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Title of the Thesis: Cloning and expression of G protein gene of group B respiratory syncytial virus in bacterial and mammalian systems and their biophysical characterization.

Research Findings:

- The two genes were optimized and chemically synthesized for G gene sequences for prototype group B hRSV (18537) and one BA strain. Clones were prepared for the full length G gene and for the truncated part of the G gene (from second in frame ORF and ectodomain region) for expression in prokaryotic and eukaryotic expression systems.

- The optimized full length clone of prototype (18537) and one BA strain (281) of the G protein did not express in both prokaryotic and eukaryotic expression systems.

- The optimized clone of prototype (18537) and one BA strain (281) of the second in frame ORF (AUG2: Met48) of the G gene shows limited expression in the prokaryotic and eukaryotic expression systems.

- The optimized clone of hRSV group B prototype (18537) and one BA strain of the ectodomain G protein gene expressed well in *E. coli* and was detected by immunoblotting with 6X his antibody. The protein expressed as inclusion body and was purified with urea denaturation method using Ni-NTA chromatography.

- The eukaryotic expression clone for ectodomain G protein of the prototype group B hRSV (18537) and BA strain was expressed in Hep-2 cells and the protein was purified from the media portion of the transfected cells.

- The ectodomain G protein of prototype group B hRSV showed three sites for N-linked glycosylation on analysis with software (Net-Nglyc 1.0 by CBS). One particular site at N100 had potential for N-glycosylation with score predictor (G score) of 0.73. Analysis of wild type (GΔTM) and mutated (GΔTM) at N100 site by replacement of Asn to Glu by western blotting revealed that there was some observable differences in the size of the two proteins shows the particular site having more prone to N-linked glycosylation in the prototype strain.

- The deglycosylation of G proteins resulted in their degradation and these degraded proteins appeared as smear on western blotting. Results of this experiment concluded that ectodomain G protein of hRSV has both N-linked and O-linked glycosylation with O-linked sugars contributing to major part of the glycosylation.

- The ectodomain G protein expressed in bacterial system was subjected to biophysical studies. The ectodomain G protein showed surface hydrophobicity of 1.77 μM^{-1} which was much lower than surface hydrophobicity of native ovalbumin (12 μM^{-1}).

- The molar absorption coefficient ε of GΔTM was estimated to be 7950 M^{-1} cm^{-1} at 280 nm and mean residue ellipticity at 222 nm ($[\theta]_{222}$) was -19701.7 deg cm^2 $dmol^{-1}$ at pH 8.0 and 25 ^{0}C.

- The secondary structure of the ectodomain G protein was determined by far UV CD spectra. It was concluded that the ectodomain G protein mainly consist of α-helix (92.3 %) with some amount of β-sheet (7.7 %).

- On thermal denaturation of the protein, the protein was stable upto 85 ^{0}C.
- Heat induced denaturation of the ectodomain G protein resulted in total loss of β-sheet where as not much change was observed in α-helix part of the secondary structure.
- Urea-, GdmCl- and acid-induced denaturation resulted in almost total loss of the overall secondary structure including both α-helix and β-sheet.

This is the first investigation of cloning, expression and characterization of G protein of BA viruses from India. Structural characterization of G protein will assist in vaccine development efforts.