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Comparison of plasmid vaccine immunization schedules using intradermal *in vivo* Electroporation

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ABSTRACT

In vivo electroporation (EP) has proven to significantly increase plasmid transfection efficiency and augment immune responses after immunization with plasmids. This study attempted to establish an immunization protocol using intradermal (ID) EP. BALB/c mice were immunized with a plasmid encoding HIV-1 p37gag, either ID with the Derma Vax™ EP device (now sold as the Agile Pulse™ *In Vivo* System), intramuscularly (IM) without EP, or with combinations of both. A novel FluoroSpot assay was used to evaluate the vaccine-specific cellular immune responses. The study showed that ID EP immunizations induced stronger immune responses than IM immunizations using a higher amount of DNA and that repeated ID EP immunizations induced stronger immune responses than IM priming followed by ID EP boost. Two and three ID EP immunizations induced immune responses of similar magnitude and a short interval between immunizations was superior to a longer interval in terms of magnitude of cellular immune responses. The data obtained in this study can aid in the design of vaccine protocols using ID EP.

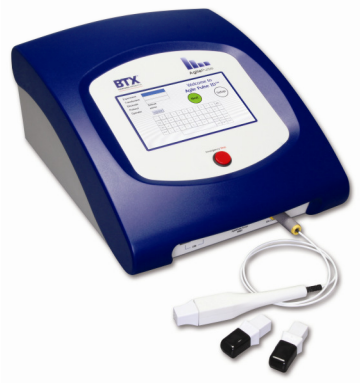
METHODS

Groups of 8-10 female BALB/c mice, 5-9 weeks old, were immunized at week 0, 4 and 8 with 15 µg of a plasmid encoding HIV-1 p37gagB intra-dermally with the Agile Pulse™ *In Vivo* System (formerly known as Derma Vax™) (BTX Holliston, MA) (on one flank of the mice as described in(24)) or with 50 µg of the same plasmid IM without EP. Ten days post the last immunization, all mice were sacrificed and spleens and sera collected. The experiment was repeated for mice receiving one, two or three ID EP immunizations with four weeks immunization intervals. These mice were bled regularly from the tail vein to obtain sera and peripheral blood mononuclear cells (PBMCs).

Table 1. Immunization protocols

Group	w0	w4	w8
1xEP	-	-	EP
2xEP (4w)	-	EP	EP
3xEP	EP	EP	EP
2xEP (8w)	EP	-	EP
2xIM+1xEP	IM	IM	EP
1xIM+1xEP	IM	-	EP
3xIM	IM	IM	IM
1xIM	-	-	IM
naïve	-	-	-

EP, id EP immunization (15µg DNA)
IM, im immunization without EP (50µg DNA)



Agile Pulse In Vivo System Cat. #47-0400N

84 October Hill Rd. Holliston, MA 01746 Toll Free Ph: 800-272-2775 or 508-893-8999
Email: techsupport.btx@harvardapparatus.com Web: www.BTXonline.com

Fig. 2. A, B, C

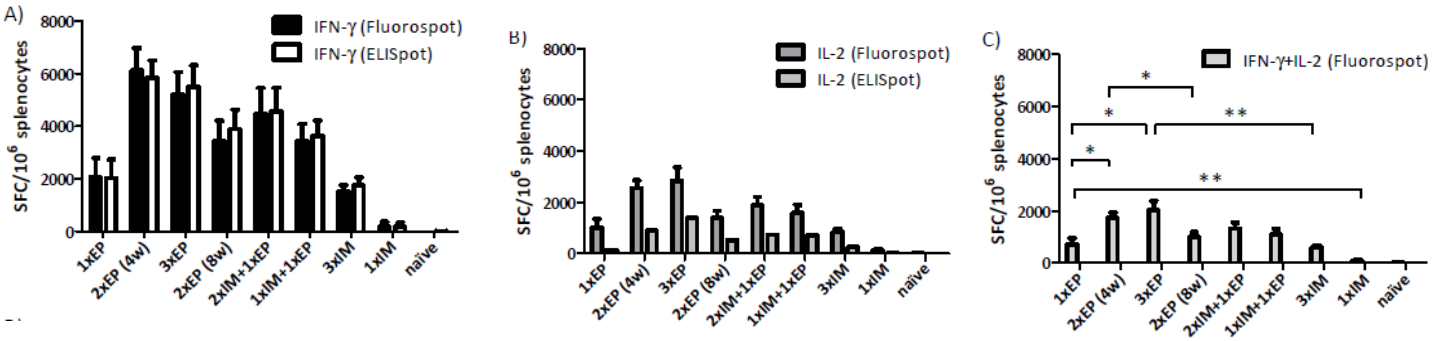


FIG. 2. Cellular responses measured by FIG. 2. IFN-g/IL-2 FluoroSpot and IFN-g and IL-2 ELISpot. Individual (FluoroSpot and IFN-g ELISpot) and pooled (IL-2 ELISpot) splenocytes were stimulated with a p24gagB peptide pool ten days post the last immunization. Important significant differences in IFN-g/IL-2 FluoroSpot (C) are marked *(p<0,05) and **(p<0,01), but applies also for IFN-g (A) and IL-2 (B) FluoroSpot. Bars and error bars represents mean and standard error of mean, respectively (n=8).

