International Journal of Pharma Medicine and Biological Sciences

ISSN 2278 – 5221 www.ijpmbs.com
Vol. 2, No. 3, July 2013

Research Paper

IN SILICO ANALYSIS OF OUTER MEMBRANE PROTEIN 31 OF *BRUCELLA* SPP. TO IDENTIFY AND CHARACTERIZE THE POTENTIAL T CELL EPITOPE

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Human vaccines for brucellosis are still not licensed and in some cases, animal vaccines are used for humans. We herein conducted a study to identify and characterize a suitable T cell epitope, which might be efficient against *Brucella* spp. We have assessed all the antigenic outer membrane proteins of *Brucella* spp recorded to date in Protegen (http://www.violinet.org/protegen/index.php) database and found that outer membrane protein (Omp31) of *Brucella melitensis* M28 was superior among all others. Analysis of Omp31 of *Brucella melitensis* M28 with NetMHCII 2.2, SYFPEITHI, ProPred and NetCTL 1.2 servers for identification of T cell epitope predicted an epitope 125-VRARLGYTATERLMV-139. These four servers also predicted that the epitope could bind with at least 6 major histocompatibility complex class-II (MHC II) and 5 MHC I molecules. The epitope shared ~86.93% identity with Omp31 of all human antigenic *Brucella* spp. Analysis with different bioinformatics tools revealed that this epitope was highly stable and capable to induce T cell-mediated immunity. Finally, molecular docking simulation showed that it could bind with the binding groove of MHC II and MHC I molecules with numerous significant hydrogen bonds. Results reported herein indicate that 125-VRARLGYTATERLMV-139 might be the target for epitope-based vaccine in humans against brucellosis.

Keywords: Adhesin, *Brucella*, Brucellosis, Epitope, Molecular docking, Outer membrane protein 31

INTRODUCTION

Brucellosis is one of the most usually occurring re-emerging zoonotic infections, with more than half a million new cases reported worldwide every year although the number of cases reported is considered to be largely underrated (Pappas *et al.,* 2006). It is a systemic infection in humans and manifest with multitudinous non-specific symptoms (e.g., fever, sweating, malaise, anorexia, headache, back pain etc.) as well as

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substantial residual disability. Because of its high incidence in developing countries, economic consequences and difficult eradication, the World Health Organization has considered brucellosis as one of the seven neglected zoonoses, a group of diseases that contribute to the perpetuation of poverty (Maudlin *et al.,* 2009). Brucellosis is caused by *Brucella* spp, which are gram-negative coccobacillus. They are facultative intracellular pathogens and localized predominantly in organs such as lung, spleen, liver, bone marrow and synovium (Akhvlediani *et al.,* 2010). Among six recognized *Brucella* species, *Brucella melitensis*, *B. abortus*, *B. suis*, and *B. canis* are pathogenic to human and *B. melitensis* is more pathogenic over others (Pappas *et al.,* 2006; Sarinas and Chitkara, 2003). In human, this bacterial infection can occur through consumption of contaminated and unpasteurized animal products, direct contact with infected animal parts and the inhalation of infected aerosolized particles, and is considered as an occupational disease in shepherds, abattoir workers, veterinarians, dairy-industry professionals and personnel in microbiology laboratories (Almuneef *et al.,* 2004; Corbel, 1997; Franco *et al.,* 2007). It can also be transmitted from person-to-person (Ertem *et al.,* 2000; Meltzer *et al.,* 2010; Mesner *et al.,* 2007).

Complete eradication of brucellosis from certain area is not possible until the natural reservoir of *Brucella* spp, i.e., cattle, sheep, goat, humans, etc., remain unprotected. There is currently no licensed vaccine against brucellosis to be used in the humans and not a single attempt of clinical trial was reported to date (Perkins *et al.,* 2010). Lack of human vaccines of brucellosis creates more complications to control animal brucellosis and development of vaccines of humans could limit the natural reservoir of *Brucella* spp. Antibiotics used for treatment of human brucellosis includes tetracycline, trimethoprim-sulphamethoxazole, aminoglycosides, rifampicin, quinolines and chloramphenicol (Al-Tawfiq, 2008). Combinations of some of these antibiotics are usually used because high incidence of brucellosis relapse in single application, and the relapse rates are habitually reported between 5% and 40% (Perkins *et al*., 2010). Therefore, a vaccine should be designed to be safe and effective for using in humans. Computer aided design of epitope based vaccines have attracted considerable concentration lately as a potential, inexpensive and easy means of vaccine development for treating infectious disease (Barh *et al*., 2010; He and Xiang, 2010; He *et al*., 2010). Now-a-days, functional annotation of several pathogenic bacteria and the advent of human genome boosts epitope-based vaccine design. An epitope-based vaccine immunizes the host with a minimal structure, consisting of a well-defined antigen that avoids potential undesirable effects. It was shown experimentally that a synthetic peptide mimicked to epitope could induce immune response against native protein of bacteria causing meningitidis and tuberculosis and of virus causing influenza (Ben-Yedidia and Arnon, 2007; Shinnick *et al*., 1984; Wang *et al*., 2012). Surface and secreted proteins of any pathogen are mostly antigenic and are responsible for pathogenicity. Hence, suitable antigenic determinants of *Brucella* outer membrane proteins (Omps) might be potential targets to develop epitope-based vaccine against brucellosis. It has been shown that outer membrane protein 31 (Omp31) is not essential for virulence in *B. melitensis* Rev.1, but this protein is essential for membrane stability (Guilloteau *et al*., 2006), iron intake from the host (Delpino *et*

al., 2006) and colonization (Caro-Hernandez *et al*., 2007). Antigenicity of Omp31 has been confirmed by applying as DNA vaccine in model animals and prefigured as a potential precursor for epitope-based vaccine (Cassataro *et al*., 2005a; Cassataro *et al*., 2007; Cassataro *et al.*, 2005b; He and Xiang, 2010). However, the characteristics of T cell epitopes of Omp31 and their interaction with the Major Histocompatibility Complex (MHC) have not been elucidated. We herein investigated the T cell epitopes of Omps from *Brucella* spp. Based on analysis with numerous bioinformatics tools, Omp31 from *B. melitensis* M28 showed the best cell epitope.

MATERIALS AND METHODS

Identification of T Cell Epitope

The antigenicity of Omps were analyzed with the antigen database and analysis server Protegen (http://www.violinet.org/protegen/index.php), protective antigens and subunit vaccines prediction server VaxiJen (Doytchinova and Flower, 2007) and vaccine target prediction and analysis server Vaxign (http://www.violinet.org/vaxign/index.php). The threshold value in VaxiJen was kept 0.5. After confirmation of highest antigenicity of Omp31, all the amino acid sequences of Omp31 of human pathogenic *Brucella* spp were retrieved from the protein database (http://www.ncbi.nlm.nih.gov/protein) and the non-identical sequences were subjected for multiple sequence alignment analysis with T-Coffee server (http://tcoffee.crg.cat/apps/tcoffee/do:regular). For identification of efficient T cell epitope from Omp31 with strong MHC II binding affinity, artificial neural network based computer program NetMHCII 2.2 (Nielsen and Lund, 2009), motif matrices based SYFPEITHI (Schuler *et al*., 2007) and quantitative matrices based ProPred (Singh and Raghava, 2001) servers were used. To predict MHC I binding ability, the epitope sequence was subjected to NetCTL 1.2 Server (http://www.cbs.dtu.dk/services/NetCTL/; Larsen *et al.*, 2007). Binding ability of the epitope was analyzed against all available MHC I HLA supertypes. Threshold value for ' Weight on C terminal cleavage', ' Weight on TAP (transport associated antigen processing) transport efficiency' and 'Epitope identification' were kept 0.15, 0.05 and 0.75, respectively. Physico-chemical property of epitope was analyzed with ProtParam computer program (http://web.expasy.org/protparam/; Wilkins *et al.*, 1999).

