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Proteins and Peptides Encoded by *M. tuberculosis*-Specific Genomic Regions for Immunological Diagnosis of Tuberculosis

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Tuberculosis (TB) is a major global health problem with about 9 million new cases and 1.7 million deaths annually [1]. The world-wide problem of TB is expected to worsen in the future due to many reasons, including the spread of drug-resistant strains of *Mycobacterium tuberculosis*, and TB-HIV co-infection [1]. In addition to new drugs at affordable prices, the global control of TB requires cost-effective reagents for specific diagnosis and protective vaccines [2]. At present, among the commonly used strategies to control TB in the world are the diagnoses of TB using the purified protein derivative (PPD) of *M. tuberculosis* in delayed-type hypersensitivity (DTH) skin responses, and immunization with *Mycobacterium bovis* BCG as a vaccine. However, the current data demonstrates that both of these modalities have failed to control the world-wide problem of TB [1].The major limitation of PPD is its inability to differentiate between individuals vaccinated with BCG, infected with *M. tuberculosis* and exposed to environmental non-tuberculous mycobacteria [3]. This is because PPD is a crude mixture of hundreds of proteins present in the culture supernatant of in vitro grown *M. tuberculosis* and contains both species-specific as well as crossreactive antigens [3]. On the other hand, BCG vaccination imparts inconsistent protection against TB in different parts of the world, in people of various age groups and against different clinical manifestations of the disease, which suggest that BCG may be lacking in some important antigens of *M. tuberculosis* required for optimal induction of protective immunity [3]. Therefore, for diagnostic as well as vaccine applications, the identification of *M. tuberculosis*-specific antigenic proteins may be essential [4].

The availability of the complete genome sequence of *M. tuberculosis* in 1998 opened up the door for comprehensive genomic comparisons between mycobacterial species to identify species-specific genomic regions, and lead to the identification of 11*M. tuberculosis* genomic regions deleted/absent in all BCG strains used for vaccination against TB in different parts of the world [5]. It was suggested that these regions could be useful for both specific diagnosis and vaccine applications [6-8]. The analyses of DNA sequence present in these regions of differences (RDs) for the presence of genes and encoded proteins, by using computer-based programs for prediction of open reading frames, have suggested that RD genomic regions deleted/absent in all BCG strains could encode 89 *M. tuberculosis*-specific proteins [9]. As given below, studies have been performed to characterize these proteins for diagnostic applications, using sera and peripheral blood mononuclear cells from TB patients and healthy subjects in antibody and cell mediated immunity assays.

To obtain the RD proteins for immunological investigations, attempts were made first to obtain them as recombinant proteins [9]. These procedures have required the in vitro amplification of RD genes using polymerase chain reaction, cloning of the amplified gens in plasmid vectors to generate recombinant plasmids, expression of the cloned genes in *Escherichia coli* after transforming them with the recombinant plasmids, and purification of recombinant RD proteins from the recombinant *E. coli* cell lysates (free of the *E. coli* components) to homogeneity [10-12]. However, the results of these experiments have shown that problems were encountered at each of the above steps, and therefore, all of the targeted proteins could not be obtained in purified form and in quantities sufficient for immunological investigations [13,14].

To overcome the problems associated with the recombinant production of RD proteins, an alternative approach has been used by employing synthetic peptide chemistry to obtain overlapping peptides covering the sequence of each RD protein [15]. This strategy was successful in synthesizing peptides covering the sequence of all targeted RD proteins absent in BCG [15]. These peptides overlapped by 10 amino acids with the neighboring peptides thus minimizing the missing of linear peptide epitopes recognized by either T cells or antibodies, which usually recognize peptides of less than 10 aa [16]. Conceptually, the use of synthetic peptides in cell mediated immunity assays has no problems because T cells (the effector/indicator cells in cell mediate immunity assays) recognize only linear epitopes, whereas it may be problematic for epitopes recognized by antibodies, because antibodies are usually considered to recognize conformational epitopes. However, recent studies have shown that antibodies may also recognize linear epitopes [17]. Therefore, in our studies, we have used synthetic peptides of RD proteins in both types of assays using 96-well micro titer plates. However, an important difference in using them for antibody and cellular reactivity has been the use of individual peptides and pools of peptides, respectively. The pooling of peptides in case of antibody assays does not work well because of the competition for binding of the peptides to solid support during the coating step of the well in enzyme-linked immunosorbent assays (ELISAs); whereas, pooling of the peptides does not seem to matter much in case of T-cell assays (cellular reactivity) because of the requirement of the binding of peptides to human leukocyte antigens on the surface of antigen presenting cells before the epitope is presented to T cells [16]. This step selects the relevant peptide from the pool and thus minimizes the effect of pooling in T-cell responses.

