Exploration studies for *Leishmania donovani* CpG DNA pattern interacting with Toll Like Receptor 9: An *in silico* approach

Chhedi Lal Gupta and Preeti Bajpai*

[1]*Department of Biosciences, Integral University, Lucknow-226026, India*

***Corresponding Author E-mail:** pbajpai@iul.ac.in, preeti2874@gmail.com

Abstract

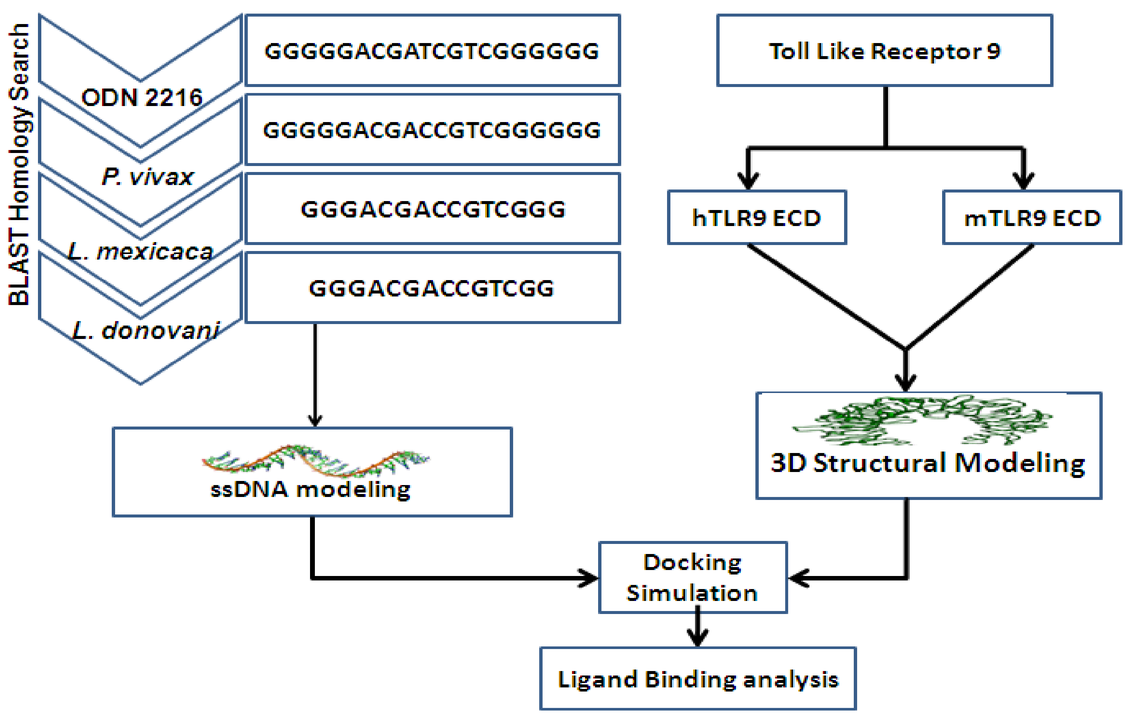
The potential involvement of Toll-like receptors in combating parasitic infections of Leishmania, an intracellular protozoan parasite causes a broad range of clinical manifestations from cutaneous lesions to fatal visceral disease has gained significant attention during the last decades. Although it is well established that Toll-like receptors 9 (TLR9) recognizes microbial CpG DNA, but the specificity of the CpG DNA pattern of Leishmania parasite interacting with endosomal TLR9 is still unexplored. Thus to identify the specific CpG DNA pattern act as TLR9 ligand in *Leishmania donovani* (*L. donovani*), the homology search was performed using known CpG ODN 2216 (synthetic) as initial template until a consistent CpG pattern in *L. donovani* was achieved. Further, we propose a reliable 3D complex model of TLR9 ectodomains (ECDs) with CpG DNA patterns utilizing homology modeling and docking approaches. The molecular docking studies revealed that identified *L. donovani* CpG pattern encompasses robust docked energy than other patterns considered for this study. The complex interaction patterns demonstrated that the interface between TLR9 and CpG DNA molecules were geometrically complementary. The computed molecular interactions indicated that the identified *L. donovani* CpG DNA pattern shows comparable binding with known CpG ODN 2216 by LRR11 region of TLR9 acting as the critical region for ligand recognition. This complex model may lead to a better understanding of the function of TLR9 and its interaction with CpG DNA and will improve our understanding of TLR9-ligand interaction in immune regulation.

Introduction

TLR9 recognizing microbial CpG-DNA/CpG-containing oligodeoxynucleotides (CpG ODN) is localized within the endosomal compartment of APCs . Internalizing CpG-DNA within the endosome initiates signaling via sequential recruitment of MyD88 and TRAF6, thereby activating downstream nuclear transcription factors NF-kB and AP-1 which instigates the induction of inflammatory cytokines viz. TNF-α, IL-6, IL-1β, and IL-12. Synthetic CpG-ODN mimics the stimulatory effect of microbial DNA inducing both lymphoid and myeloid lineage proliferation.