Retrieval of 3D-Structure

The 3D-structure of epitope was built using PyMol molecule builder as described previously (Patronov *et al*., 2011). There was no experimental structure available in Protein Data Bank (PDB) (http://www.pdb.org/pdb/home/home.do) for HLA-DRB1*0701 and HLA-DRB1*0901 molecules. The 3D structures of these two proteins were obtained using homology modeling with SWISS-MODEL (http://swissmodel.expasy.org; Schwede *et al.*, 2003). The homology models of HLA-DRB1*0701 (Uniprot KB ID P13761) and HLA-DRB1*0901 (Uniprot KB ID Q0R326) were constructed based on the experimental structure of HLA-DR1 beta chain (PDB ID, 1AQD-b) and DRB1-1 beta chain (PDB ID, 3PGD-b), respectively. The amino acid sequence identity of HLA-DRB1*0701 and HLA-DRB1*0901 with HLA-DR1 and DRB1-1 beta chains are 89.31% and 87.90%, correspondingly. To evaluate how much the structural models deviate from the template, the Root-Mean-Square Deviation (RMSD) value between the template and the homology model superposed was determined by Internal Coordinate Mechanics

method based computer program Molsoft Internal Coordinate Mechanics (ICM)-pro 3.5 (Totrov and Abagyan, 1997). The 3D structure of HLA-DRB1*1101 (Uniprot KB ID P20039) was taken from Swiss-Model Repository (Kiefer *et al*., 2009). The stereochemical quality of the homology models were assessed by the PROCHECK program through PDBsum server (Table 1) (Laskowski, 2001). The PDB ID for experimental structures of HLA-DRA (Uniprot KB ID P01903), HLA-DRB1*0101 (Uniprot KB ID P04229) and HLA-DPB1*0401(Uniprot KB ID P04440) were 1A6A-a, 1SJE-b and 3LQZ-b, respectively. One representative from each of MHC I HLA supertypes A1, A3, B7, B8 and B27 were retrieved from protein data bank. PDB ID for experimental structures of HLA supertype A1 (Uniprot KB ID P30443), A3 (P04439), B7 (P30685), B8 (P30460) and B27 (P03989) were 3BO8-a, 3RL2-a, 3BWA-a, 1M05-a and 1OGT-a, respectively.

Peptide Docking

The 3D structure of the Omp31 T cell epitope built by PyMol molecule builder was subjected for molecular docking simulation with MHC I and II molecules by Molsoft ICM-pro 3.5. The Omp31 T cell epitope could bind with MHC II (HLA-DRB1*0701, HLA-DRB1*0101, HLA-DRB1*1101, HLA-DRB1*0901, HLA-DPB1*0401 and HLA-DPB1*0402) and MHC I (HLA-A1, HLA-A3, HLA-B7, HLA-B8 and HLA-B27) molecules. According to Uniprot KB, HLA-DPB1*0401 and HLA-DPB1*0402 have the same 3D structure (PDB ID, 3LQZ-b) and for this, molecular docking of the epitope was conducted against five HLA-DRB 3D structures mentioned above except HLA-DPB1*0402. Ionizable groups in the protein structures were transformed into the protonated states and ICM default partial atomic charges were set. ICM pocket finder macro was used for pocket selection, and after receptor positioning, the ICM method based molecular docking was done as describe previously (Khan and Ranganathan, 2010). Best docking orientation was selected based on binding free energy and hydrogen bond distance.

RESULTS

Identification and Characterization of T Cell Epitope

Protegen analysis showed that 21 experimentally verified protective antigens have been reported in *Brucella* spp to date. Among the 21 *Brucella* antigenic proteins, four are Omps, seven are cytoplasmic and four are periplasmic proteins. The localizations of the rest six proteins are still unknown. Four Omps are MotY, lipoprotein Omp19, 25 kDa outer membrane immunogenic protein and Omp-like transmembrane domain (Yang *et al*., 2011). Bacterial surface proteins are potential vaccine candidates, and recently it has been shown that Omp31 (protein accession No.ADZ67646) is present in virulent *B. melitensis* M28 and in its attenuated strain, *B. melitensis* M5-90 which is used as attenuated vaccine (Wang *et al*., 2011a). Antigenicity of Omps of *Brucella* spp analyzed with Vaxign showed that the adhesin probability of Omp31 of *B. melitensis* M28 was higher than other antigenic Omps (Table 2). The antigen probability was further analyzed with VaxiJen server. The antigen probability for Omp31 of *B. melitensis* M28 was 0.7050, whereas that of Omp19 was 0.6580. These results indicated that Omp31 had higher antigenicity than Omp19. Omp31 of *B. melitensis* M28 was then subjected to the NetMHCII 2.2 web server (http://www.cbs.dtu.dk/services/NetMHCII/) and analyzed against 26 MHC II molecules available

Table 1: Ramachandran Plot Statistics Computed with PROCHECK Program

Plot Statistics	DRB1*11O1	DRB1*0701	DRB1*0901
% residues in favorable regions	77.6	89.5	89.2
% residues in additional residue regions	21.8	9.9	10.8
% residues in generously regions	0.6	0.0	0.0
% residues in disallowed regions	0.0	0.6	0.0
% of non Proline and non Glycine residues	100.00	100.00	100.00

Table 2: Comparison of antigenicity of Omps of *Brucella* spp analyzed with Vaxign computer program

	Vaxign analysis				
Protein Name	**Accession Number**	**Localization Probability**	**Adhesin Probability**	**Trans-membrane Helices**	**No. of Similar human Protein**
MotY of *B. melitensis* biovar Abortus 2308	YP_415057	OM[a] (Probability=1)	0.39	0	0
lipoprotein Omp19 of *B. melitensis* biovar Abortus 2308	YP_415269	OM (Probability=1)	0.66	0	0
25 kDa outer-membrane immunogenic protein precursor of *B. melitensis* bv. 1 str. 16M	NP_540166	OM (Probability=1)	0.55	0	0
OmpA-like transmembrane domain-containing protein of *B. melitensis* biovar Abortus 2308	YP_414995	OM (Probability=1)	0.57	0	0
Omp31of *B. melitensis* M-28	ADZ67646	OM (Probability=1)	0.67	0	0

Note: [a]OM stands for outer membrane.

in this database for T cell epitope. Omp31 showed strong binding capacity with 13 MHC II molecules (Table 3). The strongest epitope (125-VRARLGYTATERLMV-139) was found against HLA-DRB 1*0701 that showed affinity 3.5 nM, whereas below 50 nM is categorized as strong binders in NetMHCII 2.2 server. Results in Table 3 indicated that the epitope (125-VRARLGYTATERLMV-139) might be a good candidate of promiscuous peptide to develop epitope-based vaccine. The binding capability of the epitope with MHC II molecules was further assessed with ProPred (http://www.imtech.res.in/raghava/propred/) and SYFPEITHI (http://www.syfpeithi.de/home.htm) web servers. Both approaches showed the same epitope as the strongest epitope (Table 4). The epitope's ability to induce immunity was also predicted with VaxiJen that revealed the probability as 0.5641. This result indicated that the epitope could elicit strong

