

# Enhancement of Cellular Immune Response to a Prostate Cancer DNA Vaccine by Intradermal Electroporation

## INTRODUCTION

Intradermal DNA injection targets the skin for efficient delivery in vaccine research. The skin is an excellent target for DNA vaccine delivery since it is easily accessible and has abundant antigen-presenting cells for a robust immunological response. This response is further increased when injection is followed by intradermal electroporation-- a series of electrical pulses that are applied through an array of small electrodes pressed onto the tissue. This paper optimizes electroporation conditions for maximum DNA uptake and describes enhanced CD8+ lymphocyte response to prostate cancer DNA vaccination after intradermal injection with electroporation.

## RESULTS

1. Gene expression was measured in mice as luciferase production for five sets of electroporation parameters: field strength, number of pulses, pulse duration, dual or single mode (Table 1).
2. Electroporation increased gene expression 100- to 100-fold compared to intradermal injection alone for four out of five pulse protocols (Figure 2).
3. Immunological response to PSA vaccination was measured as PSA-specific CD8+ expression for intradermal vaccination with or without electroporation and compared to intramuscular vaccination (Figure 3a).
4. Intradermal electroporation using protocols D and E stimulated a robust immune response (Figure 3b).
5. The enhanced immunological response was persistent 15 days past treatment (Figure 3c).

## CONCLUSIONS

Intradermal electroporation enhances DNA vaccine delivery with 100- to 1000-fold enhanced expression and a robust immune response compared to injection alone for either intradermal or intramuscular vaccination. The level of enhancement and strength of the response depends on the pulse parameters used for electroporation where a dual-pulse protocol was most effective.

## METHODS

### *DNA injections and in vivo electroporation*

Intramuscular injections were delivered bilaterally into both tibialis anterior muscles with 10 µg DNA/20 µl PBS or 50 mg DNA/50 µl PBS. Intradermal injections with 10 µg DNA/20 µl PBS or sterile H<sub>2</sub>O were made on each flank, near the base of the tail, using a 29-gauge insulin-grade syringe (Micro-Fine U-100, BD Consumer Healthcare, Franklin Lakes, NJ, USA). Immediately after intra-dermal DNA administration, a needle array electrode was placed over the raised skin area of injection and pulses of different voltages were applied or not (nonelectroporated control). The needle array electrode consisted of two parallel rows of four 2-mm pins (1.5 x 4-mm gaps) (Cyto Pulse Sciences, Inc., Glen Burnie, MD, USA). Electroporation was performed using the PA-4000S—Advanced PulseAgile Rectangular Wave Electroporation System and software (Cyto Pulse Sciences, Inc.). Electroporation pulses were monitored and stored using a PCS64i digital oscilloscope (Velleman Components N.V., Belgium).

### *Luciferase Assay*

Balb/c mice were euthanized 24 h after DNA administration and skin biopsies removed. Skin biopsies were stored at -80°C until analysis. The skin was homogenized in 500 µl of lysis buffer (BD Biosciences, PharMingen, San Diego, CA, USA), vortexed at room temperature for 20 min, and centrifuged. Luciferase activity in cell lysates was measured using the Enhanced Luciferase Assay kit (BD Biosciences, PharMingen) on a Wallac Victor Multilabel Counter (Perkin-Elmer, Life Sciences, Upplands V7sby, Sweden). The bioluminescence of a 50-µl aliquot of each sample was counted for 10 s and recorded as counts per second (cps). Using the Enhanced Luciferase Assay kit, the specific activity of firefly luciferase protein (BD Biosciences, PharMingen) on this luminometer was 170,000 cps/ng luciferase protein. Background luminescence (skin injected intradermally with empty vector, pVax, and electroporated) was subtracted from all samples.

### *Lymphocyte Preparation*

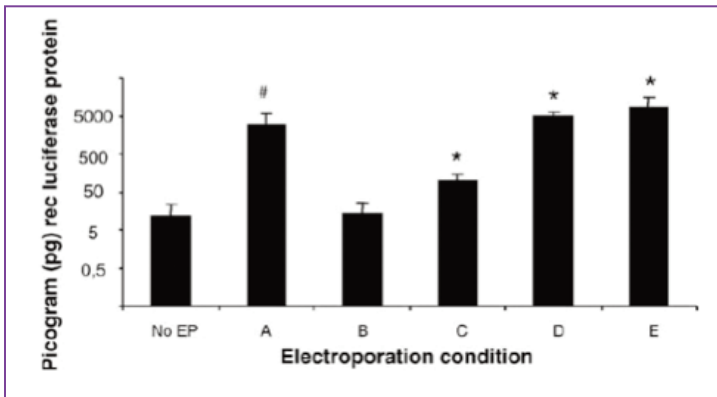
C57Bl/6 mice were bled at three different time points between day 11 and day 16 after a single immunization with the pVax-PSA plasmid. One hundred microliters of blood from the tail vein was mixed with 100 µl of CPD-A anticoagulant (Sigma, St. Louis, MO, USA). The erythrocytes were removed using the Ammonium Chloride Lysing Reagent (BD Biosciences, PharMingen) and after washes in handling medium (DMEM supplemented with 10 mM HEPES, 5 x 10<sup>-5</sup> M 2-mercaptoethanol, 25 µg/ml gentamicin, and 1% FCS) the cells were resuspended in complete medium (DMEM handling medium + 2 mM L-glutamine, 1% nonessential amino acids, and 5% FCS) and used for ex vivo intracellular staining. The peptide ppsa65-73 represents an immunodominant H-2Db-restricted CTL epitope of human PSA.

