Computational Analysis of Interaction between Mycobacterial Antigens Rv0679c and Rv0180c with Toll like Receptors of Human and Mouse

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ABSTRACT

Toll like receptors (TLRs) play a key role in the innate immune response to infectious agents. The *Mycobacterium tuberculosis* bacilli and its antigens interaction with TLR-2-1 and TLR-4-MD2 were shown to activate intracellular signalling which determines the outcome of the disease. Present study focused on two new antigens Rv0679c and Rv0180c of M.tb H37Rv and their interactions with human and mouse TLRs using computational approach. Structures of Rv0679c and Rv0180c antigens of M.tb were generated using I-TASSER and docked with TLR-2-1 and TLR-4-MD2 complexes of human and mouse using Cluspro2.0. Rv0180c antigen has better binding energy to both human and mouse TLR-2-1 and TLR-4-MD2 compared to Rv0679c and other mycobacterial antigens. The Rv0679c and Rv0180c antigens have better binding energy to TLR 4-MD2 complex compared to TLR-2-1 in both human and mouse. Both antigens has better binding energy to mouse TLRs compared to human except where Rv0180c has better binding to human TLR-2-1. Our findings suggest that Rv0180c might be the preferred for binding to TLRs than Rv0679c and within TLRs, TLR-4-MD2 for interaction.

General Terms

Invasive mechanism of mycobacterial antigens using TLRs

Keywords

Mycobacterium tuberculosis, toll like receptors, invasion, I-TASSER, docking, ClusPro

1. INTRODUCTION

Intracellular pathogen *Mycobacterium tuberculosis* (M.tb) even though the earliest to be indentified which causes disease in humans still remain a major global health threat [1] One of the reasons could be development of effective evasion strategies adopted by bacterium to overcome host defence mechanisms [2]. Sequencing of whole genome of bacterium [3] lead to better understanding of membrane bound antigens associated with host receptor interaction leading to probable invasion / entry into the host.

Two novel proteins Rv0679c and Rv0180c were identified from M.tb H37Rv, which are membrane bound and play a role in entry / invasion of pathogen into host cells [4, 5]. Rv0679c protein was classified as a membrane protein of the envelope of M.tb with a molecular mass of 16.5 KDa, consisting of 165 amino acids and contains a putative N-terminal signal sequence and a consensus lipoprotein-processing motif. Rv0679c protein exists as a tight complex with LAM, one of the major components of cell envelope involved in pro-inflammatory and anti-inflammatory responses [6]. Cifuentes et al [4] have shown that peptides of Rv0679c inhibited invasion / enrty of live M.tb bacilli into A549 and U937 macrophage cell lines in vitro. Rv0180c is a conserved transmembrane protein with a molecular mass of 47.5 KDa, consisting of 452 amino acids with unknown function however shares a high sequence similarity with putative conserved membrane and transmembrane proteins from different mycobacterial species [5]. Caceres [5] have shown that the peptides of this protein inhibited the invasion of M.tb H37Rv into U937 and A549 cell lines in vitro indicating its candidature as a subunit based vaccine against tuberculosis. Either Rv0679c or Rv0180c protein were not evaluated for the possible mechanism of blocking invasion into the cells, which could be probably through specific receptors.

Pattern recognition receptors (PRRs) like toll-like receptors (TLRs) play a pivotal role in the induction of an innate immune response to various infectious agents, including m*ycobacteria* spp [7]. The interface between M.tb and its proteins to various TLRs is not fully understood, but it appears that whole mycobacteria or distinct mycobacterial components may interact with different members of the TLR family [8]. Studies indicated that TLR2 and TLR4 play a major role in regulating innate recognition to mycobacterial infection [9]. Encounter with triacylated or diacylated lipoproteins induces heterodimerization of human TLR-2 either with TLR-1 or TLR-6 [10]. Similarly TLR-4, associates with Myeloid differentiating factor (MD-2) and it is believed to be the component of the TLR4-MD-2 complex that interacts with lipopolysaccharides of different bacteria [11].