Figure 1: Multiple alignment of protein sequences of Omp31 from *Brucella* spp. Accession numbers of Omp31 from *B. melitensis* were ACV07678 (*B. mel*_1), ACS50328 (*B. mel*_2), ADZ67646 (*B. mel*_M28), ADZ88512 (*B. mel*_M5-90), ACQ84164 (*B. mel*_3), YP_002733324 (*B. mel*_4), AAL27285 (*B. mel*_5) and ADL14702 (*B. mel*_6). Accession numbers of Omp31 from *B. suis* were AAL27290 (*B. suis*_1), AAL27289 (*B. suis*_2), AAL27287 (*B. suis*_3) and AAN30527 (*B. suis*_4), and those of Omp31 from *B. abortus* and *B. canis* were AAS84568 and AAL27296, respectively. The best epitope based on conservation and NetMHCII 2.2 analysis is shown in the box. The epitope sequence from *B. melitensis* M28 is underlined. Non-identical epitope sequences are shaded and the animo acid residues for which the epitope varies are italic and underlined

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B.mel_M28       -------------------MFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    70
B.mel_M5-90     ------------------------MAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    64
B.mel_1         --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    81
B.canis         --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    81
B.suis_1        --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    81
B.suis_2        --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDATAGG    81
B.suis_3        --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDIEQVSGSLDVTAGG    81
B.suis_4        MFSLKGTVMKTALLASVAMLFTSSAMAADIIVAEPAP---VAVDTFSWTGGYIGINAGYAGGKFKHPFSGIEQDGAQDFSGSLDVTASG    86
B.aortus        MFSLKGTVMKTALLASVAMLFTSSAMAADIIVAEPAP---VAVDTFSWTGGYIGINAGYAGGKFKHPFSGIEQDGAQDFSGSLDVTASG    86
B.mel_3         --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    81
B.mel_2         --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    81
B.mel_5         --------MKSVILASIAAMFATSAMAADVVVSEPSAPTAAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    81
B.mel_6         --------MKSVILASIAAMFATSAMAADVVVSEPSAPTVAPVDTFSWTGGYIGINAGYAGGKFKHPFSSFDKEDNEQVSGSLDVTAGG    81
B.mel_4         ------------------MLQYS--------------------DRHWS----------------PY------WAQDFSGSLDVTASG    27
                                                            *:                *:        ::.*****.**.*

B.mel_M28       FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   147
B.mel_M5-90     FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   141
B.mel_1         FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERVMVYGTGGLAY   158
B.canis         FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GPIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.suis_1        FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.suis_2        FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.suis_3        FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.suis_4        FVGGVQAGYNWQLANGLVLGGEADFQGSTVKSKLVDNGDLSDIGVAGNLSGDESFGLETKVQWFGTVRARLGFTPTERLMVYGTGGLAY   175
B.aortus        FVGGVQAGYNWQLANGLVLGGEADFQGSTVKSKLVDNGDLSDIGVAGNLSGDESFVLETKVQWFGTVRARLGFTPTERLMVYGTGGLAY   175
B.mel_3         FVGGVQAGYNWQLDNGVVLGAGTDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.mel_2         FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.mel_5         FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.mel_6         FVGGVQAGYNWQLDNGVVLGAETDFQGSSVT------GSIS-AGASG-LEG----KAETKVEWFGTVRARLGYTATERLMVYGTGGLAY   158
B.mel_4         FVGGVQAGYNWQLANGLVLGGEADFQGSTVKSKLVDNGDLSDIGVAGNLSGDESFGLETKVQWFGTVRARLGFTPTERLMVYGTGGLAY   116
                ************ **:***. :*****:*.      * :*  *.:* *.*      ****:*********:*.***:**********

B.mel_M28       GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR-- NLVDVDNS-FLESKVNFHTVRVGLNYKF      229
B.mel_M5-90     GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      223
B.mel_1         GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.canis         GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.suis_1        GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.suis_2        GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.suis_3        GKVKSAFNLGDDASALHTWPDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.suis_4        GKVKTSLSAYDDGESFSAGNSKTKAGWTLGAGVEYAVTNNWTLKSEYLYTDLGKRSFNYIDEENVNINMENKVNFHTVRLGLNYKF      261
B.aortus        GKVKTSLSAYDDGESFSAGNSKTKAGWTLGAGVEYAVTNNWTLKSEYLYTDLGKRSFNYIDEENVNINMENKVNFHTVRLGLNYKF      261
B.mel_3         GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.mel_2         GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.mel_5         GKVKSAFNLGDDASALHMWSDKTKAGWTLGAGAEYAINNNWTLKSEYLYTDLGKR--NLVDVDNS--FLESKVNFHTVRVGLNYKF      240
B.mel_6         GKVKSAFNLGDDASALHTWSDKTKAGWTLGAGAEYAFNNNWTLKSEYLYPYLGKR--NLVDVDNS--FLESKVNFHTDRFGLNYKF      240
B.mel_4         GKVKTSLSAYDDGESFSAGNSKTKAGWTLGAGVEYAVTNNWTLKSEYLYTDLGKRSFNYIDEENVNINMENKVNFHTVRLGLNYKF      202
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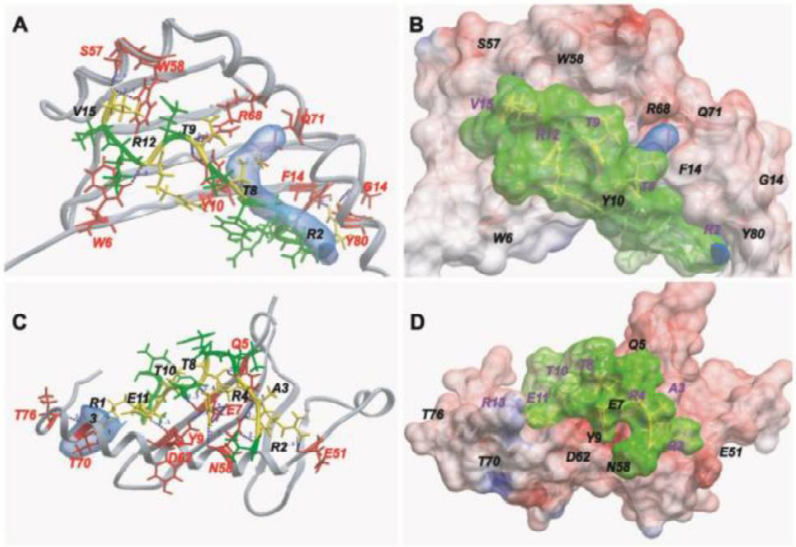
antibody production and cytotoxic T-cell response.

