

# The BTX Current

Vaccine Edition  
January 2012

## In vivo assessment of skin electroporation using square wave pulses

The application of short-duration high-voltage pulses to the skin has been shown to enhance transdermal drug delivery by several orders of magnitude and to transiently permeabilize cells in tissue. Both exponentially decaying (ED) pulses and square wave (SW) pulses have been applied. The latter have also been used for electrochemotherapy. To date, their effect on skin integrity has not been analyzed. The scope of this work was (i) to investigate the effect induced by SW pulses on the stratum corneum and the skin, (ii) to evaluate the safety issue associated with **electroporation**, (iii) to contribute to the understanding of drug transport. Biophysical techniques (transepidermal water loss, chromametry, impedance and laser Doppler velocimetry or imaging measurement) and histological methods were combined to provide a global picture of the effects. Ten SW pulses applied to the skin induced a mild impairment of the skin barrier function and a dramatic decrease in skin resistance. These changes were reversible. A transient decrease (5 min) in blood flow was observed. Neither inflammation, nor necroses were observed. These studies confirm the tolerance of the skin to square wave pulses *in vivo*.

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*Journal of Controlled Release* 79 (2002) 219–227



## Late administration of plasmid DNA by intradermal electroporation efficiently boosts DNA-primed T and B cell responses to carcinoembryonic antigen



Heterologous boost immunization is considered the most efficient way to enhance DNA-primed immune responses. We have previously shown that administration of recombinant carcinoembryonic antigen (CEA) efficiently boosts humoral responses in mice primed with CEA DNA. However, clinical grade recombinant proteins are far more intriguing to produce than plasmid DNA. Therefore, the possibility to use plasmid DNA for both priming and boosting would be beneficial. With the prospect of future use in a clinical trial, we investigated if **electroporation-mediated delivery of DNA** could be used to boost DNA-primed immune responses to CEA. The animals received either one injection of recombinant CEA or one intradermal injection of twt-CEA DNA, encoding the wild type CEA fused to a tetanus T helper epitope, in combination with **electroporation**. Boosting with rCEA protein did not enhance T cell responses to CEA but induced CEA-specific IgG in 4 of 8 mice. In contrast, intradermal delivery of twtCEA DNA by **electroporation led to a tenfold increase in IFN- $\gamma$  - producing CD8+ T cells**, compared to the levels obtained after the third priming immunization. The DNA boost also induced high CEA-specific IgG titers in all immunized animals (8/8). The data suggests that a late DNA boost, in combination with enhanced DNA delivery by **electroporation**, could be used to enhance the efficiency of DNA vaccination and substitute for a heterologous protein boost vaccination.

Andreas Bråve, David Hallengård, Lindvi Gudmundsdóttir, Richard Stout, Richard Walters, Britta Wahren, Kristian Hallermalm  
*Vaccine* 27 (2009) 3692–3696

