Chapter 27

Electroporation-Mediated Intradermal Delivery of DNA Vaccines in Nonhuman Primates

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Abstract

Strategies to improve vaccine efficacy are still required. The immunogenicity of DNA vaccines is strongly improved by electroporation (EP). The skin is populated with a wide variety of immune cells, making it an attractive tissue for vaccine delivery. Here we describe a method for the EP-mediated intradermal delivery of DNA vaccines in nonhuman primates, as a model for preclinical development of human vaccines, using noninvasive needleless electrodes.

Key words DNA, Vaccine, Electroporation, Skin, Nonhuman primate

1 Introduction

The skin is the first line of defense to protect the body from dehydration, injury, and infection [1]. It is populated with a wide variety of immune cells, including dendritic cells (DCs), making it the largest immunological tissue of the human body. Although intramuscular delivery has been the most widely used route of administration of vaccine, skin is a very attractive tissue for vaccination [2, 3]. Anti-smallpox vaccine was the most successful vaccine for humans and was administered by skin scarification or puncture; BCG and new commercial flu vaccines are injected by intradermal (ID) route [4]. At steady state, the human skin is rich in cells specialized in immune surveillance, including Langerhans cells (LCs) in the epidermis and several subsets of DCs and macrophages in the dermis [5]. These potent antigen-presenting cells sample the antigens in their environment by diverse mechanisms, can be activated by "danger signals," and migrate to the draining lymph nodes to interact with antigen-specific T and B lymphocytes, resulting in the priming and induction of adaptive immune response. ID administration of plasmids in association with electroporation (EP) is one of the most efficient nonviral methods for

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the delivery of gene into the skin [6, 7] and is suitable to deliver DNA vaccine when a Th1-oriented response is desired [8].

In a previous work conducted in nonhuman primates (NHPs), which share a very similar immune system organization with humans [9–11], allowing prediction of immunogenicity, we demonstrated that ID delivery of a plasmid, combined with EP, strongly enhanced the expression of the vaccine antigen in the epidermis [12]. The results suggested a strong involvement of LCs in the induction of Th1-oriented responses, characterized by the persistence, over 2 years, of $CD4^+$ and $CD8^+$ antigen-specific T lymphocytes. Here, we describe the method used for EP-mediated ID delivery of DNA vaccines in NHPs. A special feature of the described method is to use needleless external electrodes, by contrast with usual methods for large animal models.

2 Materials

1. Sterile phosphate-buffered saline (PBS) pH 7.2.
2. Plasmids adjusted at 1 mg/ml in sterile PBS (*see* **Note 1**). Prepare 1 ml per animal plus 10 % to compensate the lost when filling the syringes.
3. Adult cynomolgus macaques (*Macaca fascicularis*) from Mauritius, weighing 3–6 kg.
4. Ketamine chlorhydrate.
5. Ethanol 70 %.
6. Sterile syringes with needle 29 G × 1/2 in.
7. Nepa Gene CUY21 EDIT electroporator (Sonidel Ltd, Dublin, Ireland).
8. Tweezers electrodes.
9. Gel for electrodes.
10. DNA decontamination solution.

3 Methods

3.1 Intradermal Injection

1. Sedate the animals with ketamine chlorhydrate (10–15 mg/kg).
2. Shave the back of the animals.
3. Clean the shaved skin with ethanol 70 %.
4. Fill the syringe with 1 ml of plasmid solution and inject ID (*see* **Note 2**) 100 µl per site of plasmid in PBS, ten sites per animal (Fig. 1). Space the injection sites out 2–3 cm apart.

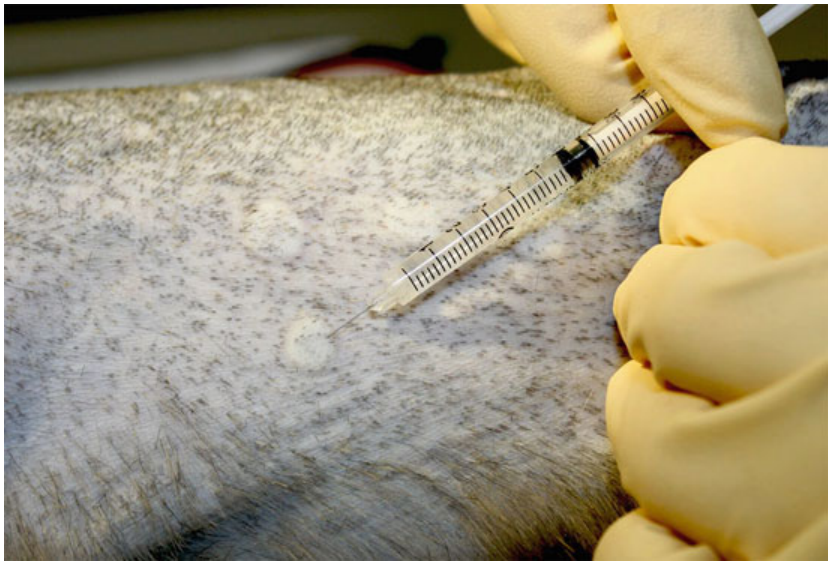

Fig. 1 Intradermal injection

3.2 Determine the Optimal Voltage for Electroporation

1. Set up the electroporator program to six square-wave pulses of 10 ms with 90 ms intervals at 110 V.
2. Save the program in the electroporator memory.
3. Put a small amount of gel (*see* **Note 3**) on two sites 2–3 cm apart of the shaved untreated skin.
4. Fold the skin between both sites (Fig. 2a).
5. Place the electrodes on each spot of gel (Fig. 2b).
6. Maintain firmly and start the electroporator.
7. Read the output current intensity, which should be comprised between 300 and 700 mA (*see* **Note 4**).
8. If necessary, adjust the voltage to reach the recommended intensity.