TLRs are highly conserved from Drosophila to humans and share structural and functional similarities. Mouse is being used as a model to study the pathogenesis of tuberculosis for better understanding of mechanisms related to bacterial pathogenicity vs. host resistance and susceptibility. Hence in the present study simultaneous assessment of the interaction of mycobacterial proteins with human and mouse TLRs was done for comparative analysis. Three different mycobacterial proteins such as LprG, Ag85 and MPT51 were used for comparison.

Antigen 85 complex (Ag85) of M.tb consists of three abundantly secretary proteins (FbpA, FbpB and FbpC2) which play a role in the pathogenesis of tuberculosis apart from exhibiting cell wall mycolyl transferase activity [12]. MPT51, a secretary protein of M.tb shares 40% identity with the Ag85 components and is involved in cell wall mycoloylation [13]. LprG is a conserved membrane Lipoarabinomannan carrier protein essential for virulence of M.tb [14]. LprG binding to LAM facilitates its transfer from the plasma membrane into the cell envelope, increasing surface-exposed LAM, enhancing cell envelope integrity, allowing inhibition of phagosome-lysosome fusion and enhancing M.tb survival in macrophages [15].

The combined approach of proteomics, protein-protein interaction and receptor-ligand interaction studies using bioinformatics approach has potential to generate information and knowledge that will enable in understanding host-pathogen interaction leading to entry / invasion into host cell. This enables the development of new therapies and interventions strategies needed to treat or control disease. In the light of above the present study assessed whether the Rv0679c and Rv0180c hypothetical invasive proteins of M.tb could bind to TLRs and if so might be associated as molecules for entry in humans and mouse system.

2. METHODOLOGY
2.1 Retrieval of Rv0679c and Rv0180c sequences and Homology modeling using I-TASSER

The sequence of Rv0679c (165 aminoacids) and Rv0180c (498 aminoacids) antigens were retrieved from TubercuList database [http://tuberculist.epfl.ch].Structures of Rv0679c and Rv0180c antigenss were generated using I-TASSER. [16,17,18]. Iterative Threading ASSEmbly Refinement (I-TASSER) is a hierarchical method for protein structure and function prediction. Briefly, the structures are generated by LOMETS (Local Meta-Threading-Server) first, which further enables to generate 3D models by collecting high-scoring target-to-template alignments from 9 locally-installed threading programs. Atomic models are then constructed by iterative template fragment assembly simulations; functional insights of the target are finally derived by threading the 3D models through protein function database BioLiP.

2.2 Retrieval of M.tb antigen structures
Structures of other mycobacterial antigens like LprG (PDB ID: 3MH8)**,** Ag85 (PDB ID: 1SFR)**,** MPT51 (PDB ID: 1R88) were obtained for comparative analysis from protein data bank (PDB) **[**http://www.rcsb.org/pdb/home/home.do]. The X-ray crystallographic structures of human TLR-2-1 heterodimer PDB (PDB id- 2z7x) and TLR-4-MD-2 dimers (**PDB ID: 3FXI)** and mouse TLR-2 (PDB Id: 2Z82) and TLR4-MD2 (PDB Id: 2Z64) dimer were also retrieved from PDB.

2.3 Structure validation using PROCHECK
Structures of Rv0679c and Rv0180c antigens generated by I-TASSER was validated using PROCHECK, which is available online at http://www.ebi.ac.uk/thornton-srv/software /PROCHECK/ to ensure zero residues in the disallowed regions. It provides a detailed check on the stereochemistry of the protein structure. PROCHECK produces a number of PostScript plots, analyzing its overall and residue-by-residue geometry. The final models obtained were used for docking analysis.

2.4 Docking Studies
Docking of antigens with human and mouse TLR's was performed using Cluspro2.0 where in antigen is considered as ligand and TLR as a receptor [http://cluspro.bu.edu /home.php]. Cluspro2.0 is a web-based automated program for the computational docking of protein structures. Once the coordinate files of the protein structures are uploaded the docking algorithms evaluate billions of putative complexes, retaining a preset number with favourable surface complementarities. The obtained structures are then filtered, selecting those with good electrostatic and desolvation free energies for further clustering.