The evaluation of RD peptides for antibody reactivity in ELISA, with sera from TB patients and healthy subjects, identified three peptides of diagnostic relevance, i.e. aa 346-370 of Rv3876, aa 241-265 of Rv1508c and aa 325-336 of Rv1516c, which had significantly strong antibody reactivty with sera from TB patients than healthy subjects ($P < 0.05$), and significantly higher positivity with TB sera (% positives = 66 to 93%) than sera from healthy subjects (% positives = 10 to 28%)

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[17]. These results suggest that the three identified peptides could be useful in sensitive and specific diagnosis of TB using antibody assays. Furthermore, testing with peripheral blood mononculaer cells for cellular reactivity in T cell assays identified three proteins of RD1, i.e. PPE68, ESAT6 and CFP10, and two proteins each of RD7 (Rv2346c and Rv2947c) and RD9 (Rv3619 and Rv3620) as the best stimulators of T helper 1 (Th1) cells in antigen-induced proliferation and/or IFN-γ secretion assays [18-23].

The diagnostic potential of four antigens encoded by *M. tuberculosis*-specific RD1 region genes (PE35, PPE68, CFP10, ESAT-6) and RD9 region gene Rv3619c was further investigated in cellular assays using DTH responses in guinea pigs [24]. The results showed that mycobacterial sonicates containing species-specific and cross-reactive antigens, induced positive DTH responses in *M. tuberculosis*, *M. bovis* BCG, *M. avium* and *M. vaccae* injected guinea pigs. However, the purified recombinant proteins PE35, PPE68, CFP10 and ESAT-6 elicited positive DTH responses in *M. tuberculosis*-injected group but not in BCG, *M. avium* and *M. vaccae*-injected guinea pigs, whereas Rv3619c elicited positive DTH responses in *M. tuberculosis* and in BCG-injected groups but not in *M. avium* and *M. vaccae*-injected guinea pigs. The overall results showed that the recombinant RD1 antigens induced *M. tuberculosis*-specific DTH responses [24]. Thus, these antigens may be useful in the specific diagnosis of tuberculosis, and therefore could provide a better alternative to the currently used PPD-based skin test in humans.

