Pneumonia, once referred to as “Captain of men of Death” by Sir William Osler in early 20th century, is still a major cause of death in the new millennium, despite the vast armamentarium of antimicrobial agents and improved medical care (Kollef, 1999). In India, pneumonia has been found to be most common cause (68.8%) of respiratory distress syndrome in neonates (Mathur, 2002). It is the sixth leading cause of death in the United States. Also, it is the second most common nosocomial infection and the leading cause of death from hospital acquired infections (Andriesse and Verhoef, 2006).

Pneumonia is characterized by an inflammation and consolidation of the lung tissue caused by infectious agents. It can be broadly classified as ‘community-acquired’ or ‘noscomial pneumonia’. Community acquired pneumonia is contracted outside the hospital and the population at risk includes elderly, infants and young children (Bartlett and Mundy, 1995). In India, community-acquired pneumonia in adults has a morbidity and mortality ranging between 10% and 25% (Dey et al., 1997; 2000). The gram positive bacterium, Streptococcus pneumoniae, is the most frequently isolated pathogen from patients with community-acquired pneumonia followed by Klebsiella pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa and Staphylococcus aureus (Bartlet and Mundy, 1995; Reechaipichitkul et al., 2004). Gram negative bacteria account for less than 2% of
community-acquired pneumonias but are responsible for most nosocomial pneumonias, including the fatal ones.

Nosocomial or hospital acquired pneumonia generally develops more than 72 hours after hospitalisation and the incidence is highest in the intensive care units (ICUs), especially in patients with intubation or mechanical ventilation (Vincent, 1995). Chastre and Fagon (2002) reported that pneumonia affects 8-28% of the patients receiving mechanical ventilation and the mortality rate of ventilator – associated pneumonia can range from 24% to 50%. The predominant organisms responsible for pneumonia were *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. In India, Merchant et al., (1998) studied the incidence of hospital acquired pneumonia and found that most frequent isolates included *Pseudomonas* species (44%) and *K. pneumoniae* (34%). Mukhopadhyay et al. (2003) described that in case of nosocomial pneumonia, 93% of the isolates were gram negative bacteria, with *Pseudomonas* species being the most predominant. More recently, Singhal et al. (2005) analysed bronchoalveolar lavage samples from ventilated patients and identified that common isolates in the decreasing order of frequency were *Acinetobactor* species, *P. aeruginosa*, *K. pneumoniae* and *E. coli*.

*Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* was first obtained in pure culture by Gessard in 1882 from cutaneous wounds which had a blue green discoloration (Forkner, 1960). *P. aeruginosa* is a gram negative aerobic rod with polar, monotrichous flagella and protein structures on the surface (pili) that are responsible for adherence. Besides *P. aeruginosa*, *P. cepacia* and *P. psuedomallei* are two important *Pseudomonas* species involved in human infections. (Leelarasameree et al., 1989; Stableforth and Smith, 1994). *P. aeruginosa* is ubiquitous and extremely versatile, biochemically. It can grow in many habitats.
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including soil, surface waters, sewage, plants and various foods consumed by man (Wilson and Dowling, 1998). In hospitals, it can be found in sinks, respirators, humidifiers and is often transmitted by medical personnel through direct health care worker – to patient transfer (Nseir et al., 2002).

*P. aeruginosa* is the epitome of opportunistic pathogen of humans. The bacterium rarely causes infection in the normal host, but is an efficient pathogen in immunocompromised individuals causing a variety of infections. These include HIV patients who have low CD4 cell counts and have a substantially higher risk for *P. aeruginosa* pneumonia and bacteremia (Afessa and Green, 2000). Patients who are immunosuppressed, particularly transplant recipients, burn patients, patients with cancer and neutropenia are at a risk of acquiring *P. aeruginosa* infections (Chatzinikoloau et al., 2000; Chastre et al., 2000; Santucci et al., 2003). It is the most frequently identified pathogen in patients who required tracheotomy for continued mechanical ventilation in the ICUs with mortality as high as 34 to 48% (Cook et al., 1998; Rello et al., 2003). Craven (2000) described that pathogenesis of ventilator-associated pneumonia (VAP) by *P. aeruginosa* is related to breach of the epithelium, which results from mechanical injury due to endotracheal intubation. This not only compromises the natural barrier between oropharynx and trachea but also facilitates the entry of colonized pathogens through micro- and macroaspiration of infected oral and gastric components. Sligl et al. (2006) identified *P. aeruginosa* as one of the most common sources of bacteremia associated with nosocomial pneumonia (48.9%).

Khan et al. (1995) reported that chronic airway inflammation with recurrent *P. aeruginosa* infections is a major cause of morbidity and mortality in patients with cystic fibrosis (CF). Wine et al. (1999) described that in CF, defective function of cystic fibrosis transmembrane conductance regulator (CFTR) in airway epithelium
and submucosal glands results in chronic disease of the respiratory tract, which manifests early in life by airway obstruction and repeated infections of the CF lung. The CF patients are particularly susceptible to *P. aeruginosa infections* and this organism plays a critical role in the development and progression of pulmonary disease in these patients. Burns *et al.* (2001) analysed bronchoalveolar lavage and showed that 97% of children with CF were colonized with *P. aeruginosa* by the age of 3 years. An enormous amount of work has been done to explain why *P. aeruginosa* (and not other opportunistic pathogens) is the major cause of disease in CF. Prince (2002) attributed the pathogenesis of this bacterium in colonization of CF lungs, to its ability to form biofilms. Donaldson and Boucher (2003) described in detail that lack of normal CFTR chloride channel function in the airway epithelium and associated defects in sodium and water transport result in dehydrated airway secretions and mucus plugging. The contamination of these mucus plugs by the ubiquitous *P. aeruginosa* and its rapid adaptation to the milieu of the airway, result in initial intermittent and then chronic infection. Weber *et al.* (2001) explained that accumulation of inflammatory cytokines and growth factors within the retained mucus account for excessive inflammation observed in CF. Karp *et al.* (2004) highlighted that a decrease in anti-inflammatory mediators could also influence an exaggerated inflammatory response in CF. Sadikot *et al.* (2005) have recently reviewed this field and have concluded that persistent inflammatory response to bacteria infecting CF airways eventually leads to lung damage and fibrosis.

The pathogenesis of *P. aeruginosa* is complex and outcome of an infection depends on the virulence factors displayed by the bacteria as well as on host response. Initial colonization of the host tissues is aided by surface appendages like flagella and pili (Fleiszig *et al.*, 1997; Feldman *et al.*, 1998). *P. aeruginosa* also encodes type III secretion system (TTSS), which is a major determinant of virulence and allows
the bacteria to inject toxins into the host cells (Hauser et al., 1999; Faure et al., 2004). The bacterium secretes different effector proteins via TTSS like Exo S, Exo T, Exo U, Exo Y and Exo A (Finck-Barbancon et al., 1997; Epelman et al., 2004; Sadikot et al., 2005). Central to the success of this bacterium as an opportunistic pathogen, is the genetic flexibility provided by its large genome (Stover et al., 2000; Wolfgang et al., 2003). Clinical isolates have been found to have pathogenicity islands which contain discrete group of several genes that directly contribute to virulence (Spencer et al., 2003). Coordination of gene expression in P. aeruginosa is also controlled by quorum-sensing system, a complex regulatory circuit involving cell-to-cell signalling through small diffusible molecules called autoinducers (Davies et al., 1998; Derenkard and Ausuble, 2002; Lesprit et al., 2003). Sadikot et al. (2005) have summarised important virulence factors responsible for pathogenesis of P. aeruginosa which include production of alginate, pyocyanin pigment, elastase, alkaline protease, hemolysins, siderophores and lipopolysaccharide.

**Animal models for P. aeruginosa pneumonia**

Several models have been described in experimental animals to study both acute as well as chronic pneumonia induced by P. aeruginosa. In earlier studies, administration of free P. aeruginosa provided a model of acute lung infection with either rapid clearance of the organism or acute sepsis and death (Jackson et al., 1967; Southern et al., 1970; Schook et al., 1977).

Chronic pulmonary infections with P. aeruginosa have been established in many experimental animals like cats, rats and mice. Cash et al. (1979) originally established chronic bronchopulmonary infection in rats using P. aeruginosa embedded in agarose beads. This agarose bead method was successfully adopted by Winnie et al. (1982) and Thomassen et al. (1984), to induce chronic pneumonia in
cats by repeated intrapulmonary inoculations. Changes in the morphology and phagocytic activity of alveolar macrophages along with quantitative inflammatory cell response were elucidated in their study. Nacucchio et al. (1984), described that free living *P. aeruginosa* in the presence of sterile agarose beads, is sufficient to cause histopathological changes in rats that closely resemble to those observed with *P. aeruginosa* laden agarose beads.

Mayer and Walzl (1983) developed an experimental *P. aeruginosa* induced acute lung infection by aerosol exposure in mice pretreated with cyclophosphamide (neutropenic mice). Sordelli et al. (1992) described the important role played by phagocytes, especially PMNs, recruited to the site of infection in their mouse model.

Starke et al. (1987) developed a mouse model of chronic pulmonary infection, by transoral intratracheal inoculation of *P. aeruginosa* enmeshed in agarose beads, to study bacteriological and pathological features of this infection. Chmiel et al. (1999) adopted this model to investigate the role of IL-10 in excessive inflammation during chronic endobronchial *P. aeruginosa* infection in mice. The agarose bead model has been used extensively, over the years, to study pathogenesis of *P. aeruginosa* induced chronic pneumonia particularly in relation to cystic fibrosis (Iwata and Sato, 1991; Song et al., 1998; Cantin and Woods, 1999; van Heeckeren et al., 1997; 2002).

