World Academy of Science, Engineering and Technology
International Journal of Medical and Health Sciences
Vol:12, No:4, 2018

Antibody Reactivity of Synthetic Peptides Belonging to Proteins Encoded by Genes Located in *Mycobacterium tuberculosis*-Specific Genomic Regions of Differences

Abu Salim Mustafa

***Abstract*—**The comparisons of mycobacterial genomes have identified several *Mycobacterium tuberculosis*-specific genomic regions that are absent in other mycobacteria and are known as regions of differences. Due to *M. tuberculosis*-specificity, the peptides encoded by these regions could be useful in the specific diagnosis of tuberculosis. To explore this possibility, overlapping synthetic peptides corresponding to 39 proteins predicted to be encoded by genes present in regions of differences were tested for antibody-reactivity with sera from tuberculosis patients and healthy subjects. The results identified four immunodominant peptides corresponding to four different proteins, with three of the peptides showing significantly stronger antibody reactivity and rate of positivity with sera from tuberculosis patients than healthy subjects. The fourth peptide was recognized equally well by the sera of tuberculosis patients as well as healthy subjects. Predication of antibody epitopes by bioinformatics analyses using ABCpred server predicted multiple linear epitopes in each peptide. Furthermore, peptide sequence analysis for sequence identity using BLAST suggested *M. tuberculosis*-specificity for the three peptides that had preferential reactivity with sera from tuberculosis patients, but the peptide with equal reactivity with sera of TB patients and healthy subjects showed significant identity with sequences present in nob-tuberculous mycobacteria. The three identified *M. tuberculosis*-specific immunodominant peptides may be useful in the serological diagnosis of tuberculosis.

***Keywords*—**Genomic regions of differences, *Mycobacterium tuberculosis*, peptides, serodiagnosis.

I. Introduction

TUBERCULOSIS (TB) is a chronic bacterial disease and world-wide public health problem with about one-third of the world population being infected with *Mycobacterium tuberculosis*. TB is the ninth leading cause of death globally and the leading cause from a single infectious agent [1]. According to the most recent estimates by the World Health Organization, about 10.4 million people fell ill with TB and 1.7 million people died of TB in 2016 [1]. In particular, TB is a serious health problem in the poor and developing countries of Asia and Africa [1]. In Kuwait, there were 837 reported cases of TB among about 4.1 million residents in 2016 with an incidence rate of ca 24 per 100,000 people [2]. In addition to availability of new drugs and vaccines to control TB [3]-[5], cost-effective methods/reagents for specific diagnosis are required for global control and eradication of TB [6]-[9].

The purified protein derivative (PPD) of *M. tuberculosis* is routinely used, as a tuberculin skin test (TST), for the diagnosis of TB all over the world. Although, TST is a cost-effective method and is widely used in the diagnosis of TB, it often fails to distinguish BCG vaccination and exposure to non-tuberculous mycobacteria from *M. tuberculosis* infection [8], [9]. Inaccuracy of the TST reflects a low diagnostic specificity due to the presence in PPD of antigens shared by BCG and other environmental mycobacterial species [8], [9]. Therefore, diagnostic tests specific for *M. tuberculosis* are required to avoid the limitations of TST.

The comparative genome analyses of *M. tuberculosis* with other mycobacteria have revealed the presence of 11 *M. tuberculosis*-specific genomic regions that are absent/deleted in all BCG substrains used as vaccines against TB [10]-[15]. These regions are known as regions of differences (RDs) and include RD1, RD4-RD7, RD9-RD13 and RD15. The genes present in these RDs are predicted to encode 89 proteins of *M. tuberculosis*, which are considered specific to this bacterium [10]. If immunoreactive is found in serological assays, these proteins/peptides may be useful in the specific serodiagnosis of TB (both active and latent).

To evaluate the proteins of RDs in specific diagnosis of TB, overlapping synthetic peptides corresponding to 39 proteins located in five *M. tuberculosis*-specific RDs (RD1, RD4, RD5, RD6 and RD7) were designed and chemically synthesized. The sero-reactivity of the synthesized peptides was determined by testing serum samples from TB patients for antibodies in enzyme-linked immunosorbent assays (ELISAs). The serological specificity of the identified immunodominant peptides was determined by testing control sera from BCG-vaccinated healthy subjects. *M. tuberculosis*-specificity visa-a-visa cross-reactivity of the major seroreactive peptides and their immunodominance was predicted using publicly available online bioinformatics servers.