3. RESULTS & DISCUSSION
3.1 Prediction of 3D structures of Rv0679c and Rv0180c antigens of M.tb
Structures of Rv0679c and Rv0180c antigens generated by I-TASSER were submitted to PBDsum database (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/ GetPage.pl?pdbcode=index.html) which provides an overview of the contents of the protein structures. Secondary structure analysis of Rv0679c using PDBSum revealed 2 helices, 16 beta turns and 7 gamma turns (Figure 1A) while Rv0180c secondary structure has 25 helices, 32 beta turns and 12 gamma turns (Figure 1B).
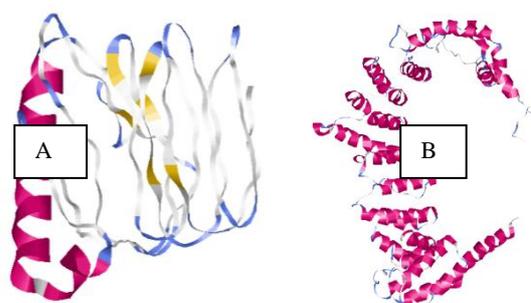
Figure 1: Structure of Rv0679c and Rv0180c antigens of M.tb generated using I-TASSER.

A: In Rv0679c antigen structure pink indicates alpha helix; yellow indicates beta sheets, blue indicates turns and white indicates coils. **B**: In Rv0180c antigen structure Pink indicates alpha helix and blue indicates turns.

3.2 Validation of antigen structures
Structures of Rv0679c and Rv0180c antigens generated by

I-TASSER were validated using PROCHECK. Figure 2 depicts Ramachandran plot for Rv0679c antigen with zero residues in the disallowed region. The Ramachandran plot for Rv0180c antigen is depicted in Figure 3, showing zero residues in the disallowed region. PROCHECK statistical data for both the antigens was shown in Table 1 (Rv0679c) and

Table 2 (Rv0180c). This study is the first report on the structures of Rv0679c and Rv0180c antigens obtained using computational approach as the crystallography studies are not reported so far.
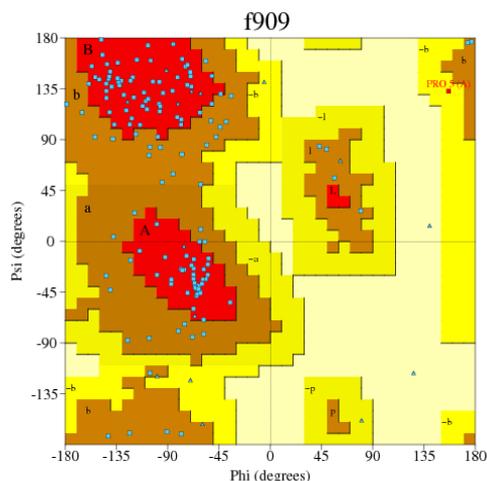
Figure 2: Ramachandran plot of Rv0679cantigen
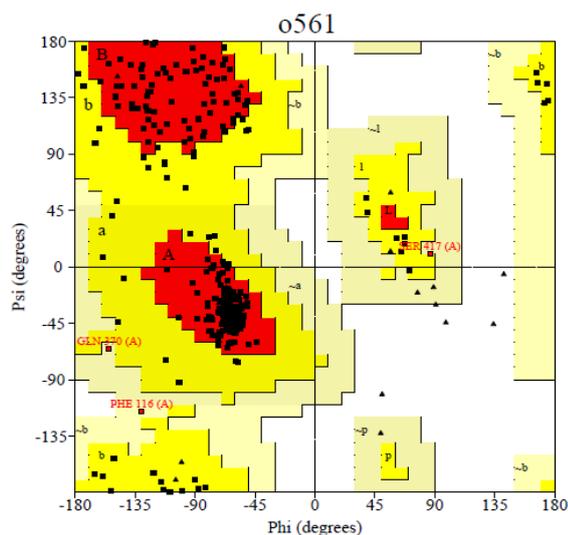
Figure 3: Ramachandran plot of Rv0180c antigen

Table 1 : PROCHECK statistics for Rv0679c antigen

Ramchandran plot statistics	No. of residues	%tage
Most favoured regions [A,B,L]	98	70
Additional allowed regions [a,b,l,p]	42	30
Generouslyallowed regions	0	0
Disallowed regions [xx]	0	0
Non-glycine and non-proline residues	140	100
End-residues (excl, Gly and pro)	2	
Glycine residues	15	
Proline residues	8	
Total number of residues	165	

3.3 Docking analysis for assessment of binding energies

TLR-2-1 and TLR-4-MD2 structures retrieved from PDB were docked with mycobacterial antigens. The binding energies and docked complexes obtained between human TLRs and antigens are depicted in Table-3 and with mouse TLRs in Table-4.