Morissette et al. (1995) developed a murine model of endobronchial infection with *P. aeruginosa* in different mouse strains. The bacteria enmeshed in agar beads was instilled intratracheally and it was observed that resistant strains (like BALB/c) cleared the infection within 3 days while susceptible strains (like DBA/J) exhibited high mortality at 3 days. Gosselin et al. (1995) employed the same model to analyze the role of several inflammatory cytokines in the lungs of both resistant and susceptible strains of mice. These
cytokines appeared within 3 to 6 hours of infection in all the groups and TNF-α expression was more in the resistant strains. Jain-Vora et al. (1998) and later, Tsai et al. (2000) adapted this mouse model to investigate the role of IL-4 and CXC chemokines in *P. aeruginosa* induced acute lung infection.

Tang *et al.* (1995; 1996) developed a neonatal mouse model of acute pulmonary infection by intranasal instillation of *P. aeruginosa* to study the role of pili as a virulence factor in eliciting inflammatory response. They also employed several mutants, like *las* R, *alg* C and *exs* A mutants, to elucidate the role of these factors in the initiation of infection. Later Pearson *et al.* (2000) investigated the role of quorum-sensing systems during *P. aeruginosa* infection in the same neonatal mouse model.

The intranasal instillation method has been successfully employed by several workers in adult mice (Allewelt *et al.*, 2000; Schultz *et al.*, 2001; 2002) to specify the role of different pathogenic determinants involved in acute pneumonia induced by *P. aeruginosa*. Recently Smith *et al.* (2004) adopted this model to establish that type III secretion system regulated by cyclic AMP (cAMP) is essential for bacterial lung colonization and pathology.

Yanagihara *et al.* (1997) established a chronic respiratory tract infection mimicking diffuse pan-bronchiolitis in experimental mice. Infection was produced by placement of a plastic tube, precoated with *P. aeruginosa*, in the bronchus. This model served to explore the pathogenesis of biofilm bacteria. These workers further analysed the intrapulmonary concentrations of inflammatory cytokines during the course of chronic infection (Yanagihara *et al.*, 2000). Role of various virulence factors and therapeutic efficacy of clarithromycin was also assessed employing this model (Yanagihara *et al.*, 2002; 2003).

Yu *et al.* (1998) described a mouse model of chronic lung
infection by repeated aerosol exposure of *P. aeruginosa* to investigate various factors contributing to *P. aeruginosa* persistence. Associated inflammatory process in the lungs of infected mice was evaluated in terms of cytokine generation both in nutritionally deficient as well as normal mice (Yu et al., 2000).

**Klebsiella pneumoniae**

Members of genus *Klebsiella* are gram negative rods, belonging to the family Enterobacteriaceae. The bacteria, initially described by a German microbiologist Edwin Klebs in the 19\(^{th}\) century, are non-motile, usually encapsulated facultative anaerobes (Umeh and Berkowitz, 2002). Two members of the genus *Klebsiella*, responsible for most of the human infections are *K. pneumoniae* and *K. oxytoca*. *Klebsiella* species are ubiquitous in nature. Their non–clinical habitats encompass the gastro-intestinal tract and mucosal surfaces of mammals such as humans, horses or swine, as well as environmental sources like surface water, sewage, soil and plant (Brown and Seidler, 1973; Seidler et al., 1975; Bagley, 1985; Edberg et al., 1986; Podschun et al., 2001). In humans, *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract (Roosenthal and Tager, 1975).

Medically, *K. pneumoniae* is an important pathogen which is associated with infections of gastro-intestinal tract and other mucosal surfaces. It is among the eight most frequently isolated pathogens from hospitalised patients (Gupta, 2002; Vincent, 2004). The spectrum of clinical syndromes includes pneumonia, septecemia, wound infection, burn infection and urinary tract infections. However, infections of respiratory tract predominate with a high rate of morbidity and mortality (Cortes et al., 2002; Struve and Krogfelt, 2004). *Klebsiella* mediated infections are far more prevalent in health care settings than in the community. Neuhauser et al. (2003) analysed over 35,000 bacterial
isolates from intensive care units in the United States and found *K. pneumoniae* to be the third most commonly isolated organism and most commonly identified species in blood cultures. Recently Lawlor *et al.* (2005) stated that systemic infections, resulting from *K. pneumoniae* caused urinary tract infections and pneumonia can have mortality rates as high as 60%.

*K. pneumoniae* is an opportunistic pathogen and primarily attacks neonates and immunocompromised hospitalised patients, suffering from severe underlying diseases such as diabetes or chronic pulmonary obstruction (Podschun and Ullman, 1998; Lai *et al.*, 2000; Regue *et al.*, 2004). The hands of health care workers and gastrointestinal tracts of hospitalized infants serve as principal pathogenic reservoir for the transmission of the organism and are responsible for multiple hospital outbreaks (Gupta, 2002). However, the presence of indwelling medical devices, like catheters or endotracheal tubes, contamination of respiratory support equipment and use of antibiotics, are factors that increase the likelihood of nosocomial respiratory tract infections with *K. pneumoniae* due to formation of biofilms or emergence of multi-drug resistant strains (Umeh and Berkowitz, 2002).

Pneumonia caused by *K. pneumoniae*, especially nosocomial pneumonia, results in high rates of mortality and morbidity, since the infection is characterized by a rapid progressive clinical course complicated by lung abscesses and multilobular involvement, which leaves little time to establish an effective antibiotic treatment (Carpenter, 1990; Cortes *et al.*, 2002). Several bacterial factors contribute to the pathogenic mechanisms of *K. pneumoniae* infections. These include, the capsular polysaccharide (CPS), fimbriae (pili), serum resistance, lipopolysaccharide (LPS) and siderophores. CPS acts as a physical barrier for the bacteria that resists phagocytosis by host alveolar macrophages and PMNs (Podschun and Ullmann, 1992).
Regue et al. (2004) have described more than 90 serotypes in Klebsiella species. There is high variation in the virulence among different capsular serotypes, K1 and K2 being the most clinically relevant serotypes (Casewell and Talsania, 1979; Mizuta et al., 1983; Podschan, 1990). LPS is a major structural and immunodominant molecule which contains lipid A moiety, core antigen and O-polysaccharide. Vinogradov and Perry (2001) described 9 different O-antigen serotypes. Virulence of LPS and O-side chain has been established by many workers (Merino et al., 1992; Cortes et al., 2002; Chhibber et al., 2003; Sinha et al. 2004). Recently, Lawlor et al. (2005) employed an intranasal mouse model of pneumonia and conducted a genetic screening of 4800 independent insertional mutants to identify novel virulence factors of K. pneumoniae.

Animal models for Klebsiella pneumoniae mediated pneumonia

Experimental models of K. pneumoniae mediated acute as well as chronic pneumonia have been established in different animal species. Sale and Wood (1947), first developed experimental pneumonia in rats by intratracheal instillation of bacteria. This model simulated the pattern of acute pneumonia in humans to some extent, but it had unusually high mortality rates.

Although there are several animal models described in literature in relation to acute pneumonia, but only limited studies are available where chronic pulmonary infection has been established in experimental animals. Berendt et al. (1977) developed chronic bronchopneumonia in rats by intranasal instillation of K. pneumoniae. However, the animals died in the early phase of infection and there was a greater variation in the bacterial concentration found in the lungs of individual rats. Moreover, bacteria were also detected in blood, spleen and liver tissue. This model was eventually abandoned by these workers.
Later, Berendt et al. (1978) described a non-human primate model for study of *K. pneumoniae* respiratory tract infection which allowed measurement of clinical signs such as fever, respiratory rate and throat cultures, that cannot be examined in a rodent model. However, rapid death rate and low frequency of abscess formation severely restricted the utility of this model. In addition, limited availability of large animals restricts the execution of large-scale experiments.

Domenico et al. (1982) established chronic lobar pneumonia in rats by intratracheal instillation using clinical isolates of *K. pneumoniae*. This model allowed the assessment of colonization and infection in the rat lungs for atleast 28 days. The workers employed two variants of a *K. pneumoniae* strain which differed in capsule size and colony morphology. The importance of appropriate strains and their virulence, in causing pneumonia, was highlighted in this study.

Bakker-Woudenberg et al. (1985) and Roosendal et al. (1987) developed a rat lobar pneumonia model to study the therapeutic efficacy of different antibiotics against *K. pneumoniae* induced infection. This rat model of acute pneumonia has been employed by several workers to investigate the pathogenetic and immunoprotective potential of *K. pneumoniae* LPS and CPS antigen (Rani et al., 1990; Chhibber and Bajaj, 1995; Bakker-Woudenberg et al., 2001; Chhibber et al., 2003).

Izawa et al. (1988) established chronic pulmonary infection in mice by aerosol inoculation of *K. pneumoniae*. These workers also investigated the effect of mouse strain differences in susceptibility to experimental infection. The course of infection was studied upto 12 weeks but significant mortality was observed from 4th week onwards.

Greenberger et al. (1995) developed a mouse model of acute pneumonia by intratracheal instillation of *K. pneumoniae* to study the
host inflammatory response generated during the course of infection. They observed that increase in lung bacterial load was accompanied by several fold increase in the generation of pro-inflammatory cytokines like TNF-α, MIP-1α and MIP-2, as compared to the controls. There was also a simultaneous increase in the lung mRNA levels of anti-inflammatory cytokine, IL-10. In a later study, these workers (Greenberger et al., 1996), employed the same animal model and demonstrated that inhibition of MIP-2 resulted in decreased bacterial clearance and attenuated the neutrophil influx as well.

Laichalk et al. (1996; 1998) also employed the intratracheal mouse model of *K. pneumoniae* mediated acute pneumonia to investigate the protective role of TNF-α production during the course of infection. Reduced survival of the animals observed by using anti-TNF-α antibody, proved the importance of TNF-α as an essential cytokine mediator of innate immunity against *K. pneumoniae* infection. Tsai et al. (1997) adopted the intratracheal mouse model to study the role of nitric oxide in the host defense against *K. pneumoniae* induced acute pneumonia. These workers reported that nitric oxide plays a critical role in antibacterial host defense against bacteria. Moore et al. (2002) employed IFN-γ knockout mice to investigate the divergent role of gamma-interferon in pulmonary versus systemic infection induced by intratracheal inoculation of *K. pneumoniae*. They concluded that IFN-γ is a critical mediator in the resolution of pulmonary infection. More recently, these workers (Moore et al., 2005) employed TNF receptor 1 (TNFR1) deficient mice to assess the importance of TNFR1 mediated signalling during *K. pneumoniae* infection. They found that despite excessive production of TNF-α, there was increased mortality in these mice, since production of cytokine was dysregulated in the absence of TNFR1 signalling.