II. Materials and Methods

A. Serum Samples from Patients and Controls

Peripheral blood samples (5 ml) were collected in plain

The study was supported by Kuwait University grant no. MI02/12 and Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

Abu Salim Mustafa is with the Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait (phone: +965-24636505, e-mail: abusalim@hsc.edu.kw).

tubes from adult pulmonary TB patients (n = 100, recruited from the Chest Diseases Hospital, Kuwait) and BCG vaccinated healthy subjects (n =100, recruited from the Central Blood Bank, Kuwait), as described previously [16]. The study was approved by the Joint Ethics Committee of the Health Sciences Centre, Kuwait University, and the Ministry of Health, Kuwait. Informed consent was obtained from all subjects included in the study. The blood was kept overnight in a fridge, and the sera were separated from the clotted blood by centrifuging the samples at 250 x g for 5 minutes. The isolated sera were kept frozen at -20 ^{0}C until use.

B. Chemically Synthetized RD Peptides

Peptides (25-mers overlapping with neighboring peptides by 10 aa) corresponding to proteins encoded by genes predicted in five RDs of *M. tuberculosis*, i.e. RD1, RD4, RD5, RD6 and RD7 were synthesized using fluonerylmethoxycarbonyl chemistry, as described previously [17]-[19]. The stock concentrations of the peptides (5 mg/ml) were prepared in sterile phosphate buffered saline (PBS, pH 7.0) [20], [21]. The stocks were kept frozen in aliquots at -20 ^{0}C until tested. The working concentrations of peptides for coating the wells of ELISA plates were prepared by diluting the stocks in PBS (pH 7.0) to a final concentration of 10 µg/ml.

C. ELISA for Antibody Reactivity

The synthetic RD peptides were used to test for antibody reactivity in ELISA with the sera obtained from TB patients and healthy controls according to procedures described previously [16], [22]. In brief, the wells of 96-well PolySorb plates (ThermoFisher Scientific, Waltham, MA, USA) were coated by adding 100 µl of the coating concentrations (10 µg/ml) of peptide solutions and overnight incubation at 37 ºC. Wells lacking the coating peptide were used as blanks. The negative controls included peptide-coated wells lacking the addition of human serum (peptide control), and peptide non-coated wells followed by addition of human serum (serum control). All the wells were blocked with 100 µl of blocking buffer (0.17 M boric acid, 0.12 M NaCl, 0.05% Tween-20, 1 mM EDTA and 0.25% BSA, pH=8.0) for 1 h at 37 ºC. After three times washing with distilled water, 50 µl of human sera (diluted 1:100 in blocking buffer) were added to appropriate wells and the plates were incubated for 1 h at 37 ºC. All the wells were washed three times with distilled water and incubated further with 50 µl of anti-human IgG conjugated to alkaline phosphatase (diluted 1:1000 in blocking buffer, ThermoFisher Scientific) for 1 h at 37 ºC. The wells were washed three times with distilled water; 50 µl of the substrate p-nitrophenyl phosphatase (ThermoFisher Scientific) was added to each well, and the plates were incubated for 1 h at 37 ºC. The reactions were stopped by adding 25 µl of 3 M NaOH to each well, and the reaction intensities were measured by determining optical densities (OD) at 405 nm using an ELISA reader (Tecan Spectra, Austria). Each sample was tested in duplicate to obtain mean OD_{405} value. The results were analyzed using Windows Software Package for Statistical Analysis (SPSS) version 17.0 (Chicago, IL, USA). The median delta OD_{405} values (OD_{405} value obtained with serum plus peptide minus OD_{405} value obtained with serum or peptide alone, whichever was higher) were calculated for individual peptides with sera from TB patients and healthy subjects. The significant differences ($P < 0.05$) between the two groups of sera were determined using Mann-Whitney test and one-way ANOVA (analysis of variance).

The ELISA results for a specimen with a given peptide were considered positive with E/C $\geq$2 [16], [20], where E/C = Mean OD_{405} value in wells with a serum sample plus peptide / Mean OD_{405} value in wells with the same serum or peptide alone (whichever was higher).