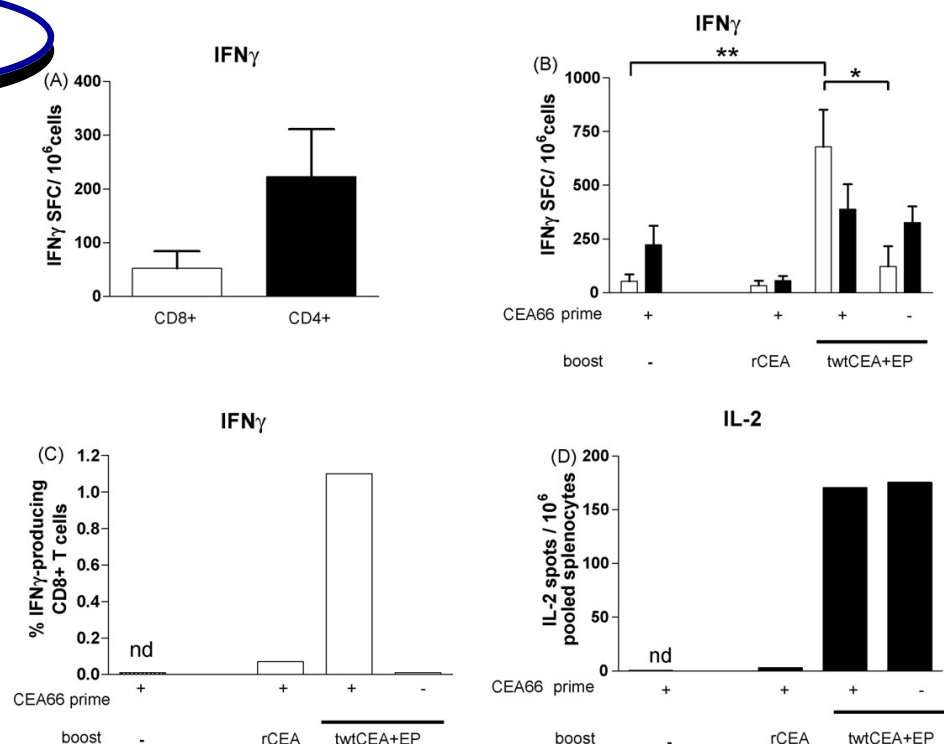


Fig. 1. CEA-specific T cell responses after immunization. (A) CD8+ and CD4+ T cell responses were assessed by IFN- $\gamma$  ELISpot on splenocytes isolated from individual mice 10 days after the third priming immunization and stimulation with MHC I or II restricted CEA peptides. Bars show the group mean of n = 8 individual mice. (B) CD8+ (white bars) and CD4+ (black bars) T cell responses assessed by IFN- $\gamma$  ELISpot on splenocytes from individual mice isolated 10 days after the last immunization of each group. Splenocytes were stimulated with MHC I or II restricted CEA peptides for CD8+ and CD4+ T cell responses, respectively. Note: The data from the CEA66 prime only group is the same as presented in (A). Bars show the group mean of (n = 8) individual mice, except in the twtCEA + EP boost only group (n = 6). (C) CD8+ T cell production of IFN- $\gamma$ , analyzed by intracellular cytokine staining of pooled splenocytes after stimulation with the MHC I restricted CEA-peptide YLGPDAPI. (D) CD4+ T cell production of IL-2 was assessed by ELISpot on pooled splenocytes taken 10 days after the boost immunization and stimulated with the MHC II restricted peptide PPSWRINGIPQQ. ND, not determined. Error bars show the standard error of the mean of data from n individual mice. \* or \*\* indicates statistical significance of p < 0.05 or p < 0.01.

