenleiter, 2000). Among the glycoproteins used, glycoprotein B (gB) is particularly interesting because it is highly immunogenic, due in part to the presence of several B cell epitopes on the PrV gB (Zaripov et al., 1998, 1999), and has been described as a potential carrier of foreign antigens (Dory et al., 2009).

DNA vaccines are generally administered intramuscularly or intradermally, using either a needle and syringe or a needle-free injector. Many strategies to improve the effectiveness of DNA vaccines have been proposed and tested. Among these, electroporation has a promising future (Luxembourg et al., 2007). Electroporation consists in the application of an electric current on both sides of the injection point. Cells at the injection site are thereby temporarily permeabilised, promoting the entry of plasmids by electric current into the cells; furthermore, the immune response to the encoded antigens is enhanced (Bachy et al., 2001; Rizzuto et al., 1999; Selby et al., 2000; Widera et al., 2000). Electroporation has been demonstrated as a powerful technique in large animals, including pigs (Babiuk et al., 2002; Bodles-Brakhop et al., 2011).

In the preliminary study presented here, we investigated whether electroporation can increase the immune response in pigs after immunisation with a single intramuscular (i.m.) injection of a plasmid encoding PrV gB, a DNA vaccine that has been successfully used in our laboratory (Dory et al., 2005a, 2009). Given our knowledge on the correlation between the induction of immune response and protection generated by PrV DNA vaccines (Dory et al., 2005a,b; Gravier et al., 2007) and the fact that electroporation has not yet been optimised for pigs, we chose not to challenge the pigs with PrV to avoid subjecting them to unnecessary suffering. The experimental protocol was approved by the ANSES, National Veterinary School of Alfort and University of Paris-Est Créteil (France) ethics committee for animal experimentation (Notice No. 10/04/13-05) and the pigs were housed and treated in accordance with local veterinary regulations (Direction des Services Vétérinaires des Côtes d'Armor, France). Four groups of four eight-week-old specific pathogen-free pigs were involved. Pigs were anaesthetised prior to vaccination, as described in some studies (Bodles-Brakhop et al., 2011; Tollefsen et al., 2003). For this purpose, 1 g of thiopental/50 kg body weight was intravenously injected. Plasmids diluted in PBS were injected into the left *biceps femoris* muscle at a dose volume of 600 µl using 0.45 mm × 12 mm needles. Groups 1 and 2 were injected with 2275 µg PrV gB-pcDNA3 (2.5×10^{14} copies), group 3 was injected with 1500 µg empty pcDNA3 (2.5×10^{14} copies) and group

* Corresponding author. Tel.: +33 2 96 01 64 42; fax: +33 2 96 01 62 83.
E-mail address: daniel.dory@anses.fr (D. Dory).

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4 was injected with PBS. Electroporation was then applied to groups 1, 3 and 4. Stainless-steel electrodes (0.2 mm wires, 10 mm long and 10 mm apart) were placed on each side of the injection point. Eighty seconds after plasmid or PBS injection (as described in Draghia-Akli et al., 2008), the pulsed electric field was applied using a BTX ECM 830 Pulse Generator (Harvard Apparatus, Holliston MA, USA). Stimulation consisted of five pulses of 150 V and 20 ms duration each with a 200 ms interval between each pulse and with no change in polarity (as described in Babiuk et al., 2002). Anaesthesia, plasmid injection and electroporation procedure were well tolerated by the pigs.

Blood samples were collected before injection and then weekly from the first to the sixth week after immunisation. Titres of serum anti-PrV-glycoprotein immunoglobulin G-type (IgG) antibodies were determined as previously described (Gravier et al., 2007). IgG titres (log 10) were expressed as the highest dilution giving an optical density (OD, measured at 450 nm) value higher than the three-fold OD of serum from a non-vaccinated and non-infected pig. As presented in Fig. 1, the PrV gB-specific antibody response was detected 2 weeks after injection of PrV gB-pcDNA3, but the difference between electroporated and non-electroporated pigs was not significant. Starting from third week after injection, pigs injected with PrV gB-pcDNA3 and electroporated produced significantly more antibodies against PrV than pigs injected with the same plasmid without subsequent electroporation. No antibodies were detected in the control groups (empty pcDNA3 or PBS). Statistics were performed using the non-parametric Mann–Whitney test (Mann and Whitney, 1947) included in the Systat 9 software package (Systat Software, Inc., Point Richmond, CA). The limit of significance was 0.05 for all comparisons. No PrV-neutralising antibodies were detected in any blood sample (not shown). In a previous study (Dory et al., 2009), significant levels of neutralising antibodies were detected only after the third injection of PrV gB-pcDNA3 in pigs (without electroporation). Nevertheless, protection against an experimental PrV-infection can be achieved in the absence of detectable PrV-neutralising antibodies and with a comparable level of specific antibodies (Gravier et al., 2007). We speculate that DNA vaccination with PrV gB-pcDNA3 accompanied by electroporation, which resulted in an increase in PrV-specific IgG antibodies, may induce protective immunity.