Analysis with NetCTL 1.2 showed that the 9-mer of the predicted epitope (125-VRARLGYTATERLMV-139) could bind significantly with five MHC I HLA supertypes namely A1, A3, B7, B8 and B27. The 9-mer epitope that could bind with A1, A3 and B27 MHC I HLA supertypes were 131-YTATERLMV-139, 128-RLGYTATER-136 and 127-ARLGYTATE-135, respectively. However, the 9-mer epitope 126-RARLGYTAT-134 could bind with both MHC I HLA-B7, B8 supertypes. To predict the universal effectiveness of the epitope, we retrieved all 202 (including 73 NCBI reference sequences) Omp31 sequences of human pathogenic *Brucella* spp from protein database and found 14 non-identical

Figure 2: Docking of the epitope with HLA-DRB1*0701 and HLA-DRA. Bird eye representation of the ribbon structures of epitope-HLA-DRB1*0701complex (A) and epitope-HLA-DRA complex (C) are shown. MHC II structures are shown as grey ribbon and the amino acid residues involved in the H-bonding network are shown as red sticks and labeled red. The epitope is shown as green ribbon and the amino acid residues involved in the H-bonding network are shown as yellow sticks and labeled black. MHC II binding pocket in the groove is shown as bluish electrostatic sphere. H-bonds are displayed as blue spheres and the H-bonding distances are labeled blue. Electrostatic views of epitope-HLA-DRB1*0701complex and epitope-HLA-DRA complex are represented in (B) and (D). MHC II structures are shown in reddish white color and the amino acid residues involved in the H-bonding network are labeled black. The epitope is shown as green and the amino acid residues involved in the H-bonding network are labeled purple. H-bonding network between the amino acid residues of the epitope and those of the MHC II are detailed in Table 5



Omp31 sequences. These non-identical sequences were then subjected to T-Coffee server for multiple sequence alignment to find out the identity and the universality of the predicted epitope among all human pathogenic *Brucella* spp (Figure 1). Figure 1 showed that all the sequences have high identity after the N-terminal region. However, except the sequence from *B. melitensis*-4, all other sequences bear high identity from N-termini to C-termini. Figure 1 revealed that the predicted strongest epitope was 100% identical in the Omp31 sequences from 10 *Brucella* spp and 86.93% identical in all 14 Omp31 sequences. Physico-chemical properties of this epitope showed that it had molecular weight of 1736.0 Da with 250 atoms ($C_{75}H_{130}N_{24}O_{21}S_1$). Analysis with the ProtParam computer program revealed that the half-life of the epitope in mammalian cells was 100 h and instability index was 10.85. This result indicated the high stability of the epitope since the ProtParam instability index smaller than 40 is predicted as stable.

Docking of Predicted T Cell Epitope to MHC Molecules

Molecular docking was performed to analyze the interaction of the epitope with MHC molecules. Docking showed that the protrusion of epitope

side chains bound into cavities within the groove of homology models of MHC II HLA-DRB1*0701 through 11 hydrogen bonds (Figures 2A and B; Table 5). Through docking simulation, it was found that the binding energy between the epitope and the HLA-DRB1*0701 was –259.58 kcal/mol whereas ≤ –32 kcal/ mol is generally considered as good score in ICM method (Totrov and Abagyan, 1997). The RMSD value between the template HLA-DR1 beta chain and the model of HLA-DRB1*0701 was 0.08 Å. As HLA-DRA does not have polymorphisms in the peptide binding part and acts as the sole alpha chain for HLA-DRB1, HLA-DRB3, HLA-DRB4 and HLA-DRB5, we performed molecular docking simulation between the epitope and HLA-DRA (Figures 2C and D). Docking analysis revealed that the epitope was bound to the groove by 15 hydrogen bonds (Table 5). The binding energy between the epitope and the HLA-DRA was -219.49 kcal/mol. Molecular docking of the epitope was further performed with HLA-DRB1*1101, HLA-DRB1*0901, HLA-DRB1*0101 and HLA-DPB1*0402 (Figure 3). Docking analysis showed that the epitope side chains bound into cavities within the grooves of HLA-DRB1*1101, HLA-DRB1*0901, HLA-DRB1*0101 and HLA-DPB1*0402 through 15, 7, 8 and 6 hydrogen bonds, respectively, and the binding energy of the epitope with these MHC II molecules were -258.77, -255.78, -172.47 and -183.54 kcal/mol, correspondingly (Table 5). The RMSD value between the template DRB1-1 beta chain and the model of HLA-DRB1-0901 was 0.072 Å. The RMSD values between the epitope and the epitope-MHC II complexes with HLA-DRB1*0701, HLA-DRA, HLA-DRB1*1101, HLA-DRB1*0901, HLA-DRB1*0101 and HLA- DPB1*0402 were 5.86, 8.80, 4.51, 5.35, 7.72 and 8.06 Å, respectively. Docking of the 9-mer of the predicted epitope was performed with MHC I molecules. Docking analysis showed that the 9-mer epitope 131-YTATERLMV-139 bound to HLA-A1 through 9 hydrogen bonds with the binding energy of -180.61 kcal/mol (Figure 4; Table 6). Epitopes 128-RLGYTATER-136 and 127-ARLGYTATE-135 bound into cavities within the grooves of HLA-A3 and HLA-B27 through 19 and 6 hydrogen bonds, respectively, and the binding energy with these MHC I molecules were -222.61 and -178.88 kcal/mol, correspondingly (Figure 4; Table 6). The epitope 126-RARLGYTAT-134 bound into cavities within the grooves of HLA-B7 and HLA-B8 through 11 and 16 hydrogen bonds, respectively, and the binding energy with these MHC I molecules were -157.94 and -196.45 kcal/mol, correspondingly (Figure 4; Table 6).

DISCUSSION

Epitope-based vaccine is specific to induce immunity against selected epitope(s) and avoid the immune responses induced by unfavorable epitopes of the antigen to create less complication (Wang *et al.*, 2011b). Along with increased safety, epitope-based vaccines give the opportunity to engineer the epitope to increase its universality, potency and breadth (Sette and Fikes, 2003). In addition, epitope-based vaccines have been shown to be successful in various infectious diseases, such as *Neisseria meningitides* infection, influenza and tuberculosis (Ben-Yedidia and Arnon, 2007; De Groot *et al.*, 2005). Small epitopes (usually 8-15 amino acid length) can be delivered easily by any of the following devices such as peptide-adjuvant conjugates (lipid-core peptides), peptide amphiphiles, lipid-based synthetic vesicles, endogenous exosomes containing peptide-loaded MHC molecules,

Table 3: Binding of Epitopes of Omp31 from *B. melitensis* M28 against MHC II Molecules[a]

MHC II Alleles	No. of Strong Binding Epitope	Strongest epitope sequence			No. of Weak Binding Epitope
		Affinity (nM)	Full Epitope Sequence (15 mer)	Core Peptide (9 mer)	
HLA-DRB1*0101	34	6.30	TGGYIGINAGYAGGK	YIGINAGYA	89
HLA-DRB1*0301	6	8.8	RNLVDVDNSFLESKV	VDVDNSFLE	13
HLA-DRB1*0701	24	3.5	VRARLGYTATERLMV	LGYTATERL	49
HLA-DRB1*1101	7	18.8	RLGYTATERLMVYGT	YTATERLMV	31
HLA-DRB5*0101	18	16	YIGINAGYAGGKFKH	INAGYAGGK	51
HLA-DPA 1*0301-DPB1*0402	4	25.7	RLGYTATERLMVYGT	YTATERLMV	20
HLA-DQA 1*0102-DQB1*0602	1	12.5	MFATSAMAADVVVSE	MFATSAMAA	43
HLA-DQA 1*0501-DQB1*0301	77	5.2	AGWTLGAGAEYAINN	TLGAGAEYA	67
HLA-DRB1*0405	2	37.4	LAYGKVKSAFNLGDD	YGKVKSAFN	52
HLA-DRB1*0901	4	41.5	RARLGYTATERLMVY	YTATERLMV	76
HLA-DRB1*1302	1	47.9	GAEYAINNNWTLKSE	YAINNNWTL	25
HLA-DRB3*0101	4	30.6	VQAGYNWQLDNGVVL	WQLDNGVVL	23
HLA-DPA 1*0301-DPB1*0401	2	33.5	RARLGYTATERLMVY	YTATERLMV	16

Note: [a] Binding of epitopes of Omp31 from *B. melitensis* M28 was predicted using NetMHCII 2.2 server in accordance with the instruction of the server. 13 MHC II molecules against which bindings were strong are shown. Binding affinity below 50 nM is considered as strong. The strongest epitope bound to HLA-DRB10701 MHC II molecule is shaded.