Held et al. (1998) developed a model of acute pneumonia by
employing intranasal instillation method of *K. pneumoniae* in normal mice. They assessed the role of granulocyte-colony stimulating factor in the host defense against the bacteria. This model was successfully adopted by Yoshida *et al.* (2000; 2001), to study the induction of IL-10 and role of capsule in local and systemic inflammatory responses of mice during pulmonary infection with *K. pneumoniae*. Recently Yadav *et al.* (2003) employed this model to study the induction as well as resolution of acute lobar pneumonia induced by *K. pneumoniae* in normal mice.

**BIOFILM CELL FORMS AND RESPIRATORY TRACT INFECTIONS**

Costerton *et al.* (1985; 1987) initially described that chronic infections, especially due to the use of medical implants and prosthetic devices, involve bacterial consortia which grows as bacterial biofilms within extended polysaccharide glycocalices. Such microcolonies condition their environment through specific enzymes and metabolic products. The glycocalyx, acting as a barrier, isolates the enclosed cells from fluctuations in the surrounding environment.

Gilebert *et al.* (1990) explained the different physiological properties of these sessile biofilm population which are distinct from their planktonic counterparts and, thus, contribute to survival within the infected host. They suggested that the slow growth rate was responsible for dormancy and stringent response of biofilm cells towards the antibiotic treatment. The slow growth rate also appears to modulate the hydrophobicity of the cell surface and thereby influence the colonization of surfaces.

Lawrence *et al.* (1991) performed the scanning confocal laser microscopy to visualize fully hydrated bacterial biofilms. The *Pseudomans* biofilms were most cell dense at their attachment surfaces and became increasingly diffuse near their outer regions.
Biofilms of different bacterial species exhibited distinctive arrangements of major structural components. In general, biofilms were found to be highly hydrated, open structures, composed of 73% to 98% extracellular materials and space.

Stewart (1994) adopted a computer model of biofilm dynamics to evaluate the plausibility of two different mechanisms of biofilm antibiotic resistance, by qualitative comparison with data from a well characterized experimental system. Both the mechanisms predicted the experimentally observed resistance of 7-day old *P. aeruginosa* biofilm compared to that of 2-day old biofilms. Reduced susceptibility of thicker biofilms because of oxygen depletion lead to very slow growth rate of bacteria in the biofilm. These workers suggested the potential of such models to enhance experimental design and analysis of biofilm in medicine.

Ketyi (1995a, 1995b) conducted a series of experiments to study the properties of *P. aeruginosa* biofilm on model medical devices. Different materials like polyethylene rings, teflon, silicon and rubber devices were infected with the bacteria and implanted subcutaneously into the mice. Number of bacteria attaching to their surface was determined after 5 days and highest bacterial count was observed on rubber and silicon devices. Moreover, the biofilm mode of growth strongly reduced the sensitivity of the strains against most of the antibiotics used especially polymyxin B. On the other hand, beta-lactum antibiotics were found to be equally effective against planktonic as well as biofilm cells of *P. aeruginosa*.

Meluleni et al. (1995) evaluated the oposonophagocytic killing of mucoid *P. aeruginosa* growing in the biofilm form. Antibodies from infected cystic fibrosis patients exhibited poor opsonic killing of biofilm bacteria but suspended, mucoid *P. aeruginosa* cells were killed less efficiently. Bacterial resistance to killing could be overcome by
enzymatic disruption of biofilm layer.

Davies et al. (1998), demonstrated, in their study, that a cell-to-cell signal is required for the differentiation of individual cells of *P. aeruginosa* into complex multicellular structures (biofilms). The control of biofilm differentiation and integrity by quorum-sensing has important implications in infections where the bacteria can colonize medical devices such as endotracheal tubes. Inhibition of these quorum-sensing signals could aid in the treatment of biofilm infections.

Costerton et al. (1999) reviewed the role of bacterial biofilms in different infections with particular reference to *P. aeruginosa* in cystic fibrosis patients. These workers explained that while growing in the biofilms, these bacteria release antigens to which very high concentrations of antibodies are observed in the blood and lungs of these patients. However, neither the bactericidal nor the opsonizing capabilities of these antibodies are realized. Antibiotics can act on the planktonic cells continuously shed by these biofilm bacteria but have no effect on the biofilm cells resulting in chronic infections observed in these patients.

Watnick and Kolter (1999) described the genetic basis of biofilm formation by *P. aeruginosa*. They described that production of alginate, an exopolysaccharide, is increased by several folds during the biofilm formation. Transcription of *alg C* is involved in the production of alginate. Furthermore, pulmonary isolates of these bacteria are mucoid, due to production of copious amounts of alginate and also show absence of flagellum. This leads to the conclusion that bacteria differentiate into a biofilm-associated cell form by repressing synthesis of flagellum that destabilizes the sessile structure and produces copious amounts of alginate which will reinforce the biofilm structure.

Anderl et al. (2000) assessed the role of antibiotic resistance to ampicillin and ciprofloxacin by *K. pneumoniae* biofilms. They observed
that a β-lactamase negative strain of *K. pneumoniae* had a minimum inhibitory concentration of 2µg/ml ampicillin in aqueous suspension. But when the same strain was grown as a biofilm, it was scarcely affected by 4 hours of treatment with 5000 µg/ml of ampicillin, a dose that eradicated free-floating bacteria.

Whitely *et al.* (2001) investigated the gene expression in *P. aeruginosa* biofilms, to gain insight into differences between free-living bacteria and those in the biofilm form, by using DNA microarrays. They observed that despite striking differences in their morphologies, only about 1% genes showed differential expression in the two growth modes; about 0.5% of genes were activated and about 0.5% were repressed in biofilms. Exposure of biofilms to high levels of antibiotic tobramycin caused differential expression of 20 genes. The results of this study brought out that gene expression in biofilm cells is similar to that in free-living cells but there are some significant differences.

Stewart and Costerton (2001) summarized the features of biofilm infection, especially in implanted medical devices or damaged tissues and proposed 3 mechanisms responsible for antibiotic resistance observed in these biofilm communities. The first hypothesis was the possibility of slow or incomplete penetration of antibiotic into the biofilm. Secondly, an altered chemical microenvironment within the biofilm with oxygen depletion leading to anaerobic niches in the deep layers of the biofilm. Thirdly, a sub-population of microorganisms in a biofilm forms a unique, and highly protected, phenotypic state – a cell differentiation similar to spore formation. This provides a powerful, and generic, explanation for the reduced susceptibility of biofilms to antibiotics.

Langstraat *et al.* (2001) reported that formation of biofilms *in vitro* by *K. pneumoniae* was affected by the production of fimbriae on the bacterial surface. However, a functional MrkD adhesion component of
fimbrial shaft, was not necessary for efficient biofilm formation. Using isogenic fimbriate and nonfimbriate strains of *K. pneumoniae*, these workers demonstrated that presence of type 3 fimbriae facilitated the formation of dense biofilms in a continuous – flowthrough chamber. Transformation of non-fimbriate mutants with a plasmid possessing an intact *mrk* gene cluster restored the fimbrial phenotype and rapid ability to form biofilms.

Drenkard and Ausubel (2002) studied that *P. aeruginosa* biofilm formation and antibiotic resistance are linked to phenotypic variation. Antibiotic resistant phenotypic variants of *P. aeruginosa* with enhanced ability to form biofilms arise at a high frequency both *in vitro* and in the lungs of cystic fibrosis patients. They identified a regulatory protein (Pvr R), that controls the conversion between antibiotic-resistant and antibiotic susceptible forms. Compounds that affect Pvr R function could have an important role in the treatment of cystic fibrosis infections.

Andrei *et al.* (2002) elucidated the role of nutrient limitation and stationary – phase existence in *K. pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. It was observed that glucose did not penetrate all the way through the biofilm, and oxygen penetrated only into the upper 100μm. The specific catalase activity was elevated in biofilm bacteria to a level similar to that of stationary-phase planktonic cells. Transmission electron microscopy revealed that bacteria were affected by ampicillin near the periphery of the biofilm but not in the interior.

Lavender *et al.* (2004) confirmed the virulence of *K. pneumoniae* biofilm formed *in vitro* and its ability to cause infection *in vivo* following construction of a bank of mini-Tn5 mutants. They emphasized that though *in vitro* biofilm assays have the potential to identify putative virulence genes, yet individual determinants have to be assessed *in
Balestrino et al. (2005) characterized the role of type 2 quorum-sensing in *K. pneumoniae* biofilm formation. *K. pneumoniae* was found to express luxS and secrete a signal related to autoinducer, AT-2 of *V. harveyi*. Potential role of luxS in the colonization in mice and biofilm formation *in vitro* was assessed by construction of luxS mutant. Microscopic analysis of the biofilm structures revealed that luxS mutant was able to form a mature biofilm but with reduced capacities in the development of microcolonies, mostly in the early stages of biofilm formation. This data suggested that a lux-S dependent signal plays a role in early stages of biofilm formation by *K. pneumoniae*.

Recently, Abdi-Ali et al. (2006) determined the biofilm-forming capacity of different clinical isolates of *P. aeruginosa* using a modified enzyme linked immunosorbent assay. Observation of the bacterial biofilms on Teflon sheets and on a catheter with scanning electron microscope showed greater biofilm formation on the catheter surface. There results also showed differences in the antibiotic susceptibility of planktonic and biofilm cell populations. Macrolides were found to be most efficient, showing 100% penetration; fluoroquinolones and beta-lactams had a high penetration rate (>75%), whereas the rates for aminoglycosides were low (59-70%) against *P. aeruginosa* biofilms.

