Summary and Conclusions

Salient features in relation to the study entitled "Effect of gram negative bacteria on the activity of resident alveolar macrophages and lung tissue in polyunsaturated fatty acids fed experimental mice" are:

1) *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains were chosen for the study since both these gram negative bacteria are commonly associated with nosocomial as well as community-acquired pneumonias. Standard virulent strains of *P. aeruginosa* PA103, a cytotoxic strain belonging to serotype 11 and *Klebsiella pneumoniae* B5055, most commonly encountered 01 and K2 serotypes in clinical situations, were two representatives strains employed in the study.

2) Acute broncho/lobar pneumonia was established in LACA (Swiss-Webster) mice following intranasal inoculation of planktonic and preformed biofilm cells (4-day old) of *P. aeruginosa* PA103 with an optimal infection dose of 2x10^9 CFU/ml.

3) The time course study of acute pneumonia induced with *P. aeruginosa* PA103 revealed that maximum lung bacterial load was observed at 6 hour post infection period. Animals infected with biofilm cells had only marginally higher cell counts. This was followed by a decline thereafter and lungs became sterile at 48
Summary and Conclusions

hours post infection time in the animals infected with planktonic cells. Infection, however, persisted till 48 hour in the mice infected with biofilm cells and lungs became sterile at 72 hour post infection time.

4) Evaluation of pathology performed at different time intervals demonstrated severe bronchopneumonia at 6 hour post infection period. This decreased to milder pneumonia noticeable at 24 hour and 48 hour in the mice infected with planktonic cell and biofilm cell forms respectively.

5) Acute inflammatory response generated during acute pneumonia was studied through neutrophil infiltration assessed by examination of bronchoalveolar lavage fluid and myeloperoxidase (MPO) estimation in lung homogenate supernatants. Maximal levels were observable at 6 hour (peak infection time). Elevated levels of malondialdehyde and lactate dehydrogenase gave the index of tissue destruction operative through lipid peroxidation and cell cytotoxicity.

6) Time dependent production of proinflammatory cytokines (TNF-α and IL-1β) and nitric oxide could be demonstrated in bronchoalveolar lavage as well as lung homogenate supernatants of infected mice with maximal levels at 6 hour post infection. In mice infected with biofilm cells, significantly higher levels of TNF-α and IL-1β were observed from 24 hour onwards. The kinetics of IL-10 production showed that the levels of this anti-inflammatory cytokine was raised towards the later stage of infection which could be playing a potential role in the resolution of acute pneumonia.

7) Chronic pulmonary infection was established with repeated intranasal infections of 2x10^5 CFU/ml each of planktonic and biofilm cells of P. aeruginosa PA103 in LACA mice. Significant
Summary and Conclusions

Bacterial load persisted throughout the study period of 6 weeks. Corresponding lung pathology study revealed progressive chronic inflammatory changes in the lung tissue after 4 weeks characterized by abscess formation and fibroblastic proliferation. Nitric oxide, malondialdehyde and lactate dehydrogenase levels remained consistently raised throughout the observation period.

8) Levels of TNF-α, IL-1β and IL-10 in bronchoalveolar lavage and lung homogenate supernatants were significantly elevated after the first infection followed by decrease to intermediate levels which persisted till end of the experiment. However, IL-10 concentrations remained significantly lower in comparison to levels of pro-inflammatory cytokines at all time points with an increase observed only towards the 6th week. Severity of infection and chronic inflammation induced by biofilm cells was comparable to that observed in case of chronic pneumonia induced planktonic cells.

9) Acute pneumonia was established in BALB/c mice by intranasal inoculation of planktonic and preformed biofilm cells (3-day old) of *K. pneumoniae* B5055 with an optimal dose of 10⁴ CFU/ml. Course of infection was studied over a period of 14 days. Peak of infection was observed on 3rd post infection day, with both the cell forms, as assessed in terms of lung bacterial load and lung pathology, but significant difference was seen from 5th day onwards.

10) Infection induced by biofilm cells persisted till 12th post infection day and lungs became sterile by 14th day. In case of acute pneumonia induced by planktonic cells, sterile lungs could be observed on 10th post infection day.