Table 4: Binding of Epitopes of Omp31 from *B. melitensis* M28 against HLA-DRB1*0701 Molecule[a]

Epitope Rank	Start Position	Sequence	Score
Predicted with ProPred			
1	128	LGYTATERL	5.92
2	80	WQLDNGVVL	5.00
3	120	WFGTVRARL	4.70
4	181	YAINNNWTL	4.60
5	130	YTATERLMV	4.50
Predicted with SYFPEITHI			
1	126	RARLGYTATERLMVY	32
2	97	GSSVTGSISAGASGL	26
3	118	KVEWFGTVRARLGYT	26
4	128	RLGYTATERLMVYGT	26
5	49	FSSFDKEDNEQVSGS	24

Note: Binding of epitopes of Omp31 from *B. melitensis* M28 was predicted by analyzing with ProPred and SYFPEITHI servers. The higher the score of an epitope the greater is the probability of binding to a given MHC molecule. The core sequence (9-mer) of the epitope 125-VRARLGYTATERLMV-139 is shaded. Five epitopes with the top scores are shown.

Table 5: Hydrogen Bonding And Binding Energy Of Epitope-MHC II Complexes[a]

Epitope-MHC II complex	No. of H-bonds	Binding energy (kcal/mol)	Receptor residues in MHC II	Ligand residues in epitope	Interacting atoms	H-bond distance (Å)
Epitope-HLA-DRB1*0701	11	-254.95	W6	R12	H-O	1.92
			Y10	T10	H-O	2.77
			S57	V15	H-O	2.19
			W58	V15	H-O	2.68
			R68	T10	H-O	1.91
			R68	T10	H-O	2.22
			Q71	T8	H-O	2.35
			Y10	T10	O-H	2.58
			F14	R2	O-H	2.77
			G17	R2	O-H	2.30
			Y80	R2	O-H	2.78
Epitope-HLA-DRA	15	-219.49	Q5	A3	H-O	2.53
			E7	T8	H-O	2.45
			Y19	E11	H-O	2.06
			N58	R2	H-O	2.70
			N58	A3	H-O	2.69
			Q5	T8	O-H	2.12
			E17	R4	O-H	2.29
			E11	R4	O-H	2.76
			E11	T10	O-H	2.33
			E55	R2	O-H	2.30
			N62	R2	O-H	2.13
			N62	R4	O-H	2.70
			D66	R4	O-H	2.55
			T74	R12	O-H	2.35
			T80	R12	O-H	2.45
Epoitope-HLA-DRB1*1101	15	-258.77	S9	Y7	H-O	2.50
			S9	A3	H-O	2.53
			F13	T10	H-O	1.87
			Q66	V1	H-O	2.75
			K67	V1	H-O	2.53
			R70	A3	H-O	2.56
			R70	V1	H-O	2.36
			Y74	L5	H-O	1.31
			C11	A9	O-H	2.61
			C11	T10	O-H	2.72
			C11	T10	O-H	2.42
			F13	R12	O-H	2.40
			N73	R4	O-H	2.64
			Y79	R12	O-H	1.98
			R89	M14	O-H	2.15
Epitope-HLA-DRB1*0101	8	-172.47	W9	A3	H-O	2.59
			Q70	R12	H-O	2.73
			T77	R12	H-O	1.82
			W9	R4	O-H	2.61
			Y60	R2	O-H	2.69
			E69	R12	O-H	2.63
			T77	M14	O-H	2.75
			Y78	Y7	O-H	2.79
Epitope-HLA-DPB1*0401	6	-183.54	Y56	R2	H-O	2.03
			Y5	R12	O-H	2.20
			D53	R2	O-H	2.42
			Y56	T8	O-H	1.93
			E67	R4	O-H	2.16
			E67	R4	O-H	2.19
Epitope-HLA-DRB1*0901	7	-225.78	C15	R4	H-O	1.64
			S60	L13	H-O	2.38
			W61	E11	H-O	2.56
			D11	Y7	O-H	2.42
			F13	G6	O-H	2.23
			L53	R12	O-H	2.58
			N82	R2	O-H	2.00

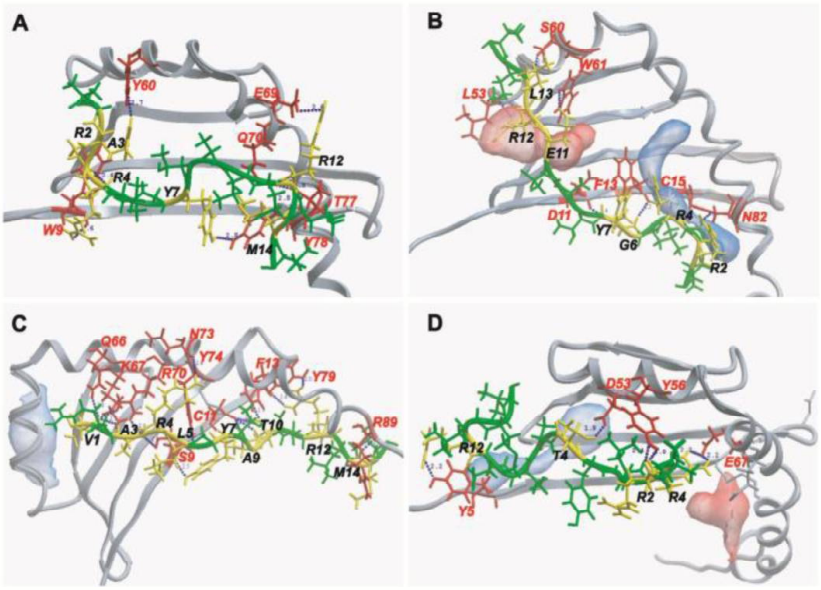
Note: [a]Hydrogen bonding distance and binding energy was predicted by Molsoft ICM-pro 3.5 computer program. Fifteen amino acid residues in the epitope (125-VRARLGYTATERLMV-139) have been numbered 1-15.

Table 6: Hydrogen Bonding and Binding Energy of Epitope-MHC I Complexes[a]

Epitope-MHC I complex	Epitope sequence	No. of H-bonds	Binding energy (kcal/mol)	Receptor residues in MHC I	Ligand residues in epitope	Interacting atoms	H-bond distance (Å)
Epitope-HLA-A1	131-YTATERLMV-139	9	-180.61	Q62 Q62 R156 R156 Y171 Q62 E63 Q155 Q155	A3 A3 V9 V9 T4 A3 T4 R6 R6	H-O H-O H-O H-O H-O O-H O-H O-H O-H	2.76 1.81 2.79 2.27 2.60 2.23 2.72 2.67 2.26
Epitope-HLA- A3	128-RLGYTATER-136	19	-222.61	Q62 N66 Q70 R114 W147 Q155 Y7 E58 Q62 T73 E63 E63 D77 D77 Y99 D116 D116 Q155 Q155	L2 L2 Y4 Y4 E8 T5 R1 R1 R1 L2 R1 L2 R9 R9 Y4 R9 R9 T5 T5	H-O H-O H-O H-O H-O O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H	2.60 2.23 2.64 2.33 1.65 2.18 2.40 2.76 2.54 2.10 2.76 2.55 1.84 2.50 2.74 2.45 2.23 2.05 2.43
Epoitope-HLA- B7	126-RARLGYTAT-134	11	-157.94	Y74 R97 W147 Y159 Y7 Y9 T73 S77 Y99 Y99	T7 G5 A8 A2 R1 R1 T9 T9 R1 R3	H-O H-O H-O H-O O-H O-H O-H O-H O-H O-H	2.41 2.69 1.60 2.32 2.76 2.66 2.21 2.60 2.24 2.26 2.38
Epitope-HLA-B8	126-RARLGYTAT-134	16	-196.45	Y159 T163 W167 Y171 T73 T73 T73 E76 E76 S77 Y99 Y116 D156 Y159 Y159 T163	T9 Y6 T7 T9 R1 R1 R1 R1 R1 R3 Y6 R3 L4 Y6 T9 A8	H-O H-O H-O H-O O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H O-H	1.06 1.96 1.81 2.35 2.72 1.99 2.06 2.15 2.75 2.61 2.53 1.65 2.47 2.64 1.84 2.76
Epitope-HLA-B27	127-ARLGYTATE-135	10	-178.88	R62 R62 K70 Y99 Y159 Y171 I66 D77 Q155 Y159	E9 A7 Y5 T8 E9 E9 R2 A1 T6 T8	H-O H-O H-O H-O H-O H-O O-H O-H O-H O-H	1.76 1.81 1.65 2.16 2.07 1.67 2.47 2.19 2.40 2.45

