# The potential of delayed type hypersensitivity-inducing *Mycobacterium tuberculosis*-specific antigens in the diagnosis of tuberculosis

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**Abstract:**

The worldwide control of tuberculosis (TB) requires affordable and easy to apply test(s), which could diagnose active/latent TB and differentiate from vaccination with *Mycobacterium bovis* Bacillus Calmette Guerin (BCG), and exposure to environmental mycobacteria. The currently used test for the diagnosis of TB is the in vivo administered tuberculin skin test that induces delayed-type hypersensitivity (DTH) skin responses in individuals infected with *M. tuberculosis*. However, this test lacks sensitivity and specificity because of the non-standardized and cross-reactive nature of the antigens used, i.e. purified protein derivative (PPD) of *M. tuberculosis*. Since PPD contains antigens shared between *M. tuberculosis*, BCG, and environmental mycobacteria, it cannot differentiate between infection with *M. tuberculosis*, vaccination with BCG, and exposure to environmental mycobacterial. To overcome the problems associated with PPD, there is a need to identify *M. tuberculosis*-specific antigens as new tuberculins for in vivo diagnostic applications in humans.

The first study to identify the defined antigens of *M. tuberculosis* inducing DTH responses was carried out by Hasløv et al. in 1995. They purified the naturally expressed antigens of *M. tuberculosis* from the culture filtrate proteins and tested the purified antigens for DTH responses in guinea pigs. Their results showed that a single antigen, named MPT64, induced positive DTH responses in guinea pigs infected with *M. tuberculosis* but not with *Mycobacterium bovis* BCG (Danish strain). Studies with overlapping synthetic peptides covering the sequence of full-length MPT64 identified a single DTH-inducing epitope of 15 residues. The screening of 56 clinical isolates of *M. tuberculosis* from Danish and Tanzanian patients demonstrated that the gene encoding MPT64 was present in all of the strains. Based on these results, the authors suggested MPT64 and its DTH-inducing peptide as possible candidates for a skin test reagent specific for the diagnosis of TB in humans.

Although, MPT64 is absent in some but present in other strains of *M. bovis* BCG. A study by Haga, showed that recombinant MPB64 (a homolog of MPT64 present in *M. bovis*) induced positive DTH responses in guinea pigs infected with live *M. tuberculosis* H37Rv or live *M. bovis* BCG Tokyo (a vaccine strain that secretes MPB64. It was found that recombinant MPB64 [rMPB64] had the same reactivity as native MPB64 (nMPB64) and nMPT64. In another study, animals sensitized with *M. bovis* BCG strains lacking the MPB64 gene failed to respond to recombinant MPT64. However, since MPT64 is present in some BCG vaccine strains, it will not be an ideal candidate for diagnostic applications throughout the world.

The identification of *M. tuberculosis*-specific antigens was accelerated with the sequencing of the *M. tuberculosis* genome and its comparison with the genome of *M. bovis* BCG. These studies identified 11 genomic regions of differences (RDs) in *M. tuberculosis* that were deleted/absent in all *M. bovis* BCG strains used for vaccination against TB in different parts of the world. Several antigens encoded by genes present in these RDs were found to be the major inducers of cellular immune responses in vitro. Among these antigens, ESAT-6 and CFP10, encoded by RD1, have been shown to induce *M. tuberculosis*-specific DTH responses in guinea pigs as shown by positive responses in animals infected with *M. tuberculosis* but negative responses in animals sensitized with *M. bovis* BCG, *M. avium*, or other non-tuberculous mycobacteria. However, all groups of animals showed positive DTH responses to PPD, which demonstrated the non-specificity of PPD. In addition to ESAT-6 and CFP10, other RD encoded antigens, i.e. PE35, PPE68 and Rv3619c have also been shown to induce positive DTH responses in guinea pigs sensitized with *M. tuberculosis*. Furthermore, a combination of antigens (ESAT-6 and CFP10 or ESAT-6 and MPT64) improved the sensitivity of the DTH response in guinea pigs. By using overlapping synthetic peptides of ESAT-6, the DTH-inducing epitope of ESAT-6 was mapped to the C-terminus of the protein. Furthermore, as compared to the individual peptides of each protein, testing with a combination of DTH-inducing peptides of ESAT-6 and MPT64 improved the sensitivity of DTH response in *M. tuberculosis*-infected guinea pigs. Thus, suggesting that a combination of DTH-inducing peptides will have better sensitivity in diagnostic applications.

The studies with ESAT-6 have been further extended in the two most important natural hosts of pathogenic *M. bovis* and *M. tuberculosis*, i.e. cattle and humans, respectively. In *M. bovis*-

infected cattle, it has been shown that sensitivity of DTH responses with ESAT-6 was as good as with PPD, with the additional advantage of improved specificity. In TB patients, testing with ESAT-6 alone or with CFP10 has shown improved specificity, but less sensitivity than PPD. Although these results are encouraging, ESAT-6 and CFP10 have limitations because they are also being used as a component of new vaccine candidates against TB in humans, and the same antigen cannot be used as a vaccine as well as for diagnostic applications. Furthermore, the use of ESAT-6 and CFP10 is already well established in diagnostic applications using in vitro assay.

If injected in vivo, these antigens may sensitize the individuals, thus jeopardizing their use for in vitro diagnostic applications. Therefore, it is imperative to identify additional *M. tuberculosis*-specific antigens active in DTH responses to prepare cocktails of antigens/peptides as new tuberculins. This will allow the use of different sets of *M. tuberculosis* antigens for vaccine and diagnostic applications. Furthermore, such antigens will also be useful in epidemiological investigations, contact tracing, and diagnostic application by in vivo testing without the problem of boosting/sensitization to the same antigen(s) upon repeated testing, as has been shown with PPD, and even in limited experience with ESAT-6.