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Tuberculin response in guinea pigs with recombinant proteins cocktail prepared from Indian strain of *Mycobacterium bovis* (3/86 Rv)

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The bovine tuberculosis caused by *Mycobacterium bovis* is a serious disease among cattle worldwide resulting in considerable economic loss. There is a need for a diagnostic test that can discriminate *M. bovis* infection from BCG vaccination and NTM sensitization in animals. In this study, we intended to find out the potential use of recombinant antigens from Indian strain of *Mycobacterium bovis* (3/86Rv) for the intradermal tuberculin test of cattle. Immunodominant proteins MPB64, MPB83 and ESAT6 from *M. bovis* (3/86 Rv) Indian strain were recombinantly overexpressed, purified and immunologically characterized (rMPB64, rMPB83 and rESAT6). Four different cocktail combinations *viz*., cocktail I of protein antigens contained rMPB64, rMPB83, rESAT6, rCFP10 with protein concentration of 0.5 µg each; cocktail II contained 0.5 µg of each of rMPB64, rMPB83, rESAT6; cocktail III with 1 µg of each rESAT6, rCFP10; and cocktail IV contained rMPB64 and rMPB83 with 1 µg concentration of each protein, were administered at a dose of 0.1 mL. The DTH response was measured in heat killed *M. bovis* and non-tuberculous mycobacteria (NTM) sensitized, bacille Calmette-Guerin (BCG) vaccinated and control guinea pigs. The first cocktail of rMPB64, rMPB83 and rESAT6 containing 1.5 µg showed almost similar to cocktails II and III but stronger DTH response even at lower individual protein concentrations (each 0.5 µg) than the rESAT6 and rCFP10 protein of third cocktail with higher individual protein concentration (each 1 µg). The fourth cocktail with rMPB64 and rMPB83 elicited less DTH response as compared to the all other formulated cocktails. Cocktail I of four protein antigens elicited highest response at 24 h. Guinea pig model sensitized with heat killed *M. bovis* was found to be an efficient model for evaluating DTH response elicited by recombinant proteins cocktails. None of the cocktails elicited positive erythematous reaction in NTM sensitized and BCG vaccinated guinea pigs. A diagnostic test based on above cocktails could discriminate *M. bovis* infection from BCG vaccinated and NTM sensitizatized cattle.

Keywords: Bovine tuberculosis, Delayed type hypersensitivity