Note: [a]Hydrogen bonding distance and binding energy was predicted by Molsoft ICM-pro 3.5 computer program. Fifteen amino acid residues in the epitope (125-VRARLGYTATERLMV-139) have been numbered 1-15.

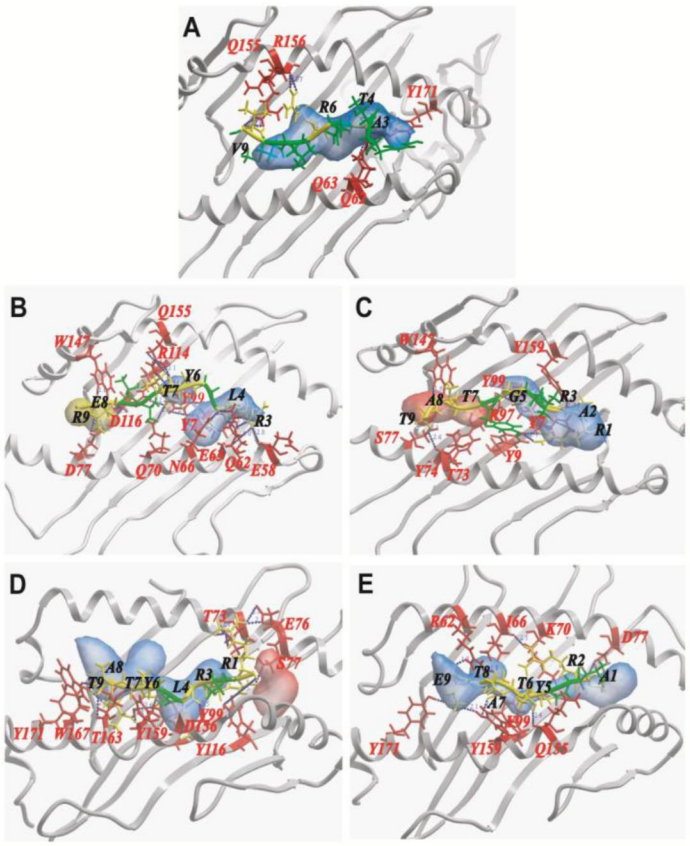
Figure 3: Docking of the epitope with HLA-DRB1*0101 (A), HLA-DRB1*0901 (B), HLA-DRB1*1101 (C) and HLA-DPB1*0402 (D) MHC II structures are shown in grey ribbon and the amino acid residues involved in the H-bonding network are shown as red sticks and labeled red. The epitope is shown as in green ribbon and the amino acid residues involved in the H-bonding network are shown as yellow sticks and labeled black. MHC II binding pocket(s) in the groove of HLA-DRB1*0901, HLA-DRB1*1101 and HLA-DPB1*0402 are shown as electrostatic sphere. H-bonds are displayed as blue spheres and the H-bonding distances are labeled blue. H-bonding network between the amino acid residues of the epitope and those of the MHC II are detailed in Table 5



noninfectious virus-like particles displaying recombinant epitopes, surface-conjugated peptides and solid-core nanobeads with conjugated peptides (Black *et al.,* 2010). In this study, using different computer-aided bioinformatics tools, we found a universal peptide (125-VRARLGYTATERLMV-139) of Omp31 from *B. melitensis* M28, which might be the most potential target among the Omps of human pathogenic *Brucella* spp to develop epitope-based vaccine for human brucellosis.

Bacterial surface proteins are potential vaccine candidates. Recently the whole genome sequences of virulent *B. melitensis* M28 and its attenuated strain *B. melitensis* M5-90 have been published (Wang *et al*., 2011a). In this report, it has been discussed that Omp31 is antigenic but not involved in virulence of *B. melitensis*. In our study, analysis with Vaxign, the vaccine target prediction and analysis server, revealed that Omp31 in more antigenic than other Omps of *Brucella* spp. However, the adhesin probability of Omp19 from *B. melitensis* biovar Abortus 2308 was 0.66, whereas that of Omp31 from *B. melitensis* M28 was 0.67. Nevertheless, Omp31 is more immunodominant than Omp19 and provokes more specific T-cell mediated immune response (Bowden *et al.*, 2000). Furthermore, comparative antigenicity analysis of Omp19 and Omp31 with VaxiJen server confirmed that Omp31 had higher antigenicity than Omp19 (data not shown). For proteins that lack obvious sequence similarity but possess similar biological

Figure 4: Docking of the epitope with HLA-A1 (A), HLA-A3 (B), HLA-B7 (C), HLA-B8 (D) and HLA-B27 (E) MHC I structures are shown in grey ribbon and the amino acid residues involved in the H-bonding network are shown as red sticks and labeled red. The epitope is shown as in green ribbon and the amino acid residues involved in the H-bonding network are shown as yellow sticks and labeled black. MHC I binding pocket(s) in the groove of HLA-A1, HLA-A3, HLA-B7, HLA-B8 and HLA-B27 are shown as electrostatic sphere. H-bonds are displayed as blue spheres and the H-bonding distances are labeled blue. H-bonding network between the amino acid residues of the epitope and those of the MHC I are detailed in Table 6



structures and functions, VaxiJen uses alignment independent method based on auto cross covariance transformation of protein sequences into uniform equal length vectors for antigen prediction that not only indicates the antigen but also its ability to induce protection (Doytchinova and Flower, 2007). Thus, results in our study obtained by analysis with Vaxign and VaxiJen servers made the speculation stronger that Omp31 is one of the effective sources of epitope-based vaccine against brucellosis. Results of analyses with NetMHCII 2.2, SYFPEITHI, ProPred and NetCTL 1.2 servers confirmed that the predicted epitope from Omp31 of *B. melitensis* M28 could bind to several MHC II and MHC I molecules and thus revealed its promiscuous nature (Khan and Ranganathan, 2010; Wilkins *et al*., 1999). NetCTL 1.2 is an integrative approach to CTL epitope prediction that predicts MHC-I binding, TAP transport efficiency and proteasomal

