ABSTRACT

Human lymphatic filariasis, a debilitating disease, caused mainly by the nematode parasites Wuchereria bancrofti, Brugia malayi and Brugia timori, afflicts 119 million people living in 73 endemic countries. The socio-economic burden of lymphatic filariasis based on labour-time loss and treatment cost is estimated to be around Rs. 3800 crores (US$842 million) every year in India. Every dollar invested in filariasis control has been estimated to produce more than $15 in benefits. The limitations of DEC chemotherapy have prompted the search for new drugs and strategies for the control and elimination of lymphatic filariasis, including immunological intervention. Analysis of the immune responses in individuals living in an endemic area indicates the existence of stage-specific immunity. Genes newly expressed or up-regulated in the infective L3 stage of the parasite are considered to be potential targets for vaccine development. Immunoscreening of the Brugia malayi L3 stage cDNA library with immune sera from an area endemic for Onchocerciasis has led to the identification of the clone TNPIBM3004 showing significant homology to Di20/22 kDa gene, a vaccine candidate of dog heart worm disease.

The nucleotide sequence of TNPIBM3004 was identical to the Brugia malayi Abundant Larval Transcript-2 (ALT-2) gene and shared a significant homology with ALT proteins of other filarial parasites. PCR analysis of the stage-specific cDNA libraries from Brugia malayi and Wuchereria bancrofti using gene-specific primers revealed that transcription of ALT-2 occurred only during the L3 stage of the parasite life cycle. Western Blot analysis with protein
extracts of the various stages of the parasite life cycle using polyclonal antibody raised in mouse against the recombinant ALT-2 protein recognized a 14kDa polypeptide only in the L3 stage of the parasite. When the human humoral response to rALT-2 was assessed by ELISA, a differential reactivity was observed among the endemic normals, (EN) with 18 of 25 (72%) demonstrating a measurable response to the protein, compared to 9 of 25 (36%) microfilaraemics (MF) and 14 of 25 (52%) of the Chronic Pathology (CP) (p = 0.01 for comparison of EN to CP as well as to MF). This humoral immune response against rBmALT-2 may be associated with the protective immunity postulated to be responsible for the resistance in ENs. IgG1 was the predominant isotype antibody involved in the recognition of rBmALT-2 protein in all the clinical groups.

In our vaccination study, mice were immunised with ALT-2 or a multiple stage antigen, SXP-1; either as a recombinant protein or DNA vaccine and protective efficacy was assessed by the micropore chamber method. In mice immunised with rBmALT-2 protein, 75% of the recovered L3 larvae were affected compared to mice immunised with PBS (p=0.02). In mice receiving the BmALT-2 DNA vaccine 46% of L3 larvae were affected compared to 24% in the control group (pVr1020) (p=0.01), indicating a statistically significant reduction in viable worm recovery. In mice immunised with rWbSXP and pVWbSXP, 67% (p=0.08) and 35% (p=0.04) of the L3 larvae were affected respectively. When compared to WbSXP-1, the ALT-2 vaccine constructs conferred statistically significant level of protection compared to respective
controls in both groups of mice immunised either with protein or DNA vaccine construct.

The observations in the present study clearly demonstrate the immunogenic and prophylactic potential of recombinant proteins and DNA vaccines of the *Brugia malayi* ALT-2 and WbSXP-1 genes. Further studies are in progress to enhance the prophylactic potential of these genes.