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Background: KSHV which is the etiological agent of Kaposi's Sarcoma encodes a protein by open reading frame (ORF) K14, called vOX2. This protein shares identity with the mammalian OX2 (CD200). Signal delivery of vOX2 occurs through binding to CD200R which contributes to maintaining the homeostasis of immune responses, in a manner similar to CTLA-4 and PDL-1/-2. Due to highly glycosylation and adhesive properties of vOX2, it failed to determine the 3D-structure of the protein by X-ray crystallography method. Therefore, probable crystal structure of vOX2 has been bioinformatically identified. Materials and Methods: In order to identify the templates, BLAST sequence homology searches (NCBI) were performed. The PD-L1 (PDB entry: 3FN3) was chosen for modeling. Multiple alignment process was carried out on the selected sequences by ClustalX2 (protein weight matrix: BLOSUM series). Model building was performed in the program MODELLER9v5 using model-ligand algorithm and models at various refinement levels were generated. Finally the refined structures were minimized under molecular mechanic AMBER method (RMS gradient = 0.5) in HyperChem7.5. All models were validated using the program ERRAT and PROCHECK at UCLA. Results: The best 3D model had an Errat score of 94%. In the modelled protein all of the glycosyl-linked residues (Asn 83, 91, 138, 157 and 166) are situated in the external region of the 3D structure with steric flexibility of their side chains. Conclusion: Our modeling supports the data which indicates that vOX2 has two Ig-like domains, located between residue 11 to 125, and 131–220, respectively and the integrin-binding motif, RGD, at residues 191–193 is exposed.
Keywords: KSHV, Kaposi's Sarcoma, vOX2

387. In Silico Analysis of a Chimeric Vaccine Candidate against Enterotoxigenic *Escherichia Coli*

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Background: Enteric infections resulting in diarrhoeal disease remain a leading global health problem. Among bacteria enterotoxigenic*Escherichia coli* (ETEC) cause the largest number of diarrhoeal cases. Based on the great health impact of infections with ETEC, there is a great interest in developing an effective ETEC vaccine.An ETEC vaccine should probably contain colonization factor antigens present on the most prevalent ETEC pathogens and LT toxoid or nontoxic LTB. Chimeric proteins carrying epitopes, linkers or adjuvant sequences increased immunogenicity of the recombinant antigen and the possibility to elicit a broad cellular or humoral immune response. *In-silico* tools are highly suited for both the discovery of new and development of existing vaccines. Materials and Methods:A synthetic chimeric 1800 bp gene encoding containing CfaB, CstH,CotA ,LTB and hydrophobic amino acid linkers, was designed; the gene, named L2C3, encoded a total of 599 amino acids. The gene was modified with regard to codon usage to optimize gene expression in *E.coli* and plant system.Modeling was done to predict the 3D structure. This model was validated with the program PROCHECK using Ramachandran plot statistics .The predicted B-cell epitopes were mapped on the surface of the model.Results:A chimeric gene containing colonization factors and LTB was designed. Tertiary structure was predicted and evaluated. Validation result showed that 97.2 % residues lies in favored and additional allowed region of Ramachandran plot.VaxiJen analysis of protein showed high antigenicity.Linear and conformational B-cell epitopeswas identified.T-cell identified epitopes were expected to bind MHC molecules.
Conclusions:The chimeric protein has epitopes that are likely to induce both the B-cell and T-cell mediated immune responses. The chimeric protein could be produce in microcapsulated form or in plant system and has potential as a valuable vaccine candidate for oral immunization against ETEC.
Keywords: Chimeric Vaccine, ETEC, Chimeric proteins

388. In Silico T-cell and B-Cell Epitope Prediction in MLV Pseudotype Virus with VSVG for Epitope Based Vaccine Design against Rabies Virus

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Background: The conventional approaches to develop vaccines are killed, live or inactivated organisms. The problems with this approach are that many of the proteins are not necessarily expressed in vitro, meaning good candidate antigens can be overlooked and it might not be possible to cultivate a particular pathogen in the laboratory, moreover, conventional approaches require longer times for identifying candidate antigens as targets. The development of epitope-based vaccines is one example of reverse vaccinology. These peptide epitopes represent the minimal immunogenic region of a protein and allow for precise direction of immune responses. A critical requirement of epitope based vaccine design is the identification and selection of T-cell and B-cell epitopes. Materials and Methods: We have produced MLV pseudotype virus with vesicular stomatitis virus glycoprotein (vsvg). We retrieved the sequence of vsvg from NCBI database. A BLAST search, subcellular localization, T and B-cell epitope prediction were performed for vsvg. Results: Homologus proteins obtained by BLAST studies were specific for vsvg. Prediction of subcellular localization of protein and presence and location of signal peptide cleavage sites that were performed using PSORT b,TMHMM, Subloc and Signal P respectively, confirmed the protein to be extracellular and transmembrane , with a signal peptide between positions 16 and 17. Characterization of epitopes on vsvg with BcePred and NetCTL-1.2 servers identified four peptide sequences NQKGNWKNVPSNYHY, PSVEQCKESIEQTKQ, TVHNST TWHSDYK and CPEGSSISAPSQTSV and three peptide sequences of ELWDDWAPY, VLRTSSGYKF and SWKSSIASFFF that are B-cell and T-cell epitopes respectively, are good candidate epitopes for vaccine design against rabies virus. Conclusion: The present study is a computational approach for identification of candidate T-cell and B-cell epitopes from vsvg using immunoinformatics tools. We report seven epitopes which have good binding affinity for MHC and antibody molecules. However, these should further be tested by lab studies for a targeted vaccine design against rabies virus.
Keywords: MLV Pseudotype Virus, VSVG, Rabies Virus

389. A Computational Study of Novel Fusion Protein (Cfab-LTB) for Use as ETEC Plant Based Vaccine

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Background: Computational studies were performed to ensure that the epitopes of the constructed novel CFA/I-LTB fusion protein were not changed during the linkage of the two molecules by 3 Dimensional structures of CfaB-LTB fusion protein and prediction of its epitopes. Materials and Methods: Protein homology based modeling was carried out using MODELLER 9 V7 software. For CfaB-LTB fusion protein homology modeling, the 3F83, 3F84 and 3F85 and the 1B44, 1LTR were selected as templates, respectively. Template models were generated for each segment of fusion protein construct based on the one template procedure of MODELLER software, and for fusion protein the multiple template procedure of MODELLER software was used. After constructing the three structures (i.e. CfaB, LTB, and CfaB-LTB), the epitopes propensity was predicted using Prediction of Antigenic Epitopes on Protein Surfaces by Consensus Scoring (EPCES) service at Chi Zhang's systems biology lab University of Nebraska-Lincoln (BMC Bioinformatics. 2009 Sep 22;10:302). In addition, the possibility of the N-Glycosylation presence in CfaB-LTB was examined by NetNGlyc 1 server. Results: Based on computational studies, secondary structure models of CfaB, LTB and CfaB-LTB fusion protein were predicted. There was no difference between the <90 EPCES score of CfaB antigenic epitopes in the fusion and independent form, indicating no significant dissimilarities between epitopes of the fusion and independent structures of CfaB. Software based figures and data predicted an independent form of LTB. The antigenic epitopes with <95 EPCES score were 59%, and these epitopes in fusion form were decreased to 53%. This may indicate that a few LTB epitopes with <95 EPCES score (%6) in the fusion structure were altered. As shown, the fusion protein may have undergon N-glycosylation in asparagines residues (residue 27, 96, 157 and 286) but these glycosylations were occurred in antigenic epitopes with low (50 EPCES) score, suggesting that the probable N-glycosylation has happened in