**ALVEOLAR MACROPHAGES**

Ozaki et al. (1989) described that alveolar macrophages along with polymorphonuclear neutrophils (PMNs) are important for primary defense against bacterial infection in lower respiratory tract. On incubation with the bacteria, alveolar macrophages released neutrophil chemotactic factor (NCF) in a dose-dependent manner. MDP-Lys (L18), a muramyl dipeptide analog, stimulated alveolar macrophages to phagocytose *P. aeruginosa* and stimulate the release of NCF from
these cells in vitro and enhanced the neutrophil response to inoculated bacteria in vivo. These results indicated that alveolar macrophages are important in initiating the neutrophil-dependent defense system against P. aeruginosa by releasing NCF.

Thepen et al. (1989) investigated in vivo role of alveolar macrophages by eliminating these cells from the lungs of an intact animal. This was accomplished by an intratracheal infection of liposome encapsulated dichloro-methylene-diphosphonate (Cl$_2$MDP). Alevolar macrophage depletion had a dramatic effect on pulmonary immune responses observed in the form of increase in antibody forming cells in lung-associated lymph nodes and a prolongation of the response. This study suggested that alveolar macrophages play a role in controlling the pulmonary immune response in a suppressive way, thereby limiting the possible damage caused by severe immune responses in the lung tissue.

Sibelle and Reynolds (1990) reviewed the defensive role played by alveolar macrophages and PMNs in case of an acute lung injury. The microbicidal efficiency of alveolar macrophages depends on the quantity of the microbial inoculum. A small number of microorganisms can be eliminated by alveolar macrophages alone, whereas an inoculum of $10^5$ or higher, induces a modest influx of PMNs to the alveoli. The most prominent cytokines produced by alveolar macrophages are TNF-$\alpha$, IFN-$\gamma$, IFN-$\beta$, oxygen radicals and nitric oxide. In addition, stimulated alveolar macrophages secrete chemotactic factors to attract granulocytes, such as IL-8, macrophage inflammatory proteins 1 and 2 (MIP-1 and MIP-2) and leukotriene B$_4$. Cytokines which are important for specific resistance against infective agents, such as IL-1$\alpha$, IL-1$\beta$ and IL-6 are also produced by stimulated alveolar macrophages.

Lohmann-Matthes et al. (1994) described that alveolar
macrophages produce oxygen metabolites, such as superoxide anion ($O_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH'), in association with phagocytosis. These reactive oxygen intermediates (ROI) play an important role in both the intracellular and extracellular defense mechanisms directed against microorganisms. This capacity to produce ROI is a prerequisite of non-specific anti-infectious defense system since a high susceptibility for infectious diseases is observed in individuals suffering from defective ROI producing enzyme systems. They confirmed the earlier findings of Myrivik et al. (1961) as well as Fels and Cohn (1986) describing the method for harvesting large number of alveolar macrophages. Broncho-alveolar lavage (BAL) collection is a technique which can be easily employed to access these cells both in humans and in animals. In a normal resting animal, alveolar macrophages represent more than 90% of the cells of BAL. These cells possess a high phagocytic and microbicidal potential. They are the only macrophages in the body exposed to air and are located in the interphase between air and lung tissue.

Frank-Ullmann et al. (1996) compared the immunological potential of interstitial macrophages and alveolar macrophages. The data showed that alveolar macrophages have a more efficient phagocytic potential, stronger microbicidal activity and secrete large amounts of reactive oxygen radicals, nitric oxide, TNF-α and IFN-γ on appropriate stimulation. They also exerted strong tumoricidal and parasiticidal activities while interstitial macrophages were more efficient in releasing immunoregulatory cytokines such as IL-1, IL-6 and also express more MHC class II molecules. The study confirmed that alveolar macrophages act as non-specific first line defense cells against infectious agents while interstitial macrophages induce a specific immune response.

Broug-Holub et al. (1997) evaluated the in vivo role of alveolar macrophages in K. pneumoniae induced pneumonia in mice. Their
study with bacterial infection in the lungs of alveolar macrophage-depleted mice revealed that there was decrease in survival accompanied by several fold increase in the number of \textit{K. pneumoniae} isolated from the infected lungs. Increased mortality was observed despite significant increase in lung PMN infiltration, as well as 10-fold increase in lung homogenate levels of TNF-\(\alpha\) and MIP-2 in alveolar macrophage-depleted mice. These results indicated the relative importance of the alveolar macrophages in the containment and clearance of bacteria.

On the contrary, Kooguchi \textit{et al.} (1998) reported that infection with \textit{P. aeruginosa} in the lungs of alveolar macrophage depleted mice resulted in reduced neutrophil recruitment, chemokine release and milder lung injury at 8 hour post infection. At 48 hour, however, depletion of alveolar macrophages decreased the bacterial clearance and resulted in delayed movement of neutrophils from the site of inflammation coupled with aggravated lung injury.

Mancuso \textit{et al.} (1998) investigated the role of specific endogenous leukotrienes in phagocytosis of \textit{K. pneumoniae} and explored the possibility that exogenous leukotrienes could restore phagocytosis in alveolar macrophages. Rat alveolar macrophages produced leukotriene B\(_4\) (LTB\(_4\)), LTV\(_4\) and 5-hydroxyeicosatetraenoic acid (5-HETE) during the process of phagocytosis, and the inhibition of endogenous leukotriene synthesis with zileuton dramatically attenuated phagocytosis. Reduction in phagocytosis was observed when alveolar macrophages were treated with antagonists to plasma membrane receptors for LTB\(_4\). Similar results were recorded in alveolar macrophages from 5-lipoxygenase-knockout mice. Hence, synthesis of all major 5-lipoxygenase reaction products play an essential role in phagocytosis.

Knapp \textit{et al.} (2002) elucidated the protective role of alveolar
macrophages during murine pneumococcal pneumonia. Significantly high mortality, along with a pronounced increase in local proinflammatory cytokine production was recorded in alveolar macrophage-depleted mice as compared to the one observed in normal mice following S. pneumoniae infection. In addition, high proportions of apoptotic and secondary necrotic PMNs in these mice, reflected the lack of efficient clearance mechanisms in the absence of alveolar macrophages. Delclaux and Azoulay (2003) have highlighted the role of alveolar macrophages during inflammatory response to infectious pulmonary injury reported by several workers (Gordon and Read, 2002; Ward, 2002). Alveolar macrophages have been shown to be important in lung defense against viruses, mycobacteria and fungi also. Interactions of pathogens with these cells is strongly influenced by soluble immune components including complement, collectins and immunoglobulins. These workers stressed that alveolar macrophage functions can be modulated by cytokines, environmental exposures, recent and chronic infections, drug therapy and gene transfer.

Rubins (2003) reviewed that alveolar macrophages stand as the guardian of the alveolar – blood interface, serving as the front line of cellular defense against respiratory pathogens. They are primary phagocytes of innate immune system, cleaning the air spaces of infectious, toxic or allergic particles that have evaded the mechanical defenses of the respiratory tract.

Arredouani et al. (2004) examined the role of class A scavenger macrophage receptor with collagenous structure (MARCO) in vivo in lung defense against pneumococcal infection by using MARCO− mice. In a murine model of pneumococcal pneumonia, MARCO− mice displayed an impaired ability to clear bacteria from the lungs, increased pulmonary inflammation and cytokine release along with diminished survival. Moreover, in vitro binding of S. pneumoniae and in vivo uptake of unopsonised particles by MARCO+ alveolar
macrophages were dramatically impaired. These findings point to an important role of MARCO in mounting an efficient and appropriately regulated innate immune response against airborne pathogens.

Recently, Beck-Schimmer et al. (2005) highlighted the anti-inflammatory effects exhibited by alveolar macrophages in early endotoxin-induced lung injury. These cells prevented neutrophil influx by controlling monocyte chemoattractant protein-1 production through alveolar epithelial cells.

**APOPTOSIS**

Apoptosis or programmed cell death is a physiological process that occurs as a part of normal development in eukaryotes (Kerr et al., 1972). Dysregulation of apoptotic pathways themselves may lead to a spectrum of human diseases which include autoimmune disorders and immunodeficiency (Thompson, 1995).

Apoptosis of mammalian host cells has been shown to be a hallmark of infection by some bacteria, viruses and parasites (Finlay and Cossart, 1997; Zychlinsky et al., 1992; 1996). Zychlinsky and Sansonetti (1997) analysed the role of apoptosis in bacterial pathogenesis and identified three pathogenic strategies involving programmed cell death. These mechanisms are—activation of apoptosis to destroy host cells, to initiate inflammation and inhibition of host cell apoptosis. These authors described that the most beneficial aspect for the pathogenic bacteria, during an infection, is induction of apoptosis in professional phagocytes like macrophages and PMNs. Several pathogens are known to induce apoptosis in macrophages before they can ingest and destroy the bacteria (Kato et al., 1995; Monack et al., 1996; Zychlinsky et al., 1997). Molecular regulators of apoptosis identified over the years include CD95, Fas ligand, bcl-2 family and caspases (cysteine proteases) (Strasser et al., 1994; Heemels, 2000).
There are a few studies cited in relation to the apoptotic potential of *P. aeruginosa* and *K. pneumoniae*. Estaquier *et al.* (1998) investigated the effect of immunomodulating glycoprotein extract from *K. pneumoniae*, RU41740 on monocyte apoptosis. This glycoprotein strongly enhanced monocyte survival *in vitro* by suppressing the apoptotic activity in these cells. A concentration ranging from 1ng/ml to 10μg/ml prevented apoptosis induced both by survival factor deprival and by gamma-irradiation. Monocytes, the precursor cells for tissue macrophages, have been shown to undergo rapid programmed death by apoptosis in cultures, unless signalled by bacterial products that enhance monocyte survival by reducing apoptosis induction. This enhanced monocyte survival by this bacterial glycoprotein, thus, represented immunostimulating effect of this compound.

