Introduction

Inspite of the recent medical advances, lower respiratory tract infections are still the most frequent cause of morbidity and mortality worldwide (Gales, 2002). Epidemiologic studies have identified gram negative bacteria, especially Pseudomonas aeruginosa and Klebsiella pneumoniae, to be the most frequently involved pathogens, both in nosocomial as well as community-acquired pulmonary infections (Merchant et al., 1998; English, 2000; Gales et al., 2002; Angles-Garav et al., 2005; Andriesse and Verhoef, 2006). Pneumonia caused by these organisms is extensive and voluminous, characterized by destruction of alveolar septa. Despite good clinical armamentarium, the emergence of multidrug resistant strains of these pathogens, especially in immunocompromised patients, leads to treatment failures (Laichalk et al., 1996; Moore et al., 2002). Apart from the arsenal of various virulence factors, P. aeruginosa and K. pneumoniae are also endowed with the ability to form biofilms, specially in mechanically-ventilated or intubated patients, in the hospital setting (Hoiby et al., 2001; Donlan et al., 2001; Lavender et al., 2004). These biofilm cell forms can successfully evade host’s immune defenses as well as elimination by the administered antibiotics (Costerton et al., 1999). This situation can lead to the establishment of persistent and chronic infections by these organisms. The development of biofilms is, thus, considered to be an important stage in the pathogenesis of infections caused by these organisms.
Introduction

Eventual outcome of any infection is decided by an interplay between the host and the pathogen’s virulence determinants. Most of the earlier interpretations, in this regard, were based on epidemiological studies. However, the development and availability of suitable animal models, which simulate human infections, can provide a detailed insight into the underlying mechanisms (Bakker-Woundenberg, 2003). Over the years, models in different animals have been described for P. aeruginosa and K. pneumoniae mediated lung infections (Domenico et al., 1982; Bakker-Woundenberg et al., 1985). Relatively recently, mice have been preferably employed, as animal models, by many workers in relation to pulmonary infections (lizawa et al., 1988; Held et al., 1998; Schultz et al., 2001; 2002; Lavender et al., 2005). In addition, many routes for inducing infection have been explored in different studies. Intranasal route of the inoculation has gained considerable importance, since it mimics the natural route of entry for pathogens in humans. It is also reproducible (Yoshida, et al., 2000; 2001). Use of this route in mice, thus, gives the preferred animal model for studying the pathogenesis of pulmonary infections in vivo (Mueller – Ortiz et al., 2004; Smith et al., 2004).

Programmed cell death or apoptosis, in vivo, is an essential mechanism in eukaryotes for normal development and homeostasis (Vaux and Strasser, 1996). Several gram negative bacteria, like P. aeruginosa and K. pneumoniae whole cells, and their cell components have been shown to infect host cells and induce apoptosis as a part of their pathogenetic mechanism (Zychlinsky et al., 1997; Hauser et al., 1999; Estaquier, et al., 1998; Jendrossek, et al., 2001; Hetz, et al., 2002). P. aeruginosa has been found to induce in vitro apoptosis in human respiratory epithelial cells, alveolar macrophages, murine dendritic cells and antigen presenting cells, which play a central role in the initiation of pulmonary host defenses against the pathogen (Rajan
et al., 2000; Worgall et al., 2002). Relevance of this process in relation to respiratory tract infections is, therefore, well recognised. Phagocytes, like polymorphonuclear leukocytes (PMNs) and alveolar macrophages, are the primary effector cells, mediating host defense against invading bacteria. PMNs, though, are not a constituent of the normal alveolar cell population, but rapidly migrate into the alveoli in response to bacterial challenge, providing critical phagocytic defense capabilities to the lung (Nelson et al., 1990). Alveolar macrophages, on the other hand, are the only intralveolar resident phagocyte population forming the first line of cellular defense in the airspaces of the lower respiratory tract (Green et al., 1977; Sibelle and Reynolds, 1990; Gwinn and Vallyathan, 2006). Activated alveolar macrophages and other cell population in the lungs, elaborate antimicrobial molecules like nitric oxide (NO) and cytokines which regulate the inflammatory response generated during the evolution of infection. In addition, production of both pro-inflammatory (like TNF-α, IL-1 and IL-6) and anti-inflammatory (like IL-4, IL-10) cytokines are reported in the infective process which is applicable to pneumonia caused by P. aeruginosa and K. pneumoniae as well (Yoshida et al., 2000; 2001; Schultz et al., 2000; 2002; Sadikot et al., 2005).