# Enhancement of Cellular Immune Response to a Prostate Cancer DNA Vaccine by Intradermal Electroporation (continued)

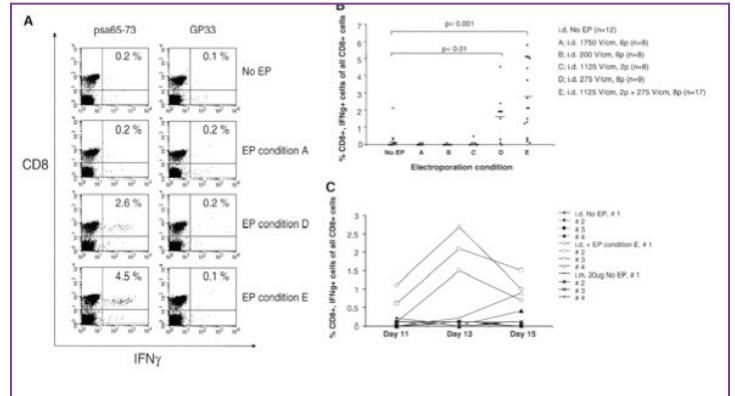
TABLE 1: Different conditions used for *in vivo* electroporation in mouse skin

Electroporation condition	Field strength (V/cm)	Group 1		Group 2		Schematic of pulsing condition <sup>a</sup>	
		Number of pulses	Pulse duration (ms)	Field strength (V/cm)	Number of pulses		Pulse duration (ms)
A	1750	6	0.1	—	—		
B	200	6	0.1	—	—		
C	1125	2	0.05	—	—		
D	275	8	10	—	—		
E	1125	2	0.05	275	8	10	

<sup>a</sup> The figures are only schematic and not proportional. The pulse interval for electroporation conditions A-B and C-E was 125 and 300 ms, respectively. The pulse interval between group 1 and 2 (condition E) was 500 ms.



**Fig. 2** Comparison of gene expression in mouse skin using different electroporation conditions. 10 µg of pVax-luc in 20 µl PBS was injected intradermally alone or in combination with one of electroporation conditions A–E (Table 1). Skin biopsies were removed after 24 h and analyzed for luciferase protein expression. Bars represent the means ± standard deviation (n = 6). \* and # indicate that the difference between the nonelectroporated group, No EP group, and other groups was statistically significant (\*P < 0.01, #P < 0.05).



**Fig. 3** Monitoring of PSA-specific CD8+ T cells in peripheral blood of mice immunized under different electroporation conditions. C57Bl/6 mice were immunized once with 10 µg pVax-PSA/20 ml PBS intradermally (i.d.) on each flank with or without electroporation (EP) or intramuscularly (i.m.) in each TA muscle. Blood was collected on days 11, 13, and 15 after immunization and the effector cells were stimulated for 4 h with 100 nM PSA-derived peptide psa65-73 or a control peptide GP33. The activated CD8+ T cells were quantified by intracellular cytokine staining for IFN<sub>γ</sub> and analyzed by flow cytometry. (A) Representative FACS plots showing the frequency of CD8+IFN<sub>γ</sub>+ T cells at day 13 after i.d. immunization. Percentages CD8+IFN<sub>γ</sub>+ T cells of all CD8+ T cells are shown in the top right corner of each dot plot. (B) Pooled results from three independent experiments are shown. Background response (0.1–0.3%) to GP33 was subtracted. The P value indicates that the difference between groups was statistically significant. (C) Kinetics of PSA-specific CD8+ T cells after DNA delivered i.m. or i.d. ± electroporation.

## REFERENCE

Roos A-K, Moreno, S., Leder, C., Pavlenko, M., King, A., and Pisa, P. (2006) Enhancement of Cellular Immune Response to a Prostate Cancer DNA Vaccine by Intradermal Electroporation. *Molecular Therapy* 13(2): 320-327. doi:10.1016/j.ymthe.2005.08.005