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In vitro characterization of five potential antigenic proteins that elicited specific immune reaction using PMBC from sensitized guinea pig models of both tuberculous and non-tuberculous *Mycobacterium* species

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A replacement antigen for PPD that improve skin test specificity has been a long-standing research goal. Description of a new reagent – of either single or multiple antigens – to replace PPD remains challenging. In response, the current study attempted to fractionate home-made short term-culture filtrate (ST-CF) of *Mycobacterium tuberculosis*, using HPLC. All obtained fractions were subjected to *in vitro* evaluation of their antigenicity by both LPA and γ - INF assay using PMBC from guinea pig models sensitized by heat killed *M. tuberculosis*. Fractions that elicited positive antigenic reactions were analyzed for its protein contents using SDS-PAGE. Multi-protein fractions were re-fractionated using shallower gradient HPLC. Obtained proteins were re-subjected to *in vitro* evaluation of their antigenic specificity using PMBC from guinea pig models sensitized by both heat killed tuberculous *Mycobacterium* (*M. tuberculosis* and *M. bovis*) and non-tuberculous *Mycobacterium* (*M. intercellularae, M. avium, M. kansasi* and *M. fortuitum*). Five proteins that elicited variable degrees of specific antigenicity only against tuberculous *Mycobacterium*-sensitized PMBC were characterized. On SDS-PAGE analysis, selected proteins ranged between ~ 5 kDa up to ~30 KDa. N-terminus sequencing of these proteins were carried out by Edman degradation using automated Gas Phase Sequencing (GPS). Obtained sequences of the N-terminus of selected proteins were used for search analysis for related *Mycobacterium* proteins using NCBI-Protein Blast. At this stage we were able to define the ORFs of the genes coding those proteins and currently we are working on the cloning of these genes for mass production of corresponding proteins for further evaluation. The future plan is to use these proteins either individually or in different combinations for *in vivo* skin testing of guinea pig models.

Biography

Amr Mohamed Abdel Fattah Mohamed has completed his PhD at the age of 35 years from University of Nebraska Medical Center, USA at 2004. He worked as associate professor of molecular diagnostics of infectious diseases at School of veterinary medicine, Assiut University, Egypt. Currently he is a full time professor of Laboratory Medicine at Umm Al-Qura University, Saudi Arabia. He is the director of Molecular Diagnostic Research Laboratory at the Central Laboratories of Collage of Applied Medical Sciences, Umm Al-Qura University. He has published more than 20 papers in reputed journals and has been serving as an editorial board member of many reputed Journals.

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