3.3 Proceed to the Electroporation of the Sites of Injection

1. Put a small amount of gel on each plasmid injection site.
2. Fold the skin between two adjacent sites and proceed to the electroporation. Repeat this step for the five pairs of injection site (*see* **Note 5**).
3. Remove the gel from the skin with ethanol 70 %.
4. When completed, unplug the electrodes and clean them with ethanol 70 % then with the DNA decontamination solution.
5. For optimum immune response, one or two boosts should be performed 6–8 weeks later.

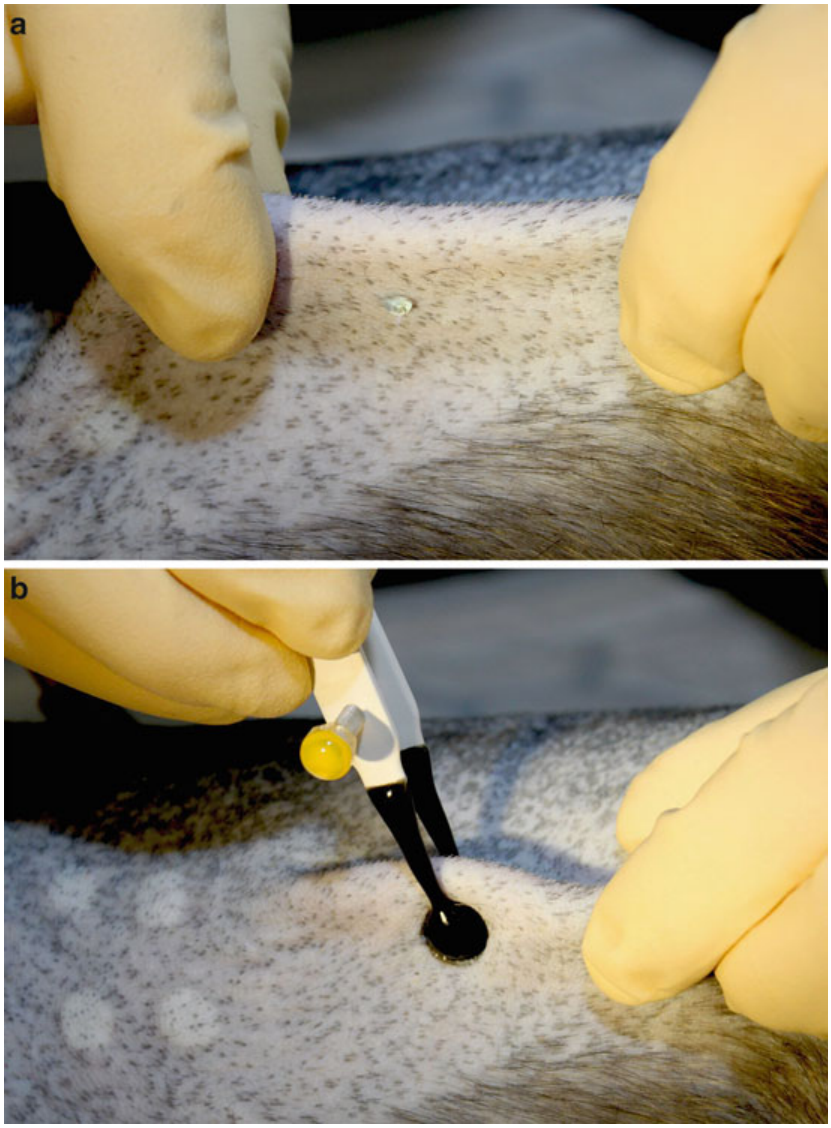

Fig. 2 Placing the tweezers electrode for electroporation. Fold the skin between the desired sites (**a**) and carefully place the electrodes on both sites (**b**)

4 Notes

1. In our hands, the best antigen expression was obtained with recombinant Auxo-GTU® plasmids [13] provided by FIT Biotech (Tampere, Finland); nevertheless, very good levels of expression were also observed with pCMV vectors.
2. This step needs practice. For best results, the intradermal injection should be as superficial as possible. For this purpose, stretch the skin, orient the needle bevel upper, and introduce the needle in the skin with a straight angle. Press the plunger slowly. The lightening of the skin and the high resistance mean that the injection is delivered in the superficial part of the skin. When reaching 100 μl, wait for the decrease of the pressure (2–3 s) before removing the needle.

3. The role of the conductive gel is to ensure good contact between the electrodes and skin. Nevertheless, use moderate amount of gel (the size of a grain of wheat for each electrode) to avoid continuity between both sites; otherwise, the current will go directly through the gel rather than through the skin.
4. The intensity of the output current is mainly dependent on three parameters: (a) the tension of the current (voltage) between both electrodes. This parameter can be easily adjusted in the operating procedure of the Nepa Gene CUY21 EDIT electroporator. (b) The thickness of the skin. It is variable between animals, avoid fat animals. (c) The pressure applied to the tweezer electrodes. The use of a rubber band may help to keep a constant pressure.

   All together, these parameters did not cause any tissue damage and increased the uptake of reporter plasmid into skin cells.
5. Although the epidermis is oriented to the cathode for one site and to the anode for the other site of the pair, we never observed any differences at the level of antigen expression between both sites.

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