SUMMARY AND CONCLUSIONS

SALIENT features of the study entitled "Immunoprotective Potential of *Klebsiella pneumoniae* O–polysaccharide–Cholera Toxin B Subunit Conjugate Against Experimental Lobar Pneumonia" where basic thrust was to explore the possibility of using polysaccharide moiety from LPS antigen of *K. pneumoniae* for prophylaxis against experimental pneumonia.

1. *K. pneumoniae* B5055 (O1 : K2) was selected for the study because the strain expresses O1 and K2 serotypes which are the most commonly encountered O and K types found in clinical situation.

2. Based on pilot experiments intranasal instillation method was selected for induction of lobar pneumonia in mice. A dose of 10^4 CFU/ml was found to be optimal for establishment of acute *Klebsiella* infection in BALB/c as well as LACA strain of mice.

3. The course of experimental pneumonia was studied in terms of induction, establishment, and resolution of infection. Findings at various time intervals showed that the lung bacterial load was highest on day 3 post infection, followed by a decrease thereafter, with lungs becoming sterile by day 10 post infection.

4. A semi-quantitative scale for pathological evaluation of lung lesions was formulated, according to which the lung tissue was scored on a scale of +0 to +3. Pathological changes corroborated the bacteriological findings. Mild bronchopneumonia observed on day 1 post infection, progressed to well developed lobar

pneumonia by day 3 post infection. Signs of resolving pneumonia were observed on day 7 post infection.

5. Experimental pneumonia in mice was accompanied by an influx of inflammatory cells (macrophages and neutrophils) assessed by bronchoalveolar lavage fluid (BALF) examination and myeloperoxidase (MPO) estimation. A compartmentalized time dependent elevation of TNF–α, MIP–2, and nitric oxide was observed in the BALF fluid as well in the lung homogenate supernatant following infection in experimental animals.

6. The course of experimental *K. pneumoniae* mediated pneumonia was studied in aged, alcohol fed and dietary lipid fed mice. No significant differences were observed in the lung bacterial load and severity of pathological lesions in the young and aged mice. Alcohol feeding had a detrimental effect on outcome of *K. pneumoniae* mediated pneumonia. Significantly higher bacterial counts and lung severity scores along with 30% mortality were observed for the alcohol fed mice as compared to the control mice. Short term dietary n-3 PUFA feeding did not alter the course of experimental pneumonia, but a long term feeding regime proved to be detrimental for the host.

7. Lipopolysaccharide antigen of *K. pneumoniae* B5055 was extracted using hot phenol extraction procedure and purified by gel filtration and ultracentrifugation. The fractionation of LPS on Sephadex G-50 column showed two peaks with major TBA reactive material detectable in first peak.

8. Purified LPS was found to be toxic as well as pyrogenic, at all doses tested. It induced a second degree Schwartzman reaction in rabbits. It was found to be immunoprotective when administered *via* the intramuscular route. However, intranasal delivery at low dose provided protection, while higher dose

caused tissue damage leading to increased bacterial colonization. The protection conferred by LPS immunization was associated with enhanced recruitment of phagocytic cells to lungs, and activation of their phagocytic capacity.

9. O–polysaccharide portion of LPS was separated from the lipid A moiety by mild acid hydrolysis. This O–polysaccharide was eluted in two poorly separated peaks on Sephadex G–50 column which were pooled and lyophilized.

10. Purified O–polysaccharide was found to be non-toxic and non-pyrogenic but failed to confer any significant protection at 50μg dose when given through intramuscular as well as intranasal route.

11. Recombinant cholera toxin B subunit (rCTB) was chosen as the carrier molecule because of its efficacy, purity, safety and stability reported with several protein/polysaccharide antigens.

12. O–polysaccharide was found to be immunoprotective when delivered intranasally along with non–conjugated rCTB subunit. A 3.22 log cycle decrease was observed in the lung bacterial load after intranasal immunization The same effect was however not observed with O–PS–rCTB mixture when injected *via* the intramuscular route.

13. O–polysaccharide was successfully conjugated to rCTB subunit by carbodiimide condensation reaction using adipic acid dihydrazide as a spacer molecule, and the conjugate was eluted in the void volume on a sephadex G–50 column.

14. A good yield of coupled material (43.5%) and protein polysaccharide ratio (1.8) was achieved through this method of conjugation. The conjugate (50 μg, 100μg) was found to be non-toxic, non-pyrogenic. It did not induce any Schwartzman reaction.

15. The prepared conjugate was found to be immunoprotective when administered by both the routes i.e. the intranasal and the intramuscular route. A significant decrease ($P<0.01$) in lung bacterial load as well as severity of lung lesions was observed for the immunized animals. A 4.87 and 2.55 log cycle decrease was observed in lung bacterial load after intranasal and intramuscular immunization respectively. Protection achieved with O–PS–rCTB conjugate delivered intranasally was significantly higher than that seen after intramuscular immunization.

16. Innate immune response studied in immunized (conjugate treated) animals showed that enhanced bacterial clearance from lungs was associated with increased influx of PMNs and macrophages with enhanced phagocytic activity. This was associated with accelerated production of proinflammatory cytokines TNF–α and MIP–2 in Bronchoalveolar lavage fluid (BALF) and Lung homogenate supernatant (LHS) of immunized animals.

17. Intranasal immunization with the conjugate evoked a good systemic as well as mucosal immune response in terms of IgG and IgA expression in serum and BALF of immunized animals as determined by ELISA. In contrast intramuscular immunization with the conjugate induced a weak mucosal immune response as low levels of IgA were detected in the BALF in this group of animals.

18. Conjugate provided protection against challenge with heterologous *K. pneumoniae* strain/isolates but was lesser than that observed with homologous strain.

19. Based on the results of this study it is concluded that immunization with *K. pneumoniae* O–polysaccharide rCTB subunit conjugate provides protection against experimental lobar pneumonia in mice. This polysaccharide conjugate vaccine falls in

the category of new generation vaccines and confers protection through activation of non-specific as well as specific defense mechanisms.

20. The study establishes the efficacy of intranasal route of immunization over the parenteral immunization. It also confirms the mucosal adjuvanticity of rCTB subunit for *K. pneumoniae* O-polysaccharide antigen.