Buommino *et al.* (1999) examined the ability of porin from *P. aeruginosa* to induce apoptosis in rat epithelial cell line (SVC1). It was observed that micro-molar concentrations of porin, purified from the outer membranes of *P. aeruginosa*, induced *in vitro* apoptosis in these cells. This phenomenon was p53 independent and associated with significant decrease of bcl-2 expression and a marked increase of c-myc transcriptional activity. The Ca²⁺ influx, caused by the porin treatment of SVC1 cells, appeared to play an important role in triggering of apoptosis.

Hauser and Engel (1999) observed *P. aeruginosa* induced type III secretion – mediated apoptosis of macrophages and epithelial cells. They infected J774 A.1 cells and primary bone marrow-derived murine macrophages with the whole cells of bacteria *in vitro*. It was found that bacteria were capable of inducing apoptosis by type-III – secretion dependent killing of macrophages within 2 hours of infection. Moreover, some laboratory and clinical isolates induced significantly higher levels of this form of cell death than the others. Such killing may contribute to the ability of this organism to persist and disseminate.
within the infected patients.

In another study, Kaufman et al. (2000) reported that *P. aeruginosa* mediated apoptosis requires the ADP-ribosylating activity of Exoenzyme S. Invasive strains of the bacterium were found to induce apoptosis at a high frequency in HeLa, and other epithelial as well as fibroblast cell lines. This apoptotic phenotype in the infected cells was determined by visual changes in cell morphology, chromatin condensation, presence of high percentage of cells with subG1 DNA content and activation of caspase-3-activity. Mutants of *P. aeruginosa exo S* failed to induce apoptosis, while complementation with wild type *exo S* restored the apoptosis – inducing capacity, demonstrating that Exo S is the effector molecule. Analysis of *exo S* activity in mutants shows that the ADP-ribosylating capacity of Exo S is essential for inducing the apoptotic pathway.

Rajan et al. (2000) demonstrated that apoptosis does not follow *P. aeruginosa* challenge to human respiratory epithelial cells unless fully virulent bacteria and damage associated with tight junction integrity are present. Droemann et al. (2000) determined the parameters of cell activation and apoptosis on neutrophils in circulation and the pulmonary compartment in patients with community – acquired pneumonia. There was a significant decrease in mean apoptosis rate in pulmonary neutrophils as compared to systemic neutrophils without concomitant changes in Fas expression. In contrast, cell-activation markers and expression of complement regulating molecules were significantly increased on pulmonary cells. This decreased rate of apoptosis in pulmonary neutrophils coupled with increased activation status in the alveolar compartment may be important for effective control of pulmonary inflammation in patients with community-acquired pneumonia.

In a later study, Jendrossek et al. (2001) showed that infection of
human conjunctiva epithelial Chang cells with \textit{P. aeruginosa} results in rapid induction of apoptosis. This form of cell death was mediated by mitochondrial alterations (depolarisation), synthesis of reactive oxygen intermediates, release of cytochrome \textit{c} as well as an activation of Jun-N-terminal kinases. Moreover, stimulation of these events was dependent on upregulation of CD95 on infected cells. Efficient apoptosis of Chang epithelial cells also required expression of the type III secretion system by \textit{P. aeruginosa}.

Usher \textit{et al.} (2002) examined the effect of pyocyanin, a penazine produced by \textit{P. aeruginosa}, upon apoptosis of neutrophils \textit{in vitro}. It was observed that pyocyanin induced a concentration and time dependent acceleration of neutrophil apoptosis. 50\text{\mu}M of pyocyanin induced apoptotic changes in 5 hours, a concentration documented in the sputum of the patients colonized with the bacteria. However, pyocyanin did not induce significant apoptosis of monocyte-derived macrophages or airway epithelial cells even upto 24 hours. Pyocyanin induced apoptosis was found to be associated with sustained generation of reactive oxygen intermediates and reduced levels of intracellular cAMP. Worgall \textit{et al.} (2002) observed apoptosis in human alveolar macrophages and dendritic cells by laboratory \textit{P. aeruginosa} strains and this response could be partially reversed by genetic modification with CD40L, a CD4+ T cell molecule that plays a central role in activating antigen presenting cells. Hence, genetic manipulation of antigen presenting cells with anti-apoptotic genes may be able to strengthen host defenses in cystic fibrosis patients.

Hetz \textit{et al.} (2002) described the cytotoxic effect of microcin, E492, a bactriocin produced by \textit{K. pneumoniae} in HeLa cells. Significant apoptosis was induced by low (5\text{\mu}g/ml) and intermediate (10\text{\mu}g/ml) concentrations of this microcin. This cytotoxic effect was completely blocked by treatment with a general caspase inhibitor,
zVAD-fmk. Induction of apoptosis was associated with release of Ca$^{2+}$, probably after microcin-triggered ion channel formation. At higher concentrations ($\geq 20 \mu g/ml$), microcin induced necrotic changes in the cell lines.

Whole cells of *P. aeruginosa* and *K. pneumoniae* as well as their individual components/products are capable of inducing apoptosis in host cells. In addition, Tateda *et al.* (2003) showed specific ability of an autoinducer (3-oxo-C$_{12}$-HSL) from *P. aeruginosa*, required for quorum-sensing, to induce apoptosis in certain types of cells. When bone marrow-derived macrophages were incubated with this molecule, significant loss of cell viability, in a concentration and time dependent manner, was observed in the form of increased apoptosis. Apoptotic potential of this autoinducer molecule was observed in neutrophil and monocyte cell lines, U-937 and P388D1, but not in epithelial cell lines CCL-185 and HEp-2.

**NUTRITION / POLYUNSATURATED FATTY ACIDS**

It has long been accepted that immunity (or susceptibility to disease) depends to some extent on nutrition. The interdependency between the disciplines of nutrition and immunology was first recognized in 1970s when immunological measures were introduced as a component of assessing nutritional status (Bistrian *et al.*, 1975). A growing body of literature demonstrates the immune benefits of specific nutrients both in relation to non-infectious and infectious diseases. Almost all the nutrients in the diet play a crucial role in maintaining an optimal immune response. Deficient as well as excessive intakes can have negative consequences on the immune status (Delafuente, 1991; Keush, 2000). An inadequate intake of some of these nutrients like vitamins A, C, E, iron and zinc is known to increase susceptibility to infectious diseases (Semba *et al.*, 1995; Hemila, 1996; Weinberg, 1996; Patruta *et al.*, 1999; Prasad, 1998;
Meydani and Beharka, 2001; Field et al., 2002). Meade and Mertin (1978) first reviewed the role of fatty acids and suggested that there may be an immunological explanation for data relating to the relationship between dietary fat and disease.

Fatty acids can be saturated, monounsaturated or polyunsaturated (PUFA). Initially, several workers (Johnstan and Marshall, 1984; Weber et al., 1986; Lewis, et al., 1986) elaborated that the metabolism of two main families of polyunsaturated fatty acids (PUFA), the n-6 and n-3 PUFA, is competitive since both the pathways employ the same set of enzymes. Major end product of n-6 metabolic pathway is arachidonic acid (Fig.A). This pathway is quantitatively the most important in PUFA metabolism in humans, because linoleic acid is easily available in almost all vegetables and vegetable oil based products which are consumed in greater quantities than alpha-linolenic acid (α-LNA) which is found only in selective foods such as flaxseed oil, walnuts, peanuts and spinach. The major end products of n-3 metabolic pathway are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Very little α-LNA proceeds along the entire metabolic pathway to give rise to DHA, though EPA is produced in considerable amounts. Important natural sources of EPA and DHA are fish oil and fatty fish like mackerel, salmon and kippers.

Hwang et al. (1989) elaborated that all fatty acids participate in vital physiological processes but cannot be designated as essential. The term ‘essential’ is applied to only to polyunsaturated fatty acids which cannot be completely synthesized de novo and, therefore, they must be obtained from the diet. Connor (1994) described that PUFA have important functions on the structure and physical properties of localized membrane domains and, in addition, are involved in eicosanoid production, signal transduction and activation of nuclear transcription factors.
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Fig. A: Metabolism of PUFA

Linoleic acid (18:2n-6) → α-Linolenic acid (18:3n-3)

GLA (18:3n-6) → 18:4n-3

EPA (20:5n-3) → DPA (22:5n-3) → DHA (22:6n-3)

DGLA (20:3n-6) → 20:4n-3

GLA: gamma-linoleic acid; DGLA: dihomo-gamma-linolenic acid

Spector (1999) brought out the fact that the modern diet, especially the western diet, is rich in n-6 PUFA (with a n-6 : n-3 ratio of 20:1) with plasma and all other tissues containing the same ratio of n-6 and n-3 PUFA. Important n-6 PUFA is linoleic acid (18:2n-6) whereas important n-3 PUFA of biological significance are α-linolenic acid (18:3n-3), Eicosapentaenoic acid (EPA, 20:5n-3) and Docosahexaenoic acid (DHA, 22:6n-3). Both linoleic acid and alpha-linolenic acid can be further elongated and desaturated in animal cells forming the n-6 and n-3 families of PUFA. Deficiency of both n-6 and n-3 fatty acids can lead to debilitating disease conditions.
Fig. B: Oxidative metabolism of arachidonic acid and eicosapentaenoic acid by the cyclooxygenase and 5-lipoxygenase pathways. 5-HPETE denotes 5-hydroperoxyeicosatetraenoic acid and 5-HPEPE denotes 5-hydroxyeicosapentaenoic acid.
Simopoulos (2002) summarised the oxidative metabolism of n-3 PUFA and n-6 PUFA to elaborate the metabolic end products of these pathways (Fig. B). When the diets are supplemented with n-3 PUFA, they partially replace n-6 PUFA in the membranes of practically all the cells (i.e. erythrocytes, platelets, endothelial cells, monocytes, lymphocytes, granulocytes, neuronal cells, fibroblasts, retinal cells, hepatic and neuroblastoma cells). EPA competes with arachidonic acid for prostaglandin and leukotriene synthesis, at the cyclooxygenase and lipoxygenase level. These metabolites play an important role as inflammatory mediators during immunological response.