D. Bioinformatics Analysis

The peptides identified as immunodominant in antibody reactivity were analyzed to predict antibody epitopes using artificial neural network based B-cell epitope prediction (ABCpred) server [23], [24]. The sequences of the immunodominant peptides were also searched for identical sequences in *M. tuberculosis* and other organisms using the online available Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI), USA [25]. (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). In addition, the current status of the proteins, to which the immunodominant peptides belonged, was searched in the *M. tuberculosis* database TubercuList [26].

III. Results and Discussion

In order to identify seroreactive peptides of RD proteins, all of the synthesized peptides were first tested in ELISA individually with sera from 10 TB patients. These experiments showed that 90 peptides, belonging to 28 proteins of RDs had ELISA positivity with one or more sera, but positivities with >50% sera were observed only with four peptides, i.e. aa 346-370 of RD1 protein Rv3876, aa 241-265 of RD6 protein Rv1508c, aa 136-160 of RD6 protein Rv1510 and aa 325-336 of RD6 protein Rv1516c (Table I). Further testing of these peptides with sera from 10 healthy subjects showed that peptide aa 346-370 of Rv3876, peptide aa 241-265 of Rv1508c, and peptide aa 325-336 of Rv1516c had weaker ELISA positivity with sera of healthy subjects, whereas the peptide aa 136-160 of Rv1510 had strong ELISA positivity with sera from healthy subjects as well (Table I).

The peptides showing stronger ELISA reactivity with TB sera but weak reactivity with healthy sera were further tested with additional sera to complete testing of 100 TB patients and 100 healthy subjects. In addition, the peptide aa 136-160 of Rv1510, which showed almost equal ELISA reactivity with sera from 10 TB patients and healthy subjects, was tested with a total of 50 sera from TB patients and 50 sera from healthy subjects. The analyses of overall results showed that sera from TB patients exhibited higher delta OD_{405} values with peptides aa 346-370 of Rv3876, aa 241-265 of Rv1508c, and aa 325-336 of Rv1516c (median delta OD_{405} values = 0.42, 0.81 and 0.37) as compared to sera from healthy subjects (median delta

OD_{405} values = 0.18, 0.22 and 0.16, respectively), and the delta OD_{405} values for all of these peptides were significantly higher with sera of TB patients than healthy subjects (P<0.0001). However, the ELISA reactivities with peptide aa 136-160 of Rv1510 were similar using sera of TB patients (median delta OD_{405} value = 0.38) and healthy subjects (median delta OD_{405} = 0.26), without a significant difference between the two groups (P >0.05). The analyses of ELISA positives (E/C ≥2) showed that sera from 70%, 93%, 46% and 66% TB patients, and sera from 28%, 28%, 50% and 10% healthy subjects were positive to peptides aa 346-370 of Rv3876, aa 241-265 of Rv1508c, aa 136-160 of Rv1510 and aa 325-336 of Rv1516c, respectively (Table II).

TABLE I
ELISA POSITIVITIES OF RD PEPTIDES WITH SERA FROM TB PATIENTS AND HEALTHY SUBJECTS

RD	Protein	Peptide	Number of TB patients positive/tested	Number of healthy subjects positive/tested
RD1	Rv3876	aa 346-370	9/10	0/10
RD6	Rv1508c	aa 241-265	8/10	1/10
RD6	Rv1510	aa 136-160	10/10	8/10
RD6	Rv1516c	aa 325-336	9/10	1/10

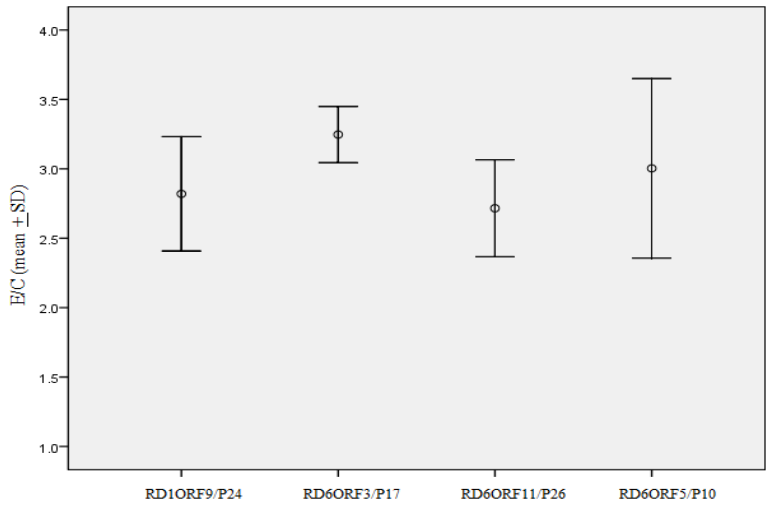
TABLE II
ELISA POSITIVITIES OF IMMUNODOMINANT PEPTIDES OF RD PROTEINS WITH SERA FROM TB PATIENTS (N=100) AND HEALTHY SUBJECTS (N=100)