cleavage (Larsen *et al.*, 2005; Larsen *et al.*, 2007). Therefore, the epitope's ability to induce the cytotoxic T cell (CD8+ mediated) and natural killer cell (inhibitory receptors mediated) mediated immunity might be satisfactory as it can bind with five MHC I HLA (two A supertypes and three B supertypes) supertypes. As intracellular organisms, human pathogenic *Brucella* spp. requires cell mediated immunity which includes CD4+ and CD8+ T cells (Golding *et al.*, 2001). The proposed epitope bears high percentage of identity among all *Brucella* spp and 100% identity among ~72% of *Brucella* spp, which indicates it as the universal epitope (Figure 1). Tyrosine (Y131 in *B. melitensis* M28) present in 11 Omp31 sequences is substituted by phenylalanine in *B. suis*_4, *B. abortus* and *B. melitensis*_4. Alanine (A133 in *B. melitensis* M28) present in 11 Omp31 sequences is substituted by proline in *B. suis*_4, *B. abortus* and *B. melitensis*_4. Leucine (L137 in *B. melitensis* M28) present in 13 Omp31 sequences is substituted by valine in *B. melitensis*_1. Phenylalanine and valine have strongly similar properties (roughly the same size and the same hydropathy) as tyrosine and leucine, respectively and proline has weakly similar properties (the size or the hydropathy has preserved in the course of evaluation) as alanine (Do and Katoh, 2008). Therefore, the substituted amino acid residues in the predicted epitope of Omp31 in only ~28% *Brucella* spp. might not alter the properties of the epitope remarkably to change its universality. ProtParam analysis of the epitope considering the substituted amino acid(s) to mimic the mutation in *B. mel*_1, *B. suis*_4, *B. abortus* and *B. mel*_4 showed almost the same half life and instability index of the predicted epitope (data not shown).

The Omp31 T cell epitope forms a number of significant hydrogen bonds with the epitope binding groove of each of the MHC II and MHC I molecules (Figures 2, 3 and 4; Tables 5 and 6). Hydrogen bonding distance less than 3Å is usually considered biologically significant. The binding energy of the Omp31 T cell epitope with each of MHC I and II molecules is also significant since binding energy below -32 kcal/mol is biologically significant (Totrov and Abagyan, 1997). The epitope has the ability to bind with residues inside the groove as well as outside the binding groove (flanking residues) of the MHC molecules that is considered as principal determinant of epitope binding affinity (Arnold *et al.*, 2002; Brusic *et al.,* 1998; Godkin *et al.*, 2001; Khan and Ranganathan, 2010; McFarland and Beeson, 2002; Patronov *et al.*, 2011; Sant *et al.*, 1999; Zavala-Ruiz *et al.,* 2004). The interaction between the predicted epitope and MHC molecules in antigen presenting cells may trigger T-Cell mediated immunity (Doytchinova and Flower, 2007; Schuler *et al.*, 2007). Data reported herein namely: (i) high percentage of identity of the epitope in all Omp31 sequences, (ii) adhesin and antigen probability of Omp31 from *B. melitensis* M28, (iii) strong binding affinity of the epitope with MHC I and II molecules, (iv) physic-chemical properties of the epitope and v) molecular docking simulation supports the notion that the proposed epitope might be a novel universal efficient epitope to produce epitope-based vaccine against humans brucellosis. However, biochemical analysis is necessary to validate the interaction between the epitope and the MHC molecules through elucidation of immunity induction.

REFERENCES

1. Akhvlediani T, Clark D V, Chubabria G, Zenaishvili O and Hepburn M J (2010), "The

Changing Pattern Of Human Brucellosis: Clinical Manifestations, Epidemiology, and Treatment Outcomes Over Three Decades in Georgia", *BMC Infect Dis* , Vol. 10, p. 346.

2. Al-Tawfiq J A (2008), "Therapeutic Options for Human Brucellosis", *Expert Rev Anti Infect Ther.,* Vol. 6, pp. 109-120.

3. Almuneef M A, Memish Z A, Balkhy H H, Alotaibi B, Algoda S, Abbas M and Alsubaie S (2004), "Importance of Screening Household Members of Acute Brucellosis Cases in Endemic Areas", *Epidemiol Infect.,* Vol. 132, pp. 533-540.

4. Arnold P Y, La Gruta N L, Miller T, Vignali K M, Adams P S, Woodland D L and Vignali D A (2002), The Majority of Immunogenic Epitopes Generate Cd4+ T Cells That Are Dependent on MHC Class II-bound Peptide-flanking Residues", *J Immunol.,* Vol. 169, pp. 739-749.

5. Barh D, Misra A N, Kumar A and Vasco A (2010), "A Novel Strategy of Epitope Design in *Neisseria gonorrhoeae*", *Bioinformation,* Vol. 5, pp. 77-85.

6. Ben-Yedidia T and Arnon R (2007), "Epitope-based Vaccine Against Influenza", *Expert Rev Vaccines* 6, pp. 939-948.

7. Black M, Trent A, Tirrell M and Olive C (2010), "Advances in the Design and Delivery of Peptide Subunit Vaccines With a Focus on Toll-like Receptor Agonists", *Expert Rev Vaccines,* Vol. 9, pp. 157-173.

8. Bowden R A, Estein S M, Zygmunt M S, Dubray G and Cloeckaert A (2000), "Identification of Protective Outer Membrane Antigens of *Brucella Ovis* by Passive Immunization of Mice With Monoclonal Antibodies", *Microbes Infect* 2, pp. 481-488.

9. Brusic V, Rudy G, Honeyman G, Hammer J and Harrison L (1998), "Prediction of MHC class II-Binding Peptides Using an Evolutionary Algorithm and Artificial Neural Network", *Bioinformatics,* Vol. 14, pp. 121-130.

10. Caro-Hernandez P, Fernandez-Lago L, de Miguel M J, Martin-Martin AI, Cloeckaert A, Grillo M J and Vizcaino N (2007), Role of the Omp25/Omp31 Family in Outer Membrane Properties and Virulence of *Brucella ovis*. *Infect Immun* 75, pp. 4050-4061.

11. Cassataro J, Estein S M, Pasquevich K A, Velikovsky C A, de la Barrera S, Bowden R, Fossati C A and Giambartolomei G H (2005a), "Vaccination with the Recombinant *Brucella* Outer Membrane Protein 31 or A Derived 27-amino-acid Synthetic Peptide Elicits a CD4+ T Helper 1 Response That Protects Against *Brucella melitensis* Infection", *Infect Immun.,* Vol. 73, pp. 8079-8088.

12. Cassataro J, Velikovsky C A, Bruno L, Estein S M, de la Barrera S, Bowden R, Fossati C A and Giambartolomei G H (2007), "Improved Immunogenicity of a Vaccination Regimen Combining a DNA Vaccine Encoding *Brucella melitensis* Outer Membrane Protein 31 (Omp31) and Recombinant Omp31 Boosting", *Clin Vaccine Immunol.,* Vol. 14, pp. 869-874.

13. Cassataro J, Velikovsky C A, de la Barrera S, Estein S M, Bruno L, Bowden R, Pasquevich K A, Fossati C A and

Giambartolomei G H (2005b), "A DNA Vaccine Coding for the Brucella Outer Membrane Protein 31 Confers Protection against *B. melitensis* and *B. ovis* Infection by Eliciting a Specific Cytotoxic Response", *Infect Immun.,* Vol. 73, pp. 6537-6546.

14. Corbel M J (1997), "Brucellosis: An Overview", *Emerg Infect Dis.,* Vol. 3, pp. 213-221.

15. De Groot A S, McMurry J, Marcon L, Franco J, Rivera D, Kutzler M, Weiner D and Martin B (2005), "Developing an Epitope-Driven Tuberculosis (TB) Vaccine", *Vaccine,* Vol. 23, pp. 2121-2131.