Apart from above mentioned factors participating in bacteria-host interaction, intake of different nutrients is considered a critical determinant for resistance and development of proper immuno-competence (Field et al., 2002; De Pablo et al., 2002). Although all the nutrients in the diet play crucial role in maintaining an optimal immune response, yet role of certain micronutrients has been highlighted in recent years in relation to non-infectious and infectious diseases (Ross, 1992; Stephenson et al., 2001; Wintergerst et al., 2005; Mahalanabis et al., 2006). The importance of potential health benefits of dietary fats has specially drawn attention of the workers (Hwang,
Introduction

Amongst these, polyunsaturated fatty acids (PUFA) are important nutritional elements for humans (Connor, 2000). n-3 and n-6 are the most commonly consumed forms of PUFA all over the world. Both these fatty acids cannot be synthesized in the body and hence have to be provided in the diet. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), two most biologically potent n-3 PUFA are found in abundance in fish oil or cod liver oil. Alphalinolenic acid, another important n-3 PUFA, is found in vegetable oils like flaxseed oil, walnuts, peanuts and spinach. Supplementation of diet with n-3 PUFA has proven to be beneficial in amelioration of cardiovascular, autoimmune and several immunological, inflammatory disorders (Stulnig, 2003; Watson, et al., 2005; Wolters, 2005; Nettleton and Katz, 2005; Damsgaard, et al., 2006). This protective effect is attributed to the anti-inflammatory properties of n-3 PUFA (Yaqoob, 2004).

The role of n-3 PUFA supplementation in relation to infectious diseases, however, is not well defined. Available data is equivocal regarding the beneficial as well as detrimental effects of these fatty acids against different infections (Anderson and Fritsche, 2002). n-3 PUFA supplementation is reported to enhance host’s survival against experimental infections induced by Group B Streptocci (Rayon et al., 1997), S. aureus (Barton et al., 1991) and E.coli (Johnson et al., 1993). On the other hand, several workers have reported impaired host resistance against L. monocytogenes (Fritsche et al., 1997; 1999), P. aeruginosa (Peck et al., 1990) and S. typhimurium (Chang, et al., 1992). However, these studies were somewhat narrow in scope, since in majority of them, survival was the primary focus. In the context of pulmonary infections, an earlier study by D'Ambola et al. (1991) reported impaired ability of n-3 PUFA fed neonatal rabbits to kill intrapulmonary S. aureus. Recently, Thors et al. (2004) observed
improved survival of mice against *K. pneumoniae* but no effect against *S. pneumoniae* in fish oil fed mice. Similarly, there was no effect of DHA supplementation against *P. aeruginosa* mediated lung infection in murine model of preexisting cystic fibrosis, developed in *cftr* knock-out mice (van Heeckeren *et al.*, 2004). In all these studies, the duration of n-3 PUFA feeding varied from 1 week to 6 weeks. The present investigation was, thus, planned to elucidate the role of n-3 PUFA feeding, on the course of acute broncho/lobar pneumonia induced by *P. aeruginosa* and *K. pneumoniae*, in normal mice with following aims and objectives:

1. To establish **acute** respiratory tract infection in normal adult mice with planktonic and biofilm cells of standard strains of *Pseudomonas aeruginosa* PA103 and *Klebsiella pneumoniae* B5055.

2. To establish **chronic** respiratory tract infection in normal adult mice with planktonic and biofilm cells of standard strains of *Pseudomonas aeruginosa* PA103 and *Klebsiella pneumoniae* B5055.

3. To study the effect of feeding (for 2 and 6 weeks duration) with three commercially available, essential polyunsaturated fatty acids, containing Eicosapentaenoic acid, Docosahexaenoic acid and alpha-linolenic acid, on the course of acute respiratory tract infection induced by **planktonic** cells of *P. aeruginosa* PA103 and *K. pneumoniae* B5055 in normal adult mice.

4. To study the efficacy of three different dietary n-3 PUFA preparations on the course of acute respiratory tract infections induced by **biofilm** cells of *P. aeruginosa* PA103 and *K. pneumoniae* B5055 in normal adult mice.
Extent of infective process (for 3. & 4.) will be assessed in terms of:

a) Assessment of bacteriological load.

b) Assessment of pathology in the lungs.

5. To study the **alveolar macrophage functions** collected from BALF of different groups of mice in terms of:

   a) Induction of apoptosis/necrosis.

   b) Phagocytic activity (non-specific immune response).

   c) Generation of pro-inflammatory (TNF-α, IL-1β) and anti-inflammatory (IL-10) cytokines (specific immune response).

6. To ascertain and compare **lung tissue damage** in different groups of mice on the basis of:

   a) Biochemical parameters (Production of Lactate dehydrogenase, Malondialdehyde and Nitric oxide).

   b) Immunological response (Assessment of pro-inflammatory : TNF-α, IL-1β and anti-inflammatory : IL-10 cytokines).