EPA and DHA from fish or fish oil lead to (1) a decreased production of prostaglandin E$_2$ (PGE$_2$) metabolites (2) a decrease in thromboxane A$_2$, a potent platelet aggregator and weak vasoconstrictor, (3) a decrease in leukotriene B$_4$ formation, an inducer of inflammation and leukocyte chemotaxis as well as adherence, (4) an increase in the thromboxane A$_3$, a weak platelet aggregator and weak vasoconstricotr, (5) an increase in prostacyclin PHI$_3$, leading to an overall increase in total prostacyclin without decreasing PGI$_2$ (both PGI$_2$ and PGI$_3$ are active vasodilators and inhibitors of platelet aggregation) and (6) an increase in leukotriene B$_5$, a weak inducer of inflammation and weak chemotactic agent.

Role of n-3 PUFA in non-infectious diseases

Initial epidemiological studies by Bang and Dyerberg (1973) as well as Kromann and Green (1980) with Greenland Eskimos showed lower mortality rates from cardiovascular diseases despite the consumption of diet that was traditionally high in fat and cholesterol. Subsequent experimental studies by different workers demonstrated that this benefit was attained from diet rich in fish and other sea food, all being abundant sources of n-3 PUFA, especially EPA and DHA. Fish oil intake has also been shown to lower blood pressure and
Experimental and clinical studies have documented the protective role of n-3 PUFA against cardiovascular diseases like atherosclerosis, angina, heart attack, congestive heart failure, arrhythmias, stroke, and peripheral vascular disease (Albert et al., 1998; Christensen et al., 1999; Oomen et al., 2000; Connor, 2001; Carrol et al., 2002; Hu et al., 2002; Shahidi et al., 2004; Zak et al., 2005). Castro et al. (2005), recently, observed in their study that n-3 PUFA from both sea food and plant sources may reduce coronary heart disease risk, with little apparent influence from background n-6 PUFA intake. This is particularly relevant for the population with low consumption or non-availability of fatty fish.

In relation to diabetes, Houtsmuller et al. (1980) initially reported beneficial effects of n-3 PUFA rich diet relative to low fat, high carbohydrate diets on glucose control and insulin sensitivity. Salmeron et al. (2001) examined the relation between dietary fat intake and the risk of type-2 diabetes. They found that saturated or monounsaturated fatty acids intake was not significantly associated with risk of type 2 diabetes but PUFA supplementation lead to a 40% lower risk for diabetes in women subjects. Recently, Nettleton and Katz (2005) reviewed that increased consumption of n-3 PUFA with reduced intake of saturated fat may reduce the risk of conversion from impaired glucose tolerance to type 2 diabetes in over-weight persons.

Kremer et al. (1989) observed significant improvement in the symptoms of rheumatoid arthritis with regular consumption of EPA and DHA (in the form of fish oil). They attributed the beneficial effect of fish oil consumption to prolonged suppression of LTB₄ which accounted for the clinical benefits. Endres et al. (1989), in their study, highlighted that fish oil supplementation induced suppression of IL-1 which contributed to the amelioration of clinical signs and symptoms of
disease activity in patients with rheumatoid arthritis. Randomized controlled clinical trials have showed benefit from n-3 PUFA supplementation against this disease (Fortin et al., 1995; James and Cleland, 2000). Recently, Fischbach and Fernandes (2005) investigated the inhibition of osteoporosis in autoimmune disease prone mice by n-3 PUFA, especially fish oil consumption. They observed that long term fish oil feeding helped in maintaining higher bone mineral density and lower synovitis in their mouse model. The workers attributed the beneficial effects, partly, to increased activity of antioxidant enzymes, decreased expression of receptor activator of NF-kappa B ligand. Hence, fish oil may serve as an effective dietary supplement to prevent bone mineral density loss in patients with rheumatoid arthritis.

In another well known autoimmune disease, psoriasis, there is marked elevation in the generation of pro-inflammatory lipoxygenase products, LTB₄ and 12-hydroxyeicosatetraenoic acid (12-HETE). Ziboh et al. (1991) studied that addition of fish oil to standard treatment produced improvement in the skin condition and resulted in a decrease in LTB₄ and 12-HETE. Allen et al. (1991) showed that in patients given UVB treatment, n-3 fatty acids prolong the beneficial effects of a course of phototherapy. Fish oil in combination with cyclosporin reduced nephrotoxicity, which is a major side effect of that drug. Wolters (2005) suggested that both experimental as well as clinical evidence shows that there is correlation between dietary n-3 PUFA consumption (especially fish oil or flaxseed oil) and aetiology as well as pathogenesis of psoriasis. There is suppression of inflammatory processes by these nutrients thus benefiting the affected persons.

Crohn’s disease and ulcerative colitis are collectively known as Inflammatory Bowel Disease. McCall et al. (1989) initially described therapeutic potential of n-3 PUFA in the treatment of ulcerative colitis. These patients have increased amounts of IL-1β and LTB₄. Recent
approach to the management of ulcerative colitis has been centred on soluble mediators of inflammation. Stenson et al. (1992), in a double-blind crossover comparison with placebo showed that fish oil supplementation increases the production of LTB5 and decreases the production of LTB4 thereby showing modest clinical improvement. Beneficial effects of n-3 PUFA supplementation in relation to inflammatory bowel disease have been reported by other workers (Belluzzi et al., 1996; Endres et al., 1999; Snider et al., 2005). However, Maclean et al. (2005) reviewed 13 controlled trials that assessed the effects of n-3 PUFA on this disease and the rates of relapse or remission. They found that only few clinical trials reported a reduction in the corticosteroid requirement or statistical significance following dietary supplementation.

Increasing evidence from animal and in vitro studies indicate that n-3 fatty acids especially EPA and DHA inhibit carcinogenesis. Numerous studies have reported beneficial effects of these fatty acids in reduced risk of breast cancer as well as colon cancer development (Linder, 1991; Bardon et al., 2005). Mechanisms whereby n-3 fatty acids modify the carcinogenesis process includes suppression of arachidonic acid derived eicosanoid biosynthesis, influence on transcription factor activity, gene expression and signal transduction pathways, alteration of estrogen metabolism, increased or decreased production of free radicals and reactive oxygen species. Zak et al. (2005) concluded that the epidemiological data on association between fish oil consumption and cancer risk are however, less consistent and more data of defined clinical groups are required for final evaluation.

Calder (2003) suggested potential application of n-3 PUFA supplementation in surgical and trauma patients. These patients show an early hyper-inflammation that can be damaging to the host. Nutritional supplementation in the form of lipid emulsions can modulate
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the inflammatory state and offer opportunity for improvement in patient outcome. Since n-3 PUFA decrease the production of inflammatory cytokines, they act as potent anti-inflammatory agents. Callo et al. (2005) concluded in their study that n-3 PUFA supplementation during hospitalization in patients undergoing coronary artery bypass graft surgery, reduced the incidence of post operative atrial fibrillation and was also associated with a shorter hospital stay.

Changes in fatty acid composition are reported to be involved in the pathophysiology of depression. Imbalance in the n-6 : n-3 cytokines and eicosanoids in this illness is reported in two studies by Maes et al. (1996;1997). Beneficial therapeutic effects of EPA and DHA in major depression have been shown in several studies (Stoll et al., 1999; Locke et al., 2001) where they reduce the risk of relapse in patients. De Vriesse et al. (2003) correlated the low levels of n-3 PUFA in maternal serum phospholipids and cholesteryl esters to the development of post partum depression in these women. They can obtain benefits from a prophylactic treatment with n-3 PUFA, especially the combination of EPA and DHA.

In relation to pulmonary diseases, continuous inflammatory processes can take place in the lungs due to several predisposing factors like smoking, allergic disorders like asthma, chronic obstructive pulmonary disease and cystic fibrosis. Early epidemiological studies showed lower incidence of developing asthma, by reducing the inflammation in the airway, in children as well as significant improvement in the lung functions of asthmatic patients following n-3 PUFA consumption (Dry and Vincent, 1991; Broughton et al., 1997; Schwartz, 2000). Recently, Oddy et al. (2004) investigated the association of childhood asthma with ratio of n-6 : n-3 fatty acids in the diet. A case control cross-sectional study designed within prospective birth cohort compared the quantities of n-6 : n-3 intake in cases with current asthma and controls with no asthma at young age. The authors
found a statistically significant association between the ratio of \( n-6 : n-3 \) fatty acids and risk for current asthma thus providing evidence that promotion of diet with increased \( n-3 \) PUFA and reduced \( n-6 \) PUFA is needed to protect children against symptoms of asthma. Wong (2005) and Micklebrough (2005) reviewed this subject based on clinical studies and observed that an overall beneficial effect of \( n-3 \) PUFA supplementation has been reported in other studies as well.

Shahar et al. (1994) studied the relation between the dietary intake of \( n-3 \) PUFA and chronic obstructive pulmonary disease (COPD) in current or former smokers in a population based study. The combined intake of EPA and DHA over a period of one year was found to be inversely related to the risk of COPD in a dose-dependent manner. These workers concluded that dietary supplements may provide protection against chronic bronchitis and emphysema due to their anti-inflammatory effects even at low doses. Schwartz, and Weiss (1994) examined the relationship between the effect of chronic dietary intake of \( n-3 \) PUFA in the form of fish on the level of pulmonary functions assessed in terms of forced expiratory volume \((FEV_1)\) in the first National Health and Nutrition Examination survey. The data collected from 2,526 adults suggested that fish consumption may be protective for occurrence of asthma and other pulmonary diseases.

