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# Enhancement of delayed type hypersensitivity in vitro reaction of antigenic cocktail ESAT6, CPF10 and MPT83 using synthetic Mycobacterium tuberculosis 19-kDa lipoprotein

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In previous study we characterized a group of antigenic proteins that elicited specific in vitro DTH in tuberculous Mycobacterium-sensitized guinea pig models but not in non-tuberculous Mycobacterium-sensitized ones. However, none of these proteins succeeded to produce DTH reaction comparable to that of conventional PPD when used for skin testing in sensitized guinea pig model. Given the complex nature of the tuberculin, it was proposed that these proteins could have represented potential candidates for skin testing not individually but in a cocktail. To enhance the DTH reaction elicited by tested antigen protein cocktails, the current study proposed for inclusion of synthetic bacterial lipoprotein, as a toll like receptor (TLR) stimulant, to provide the assumed missed pro-inflammatory stimulus from purified proteins as compared to conventional tuberculin complex. Three different cocktails of selected antigenic proteins were evaluated in the current study included cocktail 1 (ESAT6, CPF10, MPT83), cocktail 2 (ESAT6, MPT64, MPT83) and cocktail 3 (CPF10, MPT64, MPT83). Lymphocytic proliferation (LP) and gamma interferon (γ-INF) production assays were used for in vitro evaluation of antigenic cocktails using peripheral blood monocytic cells (PBMC) from sensitized guinea pigs treated with those antigenic cocktails with and without the synthetic Mycobacterium tuberculosis (Mtb) 19-kDa lipoprotein. In addition, the mRNA and protein expression levels of tumor necrosis factor-alpha (TNF-α), Interluikin-12p40 (IL-12p40) and Interluikin-1 beta (IL-1β) pro-inflammatory molecules were evaluated using real time polymerase chain reaction (RT-PCR) and ELISA, respectively. Results revealed that all evaluated cocktails produced significant LP and gamma-INF production comparable to that of conventional PPD. No significant differences were recorded between different antigenic cocktails either with or without the synthetic bacterial lipoprotein. However, at the level of pro-inflammatory molecules, all 3 cocktails revealed significant elevation of mRNA expression and higher protein levels of the 3 pro-inflammatory molecules when used in combination with the synthetic bacterial lipoprotein as compared to the cocktails without the lipoprotein. Interestingly, antigenic cocktail 1 with lipoprotein showed higher significant production of pro-inflammatory molecules at the level of gene expression and protein concentration as compared to other evaluated cocktails. In conclusion, the current results revealed the significant augmentation of pro-inflammatory stimulus and hence the delayed type hypersensitivity reaction with the inclusion of TLR legend to a cocktails of Mtb-specific antigens. The study also revealed antigenic protein cocktail (ESAT6, CPF10, MPT83) as a potential alternative skin-testing reagent when used in combination with synthetic Mtb 19 kDa lipoprotein.

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