Rv0180c and Rv0679c antigens has better binding energy of -1219 Kcal/mol and -988.6 to TLR-4-MD2 complex compared to TLR-2-1 with binding energy of -1044 Kcal / mol and -849.1 respectively in humans (Table 3). Rv0180c antigen has better binding energy compared to Rv0679c and other mycobacterial antigens like LprG, Ag85 and MPT51 used for comparative analysis (Table 3) for both TLR-2-1 and TLR-4-MD2. In general the antigens of mycobacteria preferentially had better binding affinity to human TLR-4-MD2 compared to TLR-2-1, except in the case of LprG antigen.

In mouse, antigens Rv0180c and Rv0679c had better binding energy of -1393.8 Kcal/mol and -1138 Kcal/mol to TLR-4-MD2 complex compared to TLR-2-1 with binding energy of -987.2 Kcal/mol and -979.4 Kcal/mol respectively (Table-4). Rv0180c antigen has better binding energy compared to Rv0679c and other mycobacterial antigens like LprG, Ag85, MPT51 used for comparative analysis (Table-4)

for both TLR-2-1 & TLR-4-MD2. This indicates that antigens

Table 2: PROCHECK statistics for Rv0180c antigen

Ramchandran plot statistics	No. of residues	%tage
Most favoured regions [A,B,L]	324	84.6
Additional allowed regions [a,b,l,p]	56	14.6
Generouslyallowed regions	3	0.8
Disallowed regions [xx]	0	0.0
Non-glycine and non-proline residues	383	100
End-residues (excl, Gly and pro)	1	
Glycine residues	41	
Proline residues	27	
Total number of residues	452	

of mycobacteria preferentially had better binding affinity even to mouse TLR-4-MD2 compared to TLR-2-1 except in the

case of LprG antigen.

TLRs are the type 1 transmembrane glycoproteins characterized by the presence of an extracellular domain (ectodomain ECD) containing leucine rich repeats (LRR) which is primarily responsible for mediating ligand interaction [19]. Protein structure and nature of binding of mycobacterial proteins in the right pockets of TLRs determine / influence the outcome of interaction which could be beneficial or detrimental. Rv0180c protein was binding to TLR-2-1 and TLR-4-MD2 of humans in the conventional way interacting predominantly in the ectodomain and central domain of the receptor compared to other mycobacterial antigens which were binding to C-terminal end (Table-3)

except in the case MPT51 and Rv0679c protein to TLR-4-MD2. This suggests that membrane proteins such as Rv0180c, Rv0679c and MPT51 are binding in one pattern compared to the other two - Ag85 and LprG which are secretary proteins of M.tb. Antigen 85 was shown to interact with fibronectin domain regardless of specificity of the domain [20] and leads to enhancement of phagocytosis of M. *leprae* in monocyte-derived-macrophages [21]. Taken the findings of the present study, Ag85 binding to TLRs was non-conventional and Ag85 preferring fibronectin domain in case of mycobacteria as shown by Naito M [20], one would assume similar fibronectin domain interaction could be possible or preferred by M.tb.

Table 3: Docking of Mycobacterium tuberculosis antigens Rv0180c, Rv0679c, LprG, Ag85, MPT51 with <u>human</u> TLR-2-1 Heterodimer and TLR-4-MD2 dimer