Two and three weeks after immunisation, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient centrifugation from blood samples as previously described (Dory et al., 2005b). Porcine IFN-γ and IL-4 mRNA produced by PBMCs in response to PrV stimulation were quantified. IFN-γ has several immunoregulatory roles and effector functions involved in Th1 responses, and IL-4 plays a key role in Th2 responses (Finkelman et al., 1988; Wood and Seow, 1996). Cells, at a density of 5×10^6 per well in a 24-well plate in complete RPMI medium, were incubated *in vitro* for 16 h at a multiplicity of infection (MOI) of 10 (Dory et al., 2005b). The relative expression of IFN-γ and IL-4 mRNAs were determined by quantitative real-time PCR as described previously (Dory et al., 2005b). Both PrV gB-pcDNA3 immunised groups (with and without electroporation) presented significantly positive IFN-γ responses after PrV stimulation 2 and 3 weeks after plasmid injection (Fig. 2A). However, no significant differences in production between the electroporated and the non-electroporated groups were detected. In contrast, production of IL-4 mRNA was low and not significant (Fig. 2B). These results are consistent with those obtained without the use of electroporation (Gravier et al., 2007).

The proliferation of PBMCs (at a density of 10^5 per well in a 96-well plate) was measured in response to stimulation with purified PrV glycoproteins or phytohaemagglutinin (PHA). Each culture condition was performed in triplicate. After 5 days of culture at 37 °C, 20 µl of Alamar Blue® dye (AbD Serotec, Oxford, UK) was added and the plates were further incubated for 10 h at 37 °C. The proliferation rates were determined according to manufacturer's instructions. Two weeks after injection, cells were incubated with PrV glycoproteins at a concentration equal to or higher than 100 ng/ml or with live PrV. High cell mortality was observed and no proliferation rates could be determined. Therefore, the concentration of PrV glycoproteins was therefore reduced to 10 ng/ml for subsequent samples from which results are reported below. Three weeks after injection, PBMCs of pigs from all four groups showed similar proliferation indices in response to PHA (Fig. 2C). Interestingly, proliferation of PBMCs in response to PrV-glycoprotein stimulation was observed only for the group injected with PrV gB-pcDNA3 and electroporated, and not in the other three groups, including the one injected with the same plasmid but not electroporated.

Taken together, these results show that PrV gB has the capacity to induce Th1 and Th2 responses, and that electroporation increases production of PrV-specific antibodies as well as proliferation of PBMCs in response to PrV-glycoprotein stimulation. Nevertheless, the increase in immune response observed here is relatively modest compared to the response obtained in other large animal models (Babiuk et al., 2002). The study presented provided proof of concept. We have demonstrated that electroporation is useful for enhancing immune responses in the PrV DNA vaccination model;

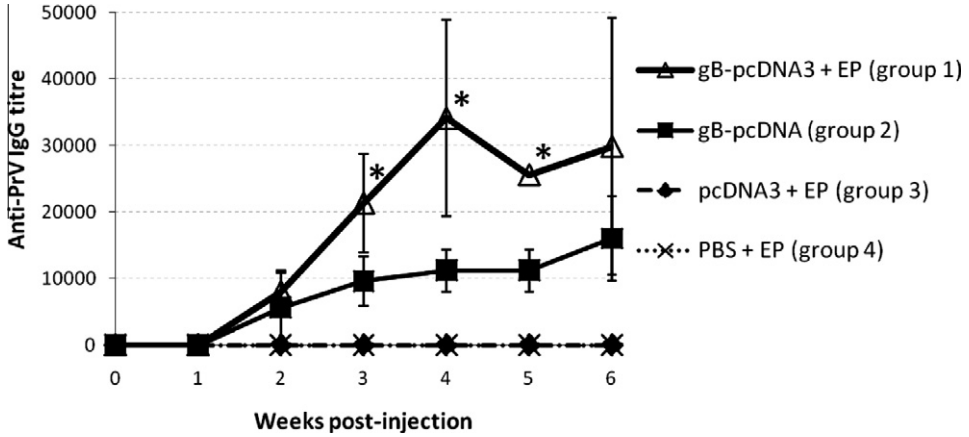

Fig. 1. Anti-PrV IgG in serum. The anti-PrV glycoprotein was determined by indirect enzyme-linked immunosorbent assay using microtitration plates coated with PrV glycoproteins as the antigen. Mean titres, including standard error bars, for each group of pigs are expressed as the highest serum dilution giving an OD value > 3 × OD of a pool of control serum from unvaccinated pigs. *$p < 0.05$.