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References

1. World Health Organization (2010) Global Tuberculosis Control. WHO Report 2010, WHO/HTM/TB/2010.7.
2. Lönnroth K, Raviglione M (2008) Global epidemiology of tuberculosis: prospects for control. Semin Respir Crit Care Med 29: 481-491.
3. Mustafa AS, Al-Attiyah R (2003) Tuberculosis: looking beyond BCG vaccines. J Postgrad Med 49: 134-140.
4. Mustafa AS (2001) Biotechnology in the development of new vaccines and diagnostic reagents against tuberculosis. Curr Pharm Biotechnol 2: 157-173.
5. Behr MA, Wilson MA, Gill WP, Slamon H, Schoolink GK, et al. (1999) Comparative genomics of BCG vaccines by whole-genome DNA microarray. Science 284: 1520-1523.
6. Mustafa AS (2002) Development of new vaccines and diagnostic reagents against tuberculosis. Mol Immunol 39: 113-119.
7. Mustafa AS, Al-Attiyah R (2004) *Mycobacterium tuberculosis* antigens and peptides as new vaccine candidates and immunodiagnostic reagents against tuberculosis. Kuwait Med J 36: 172-176.
8. de Jonge MI, Brosch R, Brodin P, Demangel C, Cole ST (2005) Tuberculosis: from genome to vaccine. Expert Rev Vaccines 44: 541-551.
9. Mustafa AS (2005) Mycobacterial gene cloning and expression, comparative genomics, bioinformatics and proteomics in relation to the development of new vaccines and diagnostic reagents. Med Princ Pract 14: 27-34.
10. Amoudy HA, Mustafa AS (2008) Amplification of six putative RD1 genes of Mycobacterium tuberculosis for cloning and expression in *Escherichia coli* and purification of expressed proteins. Med Princ Pract 17: 378-384.
11. Hanif SN, Al-Attiyah R, Mustafa AS (2010) Molecular cloning, expression, purification and immunological characterization of three low molecular weight proteins encoded by genes in genomic regions of difference of *Mycobacterium tuberculosis*. Scand J Immunol 71: 353-361.
12. Hanif SN, Al-Attiyah R, Mustafa AS (2011) The natural expression of genes encoding major antigens of Rd1 and Rd9 in *M. tuberculosis* and other mycobacteria. Mycobact Diseases 1:105.
13. Mustafa AS (2005) Recombinant and synthetic peptides to identify *Mycobacterium tuberculosis* antigens and epitopes of diagnostic and vaccine relevance. Tuberculosis (Edinb) 85: 367-376.
14. Mustafa AS (2009) Vaccine potential of *Mycobacterium tuberculosis*-specific genomic regions: *in vitro* studies in humans. Expert Rev Vaccines 8: 1309–1312.
15. Mustafa AS (2012) What's New in the Development of Tuberculosis Vaccines. Med Princ Pract 21: 195-196.
16. Mustafa AS (2000) HLA-restricted immune response to mycobacterial antigens: relevance to vaccine design. Hum Immunol 61: 166-171.
17. Al-Khodari NY, Al-Attiyah R, Mustafa AS (2011) Identification, diagnostic potential and natural expression of immunodominant seroreactive peptides encoded by five *Mycobacterium tuberculosis*-specific genomic regions. Clin Vaccine Immunol 18: 477-482.
18. Mustafa AS, Cockle PJ, Shaban F, Hewinson RG, Vordermeier HM (2002) Immunogenicity of *Mycobacterium tuberculosis* RD1 region gene products in infected cattle. Clin Exp Immunol 130: 37-42.
19. Al-Attiyah R, Mustafa AS (2008) Characterization of human cellular immune responses to novel *Mycobacterium tuberculosis* antigens encoded by genomic regions absent in *Mycobacterium bovis* BCG. Infect Immun 76: 4190-4198.
20. Hanif SN, El-Shammy AM, Al-Attiyah R, Mustafa AS (2008) Whole blood assays to identify Th1 cell antigens and peptides encoded by *Mycobacterium tuberculosis*-specific RD1 genes. Med Princ Pract 17: 244-249.
21. Mustafa AS, Al-Attiyah R, Hanif SNM, Shaban FA (2008) Efficient testing of large pools of *Mycobacterium tuberculosis* RD1 peptides and identification of major antigens and immunodominant peptides recognized by human Th1 cells. Clin Vaccine Immunol 15: 916-924.
22. Mustafa AS, Al-Attiyah R (2009) Identification of *Mycobacterium tuberculosis*-specific genomic regions encoding antigens inducing protective cellular immune responses.Indian J Exp Biol 47: 498-504.
23. Mustafa AS, Al-Saidi F, El-Shamy AS, Al-Attiyah R (2011) Cytokines in response to proteins predicted in genomic regions of difference of *Mycobacterium tuberculosis*. Microbiol Immunol 55: 267-278.
24. Hanif SN, Al-Attiyah R and Mustafa AS (2010) Species-specific antigenic *Mycobacterium tuberculosis* proteins tested by delayed-type hypersensitivity response. Int J Tuberc Lung Dis 14: 489-494.