Antigen Structure	Binding Energy Scores & Docked complex of Antigens TLR-2-1 Dimer (Kcal/mol)	Binding Energy Scores & Docked complex of Antigen-TLR4-MD2 dimer (Kcal/mol)
LprG	**-915.6**	**-865.5**
AG85	**-955.8**	**-1062.9**
MPT51	**-882.7**	**-933.0**
Rv0679c	**-849.1**	**-988.6**
Rv0180c	**-1044.0**	**-1219.2**
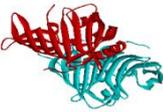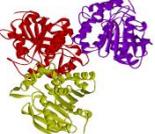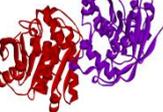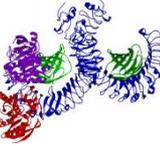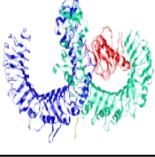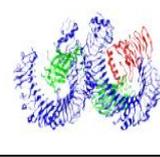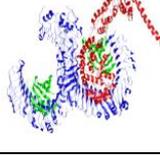
In TLR 2-1 docked complex dark blue indicates TLR-1; Sky blue and cyan indicates TLR-2. In TLR4-MD2 peptide docked complex dark blue indicates TLR4 dimers, while green indicates MD2.

Table 4: Docking of Mycobacterium tuberculosis antigens Rv0180c, Rv0679c, LprG, Ag85, and MPT51 with <u>mouse</u> TLR-2 and TLR-4.

Antigen Structure	Binding energy Scores & Docked complex of Antigen-TLR2- (Kcal/mol)	Binding energy Scores & Docked complex of Antigen-TLR4-MD2 (Kcal/mol)
LprG	**-897.4**	**-852.1**
Ag85	**-832.6**	**-856.2**
MPT51	**-882.8**	**-903.2**
Rv0679c	**-979.4**	**-1138.0**
Rv0180c	**-987.2**	**-1393.8**
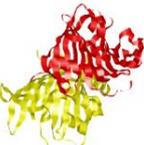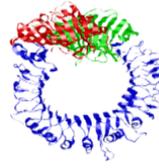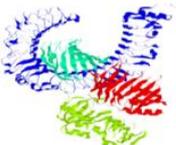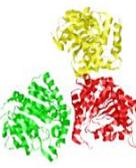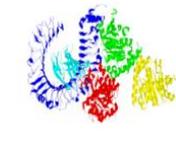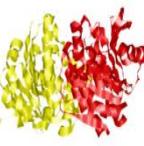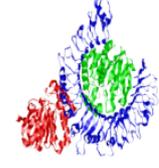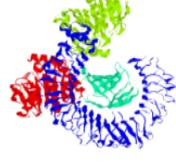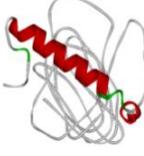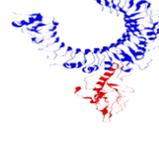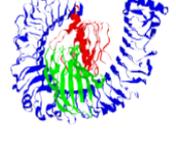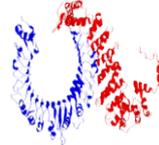
In TLR2 docked complex dark blue indicates TLR-2; In TLR4-MD2 peptide docked complex dark blue indicates TLR4 dimer, while green indicates MD2.

Stimulation studies using different lipoproteins of Mycobacteria such as LpqH (Rv3763) [22] , LprG (Rv1411c) [23], LprA (Rv1270c) [24] etc on murine bone marrow derived macrophages, human monocytic cell line and primary human macrophages suggested the ability of these proteins to inhibit the antigen processing, MHC expression and production of interferon-gamma in respective experiments.

They observed that it was predominantly due to the interaction of these proteins with either TLR-2 or TLR-4 leading to redundant / diminished mechanisms affecting the recognition of infected macrophages by T cells and thus evading the immune system.

This study is the first report to indicate Rv0679c and Rv0180c proteins of M.tb interaction with TLRs, using computational approach. However, one could not comment on the functional outcome of Rv0679c and Rv0180c proteins interaction with TLRs, but speculate based on other lipoproteins study of M.tb. TLRs interaction with Rv0679c and Rv0180c proteins could lead to invasion / entry of pathogen inside the host subsequently modulating immune system for evading purpose.

4. CONCLUSION

The Rv0679c and Rv0180c proteins could bind to TLRs where the binding affinity / energies are high for human and mouse TLR-4 compared to TLR-2. Rv0180c protein has better binding energy than Rv0679c indicating Rv0180c might be more effective in invasion / entry compared to Rv0679c protein. The findings of both antigens interaction with TLRs in mouse and human are related; suggesting it could be a preferred model for addressing fundamental questions of TB pathogenesis *in vivo.*

5. ACKNOWLEDGMENTS

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