RD	Protein	Peptide	Sero-positives (%) with sera from TB patients	Sero-positives (%) with sera from healthy subjects
RD1	Rv3876	aa 346-370	70	28
RD6	Rv1508c	aa 241-265	93	28
RD6	Rv1510	aa 136-160	46	50
RD6	Rv1516c	aa 325-336	66	10

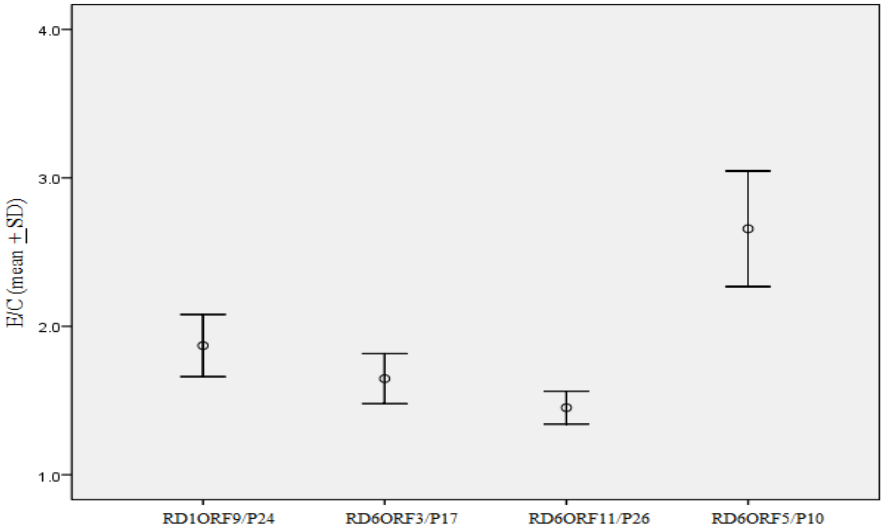
The E/C values (mean ± standard deviation) were analyzed using one-way ANOVA test for significant differences in reactivities to the above peptides in TB patients and healthy subjects. The analysis showed that ELISA reactivities were statistically similar to all the four peptides, i.e. aa 346-370 of Rv3876, aa 241-265 of Rv1508c, aa 325-336 of Rv1516c and aa 136-160 of Rv1510 in TB patients and to aa 346-370 of Rv3876, aa 241-265 of Rv1508c, aa 325-336 of Rv1516c in healthy subjects (P >0.05), whereas, as compared to other peptides, aa 136-160 of Rv1510 showed significantly higher reactivity in healthy subjects (P <0.0001) (Fig. 1).

The antibody reactivity of the four immunodominant peptides (aa 346-370 of Rv3876, aa 241-265 of Rv1508c, aa 136-160 of Rv1510 and aa 361-372 of Rv1516c) reactive with TB patient's sera was also predicted by analyzing the sequences using artificial neural network based B-cell epitope prediction (ABCpred) server [23], [24]. The server performs ranking of the predicted B cell epitopes of 10 to 20 aa length according to the score obtained by trained recurrent neural network, and higher score of the peptide means the higher probability to be an epitope [23]. It has been shown previously that the ABCpred is able to predict epitopes with 66% accuracy [23]. The results of the epitope prediction analysis suggested the presence of several high scoring epitopes (above the threshold value of 0.51) of varying length in all of the three peptides (Table III), thus strengthening the observation of their immunodominant recognition by antibodies in human sera.

