ABSTRACT

Lymphatic filariasis is a cause of widespread chronic disease associated with severe immunopathology. Filarial worms of the genus Brugia and Wuchereria are the causative agents of lymphatic filariasis (LF), a debilitating mosquito-borne infection of the tropics, which contributes significantly to the socio-economic burden faced by endemic countries. Though antifilarial drugs are available several rounds of mass treatment are necessary to reduce the levels of infection below those necessary to sustain transmission. The outstanding successes achieved in the control of bacterial and viral infections through vaccination have so far not been transferred to any human parasitic infections including nematode infections mainly due to the complicated life cycle of the parasites.

Parasites often elicit inappropriate and ineffective immune responses in the host, or dampen the host immune system, thereby preventing a robust and effective immune response by the host. Additional preventive measures such as vector control and vaccine development are crucial to control the infection in endemic regions. Antigens of the infective third stage larvae of filarial parasites are of special interest since they represent the first larval stage that enters into the human host which can be ideal targets as vaccine candidate. The Abundant Larval Transcript (ALT) protein vaccine conferred ~64-75% protection consistently in mice as well as in the permissive animal model, gerbils. The bimodal vaccination using the prime boost strategy where DNA prime and protein boost is administered was less effective with 64% protection compared to protein alone that showed 75%.

In this study Wb20/22 homologue of ALT which showed 86% similarity with ALT was cloned form L3 cDNA library of W.bancrofti. Mutants of Wb20/22, lacking signal sequence (WOSS) and acidic domain
(WOAD) were cloned to analyze the role of these regions in the immune response and immunoprophylactic efficacy. The high antibody titer, isotype profile indicating elevated levels of IgM, IgG1 and IgG2b along with cytokines level and the immunoprophylactic efficacy of WOAD which was 62.26% significantly high compared to Wb20/22 (49.82%) revealing the immunomodulatory role of the glutamic acid rich region of Wb20/22.

Multiple antigenic peptide (MAP) for many parasitic disease like schistosomiasis, malaria and other disease have made an impact in the vaccination. MAPS containing multiple of the immunodominant B cell epitope plus a T-helper (Th) cell epitope or Th epitope alone, all from the *P.berghei* circumsporozoite (CS) protein have shown the capacity to significantly protect immunized mice. The immunodominant epitopes of ALT were previously mapped and studied in mice models. The epitope mapping studies carried out in ALT was instrumental in designing the ALT MAP construction, first of its kind for filariasis. The promising results in the preliminary studies in experimental filariasis and the tremendous success of peptide vaccines in other diseases along with the need for prevention of this disease was the rationale behind this construction and evaluation of ALT MAP vaccines for lymphatic filariasis.

The ALT MAP was constructed by solid phase peptide synthesis using Fmoc chemistry. ALT MAP was encapsulated in PLGA microparticles since optimal immune responses require their efficient delivery and presentation to the immune system which is achieved by the encapsulation of antigens into polymeric microspheres. The clinical sera reactivity showed significantly (P<0.0001) higher reactivity with endemic normal individuals in both ALT and ALT MAP groups compared to MF, CP, and NEN indicating that the epitope portion of ALT used in ALT MAP construction was immunodominant.
The PBMCs showed significantly high proliferation (P<0.007) when stimulated with ALT MAP compared to ALT. The immunomodulatory regions in ALT play a crucial role in the diminishing of the ALT specific proliferation. For a prophylactic vaccine to be effective a balanced Th1/Th2 response is required. Multiple epitopes covering regions of immunological hot spots when delivered simultaneously as a single chimeric construct are known for induction of a broad spectrum of immune responses. Immunoprophylactic efficacy of the ALT, ALT MAP was tested by protection studies using micropore chamber method with L3 larvae in jirds which revealed 78.18% for ALT MAP and 74.17% ALT. ALT MAP yielded comparable and higher protection percentage than ALT, which can be used as multi antigenic peptide vaccine which is first of its kind for filariasis.

Adjuvants are required to assist new vaccines to induce potent and persistent immune responses, with the additional benefits that less antigen and fewer injections are needed. Since ALT has already been established as a putative vaccine candidate that has given the highest protection of 74% as a single antigen we decided to enhance and study the immune response by administering them with different adjuvant formulations.

Lymphatic filariasis is characterized by hyporesponsiveness, which indicates the need for strong cellular response along with humoral response for a successful adjuvant formulation. The adjuvants included in this study were alum, QS21, MPLA, MDP, Inulin and Chitosan. In this study the pattern of immune response elicited by different adjuvant formulations was analysed as a stepping stone towards the development of a prophylactic vaccine formulation for lymphatic filariasis at a preliminary level. Though MPLA and MDP are good immunopotentiators, adjuvant formulation failed to induce proliferation and recall-response to the ALT antigen. This could be due to (1) low expression of co stimulatory signals on APC (2) low production of pro-
inflammatory cytokines (3) in sufficient production of memory cells, which result in impaired T cell proliferation, rendering T cells tolerant.

Both chitosan and inulin formulations with ALT elicited a high peak titer and lymphoproliferative response thereby striking a Th1/Th2 balance. While the QS21 formulation elicited high IgG2b, IgG2a followed by IgG1 which reflected its ability to induce Th1 response. Carbohydrates and saponin based adjuvant formulation with ALT proved to be successful in eliciting both humoral and cellular response in mice model. This can be further studied for its immunoprophylactic efficacy in clearing the parasites.