Development of DNA constructs expressing *Mycobacterium avium* subsp. *paratuberculosis* proteins in mammalian cell line and study on their immunogenicity in murine model

PP Goswami, S Chakravarti, S Chandra Sekar, Rajib Deb

*Indian Veterinary Research Institute, Izatnagar India*

Several antigens of *Mycobacterium avium* subsp. *paratuberculosis* (Map) were studied as vaccine candidate and their immunogenicity has been evaluated. Previous studies in our laboratory revealed that 35 kDa protein (P35) expressed in prokaryotic expression vector could induce significant T cell immune response as well as secretion of Th1 associated cytokine interferon gamma (IFNγ). We have further cloned P35 (35 kDa), PE (10 kDa) and PPE (34.9 kDa) protein genes of Map alone and with murine IFNγ into the eukaryotic expression plasmid pIRES 6.1 and transfected in mammalian cell line which showed eukaryotic expression of recombinant proteins detected with hyperimmune sera raised against respective recombinant proteins in western blot and immunofluorescent assay. These monocistronic and bicistronic constructs were used as DNA vaccine in mice and their immunogenicity was studied by delayed type hypersensitivity (DTH), lymphocyte proliferation, nitric oxide (NO) determination and IFNγ assay. Significant DTH responses were evoked in mice immunized with bicistronic constructs than the monocistronic constructs. Also higher proliferation of the splenocytes and enhanced production of NO exposed to respective antigen was found in bicistronic groups. Significantly higher amount of IFNγ was also released in bicistronic groups. Flow cytometry analysis revealed higher CD4+ and CD8+ T cell response to the recombinant antigens. Studies also showed that co-expression of IFNγ with Map genes enhanced the immunogenicity. These results indicate the T cell epitopic nature of the antigens which could potentially be used in the development of effective DNA vaccine against paratuberculosis infection.