As stated above, three of the four immunodominant peptides showed strong reactivity with TB patients' sera but weak reactivity with sera of BCG-vaccinated healthy subjects, whereas one of the peptides (aa 136-160 of RD6ORF Rv1510) showed equally good reactivity with sera from both groups of subjects (Table I). We have previously reported a similar finding with ESAT6, CFP10 and PPE68 in antigen-induced proliferation and IFN-ɣ secretion assays using peripheral blood mononuclear cells, i.e. ESAT-6 and CFP10 induced strong responses in TB patients and weak response in BCG-vaccinated healthy subjects, but PPE68 induced equally strong responses in both groups [19]. The BLAST search in NCBI database suggested that the strong reactivity to PPE68 in healthy subjects was due to sharing of its immunodominant sequence (aa 121-145) with proteins in BCG and environmental mycobacteria, whereas the sequences of ESAT-6 and CFP10 were specific for *M. tuberculosis* [14], [27].



(a)



(b)

Fig. 1 One-way ANOVA comparison of E/C values in (a) TB patients' sera and (b) healthy subjects' sera with P24 of R1ORF9 (aa 346-370 of Rv3876), P17 of RD6ORF3 (aa 241-265of Rv1508c), P26 of RD6ORF11(aa 325-336 of Rv1516c) and P10 of RD6ORF5 (aa 136-160 of Rv1510c)

TABLE III
EPITOPE PREDICTION, BY USING THE ABCPRED SERVER, IN THE SEQUENCE OF IMMUNODOMINANT PEPTIDES OF RV1510, RV3876, RV1508C AND RV1516C

Peptide	Predicted epitope	Prediction score
136-160 of Rv1510	FVEGRWLSVGLLSVGVAGFCAQATL	
	FVEGRWLSVGLLSVGVAGFC-----	0.65
	FVEGRWLSVGLLSVGVAG-------	0.74
	FVEGRWLSVGLLSVGV---------	0.57
	---------GLLSVGVAGFCAQATL	0.54
	-------SVGLLSVGVAGFCA----	0.65
	FVEGRWLSVGLLSV----------	0.58
	------LSVGLLSVGVAG-------	0.55
	--------VGLLSVGVAG-------	0.57
346-370 of Rv3876	TQKSLRPAAKGPKVKKVKPQKPKAT	
	TQKSLRPAAKGPKVKKVKPQ-----	0.83
	------PAAKGPKVKKVKPQKP---	0.78
	----LRPAAKGPKVKKVK-------	0.70
	---------KGPKVKKVKPQKPK—	0.66
	----------GPKVKKVKPQKP---	0.80
	----LRPAAKGPKVKK---------	0.75
	TQKSLRPAAK---------------	0.82
	------PAAKGPKVKK---------	0.76
241-265 of Rv1508c	ARGLERVVIFVTLGAAAIPAWGVID	
	----------VTLGAAAIPAWGVI-	0.81
	---LERVVIFVTLGAAAIP------	0.72
	---------FVTLGAAAIPAWGVID	0.67
	-RGLERVVIFVTLGAAAIP------	0.61
	----ERVVIFVTLGAAAIPAWGVI-	0.57
	-------VVIFVTLGAA--------	0.64
	ARGLERVVIF---------------	0.62
	------VVIFVTLGAAAI-------	0.67
	--GLERVVIFVTLG-----------	0.59
	----------VTLGAAAIPAWG---	0.51
325-336 of Rv1516c	VWPSRLRRGCRA	
	VWPSRLRRGCRA	0.62
	VWPSRLRRGC--	0.62

To determine if similar situation existed for the seroreactive peptides identified in this study, the sequences of all four peptides were searched for identity using BLAST search in the NCBI database. The results showed that, in addition to various isolates of *M. tuberculosis*, the sequences of peptide aa 136-160 of Rv1510 had appreciable identity with proteins in various strains of *M. bovis* BCG (72% identity) and 11 different species/strains of environmental and non-tuberculous mycobacteria (76% to 84% identity) (Table IV). These findings suggest that strong sero-reactivity of the peptide aa 136-160 of Rv1510 with healthy sera could be due to BCG vaccination and/or exposure of donors to environmental

mycobacteria. These results further show that at the level of genomic regions, the RDs of *M. tuberculosis* may be specific [10]-[14], but at the immunodominant peptide level substantial sequence identity may occur leading to immunological crossreactivity [14]. Thus, all immunodominant proteins/ peptides encoded by *M. tuberculosis*-specific RDs may not have diagnostic relevance.