Parasitic protozoans of Leishmania genus afflict ~14 million people in 88 countries and have been a matter of serious concern. This intracellular parasite is capable of creating a broad range of clinical manifestations from cutaneous lesions to fatal visceral disease. The immune status of the host largely manipulates the outcome of disease . Current therapeutic strategies enforce limited cure due to soaring prices and restricted use. The upcoming drug resistance has added to the issues. The innate immune response has been the preferential target to the researchers towards vaccine/drug development against this neglected disease. TLRs are considered to be the first one to hamper the pathway of Leishmania parasite triggering Th1 immune response. The parasitic components LPG, GPIL, and gp63 are thought to be the first substrates to encounter the innate immune system via TLR2/4. The activation of myeloid and plasmacytoid DCs in a strictly TLR9-dependent manner against the *Leishmania spp.* has also been observed in few upcoming reports. Another study demonstrated the increased expression of TLR9 after exposure of macrophages to Leishmanial DNA thereby establishing the role of TLR9 as a role model for host innate immune response against the parasite. However, the specificity of the parasitic DNA pattern subject to TLR9 is still unexplored. The present study was therefore focused on the prediction of a common structural pattern (CpG DNA) for TLR9 recognition in *Leishmania spp.* by applying CpGODN-2216 as initial template using homology searching method. Further molecular modeling and docking studies were employed to model the TLR9 ECD−CpG DNA complexes to elucidate the critical residues of TLR9 ECD implicated for molecular interaction.

Methodology



Results

Identification of *L. donovani* CpG DNA pattern using homology searching method

Similarity matrix of CpG DNA patterns.

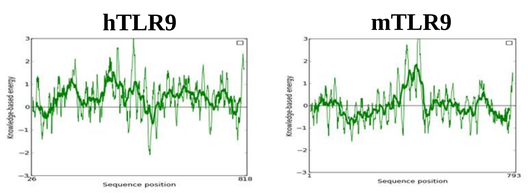
CpG DNA pattern	% Similarity			
	ODN 2216	*P. vivax*	*L. mexicana*	*L. donovani*
ODN 2216	100	95.0	93.33	92.86
P. vivax	95.0	100	100	100
L. mexicana	93.33	100	100	100
L. donovani	92.86	100	100	100

Multiple sequence alignment of CpG ODN 2216 and DNA patterns of *P. vivax*, *L. mexicana* and *L. donovani* using Clustal W tool. The asterisk represents conserved sequences.

```
L. mexicana  --GGGACGACCGTCGGG--- 15
L. donovani  --GGGACGACCGTCGG---- 14
P. vivax     GGGGGACGACCGTCGGGGGG 20
ODN 2216     GGGGGACGATCGTCGGGGGG 20
             *******  ******
```

Structural analysis of developed models of human and mouseTLR9 ectodomains

The energy profile of hTLR9 ECD and mTLR9 ECD obtained from ProSA web server.

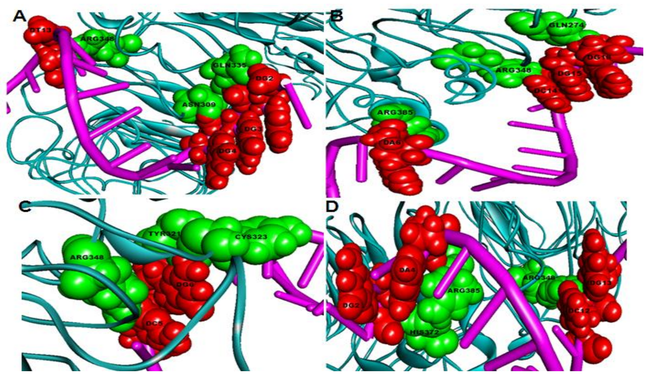


3D model validation of TLR9 ECDs through various tools.

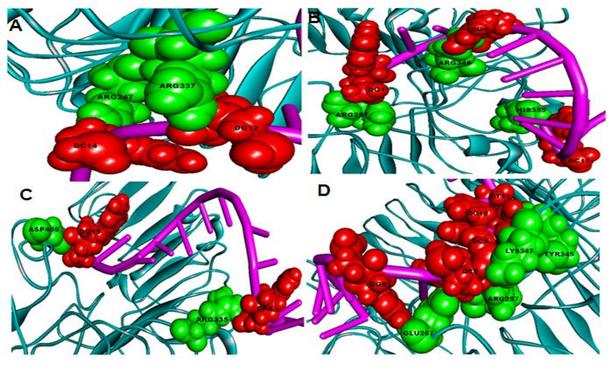
Protein	Procheck (Ramachandran Plot)				ProQ		ProSA (Z Score)
	Most favored region (%)	Additional allowed region (%)	Generously allowed region (%)	Disallowed region (%)	Predicted LG score	Predicted MaxSub	
hTLR9	75.0	24.1	0.9	0.0	4.330	0.237	-3.51
mTLR9	76.3	22.7	1.0	0.0	5.498	0.399	-6.76

Identification of ligand recognition sites of TLR9

Molecular interactions between hTLR9 and CpG DNA. The hTLR9 is shown in cyan color and CpG DNA in magenta color. The interacting residues are shown in CPK (green color for hTLR9 and red for CpG DNA). (A) Interactions between hTLR9 and CpG ODN 2216, (B) hTLR9 and *P. vivax* CpG DNA, (C) hTLR9 and *L. mexicana* CpG DNA and (D) hTLR9 and *L. donovani* CpG DNA.