11) There was an intense neutrophil infiltration in the bronchoalveolar lavage indicated by maximal MPO levels in lung...
Summary and Conclusions

homogenates on the 3\textsuperscript{rd} post infection day. Peak of nitric oxide production also correlated with the highest bacterial load in the lungs. At the same time, elevated malondialdehyde and lactate dehydrogenase levels coincided with maximum tissue damage.

12) Time dependent localized generation of TNF-\(\alpha\) and IL-1\(\beta\) was observed in the bronchoalveolar lavage as well as lung homogenate supernatants, with highest levels detected on the 3\textsuperscript{rd} post infection day. Significantly high levels of these pro-inflammatory cytokines were observed from 3\textsuperscript{rd} day onwards in the biofilm cells infected mice as compared to their levels in the mice infected with planktonic cells. Increase in IL-10 production was observed from 7\textsuperscript{th} day onwards in mice infected with both cell forms.

13) Chronic pneumonia with \textit{K. pneumoniae} B5055 was induced in BALB/c mice with three repeated infections (10\textsuperscript{4} CFU/ml each) at weekly intervals. Course of infection, studied over a period of 30 days was similar in case of planktonic as well as biofilm cells infected groups. Maximum lung bacterial load was found on 18\textsuperscript{th} post infection day. Corresponding lung pathology revealed severe chronic inflammation with abscess and fibroblastic proliferation in the lung tissues. Elevated levels of nitric oxide, malondialdehyde and lactate dehydrogenase at this time, confirmed the extent of tissue damage.

14) In the chronic infection model, the maximal levels of TNF-\(\alpha\) and IL-1\(\beta\) in bronchoalveolar lavage and lung homogenate supernatants correlated with the highest lung bacterial load. Kinetics of IL-10 production showed slow but steady rise in its levels till the end of observation period. The pattern of cytokine production was matching in the planktonic and biofilm cell infected groups.
15) Oral supplementation with three different n-3 PUFA preparations (Cod Liver Oil, Maxigard and Flaxseed oil) was carried out for a duration of 2 and 6 weeks. Course of acute pneumonia, induced by planktonic and biofilm cells of the two gram negative bacterial strains, was compared in different groups of experimental mice. Olive oil and normal saline fed mice served as oil and saline controls, respectively.

16) No effect on the course of infection was observed when acute pneumonia was induced in animals after 2 weeks of fatty acid feeding. On the other hand, six weeks of n-3 PUFA administration was found to give benefit to the mice against acute infection by both bacterial strains. There was a decrease of 1.2 to 1.6 log cycles in bacterial counts, coupled with significant improvement in pathology. Cod liver oil gave maximal benefit while with Maxigard and Flaxseed oil, advantage was almost matching.

17) Alveolar macrophages collected from all three groups of n-3 PUFA fed mice, exhibited significant decrease in the level of apoptosis following infection. Biofilm cells had an overall lower apoptotic potential than planktonic cells observable in all the three n-3 PUFA administered groups. Study was carried out in vitro with planktonic as well as biofilm cells (whole cells) of the two bacterial strains.

18) The alveolar macrophages obtained from all the three groups of n-3 PUFA fed mice showed significantly enhanced phagocytic activity for both the cell forms of P. aeruginosa PA103 and K. pneumoniae B5055. However, overall phagocytosis of biofilm cells was lower than that for planktonic cells.

19) Beneficial effect against acute pneumonia induced by planktonic and biofilm cells of the two bacteria, in n-3 PUFA fed mice, was
demonstrable through lower lung levels of nitric oxide, malondialdehyde and lactate dehydrogenase which was associated with decrease in severity of tissue damage. There was also a significant increase in the levels of pro-inflammatory cytokines (TNF-α and IL-1β) coupled with a marginal increase in the levels of anti-inflammatory cytokine (IL-10) in the n-3 PUFA fed and infected mice, as compared to the control animals.

20) This study brings out that dietary n-3 PUFA supplementation exerts an overall beneficial effect against acute pneumonia induced by both planktonic as well as biofilm cell forms of *P. aeruginosa* and *K. pneumoniae*. This is operative through upregulation of non-specific and specific immune defenses of the host. However, for this purpose, minimal duration required for n-3 PUFA administration, is 6 weeks. Out of three preparations of n-3 PUFA checked, cod liver oil gave maximal benefit in relation to all the parameters.