The BLAST search for the remaining *M. tuberculosis*-specific peptides (aa 346-370 of Rv3876, aa 241-265 of Rv1508c, aa 325-336 of Rv1516c) showed that these immunodominant peptide sequences were 100% identical in various laboratory and clinical strains of *M. tuberculosis* but absent in BCG and other mycobacteria (Tables V and VI), except two species of non-tuberculous mycobacteria, i.e. *M. kansasii* (80% identity) and *M. marinum* (68% identity) in case of aa 241-265 of Rv1508c (Table VII). These findings suggest that differences in infecting *M. tuberculosis* strains found in different geographical locations may not affect the antibody reactivity to the identified peptides.

The search in Tuberculist database for descriptions and functions performed by the three proteins containing *M. tuberculosis*-specific immunodominant peptides suggested that Rv3876/EspI is a conserved hypothetical proline and alanine rich protein of unknown function (Table VIII), Rv1508c is a probable membrane protein, predicted to be in the GT-C superfamily of glycosyltransferases and Rv1516c is a probable sugar transferase, involved in cellular metabolism (Table VIII).

TABLE IV
RESULTS OF BLAST SEARCH FOR SEQUENCE IDENTITY OF IMMUNODOMINANT 25-MER PEPTIDE AA 136-160 OF RD6 PROTEIN RV1510 IN VARIOUS STRAINS OF *M. TUBERCULOSIS* AND OTHER MYCOBACTERIA

Mycobacterial species and strains	Sequence	Identity
M. tuberculosis H37Rv, H37 Ra, K85, CPHL_A, KZN, 605, C, CDC1551, F11, KZN1435, T92, T85, str. Haarlem	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVEGRWLSVGLLSVGVAGFCAQATL Sbjct 127 FVEGRWLSVGLLSVGVAGFCAQATL 151	25/25 100%
M. bovis AF2122/97, BCG Pasteur 1173P2	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 F E RWLSV LLS+G+AGFC ATL Sbjct 135 FAEARWLSVALLSIGLAGFCLHATL 159	10/25 72%
M. avium 104, Paratuberculosis K-10	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE R LSV LLSVG+AGFC ATL Sbjct 117 FVEARPLSVLLLSVGLAGFCVHATL 141	19/25 76%
M. smegmatis str. MC2 155	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE R LSV LLSVG+AGFC ATL Sbjct 129 FVESRALSVALLSVGLAGFCLHATL 153	19/25 76%
M. sp. KMS, *M. sp*. JLS	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE RWLSV LLS G+AGFC ATL Sbjct 117 FVEARWLSVLLLSAGLAGFCVHATL 141	19/25 76%
M. marinum, *M. ulcerans* Agy99	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE RWLSVGLLSVG+AGFC ATL Sbjct 103 FVEDRWLSVGLLSVGLAGFCLHATL 127	21/25 84%
M. kansasii ATCC 12478	Query 1 FVEGRWLSVGLLSVGVAGFCAQATL 25 FVE RWLSVGLLSVG+AGFC ATL Sbjct 132 FVEARWLSVGLLSVGLAGFCLHATL 156	21/25 84%

TABLE V
RESULTS OF BLAST SEARCH FOR SEQUENCE IDENTITY OF IMMUNODOMINANT 25-MER PEPTIDE AA 346-370 OF RD1 PROTEIN RV3876 IN VARIOUS STRAINS OF *M. TUBERCULOSIS* AND OTHER MYCOBACTERIA

Mycobacterial species and strains	Sequence	Identity
M. tuberculosis H37Rv, C, CDC1551, H37Ra, F11, KZN 1435	Query 1 TQKSLRPAAKGPKVKKVKPQKPKAT 25 TQKSLRPAAKGPKVKKVKPQKPKAT Sbjct 346 TQKSLRPAAKGPKVKKVKPQKPKAT 370	25/25 100%
M. bovis AF2122/97	Query 1 TQKSLRPAAKGPKVKKVKPQKPKAT 25 TQKSLRPAAKGPKVKKVKPQKPKAT Sbjct 346 TQKSLRPAAKGPKVKKVKPQKPKAT 370	25/25 100%

