Exploring chaperone and immunological functions of Mycobacterium indicus pranii protein MIP_05962

Tuberculosis is still a major threat to mankind, causing nearly about two million deaths each year\(^1\). At present live *M. bovis*, bacilli Calmette-Guerin (BCG) is the only vaccine against TB which is however only effective in preventing TB in childhood and does not confer protection against disease in adulthood or during reactivation of latent TB. Rigorous efforts are on for new TB vaccines, which can bestow constant long-standing protection, and develop new drugs to prevent reactivation of latent infection of TB.

*Mycobacterium indicus pranii* (MIP), a strong immunomodulator has been recently demonstrated to share a greater number of putative *M. tb* antigens as compared with *M. vaccae*, another saprophyte which failed to impart protection in various trials. MIP has been shown to share a large number of common B and T cell epitopes with *M.leprae* and *M. tuberculosis*. Consequently *MIP* has also been found to confer protection against tuberculosis in animal model and is currently under human clinical trials. Moreover, *MIP* is being now extensively investigated as an immunomodulator against a number of other human diseases in addition to its clinical use as an intervention against leprosy under a commercial name of ‘Immuvac’. Comparative genomic analysis revealed the presence of 36 genes in *MIP* which are absent in *M. bovis BCG*. Most of these genes have high antigenicity index, despite being a non-pathogenic strain, thereby rendering them candidates of prime interest. One of these putatively immunogenic proteins, MIP_05962 is a member of HSP20 family (heat shock proteins) due to presence of \(\alpha\)-crystallin domain, and has amino acid similarity with *M.Leprae* HSP18 protein and antigen2 of *M.tb*.

HSP18 of *M.leprae* is a highly antigenic protein and carries a number of T-cell epitopes which elicits good CD4+T-cell response and also shares various common T cell epitopes with *M.tuberculosis*. HSPs have been found to be up-regulated under different environmental stress conditions and function as molecular chaperones by assisting in proper folding of nascent polypeptide, preventing them from thermal or chemically induced aggregation, denaturation, unfolding, thereby playing a major role in maintaining cellular homeostasis and protecting undue cell death. The heat shock proteins in addition to maintaining cell stability and integrity also have been found to play a major role in antigen presentation along with lymphocyte and macrophages activation due to their antigenic nature. Up regulation of these chaperones during host stress enables a pathogen to survive and antagonistically also make them a potential antigenic target. Thus, it seems imperative to elucidate the putative chaperones in order to devise anti-mycobacterial strategies as well as to determine the potential immunomodulation pathways. Recently the protection imparted by latency associated HspX in mice model was shown to involve Th1-type cell mediated response, critical for controlling TB infection, therefore new generation vaccines against TB are focusing on activation of this arm of the immune system\(^2\).

With this background, the chaperone and immunological functions of *MIP_05962* gene were investigated. Purified recombinant MIP_05962, expressed in *E.coli* and purified, showed thermal aggregation prevention and *in-vitro* refolding of substrate proteins Maltodextrin glucosidase and citrate synthase, prevention of thermal inactivation of Nde1, exhibited interaction with non-native protein, provided *in-vivo* viability of *E.coli* and also showed *in-vivo* assisted refolding of substrate protein. MIP_05962 exits in a large oligomeric unit and has numerous hydrophobic patches on its surface. MIP_05962 is highly stable at higher temperatures and have tremendous spontaneous ability to attend its native like conformation after removal of stress. These properties of *MIP_05962* protein lead us to conclude that the protein resembles molecular chaperone. Immunological properties of this protein were investigated in mice model. MIP_05962 showed significant proliferation of lymphocytes which confirmed that MIP_05962 is highly antigenic and induced significant Th1 type of T cell mediated response both *in-vitro* and intracellularly. It also evoked significant effector memory response in CD4+T cells. Th1-type cell mediated response is critical for controlling TB infection; therefore new generation TB vaccines are focusing on activation of this arm of the immune system. Therefore MIP_05962 has a potential for use as a subunit vaccine or booster with BCG against tuberculosis.