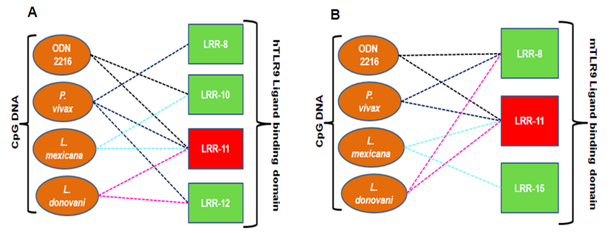
Molecular interactions between mTLR9 and CpG DNA. The mTLR9 is shown in cyan color and CpG DNA in magenta color. The interacting residues are shown in CPK (green color for mTLR9 and red for CpG DNA). (A) Interactions between mTLR9 and CpG ODN 2216, (B) mTLR9 and *P. vivax* CpG DNA, (C) mTLR9 and *L. mexicana* CpG DNA and (D) mTLR9 and *L. donovani* CpG DNA.



Docking energies af CpG DNA patterns with TLR9. obtained by molecular docking studies.

Complexes	Docking energy (Kj/mol)
hTLR9–CpG ODN 2216	-423.76
hTLR9–*P. vivax* CpG DNA	-420.98
hTLR9–*L. mexicana* CpG DNA	-368.01
hTLR9–*L. donovani* CpG DNA	-553.33
mTLR9–CpG ODN 2216	-397.92
mTLR9–*P. vivax* CpG DNA	-320.70
mTLR9–*L.mexicana* CpG DNA	-373.76
mTLR9–*L.donovani* CpG DNA	-552.94

Representation of LRR regions present in TLR9 ECD having molecular interactions with ligands (CpG DNA) (A) hTLR9 LRR region (B) mTLR9 LRR region. The black dashed lines showed hydrogen bond interactions between TLR9 LRR region and CpG ODN 2216; blue dashed lines for *P. vivax* CpG DNA; cyan dashed lines for *L.mexicana* CpG DNA and magenta dashed lines for *L. donovani* CpG DNA. The LRR11 region of TLR9 (highlighted in red color) was found to be critically involved in all the interaction.



Conclusion

The projected Leishmanial DNA sequence acting as a ligand for TLR9 exhibiting the comparable interaction versus known synthetic ODN can be taken up as an ideal innate immune generator against this fatal organism. The derivation that LRR11 of TLR9 ECD is the crucial region for ligand binding provides the platform for designing of novel immunomodulatory molecules as well as a structural framework for interpreting experimental data. The study also facilitates the better understanding of involvement of Toll like receptors in signal transduction process evoking resistance against the parasitic infections.

References

1.Pan X, Yue J, Ding G, Li B, Liu X, Zheng X, Yu M, Li J, Jiang W, Wu C, Zheng J, Zhou H. Leucine-rich Repeat 11 of Toll-like Receptor 9 Can Tightly Bind to CpG-containing Oligodeoxynucleotides, and the Positively Charged Residues Are Critical for the High Affinity. J Biol Chem 2012; 287:30596−30609.

2.Uematsu S, Akira S. Toll-Like receptors (TLRs) and their ligands. Handb Exp Pharmacol 2008; 183:1–20.

3.Chuang Tsung-Hsien, Lee J, Kline L, Mathison JC, Ulevitch RJ. Toll-like receptor 9 mediates CpG-DNA signaling. J Leukoc Biol 2002; 71: 538−544.

4.Tuon FF, Fernandes ER, Pagliari C, Duarte MIS, Amato VS. The expression of TLR9 in human cutaneous leishmaniasis is associated with granuloma. Parasite Immunol 2010; 32:769–772.

5.Schleicher U, Liese J, Knippertz I, Kurzmann C, Hesse A, Heit A, Fischer JA, Weiss S, Kalinke U, Kunz S, Bogdan C. NK cell activation in visceral leishmaniasis requires TLR9, myeloid DCs, and IL-12, but is independent of plasmacytoid DCs. J Exp Med 2007; 204:893–906.

Acknowledgement

The Department of Science & Technology, New Delhi, India is gratefully acknowledged for the financial support of this investigation as INSPIRE Fellowship to C.L.G (IF120575). The authors are also thankful Integral University, Lucknow, India for providing the Infrastructural facilities.