TABLE VI
RESULTS OF BLAST SEARCH FOR SEQUENCE IDENTITY OF IMMUNODOMINANT 25-MER PEPTIDE AA 241-265 OF RD6 PROTEIN RV1508C IN VARIOUS STRAINS OF *M. TUBERCULOSIS* AND OTHER MYCOBACTERIA

Mycobacterial species and strains	Sequence	Identity
M. tuberculosis H37Rv, CDC1551, H37Ra, F11, KZN 605, K2M 1435, C, str. Haarlem, EAS054, 98-R604, T17, K85, 'INH-RIF-EM', KZN 4207, GM1503, EAS054, T85, T92, T46, CPHLA, 021987	Query 1 LTMAVISPAIWAARGARGLERVVIF 25 LTMAVISPAIWAARGARGLERVVIF Sbjct226 LTMAVISPAIWAARGARGLERVVIF 250	25/25 100%
M. kansasii ATCC 12478	Query 1 LTMAVISPAIWAARGARGLERVVIF 25 LT AV+ PA+WAARGARGLERVV+F Sbjct149 LTGAVFTPAVWAARGARGLERVVVF 173	20/25 80%
M. marinum	Query 1 LTMAVISPAIWAARGARGLERVVIF 25 L +AV++PA+WA RGARGLER+V F Sbjct142 LVVAVLTPALWAVRGARGLERLVTF 166	17/25 68%

TABLE VII
RESULTS OF BLAST SEARCH FOR SEQUENCE IDENTITY OF IMMUNODOMINANT 12-MER PEPTIDE AA 361-372 OF RD6 PROTEIN RV1516C IN VARIOUS STRAINS OF *M. TUBERCULOSIS* AND OTHER MYCOBACTERIA

Mycobacterial species and strains	Sequence	Identity
M. tuberculosis H37Rv, H37Ra, F11, KZN 1435, str. Haarlem, CDC1551, C, 02 1987, 94 M4241A	Query 1 VWPSRLRRGCRA 12 VWPSRLRRGCRA Sbjct 362 VWPSRLRRGCRA 373	12/12 100%

TABLE VIII
THE DESCRIPTIONS AND FUNCTIONS OF RD1 PROTEIN RV3876 AND RD6 PROTEINS RV1508C AND RV1516C USING TUBERCULIST SERVER

Protein (no. of amino acids)	Description / Function
Rv3876/EspI (666 aa)	Conserved hypothetical proline and alanine rich protein of unknown function
Rv1508c (599 aa)	Probable membrane protein, predicted to be in the GT-C superfamily of glycosyltransferases
Rv1516c (336 aa)	Probable sugar transferase, involved in cellular metabolism

IV. CONCLUSION

Three peptides (aa 346-370 of Rv3876, aa 241-265 of Rv1508c and aa 325-336 of Rv1516c) of RD proteins showed strong reactivity with TB patients' sera, but weak reactivity with non-TB sera. The bioinformatics analyses suggested that these peptides have multiple antibody epitopes and are specific for *M. tuberculosis*. These observations suggest that the *M. tuberculosis*-specific peptides may have a role in the serodiagnosis of TB.

ACKNOWLEDGMENT

The technical support by Fatima Hussain and Noura Al-Khodary for conducting ELISA experiments is gratefully acknowledged.

