ABSTRACT

Human lymphatic filariasis is an incapacitating vector borne disease and is the world’s second leading cause of long-term disability. To worsen the condition there are no vaccines yet and vector control programs have limitations of insect resistance. The current drugs have limited ability in removing larval stages for preliminary control measures. Further their broad use would increase the likelihood of accelerated drug resistance. With this distressing scenario there is a growing demand to identify new molecular targets for lymphatic filariasis towards development of prophylactics and drugs that act on all the stages of parasites.

The first part of the thesis deals with strategies to enhance the immunoprophylactic efficacy of filarial vaccine targets. Accordingly, the immune responses of recombinant cuticular glycoprotein (GP29) using different expression systems were evaluated in mice and Mastomys models to validate its effectiveness as a vaccine candidate either alone or in combination. The humoral and cellular immune responses of cuticular glycoprotein (Bm-GP29) were studied in multivalent combination with the antigens ALT or VAH. Both the antigens were found to increase the titer of GP29 in a uniform manner. The GP29+VAH cocktail elicited significant antibody titres against VAH, whereas GP29+ALT cocktail were suppressive for ALT. The immunological significance of expression system in terms of proper folding and secondary structure modifications resembling native
antigens, were studied by comparing E.coli and Pichia expressed GP29 and VAH. The sera reactivity and proliferative responses of EN samples for both E.coli and Pichia expressed GP29 and VAH were significantly high compared to MF and CP. The immune responses of Pichia (P.GP29/P.VAH) or E.coli expressed (E.GP29/E.VAH) proteins either alone or in combination was evaluated in Mastomys model. The humoral and cellular responses were significantly high for Pichia expressed VAH or GP29 compared to E.coli expressed proteins especially in the P.GP29+VAH group. The percentage protection against B.malayi L3 infective larvae in Mastomys for E.GP29 and E.VAH either alone or in combination were 62.33%, 56.67% and 65.75% respectively. Comparably, the protective efficacy of P.GP29 and P.VAH either alone or in combination were 66.50%, 65.75% and 81.75% respectively. The protection results correlates with humoral and cellular responses indicating the role of eukaryotic expression systems like P.pastoris in enhancing the immunogenicity of vaccine antigens.

The second part of the thesis deals with X-ray crystallographic elucidation of Glutathione-S-transferase (GST) 3-D structure and conformational epitope analysis of Thioredoxin (TRX) 3-D structure for therapeutic intervention. Therefore, the recombinant Wb-GST was expressed, purified and co-crystallised along with its native substrate glutathione for structural characterization. The structure was solved at a resolution of 2.3Å by X-ray diffraction methodologies, which resembled \( \pi \)-class GSTs and was deposited in PDB (3T2U). The superimposed structures of Wb-GST and Hu-GST (human host) monomers showed an r. m. s. deviation of 1.2Å for all C\( \alpha \)
atoms. The G-site residues were highly conserved (differed by 8%), whereas the H-site residues revealed a significant difference (62%) between Wb-GST and Hu-GST. The H-site of Wb-GST showed greater accessibility for electrophilic substrates compared to Hu-GST. The electron density map of Wb-GST showed that the catalytic residue Tyr\(^7\) swings off and works as a proton shuttle for catalytic stabilization. The Wb-GST structure also revealed the presence of non-catalytic ligand binding sites (ligandin function) in the inter-subunit cleft, which can serve as a binding site for hydrophobic ligands. These crucial insights from structural data could be exploited for developing parasite-specific inhibitors.

The recombinant Wb-TRX was sub-cloned, expressed and purification strategies were optimised for structural studies. Although TRX is a potential therapeutic target, it shares sequence homology with host enzyme which may lead to cross-reactivity. Hence, two peptide regions that are non-homologous to host TRX proteins were conjugated (peptide conjugate-PC1) and used for the conformational epitope analysis. Peptide-specific antibodies against these regions of Wb-TRX were found to inhibit enzyme activity significantly compared to total anti-TRX antibodies whereas the human TRX activity was inhibited only by anti-TRX antibodies and not by anti-PC1 antibodies. The conformational significance of these protective epitopes (PC1) in enzyme inhibition of Wb-TRX was validated by structural analysis, which revealed PC1 to be part of a larger conformational epitope. Hence, this may propose the use of enriched conformational epitopes of Wb-TRX as vaccine candidates for host protection.
Since filariasis is a complex parasitic disease, a multi-pronged approach that incorporates both vaccine and drug development would be an ideal step towards therapeutic intervention and eradication. (1) The current study for the first time reports that pichia expressed antigens combined with multiple-antigen mode is a promising vaccine strategy by virtue of ~80% reduction in worm burden. (2) In regard with drug development approach, this is the first report on X-ray crystallographic determination of Wb-GST structure suggesting its potential as a drug target. Further, the study validates that the earlier reported protective epitopes (PC1) are a part of a dominant conformational epitope of Wb-TRX and also confirms its role in protective response by biochemical and structural analysis.