In a clinical trial conducted by Katz et al. (1996), 12 cystic fibrosis patients were randomly given intravenous infusion of fish oil emulsion or linoleic acid emulsion for one month. Improved lung functions were observed in fish oil group while lung functions decreased in the control group. DiVizia et al. (2003) evaluated the clinical response of fish oil (rich in EPA and DHA) supplementation in cystic fibrosis patients. Fish oil administration increased EPA concentration in erythrocyte membrane phospholipids after 4 to 8 months of supplementation. Pulmonary functions showed significant improvement in \( FEV_1 \) and lung inflammation was reduced in these
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patients. Recently, Cawood et al. (2005) have presented an updated systematic review of fish oil supplementation in cystic fibrosis patients. They have stressed a need for long term randomised controlled studies to evaluate the therapeutic benefit of fish oil administration in cystic fibrosis patients in order to draw a firm conclusion.

Role of n-3 PUFA in relation to infectious diseases

In relation to parasitic infections, Blok et al. (1992) reported beneficial effect of fatty acid feeding in a murine model of cerebral malaria induced by *Plasmodium berghei*. Mice in different groups were administered fish oil, corn oil or palm oil by gastric instillation either as 0.2ml concentrate or 15% (wt/wt.) with dry feed for 6 weeks before the induction of infection. Cerebral malaria occurred in only 23% of fish oil fed mice while 61% of corn oil, 81% of palm oil and 78% normal chow fed animals developed malaria respectively. The increased resistance to *P. berghei* infection was found to be associated with an enhanced *ex-vivo* production of IL-1α, IL-1β and TNF-α by peritoneal macrophages of fish oil fed mice. Allen et al. (1996), in their study, brought out that short term feeding of diets rich in n-3 PUFA (10% fish oil, 10% flaxseed oil, or 10% linseed oil) for 3 weeks to young chicks reduced the severity of *Eimeria tenella* infections in them. Later Allen et al. (1998) attributed the protective effect to the dietary - induced oxidative stress, which is an effective deterrent against cecal coccidiosis in chicken. Blok et al. (2002) reported no influence of fish oil diet on the course of *Leishmania amazonensis* infection induced in experimental mice. However, LPS induced TNF-α production was significantly enhanced in fish oil fed mice. Muturi et al. (2005) examined the effect of dietary PUFA on the mixed nematodal infection of calves. Calves were fed 25g/day of either fish oil or a mixture of palm oil and rapeseed oil as a supplement in milk replacer. They were infected orally with *Ostertagia ostertagi* and *Cooperia oncophora*, three
times per week for eight weeks. Fecal egg counts were marginally lower in fish oil fed group. But the number of intestinal immature worms, mucosal most cells and eosinophil numbers were significantly lower in the intestinal tissue of the fish oil supplemented group. These workers concluded that fish oil could be employed as immunonutritional strategy for potentiating the immune response to nematode infection in calves.

In the context of viral infections, few studies are available with either a protective or detrimental effect of n-3 PUFA supplementation. Rubin et al. (1989) found that feeding autoimmune prone (NZB x NZBW) mice with either n-3 fatty acid enriched fish oil or beef tallow did not affect the survival of animals after a lethal challenge with murine Cytomegalovirus. Fritsche and Johnston (1989) reported that feeding normal mice with diet high in linolenic acid (n-3 PUFA) enhanced viral-specific cell-mediated cytotoxicity after a live challenge with Vaccinia virus. Byleveld et al. (1999) observed that fish oil feeding diminished host defense against influenza A virus. Mice given dietary supplementation with fish oil or beef tallow for 2 weeks, were infected with influenza A virus. Assessment of sera and lungs from these animals exhibited significantly high viral titres and lower IFN-γ levels in fish oil fed groups of mice. In their follow-up study, keeping the same experimental design, these workers observed significantly lower virus-specific cytotoxicity activity in the fish oil fed mice (Byleveld et al., 2000).

In relation to human acquired immuno-deficiency syndrome virus (HIV) infection, only limited information is available. Fernandes et al. (1992) fed mice with experimental diets containing either 5% or 20% fat in the form of fish oil or corn oil for 4 weeks, following which the animals were injected with murine retrovirus – induced immunodeficiency syndrome that mimics human AIDS. It was found that mice fed with 20% fish oil diet survived significantly longer than
mice fed with 20% corn oil diet. Hellerstein et al. (1996) conducted a 10-week trial of dietary fish oil supplementation in 20 men with weight loss due to AIDS. Baseline food intake, body weight and concentrations of IFN-α remained unchanged following the diet supplementation. In vitro production of IL-1 and TNF-α by peripheral blood mononuclear cells (PBMC) was markedly reduced in fish oil supplemented males as compared to normal controls. Bell et al. (1996), in their randomised double blind study, reported that fish oil supplementation increased the incorporation of DHA into the phospholipids of plasma and there was a significant increase in the release of IL-6 from PBMC in this group of men as compared to safflower oil supplemented group. Xi et al. (1998) observed that intraperitoneal infection with murine AIDS in fish oil fed group of mice exhibited suppression in the levels of TNF-α and IL-1β, which were otherwise elevated during the infection. Woods (2005) has highlighted the beneficial effects of n-3 PUFA, EPA and DHA in improving hypertriglyceridemia and insulin resistance in patients with HIV.

In relation to bacterial infections Rubin et al. (1989) observed no effect of dietary fish oil given at 0.2g/day for 4 to 5 weeks on host survival against L. monocytogenes and P. aeruginosa induced intraperitoneal infections in autoimmune prone mice.

Peck et al. (1990) evaluated the effect of n-3 PUFA feeding on the outcome of P. aeruginosa infection in murine burn wound model. Normal mice were fed with MaxEPA (n-3 PUFA) or safflower oil (n-6 PUFA) for 2-3 weeks and subjected to 20% flame burn followed by infection with P. aeruginosa. Reduced survival rate of mice given n-3 PUFA supplementation was observed as compared to n-6 fatty acid fed group.

D'Ambola et al. (1991) studied the effect of fish oil supplementation on lung clearance of S. aureus in neonatal rabbits.
Feeding the animals with low dose of fish oil (0.22g/kg) for 7 days did not show any effect. On the other hand, high dose of fish oil (5g/kg) diminished the lung clearance of *S. aureus* by approximately 50% but the lung neutrophil recruitment or alveolar macrophage bactericidal activity remained unaltered.

Barton *et al.* (1991), observed decreased mortality with n-3 PUFA rich diet in a sepsis model that involved *S. aureus* challenge. In their study, experimental rats were fed with fish oil or safflower oil in the form of a liquid diet for 5 days. Following dietary supplementation, these rats were infected intraabdominally with *S. aureus*. Fish oil fed rats had a better survival rats (35%) as compared to safflower oil fed rats (16%).

Chang *et al.* (1992) reported decreased resistance of mice given fish oil supplementation to *S. typhimurium* infection. Mice were fed with 4% fat in the form of fish oil, corn oil or coconut oil along with the normal diet for 4 weeks followed by peroral infection with *S. typhimurium*. Significantly higher mortality rates were observed in fish oil fed mice as compared to those given corn oil or coconut oil supplementation. Fish oil fed group also exhibited highest bacterial load in their spleens.

Blok *et al.* (1992) observed increased survival against intramuscular *K. pneumoniae* infection in mice given 15% fish oil as compared to mice given 15% corn oil or palm oil as dietary supplement for 6 weeks. The beneficial effects were attributed to significantly enhanced levels of IL-1α, IL-1β and TNF-α by peritoneal macrophages in fish-oil fed group of mice. However, no protection was observed when fatty acid fed mice were infected with a higher dose of *K.pneumoniae*.

Clouvya-Molyvdas *et al.* (1992) investigated the effect of fish oil feeding in two experimental models of murine peritonitis, with *P.*
aeruginosa and S. typhimurium. No significant differences in the survival of mice against the two bacteria were observed as compared to the control groups.

Mayatepek et al. (1994) found detrimental effect of fish oil feeding in guinea pigs against experimental infection induced by *Mycobacterium tuberculosis*. Increased number of viable organisms in the spleens of fish oil fed mice were observed when intramuscular infection with *M. tuberculosis* was induced following 13 weeks of dietary supplementation. Similar findings have been reported by Paul et al. (1997). These experimental data are consistent with the epidemiologic findings in native Eskimos reported earlier (Kaplan, 1971; Bang et al., 1980), who were found to have a higher n-3 PUFA intake and a high incidence of tuberculosis.

Fritsche et al. (1997), in a series of experiments, fed C3H/HeN mice with experimental diets containing either lard oil, soybean oil or a mixture of menhaden and corn oil (n-3 plus n-6 PUFA) for 4 weeks. Infection with *L. monocytogenes* resulted in significant delay in bacterial clearance as well as decreased survival in n-3 PUFA fed mice. Fritche et al. (1999) observed reduced levels of serum IL-12 and IFN-γ at 24 h post infection, in an intraperitoneal infection induced by *L. monocytogenes* in mice given dietary fish oil supplementation for 4 weeks. These findings were confirmed by De Pablo et al. (2000) who reported reduced survival and bacterial clearance in n-3 PUFA fed mice after an intravenous infection with *L. monocytogenes*.

Bjornsson et al. (1997) also reported improved survival of fish oil fed mice (40% survival) when compared to olive oil (25% survival) or standard lab chow diet (20%) upon intramuscular infection induced with *K. pneumoniae*. In all the groups, mice were given dietary fatty acid supplementation for 6 weeks before induction of infection.

Rayon et al. (1997) studied the effect of maternal dietary
supplementation with different fatty acids on group B Streptococcal (GBS) infection in neonatal rat pups. Pregnant dams were fed with purified diet containing fat source (22% energy) in the form of corn oil or fish oil throughout pregnancy and lactation. On 7th postnatal day, pups were infected with GBS. Pups from fish oil fed group showed better survival rate (79%) as compared to the pups in the corn oil fed group (49%). These workers concluded that fatty acid composition of maternal diet affects the survival in neonatal pups against GBS infection.