REFERENCES

[1] WHO Report. 2017. Global tuberculosis report. http://www.who.int/tb/publications/global_report/en/.
[2] World Health Organization. 2017. Tuberculosis country profiles. TB burden estimates and country-reported TB data. Kuwait. http://www.who.int/tb/country/data/profiles/en/.
[3] Mustafa AS. 2009. Vaccine potential of *Mycobacterium tuberculosis*-specific genomic regions: in vitro studies in humans, *Expert Rev Vaccines* 8: 1309-1312.
[4] Mustafa AS. 2012. What's New in the Development of Tuberculosis Vaccines. *Med Princ Pract* 21:195-196.
[5] Mustafa AS. 2012. Proteins and peptides encoded by *M. tuberculosis*-specific genomic regions for immunological diagnosis of tuberculosis. *J Mycobac Dis* 2:e114.
[6] Mustafa AS. 2013. Diagnostic and vaccine potentials of ESAT-6 family proteins encoded by *M. tuberculosis* genomic regions absent in *M. bovis* BCG. *J Mycobac Dis* 3:129.
[7] Mustafa AS. 2013. In silico analysis and experimental validation of *Mycobacterium tuberculosis*-specific proteins and peptides of *Mycobacterium tuberculosis* for immunological diagnosis and vaccine development. *Med Princ Pract* 22 (Suppl 1): 43-51.
[8] Mustafa AS. 2014. The future of *Mycobacterium tuberculosis*-specific antigens/peptides in tuberculin skin testing for the diagnosis of tuberculosis. *J Mycobac Dis* 4:3.
[9] Mustafa AS, Al-Attiyah R. 2004. *Mycobacterium tuberculosis* antigens and peptides as new vaccine candidates and immunodiagnostic reagents against tuberculosis. *Kuwait Med J* 36: 171-176.
[10] 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.
[11] Al-Attiyah R, Mustafa AS. 2010. Characterization of human cellular immune responses to *Mycobacterium tuberculosis* proteins encoded by genes predicted in RD15 genomic region that is absent in *Mycobacterium bovis* BCG. *FEMS Immunol Med Microbiol* 59: 177-87.
[12] Mustafa AS, Al-Saidi F, El-Shamy ASM, Al-Attiyah R. 2011. Cytokines in response to proteins predicted in genomic regions of difference of *Mycobacterium tuberculosis*. *Microbiol Immunol* 55:267-278.
[13] Mustafa AS. 2014. T-helper 1, T-helper 2, pro-inflammatory and anti-inflammatory cytokines in tuberculosis. *IJPMB* 3:1-14.
[14] Mustafa AS. 2014. Characterization of a cross-reactive, immunodominant and HLA-promiscuous epitope of *Mycobacterium tuberculosis*-specific major antigenic protein PPE68. *PLoS One* 9:e103679.
[15] Hanif SNM, 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.
[16] Al-Khodari NY, Al-Attiyah R, and Mustafa AS. 2011. Identification, diagnostic potential and natural expression of immunodominant seroreactive peptides encoded by five *Mycobacterium tuberculosis*-specific genomic regions. *Clin Exp Immunol* 18:477-482.
[17] Mustafa AS. 2009. HLA-promiscuous Th1-cell reactivity of MPT64 (Rv1980c), a major secreted antigen of *Mycobacterium tuberculosis*, in healthy subjects. *Med Princ Pract* 18:385-392.
[18] Mustafa AS, Al-Attiyah R 2009 Identification of *Mycobacterium tuberculosis*-specific genomic regions encoding antigens those induce qualitatively opposing cellular immune responses. *Ind J Exp Biol* 47:498-504.
[19] 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
[20] Mustafa AS. 2009. Th1-cell reactivity and HLA-DR binding prediction for promiscuous recognition of MPT63 (Rv1926c), a major secreted protein of *Mycobacterium tuberculosis*. *Scand J Immunol* 69:213-222.
[21] Al-Attiyah R, Mustafa AS. 2004. Computer-assisted prediction of HLA-DR binding and experimental analysis for human promiscuous Th1-cell peptides in the 24 kDa secreted lipoprotein (LppX) of *Mycobacterium tuberculosis*. *Scand J Immunol* 59:16-24.
[22] El-Shazly S, Mustafa AS, Ahmad S, Al-Attiyah R. 2007. Utility of three mammalian cell entry proteins of *Mycobacterium tuberculosis* in serodiagnosis of tuberculosis. *Inter J Tuber Lung Dis* 11:676-682.
[23] Saha S, Raghava GPS. 2006. Prediction of continuous B-Cell epitopes in an antigen using recurrent neural network. *Proteins* 65:40–48.
[24] IEDB. June 2010, updating date. Epitope prediction and analysis tools. http://tools.immuneepitope.org.
[25] Protein BLAST: search protein databases using a protein query - NIH. https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins (accessed on December 10, 2017).
[26] TubercuList - GenoList - Institut Pasteur. http://genolist.pasteur.fr/TubercuList/ (accessed on December 17, 2017).
[27] Okkels LM, Brock I, Follmann F, Agger EM, SArend SM, Ottenhoff THM, Oftung F, Rosenkrands I, Andersen P. 2003. PPE protein (Rv3873) from DNA segment RD1 of *Mycobacterium tuberculosis*: strong recognition of both specific T-cell epitopes and epitopes conserved within the PPE family. *Infect Immun* 71:6116-6123.