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Predicting conserved epitopes in the variants of amastin protein of Leishmania major and designig an epitope based immunogenic DNA vaccine

P. Slathia*, P. Sharma

Shri Mata Vaishno Devi University, Katra, Jammu and Kashmir, India

Background: Efforts for designing vaccines against leishmaniasis are of immense importance because of increasing resistance to the current drugs and lack of any vaccine against the parasite. Amastin one of the major proteins expressed in amastigote stage has been shown to be as an important vaccine candidate. In the post genomic era sequence data is available and this may be exploited for designing epitope based vaccines. The present study deals with prediction of conserved epitopes in the variants of amastin protein in *Leishmania major* and utilizing them for designing an immunogenic DNA vaccine.

Methods: Almost all amastin (83) sequences of *Leishmania major* available in the NCBI protein database were aligned using ClustalW tool of DNASTAR 8 software module. The bootstrapping of phylogenetic tree was done by MegAlign software. Conserved regions having a length of more than 9 amino acid residues were used for prediction of MHC binding epitopes. NetMHCPan and IEDB servers were used for predictions and the predicted epitopes were joined in tandem and reverse translated using EMBOSS server. The reverse translated sequences were subjected to CpG optimization by DynaVacs server. This sequence was conceptually cloned in pBI-CMV vector.

Results: Multiple sequence alignment did not show any conserved regions that may be present in all the 83 sequences therefore the sequences which formed a part of similar node were used to find conserved peptides. The conserved peptides of each node when used for prediction resulted in maximum of 7 epitopes for MHC I and 6 epitopes for MHC II whereas a minimum of 1 epitope in both MHC I and MHC II. BLAST was used to remove any self epitopes showing homology to human proteins and any such epitope was discarded. The peptide properties were analysed by Protfun server, ExPasy tools and Protean module of DNASTAR 8. DNA sequence obtained by reverse translation should be optimized for CpG islands as they have an important role to play in immunogenicity. Sequence manipulation to construct plasmid vector resulted in development of an effective DNA Vaccine.

Conclusion: The DNA vaccine designed using the reverse vaccinology approach described above may provide long term immunity against cutaneous leishmaniasis.

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Update: serotype distribution and drug susceptibility of invasive pneumococcal diseases in central Thailand

S. Srifuengfung[1,*], C. Tribuddharat[1], W. Phongsamart[2], K. Chokephaibulkit[2], T. Chatsuwan[3], V. Treerauthanaweeraphong[4], P. Rungnobhakhun[5]

[1] *Mahidol University, Bangkok, Thailand*
[2] *Siriraj Hospital, Mahidol University, Bangkok, Thailand*
[3] *Chulalongkorn University, Bangkok, Thailand*
[4] *Children Hospital, Bangkok, Thailand*
[5] *Bhumipol Hospital, Bangkok, Thailand*

Background: *Streptococcus pneumoniae* is an important pathogen accounting for significant morbidity and mortality worldwide. At present, 92 immunologicaly distinct serotypes of *S. pneumoniae* have been described, each varying in the structure of their polysaccharide capsule. To predict potential benefit of 7-, 10- and 13-conjugate vaccines (PCV), we evaluated serotype distribution, serotype coverage over *S. pneumoniae* and drug susceptibility. 7-PCV contains 4-6B-9V-14-18C-19F-23F. 10-PCV is 7-PCV plus 1-5-7F. 13-PCV is 10-PCV plus 3-6A-19A.

Methods: The serotypes of 55 *S. pneumoniae* isolates from normally sterile sites obtained from different patients admitted to the hospitals with invasive diseases (from 17 hospitals) were evaluated. The catchment area was 5 provinces. They were serotyped by Quellung reaction, using various specific group and factor antisera of Pneumotestâ kit, a phase contrast microscope and tested for drug susceptibility by Etest/disk diffusion methods.

Results: The male:female ratio was 1.89: 1. The age range was 2 mo-87 yr. The specimen sources were 48 blood (87.27%), 3 CSF (5.45%), 3 pleural fluid (5.45%), 1 abdominal cavity (1.83%). The serotype coverages of 7-, 10-, 13-valent PCV were 46.15%, 53.85%, 84.15% in children < 5 yr (n = 26) and 51.95%, 59.26%, 74.07% in adults 15-87 yr (n = 27). Three most common serotypes in children were 6B (26.92%), 19A (19.23%), 14 (11.54%) and adults were 6B (22.22%), 6A (11.11%), 7F, 18C, 23F (7.41% each). Overall, *S. pneumoniae* was sensitive to penicillin (94.29%), cefotaxime (97.14%), chloramphenicol (71.43%), clindamycin (66.67%), co-trimoxazole (22.22%), erythromycin (60%), linezolid (100%), ofloxacin (100%), tetracycline (42.86%) and vancomycin (100%).

Conclusion: The information from this study may guide vaccine developement and the decision for the use of pneumococcal vaccines as well as appropriate empirical drug regimen for patients with invasive pneumococcal disease in Thailand.

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