Results summary:

1. GITRL and TWEAK expressing constructs have conferred increased immune responses when co-vaccinated at sub optimal levels.
2. The humoral response produced by 10µg GITRL co-immunized group was remarkably and significantly ($p=0.02$, 95% confidence level) higher than that produced by HIV-1 parent vaccine.
3. A 2 and 2.5 fold increased cellular response was produced by 7.5µg T and 10µg G co-vaccinated groups respectively, when compared to the response produced by DNA alone administered group.

Conclusive remarks:

With all these findings, this study gains greater importance with the current focus in the field of vaccines being the urge to develop a higher immune response to the corresponding disease causing antigen, due to the challenges posed by the newly emerging strains of pathogens as a result of several mutations.
Taking this study to the next level, it will be interesting to determine the immuno modulatory effects produced, in a small group of macaque monkeys.

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I would like to extend my sincere thanks to my supervisor, my lab technician Colleen Lucke and all Weiner lab members for supporting me throughout the duration of my thesis.

Enhanced immunogenicity conferred by TNFR superfamily members ligation *in vivo*

a thesis by

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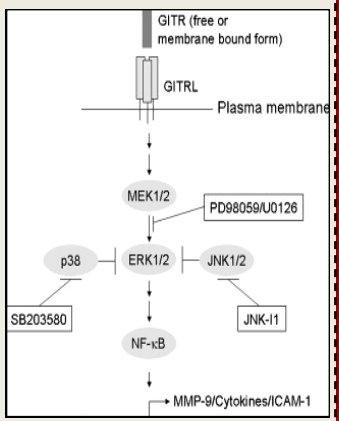
Background:

One of the strategies that have been used to enhance the immunogenicity of DNA vaccines was co-administration of co-stimulatory molecules such as potential molecular adjuvants. Unlike conventional adjuvants, molecular adjuvants are delivered as plasmid vector along with the vector encoding the antigen of interest.

Tumor Necrosis Factor superfamily ligands (TNFSFL) are co stimulatory molecules that have been reported to be involved in T cell activation and have also been used as adjuvants in several vaccination studies.

GITRL is a member of TNFR superfamily that modulates natural and acquired immune response.
It was concluded from a study that GITRL induced signaling is mediated by ERK1/2, which then triggers the activation of the transcription factor NF-κB. NF-κB controls the expression of several pro-inflammatory mediators such as chemokines and cytokines.



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TWEAK, a novel member of TNFSF has been reported to activate a large number of cellular functions including migration and proliferation TWEAK shares a common feature with GITRL that it plays a vital role in the signaling pathways that involves an activation of the transcription factor NF-κB.

Aim of the study:

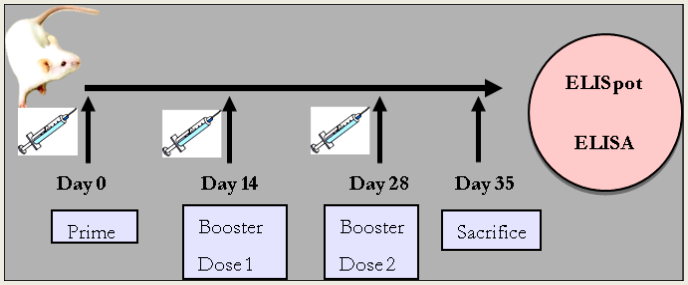
To evaluate the immune modulatory effects produced when the DNA plasmids encoding full length human GITRL and TWEAK were used as molecular adjuvants, in combination with HIV-1 Tier 2 (ZM53M.PB12) DNA vaccine.

Methods:

Construction of GITRL and TWEAK expression vectors:
Human full length GITRL and TWEAK DNA sequences were cloned into two pVax expression vectors at BamH1-EcoRI restriction site and (**G**: GITRL expressing plasmid and **T**: TWEAK expressing plasmid) were amplified to a concentration of 4mg/mL (GenScript Inc., USA).

Transient transfection and Western Blotting of protein constructs:
The DNA constructs were transfected in adherent vero cells (ATCC CRL-1587) and 48 hours following the transfection, the cells were lysed and western blotting was carried out using GITRL and TWEAK antibodies. The membranes were developed using autoradiography technique.

Mice and vaccinations:



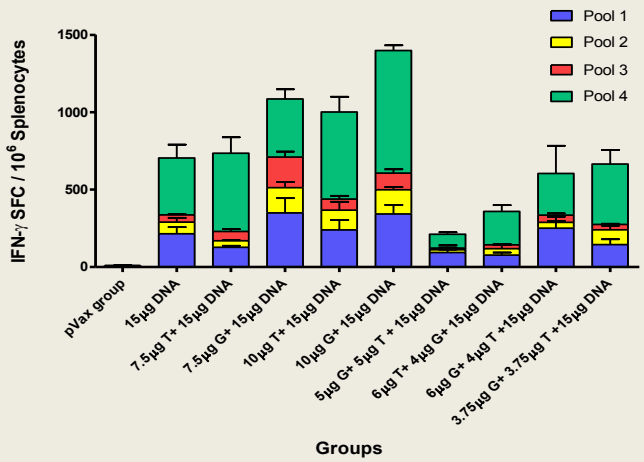
6-8 weeks old female BALB/c mice (10 groups) were immunized with various combinations of adjuvant and HIV-1 DNA in the quadriceps muscles of hind limb using Minimally Invasive Electroporation Delivery (MID-EP) system.

Humoral response, ELISA:
Recombinant HIV-1 Clade C protein (Immune Technology, IT-001-CONCp) was used to coat the 96-well Nunc-Immuno MaxiSorp plates. The plates were read at 450 nm using Biotek EL312e Bio-Kinetics reader. All the serum samples were tested in triplicates.

Cellular response, ELI Spot:
The splenocytes were stimulated overnight with specific antigenic peptides. The spots were counted using an automated reader (ImmunoSpot II; Cellular Technologies Inc., Cleveland, OH).

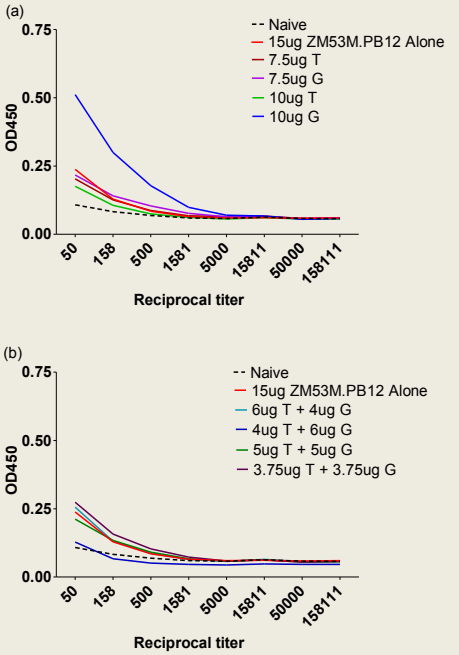
Results:

Cellular response, ELI Spot data:



Pool 1, Pool 2, Pool 3 and Pool 4 refer to the peptide pools (that were used to stimulate the splenocytes) of 15mer peptides that were specific against the HIV-1 antigen used in the study. A 2 and 2.5 fold increase in the level of cellular response was conferred by the 7.5µg T & 10µg G co-immunized group of animals .

Humoral response, ELISA data:



This graph shows the humoral responses obtained when 'G' and 'T' were used at various doses (a) separately; (b) in combination along with HIV-1 DNA vaccine. It can be noted from (a) that a higher level of response is obtained from 10µg G co-vaccinated group.