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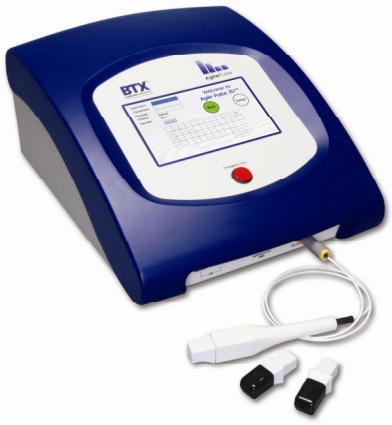
Tech-Trends Application Note Volume 4, Series 11

Intradermal Electroporation of Naked Replicon RNA Elicits Strong Immune Responses

Abstract
RNA-based vaccines represent an interesting immunization modality, but suffer from poor stability and a lack of efficient and clinically feasible delivery technologies. This study evaluates the immunogenic potential of naked *in vitro* transcribed Semliki Forest virus replicon RNA (RREP) delivered intradermally in combination with electroporation.

Methods
C57BL/6 and 129sv/ew mice were bred and used at the age of 6–9 weeks. Prior to immunization, mice were shaved on their lower back and anesthetized with 4% isoflurane. 20 µl of RNA or DNA diluted in PBS were injected intradermally (i.d.) to each flank in the lower part of the back followed by immediate electroporation (E.P.) with the Agile Pulse *In Vivo* System formerly known as Derma Vax™ (BTX, Holliston, MA) at the injection sites. Electroporation consisted of 2 pulses of 1.125 V/cm for 50 µs, and 8 pulses of 275 V/cm for 10 ms. The needle-array electrodes (NE-4-4) with two parallel rows of four 2-mm pins (1.564 mm gaps) were used for electroporation.

Results
Replicon-immunized mice showed a strong cellular and humoral response, contrary to mice immunized with regular mRNA. RREP-elicited induction of interferon-c secreting CD8+ T cells and antibody responses were significantly increased by electroporation. CD8+ T cell responses remained substantial five weeks post vaccination, and antigen-specific CD8+ T cells with phenotypic characteristics of both effector and central memory cells were identified. The immune response during the contraction phase was further increased by a booster immunization, and the proportion of effector memory cells increased significantly. These results demonstrate that naked RREP delivered via intradermal electroporation constitute an immunogenic, safe and attractive alternative immunization strategy to DNA-based vaccines.



Agile Pulse In Vivo System
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Figure 2

