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Enhancement of immune response by Priming with DNA Vaccine

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Introduction: Immunization against human chorionic gonadotropin (hCG) prevents pregnancy in sexually active women of proven fertility, as shown by previous Phase II efficacy trials. In order to make the vaccine consistent in its linkage to the carrier, we developed a recombinant vaccine linking hCGβ to LTB, B subunit of heat labile enterotoxin of *E. coli* which is a potent mucosal adjuvant. The hCGβ-LTB vaccine was fairly immunogenic in mice of different genetic strains. Since a vaccine for control of fertility should ideally be effective in every recipient and be potent enough to generate above protective threshold antibody titres to prevent pregnancy, it was decided to investigate if prime-boost approach employing a combination of anti-hCG DNA and protein vaccines, can enhance the immune response.

Methodology: hCGβ-LTB protein vaccine was made and purified using yeast *Pichia pastoris* pPIC9k/GS115 host-vector system. DNA version of the vaccine was prepared by incorporating the gene encoding hCGβ-LTB in eukaryotic plasmid VR1020 (DJ). *Mycobacterium indicus pranii* (MIP) was used as an immuno-modulator. Female inbred Balb C mice received 100 µg of DNA vaccine in saline along with 5x106 cells of MIP/animal/dose route twice fortnightly followed by 2 µg of alum adsorbed hCGβ-LTB along with 5x106 cells of MIP by intramuscular route. Second group of mice was immunized by only protein version of the vaccine along with MIP.

Results and Conclusions: Immunization with the DNA form of the recombinant hCGβ-LTB vaccine twice at fortnightly interval followed by the proteinic form of the vaccine induced distinctly higher antibody response than the proteinic vaccine alone. DNA is not only cheaper to make, it is thermostable and does not require cold chain. Hence the employment of DNA for primary immunization is expected to reduce the cost besides the benefit of enhancing antibody response.

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In *Silico* prediction of immunogenic T cell epitopes of L*eishmania* donovani gp63 protein: an alternative approach for anti-parasite vaccine development

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Visceral leishmaniasis (VL) is a major parasitic childhood disease in sub-Saharan Africa. Expensive and toxic anti-leishmanial drugs are current control methods. Safe, effective and cheap vaccines are potentially powerful strategies to control VL. Traditional vaccine development techniques have failed to deliver an effective vaccine. Leishmania vaccine development may benefit from immunoinformatics tools. This paper describes an improved in silico prediction method for immunogenic *Leishmania donovani*-GP63 T cell epitopes as VL candidate vaccines. Using the EpiMatrix algorithm, the amino acid sequence of *L. Donovani donovani* GP63 protein (GenBank accession: ACT31401) was screened for putative T cell cluster epitopes that would bind to the most common HLA class II alleles among at-risk populations. Nine epitopes were initially identified using EpiMatrix. Based on cluster score, number of EpiMatrix hits, hydrophobicity, and number of EpiBars (an EpiBar is a 9 amino acid frame predicted to bind at least 4 different HLA molecules), four peptides (P1-P4) were selected for synthesis. In a proof of concept study, blood samples from consenting healthy, leishmanin skin test (LST) reactive and non-reactive volunteers were stimulated and IFN-γ, IL-4, and IL-10 were measured. IFN-γ and IL-4 levels were similar in both groups. However, mean IL-10 levels were significantly reduced in LST reactive individuals. To evaluate whether cross-reactivity with the human genome (HG), the human gut microbiome (HM) and common human pathogens (HP) was responsible for these differences, the sequences of the evaluated peptides were screened using JanusMatrix. One of the peptides (P1), which increased IL-10 in the LST reactive volunteers, showed high cross-reactivity with HG, suggesting that P1 might induce a regulatory immune response in humans. In conclusion, immunoinformatics tools provide a promising alternative approach for anti-parasite vaccine development. Data obtained can be used in the development of epitope-based *Leishmania* vaccine.

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