Skin Electroporation: Effects on Transgene Expression, DNA Persistence and Local Tissue Environment

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 studies the effect of intradermal electroporation on the kinetics of transgene expression and DNA persistence and reports on the response of the local tissue environment.

RESULTS

1. Intradermal electroporation speeds the onset of transgene expression to $<$ 1 hour compared to $>$ 24 hours for injection alone (Figure 1a). Closer examination shows especially fast expression for some of the injection sites, at less than 17 minutes (Figure 1b).
2. Electroporation-enhanced DNA delivery shows no change in plasmid persistence over several months (Figure 1a).
3. There are no visible differences in the skin that might suggest inflammation of the electrovaccinated mice.
4. Gene profiling of the treated vs. untreated skin shows an up-regulation of genes that are associated with an immunological response in the treated skin (Figure 5). Both DNA injection and electroporation alone showed an up-regulation of these pro-inflammatory genes but the combination of DNA injection and electroporation produced a 10-fold further up-regulation.

CONCLUSIONS

Intradermal electroporation enhances DNA vaccine delivery with faster expression and stronger immunological response. The persistence of DNA expression is not affected by electroporation.

METHODS

DNA injections and in vivo electroporation

Intradermal injections in mice (10–50 µg DNA/20 µl PBS) were made near the base of the tail using a 29 G insulin grade syringe (BD Consumer Healthcare, Franklin Lakes, NJ). Immediately after DNA administration, an electrode was placed over the injection site and voltage was applied (2 pulses, 1125 V/cm, 50 µsec+8 pulses, 275 V/cm, 10 msec). Electrodes had two parallel rows of four 2-mm pins (1.5 x 4 mm gaps) (Cyto Pulse Sciences, Inc., Glen Burnie, MD). Electroporation was performed using the PA-4000S-Advanced PulseAgile Rectangular Wave Electroporation System (Cyto Pulse Sciences, Inc.).

Kinetic Assay

The kinetics of transgene expression was assayed using in vivo luciferase bioluminescence imaging after intradermal luciferase plamid DNA injection of mouse skin, with or without electroporation.

Gene Profiling

Gene profiling of the local environment using a gene array analysis identified hundreds of genes affected by the treatment. A qPCR analysis of specific genes shown in Figure 5 was performed to quantify the regulation of immunological active genes.

Skin Electroporation: Effects on Transgene Expression, DNA Persistence and Local Tissue Environment (continued)

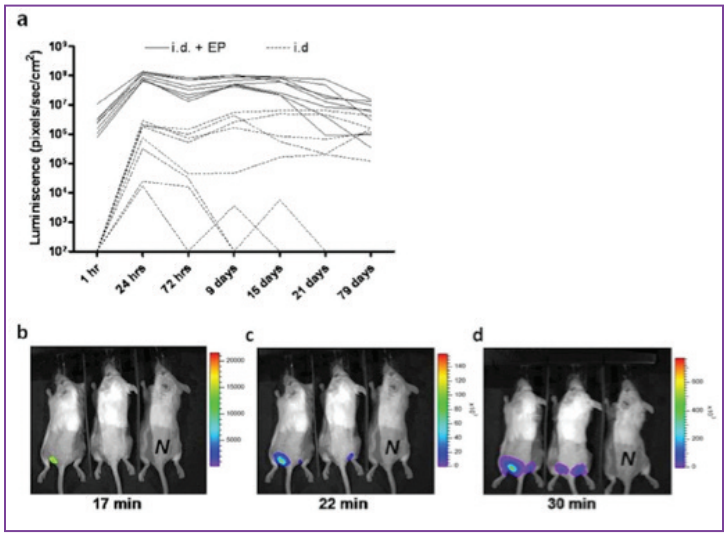

Fig. 1 *Time kinetics of transgene expression in skin after DNA electrovaccination. (a) Time course of in vivo luciferase expression after intradermal (i.d.) DNA delivery alone (dotted line) and after i.d. DNA delivery followed by electroporation (filled line). One representative experiment of two is shown (n = 8). (b–d) Immediate monitoring of gene expression after DNA electrovaccination. Representative bioluminescent images showing luciferase expression in skin at different time points after DNA electro-vaccination. N denotes the negative control (non-injected). The scale shows intensity of luminescence (photons/sec/cm2). The experiment was repeated three times.*

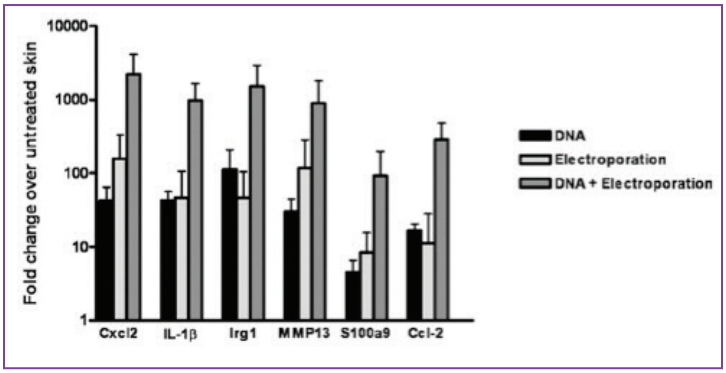

Fig. 5 *Genes up-regulated at the DNA electrovaccination site. Histogram showing fold increase in gene expression, compared to non-treated control skin, of the indicated genes after the specified treatments. Bars represent mean6SD. The number of independently analyzed samples varied from 4–6; and each sample was run in duplicates or triplicates for each QPCR. The QPCR analysis was run three times.*

REFERENCE

Roos A-K, Eriksson F, Timmons JA, Gerhardt J, Nyman U, et al. (2009) Skin Electroporation: Effects on Transgene Expression, DNA Persistence and Local Tissue Environment. PLoS ONE 4(9): e7226. doi:10.1371/journal.pone.0007226

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84 October Hill Road • Holliston MA, 01746
toll free 800.272.2775 • local 508.893.8999 • fax 508.429.5732
email techsupport.btx@harvardapparatus.com • web www.btxonline.com