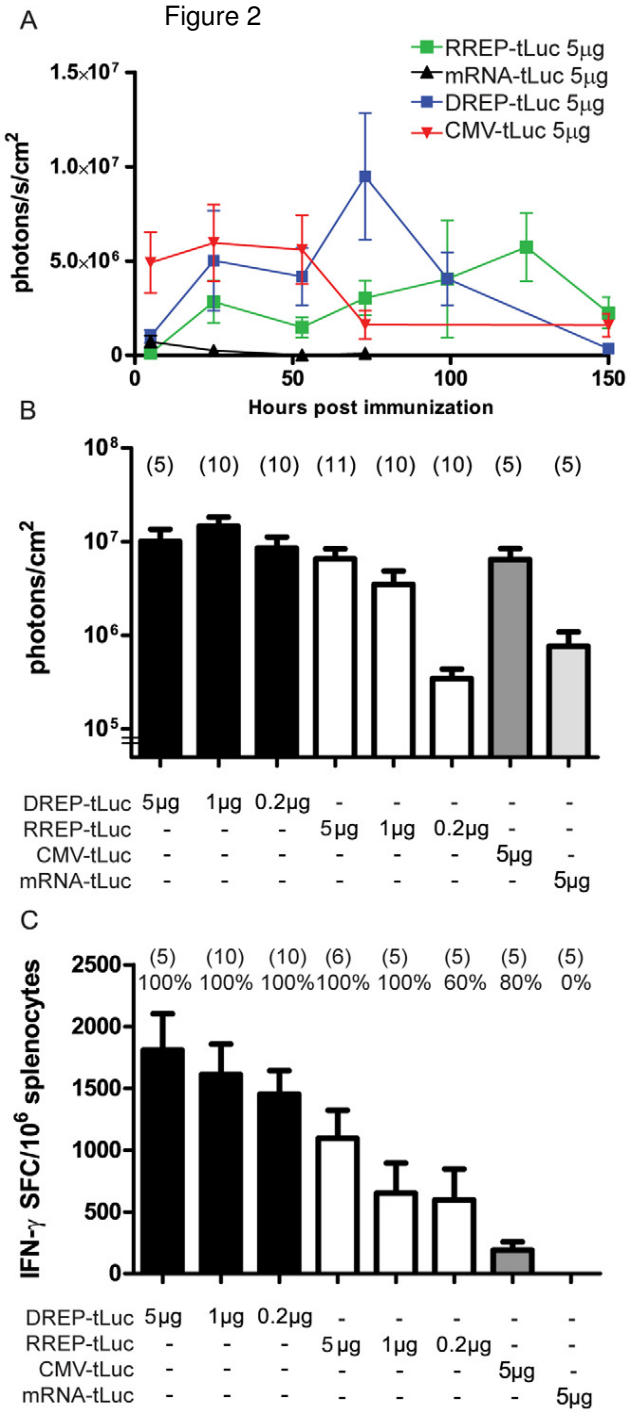

Figure 2. *In vivo* antigen expression and the immune response after electroporation. (a) Luciferase activity at different time points measured in photons/s/cm2. Data shows average activity for each group and error bars indicate SEM (number of mice per group indicated in Figure 2b). (b) Cumulative luciferase activity measured in photons/cm2. Data shows average accumulated luciferase activity up to 150 hours post immunization for each group (number of mice per group indicated in parentheses above each bar) with error bars showing standard error of the mean. (c) Antigen-specific IFN-c positive CD8+ T cells per million splenocytes 10 days post-immunization. Data shows average number of positive cells with error bars showing standard error of the mean. The total number of mice analyzed is indicated in parenthesis and the percentage of responding mice is indicated above each group.

Figure 3

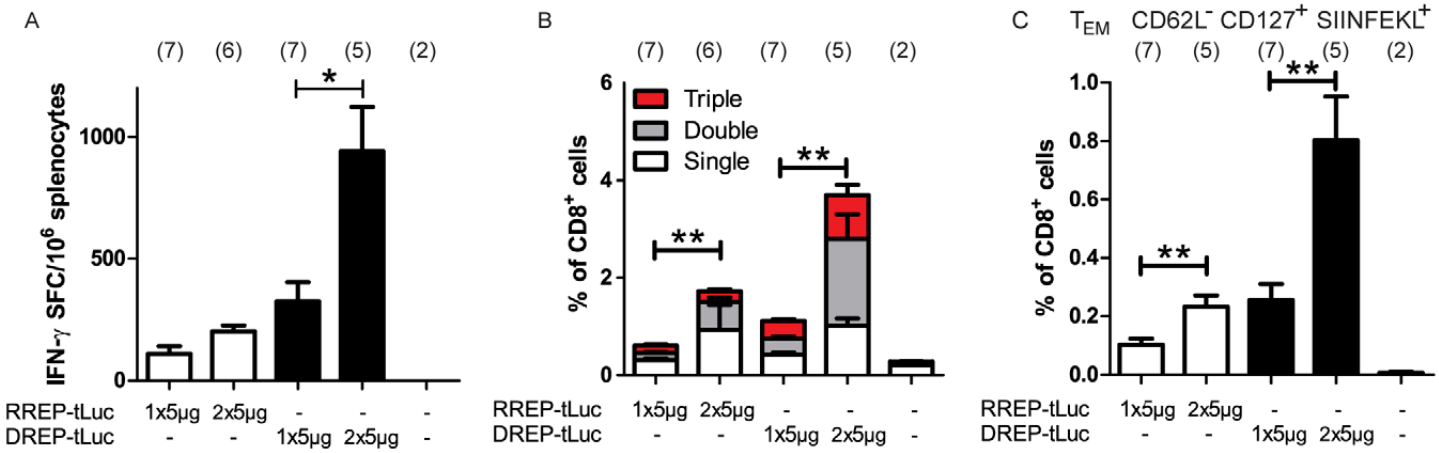

Figure 3. Cellular immune response 5 weeks post immunization. (a) Antigen-specific IFN-c positive CD8+ T cells per million splenocytes, (b) Proportion responding CD8+ T cells as determined by intracellular staining of IFN-c, IL-2 and TNF after SIINFEKL-peptide stimulation, or (c) proportion effector memory CD8+ T cells (pentamer H-2Kb/SIINFEKL positive CD8+CD62L2CD127+ cells) 5 weeks after the last intradermal immunization in combination with electroporation. Mice were either given one immunization (165 µg) or two immunizations 5 weeks apart (265 µg). Data shows average number of positive cells with error bars showing standard error of the mean. A booster immunization significantly increased the cellular memory response for both RREP-tLuc and DREP-tLuc (p,0.01). The total number of mice per group is indicated in parenthesis above each bar.

Reference: Johansson DX, Ljungberg K, Kakoulidou M, Liljestro¨m P (2012) Intradermal Electroporation of Naked Replicon RNA Elicits Strong Immune Responses. PLoS ONE 7(1): e29732. doi:10.1371/journal.pone.0029732