RESULTS

This study reported that repeated ID EP immunizations can induce strong immune responses when used as a single vaccine modality. Using this approach instead of heterologus prime-boost immunizations with microbial vectors or recombinant proteins for boosting immunizations would be beneficial due to less regulatory and safety concerns, as well as ease of development and manufacturing. It was previously reported, in mice, that boosting ID DNA priming immunizations with EP-augmented DNA immunization generate superior immune responses to carcinoembryonic antigen (CEA) when compared to boosting with recombinant CEA (6). Plasmid based vaccines delivered by EP have in pre-clinical experiments also proved to be superior to viral vectors in some settings. This was demonstrated in a study where Rhesus Macaques were immunized with either DNA or adenovirus serotype 5 (Ad5) encoding similar SIV antigens. The authors observed that the DNA approach induced higher magnitudes, and more polyfunctional profiles, of cellular immune responses than the Ad5 approach (14).

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CONCLUSION

This study showed that a straight forward protocol using repeated ID EP immunizations with a rather short immunization interval induced strong and long-lived immune responses, and the authors believe that this data can facilitate the design of immunization protocols using EP-augmented ID DNA immunization.

Fig. 4. A, B, C

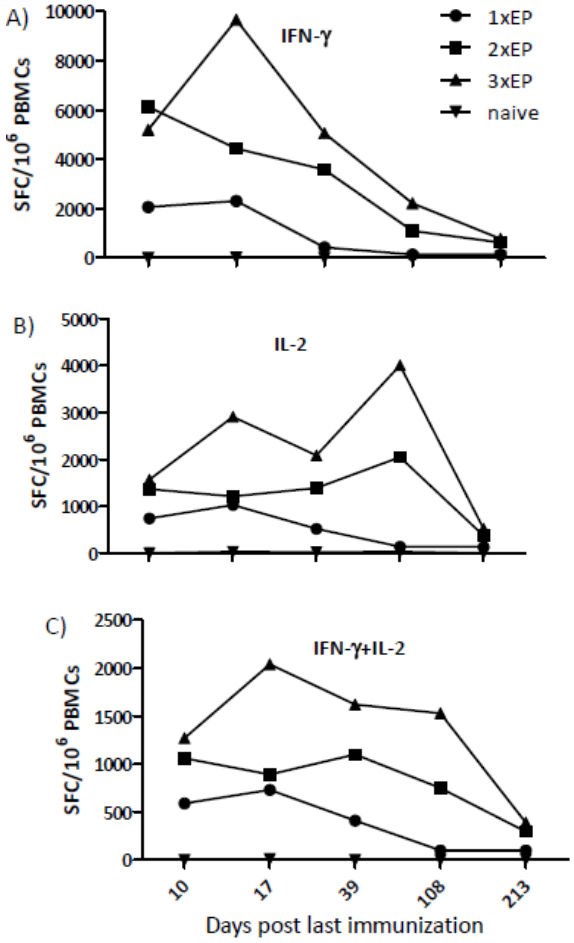
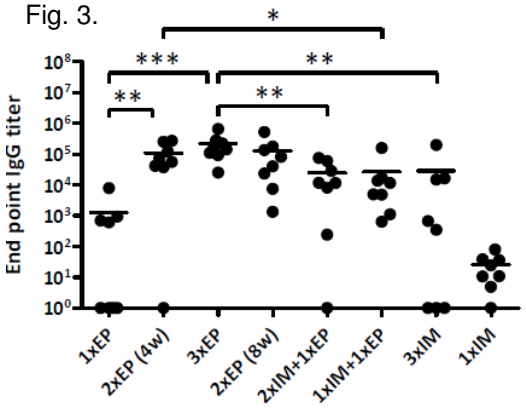


FIG. 4. Kinetics of the IFN-g (A), IL-2 (B) and IFN-g+IL-2 (C) responses as measured by IFNg/ IL-2 FluoroSpot on pooled PBMCs (n=10) at different time points after the last immunization. Cells were stimulated with a p24gagB peptide pool.

FIG. 3. Antibody responses measured by p24gagB ELISA on sera from individual mice (n=8) collected ten days post the last immunization. Important significant differences are marked *(p<0,05), **(p<0,01) and ***(p<0,001).

Fig. 3.



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