Pscheidl et al. (2000) reported reduced number of viable bacteria in the mesenteric lymph nodes and livers of rats, given fish oil supplementation, after instillation of \textit{E. coli} into the gut. The authors attributed the beneficial effect of fish oil feeding to improved killing of the bacteria through phagocytosis and not via the translocation process.

Irons et al. (2003) demonstrated that dietary fish oil can significantly impair host resistance to primary as well as secondary \textit{L. monocytogenes} infection, although the impairment of immunological memory response is much less severe. Weanling mice were fed with diets containing either lard oil or fish oil for 28 days. Animals were then immunized with the bacteria and 35 days post immunization, these mice were challenged with \textit{L. monocytogenes}. It was observed that naive mice fed with fish oil had higher bacterial loads in their liver and spleen as compared to lard oil fed mice. Fish oil and lard oil fed immunized and infected mice had lower bacterial counts than their unimmunized counterparts.

Liu et al. (2003) investigated the effects of long term fish oil supplementation on immunological response, following \textit{E. coli} LPS challenge in pigs. Alteration in the production of pro-inflammatory cytokines was considered to be responsible for improved
immunological and adrenal responses in these animals.

Puertollano et al. (2004) fed different groups of mice with diet containing low fat, olive oil, fish oil or hydrogenated coconut oil for 5 weeks duration. Fish oil fed mice exhibited reduced survival as well as increase in the number of viable bacteria in their spleen. In addition, these mice showed reduced type-1 response, inefficient action of TH2-type response and no modification of pro-inflammatory lipid mediator production following intravenous *L. monocytogenes* infection.

van Heeckeren et al. (2004) evaluated the effect of DHA supplementation against *P. aeruginosa* induced lung infection in cftr-knockout mice having cystic fibrosis. The cftr-knockout mice were given DHA supplementation in liquid diet Peptamen (40mg/day/os) and subsequently infected with intratracheal inoculation of *P. aeruginosa*-laden agarose beads. There were no significant differences in the cumulative survival rates of DHA fed cftr knock-out mice and their wild-type counterparts.

Thors et al. (2004) reported beneficial effect of dietary fish oil supplementation on survival of mice after experimental pneumonia with *K. pneumoniae*. The survival of mice fed on fish oil for 6 weeks and subsequently infected with *K. pneumoniae* in the lungs was found to be significantly higher compared to mice fed on corn oil.

Recently, Irons et al. (2005) investigated the impact of n-3 PUFA (fish oil) on adaptive immune response to *L. monocytogenes*. Fish oil fed mice were induced infection with the bacterium, following 4 weeks of dietary supplementation. The number of *Listeria* – specific CD4+ and CD8+ effector and memory T cells in the spleen was not affected by n-3 PUFA. Also, the effector cells from fish oil fed mice were equally capable of conferring protective immunity upon adoptive transfer to native recipients.
Role of n-3 PUFA in inflammation and immunomodulation

Lokesh et al. (1986) demonstrated that peritoneal macrophages from mice fed on diets rich in n-3 PUFA (fish oil) for two weeks incorporated significant amounts of n-3 fatty acids into cellular lipids which lowered their content of arachidonic acid. These macrophages also secreted lesser amounts of prostaglandin E, thromboxane B and 6-keto-prostaglandin F\textsubscript{1\alpha} following stimulation with opsonized zymosan.

Yoshino and Ellis (1987) evaluated the effect of diet rich in EPA and DHA on carrageenin-induced footpad oedema, antibody production and a delayed type hypersensitivity reaction (DTH) in rats. They found no influence of diet on oedema induced in these animals. However, there was a suppression in DTH reaction in animals due to decrease in the production of arachidonic acid metabolites E\textsubscript{2} and LTB\textsubscript{4}.

Billiar et al. (1988) studied that Kupffer cells from rats fed on fish oil for 2 weeks or 6 weeks produced less amounts of thromboxanes and prostaglandins than the kupffer cells from rats fed on corn oil or safflower oil (n-6 PUFA), following exposure to LPS. Kupffer cells from the fish oil group produced less amounts of IL-1 and TNF-\(\alpha\) when stimulated by LPS. There was also significant increase in the liver n-3 fatty acid content with a concomitant decrease in n-6 fatty acids when compared to control diet of corn oil after 6 weeks of feeding.

Endres et al. (1989) examined the effect of diet supplemented with n-3 PUFA on synthesis of two proinflammatory cytokines (IL-1 and TNF-\(\alpha\)) by mononuclear cells. 9 healthy volunteers received 10g of fish oil concentrate per day in their normal diet for 6 weeks. It was observed that synthesis of IL-1\(\beta\) was suppressed by 43% (7.4ng/ml to 4.2ng/ml). The production of TNF-\(\alpha\) also responded in the same manner. This study highlighted the anti-inflammatory properties of n-3 PUFA.
Huang et al. (1992) investigated the influence of n-3 and n-6 PUFA feeding for 6 weeks on la antigen expression and immune cell population in murine peritoneum and spleen. Dietary fat did not affect the total cell yield or percentage of B cells, macrophages or la$^+$ cells but n-3 PUFA fed group had a greater percentage of T-cells. Following infection with *L. monocytogenes*, n-3 PUFA fed group had the highest percentage of B cells, lowest number of T cells, macrophages and la$^+$ cells in their peritoneum. In the spleen, la$^+$ splenocytes were predominantly B cells. The workers concluded that effects of diet observed on immune response of one tissue or cell type may not be readily extrapolated to others.

Fowler et al. (1993) examined the effects of a low dose, short term dietary supplementation for 10 days with highly purified 1% n-3 ethyl esters on murine splenic T lymphocyte functions. Fatty acid analysis of the lymphocyte membranes showed that they were enriched with EPA and DHA in n-3 PUFA fed mice. Con A - induced lymphoproliferative assays revealed enhancement of lymphoproliferation in n-3 PUFA fed group relative to n-6 fatty acid supplementation. Mice were also immunized with *M. bovis* vaccine after dietary supplementation. The immunized n-3 PUFA fed mice exhibited significantly decreased DTH reaction to footpad testing as compared to n-6 PUFA fed group of mice. Dietary supplementation with n-3 PUFA, thus, resulted in significant changes in T cell functions.

Tappia et al. (1995) studied the *in vitro* effect of unsaturated fatty acids on production of TNF-$\alpha$ and IL-6 in thioglycollate induced rat peritoneal macrophages. These cells were cultured in the presence of either 10$\mu$M DHA/EPA (n-3 PUFA) or linoleic acid (n-6) PUFA. Macrophages incubated in the presence of EPA showed increased production of TNF-$\alpha$ by 21% and IL-6 by 69%. Linoleic acid diminished TNF-$\alpha$ production by 16% and IL-6 production by 22%. Variable
effects of unsaturated fatty acids are observed on production of different cytokines.

Jolly et al. (1997) observed in their study, that Con A-induced lymphocyte proliferation in splenocyte cultures was significantly suppressed by dietary EPA and DHA while arachidonic acid had no effect in mice fed on 2% of either n-3 or n-6 fatty acids for 10 days. The suppressed proliferative response was preceded by significant reduction in IL-2 secretion. Production of essential lipid messengers, diacyl-sn-glycerol and ceramide was also reduced.

Caplan and Jilling (2001) investigated the specific role of PUFA (n-3 and n-6) supplementation in neonatal necrotizing enterocolitis induced in mice. Neonatal rats were fed infant formula supplemented with arachidonic acid and docosahexaenoic acid (23mg/100ml). It was found that PUFA administration in this model markedly reduced the symptoms of this disease and death compared with control formula. Furthermore, PUFA significantly reduced intestinal gene expression of phospholipase A₂ (at 24 hours) and platelet activating factor receptor (at 48 hours), but had no effect on inducible nitric oxide synthase at any time point. Thus, diet rich in n-3 PUFA reduced the risk of necrotizing enterocolitis in this model.

Arrington et al. (2001) studied the effect of n-3 PUFA on murine T-cell activation. Experimental mice were fed with diets containing 2% fish oil or 2% safflower oil for 14 weeks. Splenic CD⁴⁺ and CD⁸⁺ T lymphocytes were isolated from blood samples of mice in each group. The mitogen induced proliferation of T-cell subsets was enhanced by dietary n-3 PUFA supplementation. The IL-2 production by CD⁴⁺ T-cells was reduced in fish oil fed group while it remained unchanged by CD⁸⁺ T-cells in either of the two groups of mice.

Mayer et al. (2003) conducted a randomised controlled trial by providing parenteral nutrition containing either n-3 PUFA emulsion
(containing EPA and DHA) or n-6 PUFA emulsion (containing arachidonic acid) for 5 days in 21 patients with sepsis. Plasma-free fatty acid concentration, which was previously rich in arachidonic acid now contained greater concentration of EPA and DHA with rapid incorporation of n-3 fatty acids into mononuclear leukocyte membranes. Generation of pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8) by these mononuclear leukocytes was markedly amplified during n-6 fatty acid infusion and was suppressed during n-3 fatty acid application. These workers suggested that n-3 PUFA could be used as parenteral nutrition to improve immunological functions in patients with sepsis.

Stulnig and Zeyda (2004) have reviewed the immunomodulatory functions of n-3 PUFA. These include lymphocyte proliferation, cytokine production, phagocytic activity, adhesion molecule expression and NK cell activity which are susceptible to modification by the action of these dietary lipids in both animals and humans.

In a recent clinical trial conducted by Vaisman et al. (2005), cytokine production by peripheral blood mononuclear cells (PBMC), with and without LPS stimulation, in children given n-3 PUFA supplementation was assessed. 21 children (8-12 years) were randomised to receive fish oil or canola oil for 12 weeks. PBMC separated from their blood samples were cultured. The levels of pro-inflammatory cytokines IL-1β, TNF-α and IL-6 as well as anti-inflammatory cytokine IL-10 were significantly higher in non-stimulated and stimulated cultures of n-3 PUFA fed group as compared to control group.