FIG. 2

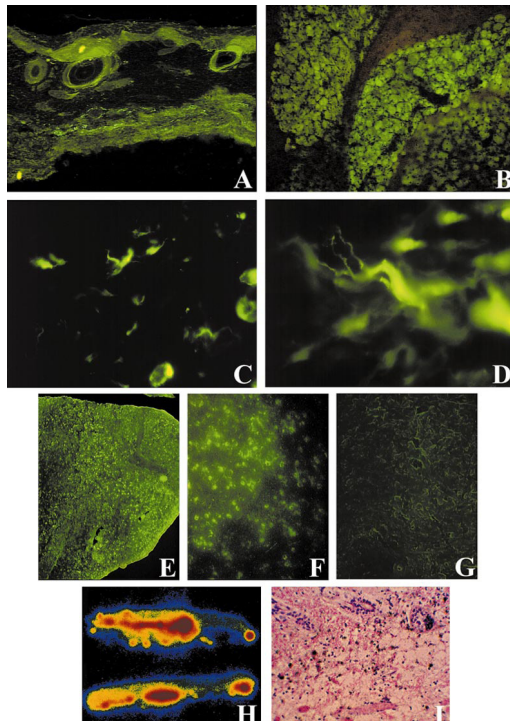


FIG. 2. Effect of EP on qualitative gene expression and distribution in skin. Histopathologic analysis with EGFP and LacZ expression demonstrating a variety of morphologic phenotypes of transfected cells in electroporated pig skin: (A) whole skin at 43, (B) EGFP transfected adipocytes at 103, (C) various EGFP-transfected dermal cells at 403, (D) magnified views of EGFP-transfected dermal cells with fine cytoplasmic processes (consistent with dendritic cells) at 1003. EGFP expression in unfixed draining lymph nodes of EGFP at 43 (E) and 403 (F) versus (G) HepB (control) transfected limbs at 403. In situ hybridization using labeled probe for hepatitis B mRNA in mouse skin transfected by electroporation: (H) phosphor image analysis demonstrating distribution of injected DNA and transgene expression in EP-treated tissues, (I) collection of photoemulsion parti-

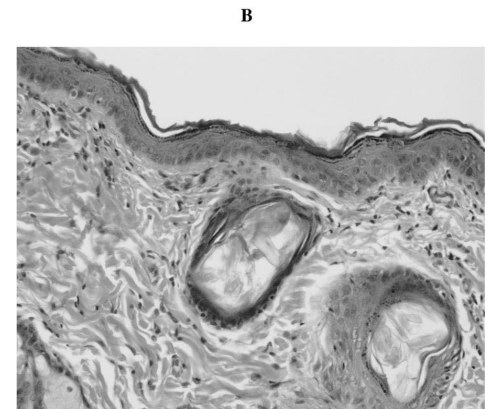
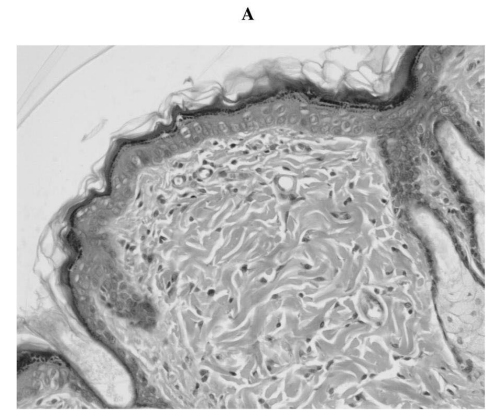


Fig. 5. Hematoxylin-eosin stained slides of control skin (A) and skin 4 days after electroporation 103(1000 V—100 ms) (B).

## Cutaneous Transfection and Immune Responses to Intradermal Nucleic Acid Vaccination Are Significantly Enhanced by *in Vivo* Electroporation



Naked DNA injection with **electroporation-mediated delivery (EP)** is a promising method for nucleic acid vaccination (NAV) and *in vivo* gene therapy. Skin is an ideal target for NAV due to ease of administration and the accessibility of large numbers of antigen-presenting cells within the tissue. This study demonstrates that ***in vivo* skin EP may be used to increase transgene expression up to an average of 83-fold relative to naked DNA injection** (50 mg DNA per dose, P < 0.005). Transfected cells were principally located in dermis and included adipocytes, fibroblasts, endothelial cells, and numerous mononuclear cells with dendritic processes in a porcine model. Transfected cells were also observed in lymph nodes draining electroporated sites. A HBV sAg-coding plasmid was used to test skin EP-mediated NAV in a murine model. Analysis of humoral immune responses including immunoglobulin subclass profiles revealed **strong enhancement of EP-mediated NAV relative to naked DNA injection**, with a Th1-dominant, mixed-response pattern compared to immunization with HBV sAg protein that was exclusively Th2 (P = 0.02). Applications for these findings include NAV-based modulation of immune responses to pathogens, allergens, and tumor associated antigens and the modification of tolerance.

Joseph J. Drabick, Jill Glasspool-Malone, Stella Somari, Alan King, and Robert W. Malone  
*Molecular Therapy* Vol. 3, No. 2, February 2001

FIG. 3. Antibody responses to hepatitis B surface antigen immune responses over time in mice transfected with/without EP and with/without boosting. Bars represent mean IgG titers for different groups. Serum for each animal was run separately (not pooled) and mean titers plus standard errors of the mean were calculated.