16. Delpino M V, Cassataro J, Fossati C A, Goldbaum F A and Baldi P C (2006), "Brucella Outer Membrane Protein Omp31 is a Haemin-binding Protein", *Microbes Infect.,* Vol. 8, pp. 1203-1208.

17. Do C B and Katoh K (2008), "Protein Multiple Sequence Alignment", *Methods Mol Biol.,* Vol. 484, pp. 379-413.

18. Doytchinova I A and Flower D R (2007), "VaxiJen: A Server for Prediction of Protective Antigens, Tumour Antigens and Subunit Vaccines", *BMC Bioinformatics,* Vol. 8, p. 4.

19. Ertem M, Kurekci A E, Aysev D, Unal E and Ikinciogullari A (2000), "Brucellosis Transmitted by Bone Marrow Transplantation", *Bone Marrow Transplant,* Vol. 26, pp. 225-383.

20. Franco M P, Mulder M, Gilman R H and Smits H L (2007), "Human Brucellosis", *Lancet Infect Dis.,* Vol. 7, pp. 775-786.

21. Godkin A J, Smith K J, Willis A, Tejada-Simon M V, Zhang J, Elliott T and Hill A V (2001), "Naturally processed HLA class II Peptides Reveal Highly Conserved Immunogenic Flanking Region Sequence Preferences That Reflect Antigen Processing Rather Than Peptide-MHC Interactions", *J Immunol.,* Vol. 166, pp. 6720-6727.

22. Golding B, Scott D E, Scharf O, Huang L Y, Zaitseva M, Lapham C, Eller N and Golding H (2001), "Immunity and Protection Against *Brucella abortus*", *Microbes Infect.,* Vol. 3, pp. 43-48.

23. Guilloteau LA, Laroucau K, Olivier M, Grillo M J, Marin C M, Verger J M and Blasco J M (2006), "Residual Virulence and Immunogenicity of CGV26 and CGV2631 *B. melitensis* Rev. 1 Deletion Mutant Strains In Sheep After Subcutaneous Or Conjunctival Vaccination", *Vaccine,* Vol. 24, pp. 3461-3468.

24. He Y and Xiang Z (2010), "Bioinformatics Analysis of Brucella Vaccines And Vaccine Targets Using VIOLIN", *Immunome Res.,* Vol. 6, pp. Suppl 1 S5.

25. He Y, Xiang Z and Mobley H L (2010), "Vaxign: The First Web-based Vaccine Design Program For Reverse Vaccinology And Applications For Vaccine Development", *J Biomed Biotechnol.,* pp. 297-505.

26. Khan J M and Ranganathan S (2010), "pDOCK: A New Technique for Rapid and Accurate Docking of Peptide Ligands to Major Histocompatibility Complexes", *Immunome Res.,* Vol. 6, pp. Suppl 1 S2.

27. Kiefer F, Arnold K, Kunzli M, Bordoli L and

Schwede T (2009), "The SWISS-MODEL Repository and Associated Resources", *Nucleic Acids Res.,* Vol. 37, pp. D387-392.

28. Larsen M V, Lundegaard C, Lamberth K, Buus S, Brunak S, Lund O and Nielsen M (2005), "An Integrative Approach tTo CTL Epitope Prediction: A Combined Algorithm Integrating MHC Class I Binding, Tap Transport Efficiency, And Proteasomal Cleavage Predictions", *Eur J Immunol.,* Vol. 35, pp. 2295-2303.

29. Larsen M V, Lundegaard C, Lamberth K, Buus S, Lund O and Nielsen M (2007), "Large-scale Validation Of Methods For Cytotoxic T-lymphocyte Epitope Prediction", *BMC Bioinformatics,* Vol. 8, pp. 424.

30. Laskowski R A (2001), "PDBsum: Summaries and Analyses of PDB Structures", *Nucleic 415 Acids Res.,* Vol. 29, pp. 221-222.

31. Maudlin I, Eisler M C and Welburn S C (2009), "Neglected and Endemic Zoonoses", *Philos Trans R Soc Lond B Biol Sci.,* Vol. 364, pp. 2777-2787.

32. McFarland B J and Beeson C (2002), "Binding Interactions Between Peptides and Proteins of The Class II Major Histocompatibility Complex", *Med Res Rev.,* Vol. 22, pp. 168-203.

33. Meltzer E, Sidi Y, Smolen G, Banai M, Bardenstein S and Schwartz E (2010), "Sexually Transmitted Brucellosis in Humans", *Clin Infect Dis.,* Vol. 51, pp. e12-15.

34. Mesner O, Riesenberg K, Biliar N, Borstein E, Bouhnik L, Peled N and Yagupsky P (2007), "The Many Faces Of Human-to-human Transmission of Brucellosis: Congenital Infection and Outbreak Of Nosocomial Disease Related to An Unrecognized Clinical Case", *Clin Infect Dis.,* Vol. 45, pp. e135-140.

35. Nielsen M and Lund O (2009), "NN-align. An Artificial Neural Network-based Alignment Algorithm For MHC Class II Peptide Binding Prediction", *BMC Bioinformatics,* Vol. 10, p. 296.

36. Pappas G, Papadimitriou P, Akritidis N, Christou L and Tsianos E V (2006), "The New Global Map Of Human Brucellosis", *Lancet Infect Dis.,* Vol. 6, pp. 91-99.

37. Patronov A, Dimitrov I, Flower D R and Doytchinova I (2011), "Peptide Binding Prediction for the Human Class II MHC Allele HLA-DP2: A Molecular Docking Approach", *BMC Struct Biol.,* Vol. 11, pp. 32.

38. Perkins S D, Smither S J and Atkins H S (2010), "Towards a Brucella Vaccine for Humans", *FEMS Microbiol Rev.,* Vol. 34, pp. 379-394.

39. Sant A J, Beeson C, McFarland B, Cao J, Ceman S, Bryant P W and Wu S (1999), "Individual Hydrogen Bonds Play a Critical Role in MHC Class II: Peptide Interactions: Implications For The Dynamic Aspects of Class II Trafficking And Dm-mediated Peptide Exchange", *Immunol Rev.,* Vol. 172, pp. 239-253.

40. Sarinas P S and Chitkara R K (2003), "Brucellosis", *Semin Respir Infect,* Vol. 18, pp. 168-182.

41. Schuler M M, Nastke M D and Stevanovikc S (2007), "SYFPEITHI: Database for Searching And T-cell Epitope Prediction",

Methods Mol Biol., Vol. 409, pp. 75-93.

42. Schwede T, Kopp J, Guex N and Peitsch M C (2003), "SWISS-MODEL: An Automated Protein Homology-modeling Server", *Nucleic Acids Res.,* Vol. 31, pp. 3381-3385.

43. Sette A and Fikes J (2003), "Epitope-Based Vaccines: An Update on Epitope Identification, Vaccine Design And Delivery", *Curr Opin Immunol.,* Vol. 15, pp. 461-470.

44. Shinnick T M, Sutcliffe J G, Green N and Lerner R A (1984), "Peptide-Elicited Protein-Reactive Antibodies in Molecular Biology and Medicine", *J Invest Dermatol.,* Vol. 83, pp. 112s-115s.

45. Singh H and Raghava G P (2001), "ProPred: Prediction of HLA-DR Binding Sites", *Bioinformatics,* Vol. 17, pp. 1236-1237.

46. Totrov M and Abagyan R (1997), "Flexible Protein-ligand Docking by Global Energy Optimization In Internal Coordinates", *Proteins Suppl.,* Vol. 1, pp. 215-220