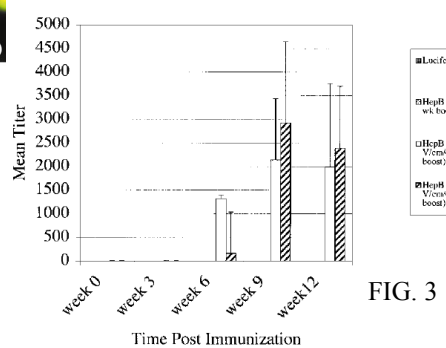


FIG. 3

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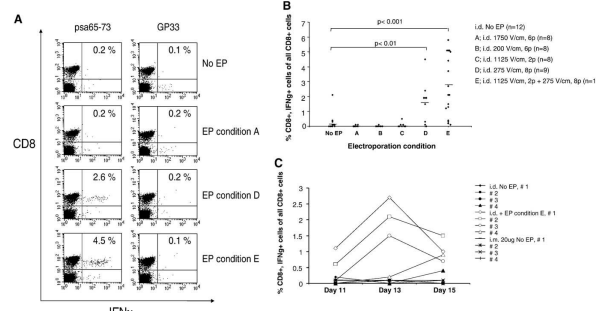
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Performed with the AgilePulse<sup>®</sup> In Vivo system

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

Recently it has become clear that more potent methods for DNA vaccine delivery need to be developed to enhance the efficacy of DNA vaccines. In vivo **electroporation** has emerged as a potent method for DNA vaccine delivery. In a mouse model, we evaluated the CD8+ T lymphocyte response to a prostate cancer DNA vaccine encoding prostate-specific antigen (PSA) after intradermal **electroporation**. A significantly increased gene expression (100- to 1000-fold) and higher levels of PSA-specific T cells, compared to DNA delivery without electroporation, was demonstrated. Interestingly, investigation of a panel of different **electroporation** conditions showed that only some conditions that induce high levels of gene expression additionally induced cellular immunity. This suggests that **electroporation** parameters should be carefully optimized, not only to enhance transfection efficiency, but also to enhance the immune response to the vaccine. This study demonstrates the applicability of intradermal **electroporation** as a delivery method for genetic cancer vaccines and other DNA vaccines relying on antigen-specific T cell induction.

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 Ag pVax-PSA/20 Al 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 $\gamma$  and analyzed by flow cytometry. (A) Representative FACS plots showing the frequency of CD8+IFN $\gamma$ + T cells at day 13 after i.d. immunization. Percentages CD8+IFN $\gamma$ + 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



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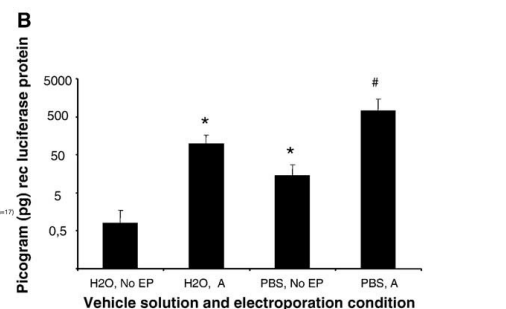
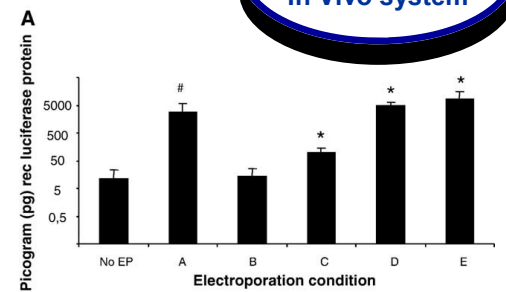
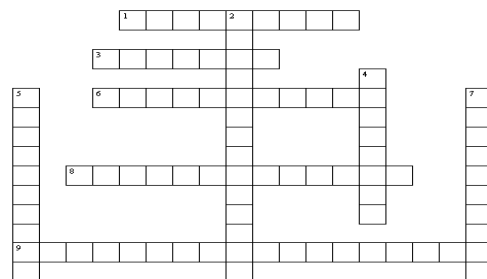


FIG. 2. Comparison of gene expression in mouse skin using different electroporation conditions and different vehicle solutions at time of DNA administration. (A) 10 Ag of pVax-luc in 20 Al PBS was injected intradermally alone or in combination with one of electroporation conditions A–E (Table 1). (B) 10 Ag of pVax-luc in 20 Al PBS or 20 Al sterile water was injected intradermally and electroporation (electroporation condition A, 1750 V/cm, 6 pulses, 100 As) was applied. Skin biopsies were removed after 24 h and analyzed for luciferase protein expression. Bars represent the means  $\pm$  standard deviation (n = 6). \* and # indicate that the difference between (A) the nonelectroporated group or (B) the H<sub>2</sub>O, No EP group and other groups was statistically significant (\*P < 0.01, #P < 0.05).

### BTX Crossword



#### DOWN

- A general term for all antibody molecules. Each unit is made up of two heavy chains and two light chains and has two antigen-binding sites.
- Serum protein formed in response to immunization and are generally defined in terms of their specific binding to the immunizing antigen.
- Literally, coming from a single clone. A clone is the progeny of a single cell. In immunology, monoclonal generally describes a preparation of antibody that is monogenous, or cells of a single specificity.
- Small cell with virtually no cytoplasm, found in blood, in all tissue, and in lymphoid organs, such as lymph nodes, spleen, and Peyer's patches, and bears antigen-specific receptors.

#### ACROSS

- A hybrid cell that results from the fusion of an antibody-secreting cell with a malignant cell, the progeny secrete antibody without stimulation and proliferate continuously both in vivo and in vitro.
- A tumour of plasma cells, generally secreting a single species of immunoglobulin.
- Any immunization against a pathogen.
- Using electrical pulses to destabilize cell membranes to create pores and fuse cell membranes together to create a hybrid cell.
- An oscillating dielectrophoretic current in which an electrical current rises to a maximum point in one direction and falls to zero and then rises in the opposite direction and then repeats.

### BTX News

#### NEW BTX Website

BTX, the Electroporation Experts, are proud to announce the launch of their new and improved website. [www.BTXonline.com](http://www.BTXonline.com).

The new website is completely revamped and now includes many new features and tools designed to keep users abreast of all the latest in today's research climate. New Features Include:

- Streamlined Navigation
- Product Search by Application
- New Training Videos & Resources
- Improved & Updated Protocols Database

To learn more about BTX products, visit the NEW [www.BTXonline.com](http://www.BTXonline.com) today, and search for products by application; download new protocols or application notes, view new training videos and tutorials and much more!

#### NEW Training Videos

Training videos are now available for the set-up and use of the Agile Pulse<sup>®</sup> In vivo system, Agile MAX<sup>®</sup> System and the Hybrimmune<sup>®</sup> Fusion System. Videos include tutorials on cell preparation pre and post fusion; in vivo electroporation and tissue targeting, all in an easy-to-learn format. Visit [www.btxonline.com](http://www.btxonline.com) to view these new additions to the BTX Resource Library.

#### NEW Products

The New AgilePulse<sup>®</sup> line of electroporation generators possess **unique variable Pulse amplitude technology** which promotes greater cell membrane poration resulting in maximum transfection efficiency with minimal damage to the cells or tissues.

AgilePulse<sup>®</sup> technology is ideal for applications in research including:

- In vivo Vaccine Delivery
- Gene Therapy Research
- Large Volume Cell Transfection

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- Hybridoma Production
- Cell Fusion
- Nuclear Transfer



47-0200 Agile Pulse<sup>®</sup> MAX Sys-



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