Advances in technology have increased the understanding of illness and disease, expanded workforce and greater resources have paved a way to provide more services of higher quality. However still several mechanism in pathological conditions are yet to be elucidated.

1. Introduction

Acute lung injury (ALI) or its most severe form, acute respiratory distress syndrome (ARDS) is an important cause of mortality in the human population and continues to be a significant challenge for the critical care by the physicians. It is clinically devastating and life threatening syndrome, it persists to carry ~40–50% mortality rate [Ware and Matthay, 2000; Matthay and Zemmens, 2011]. Hence understanding the molecular pathogenesis of ARDS is essential.

Table.1. ARDS state of affairs in India

ARDS Prevalence in India	2,33,79,578/ year
	19,48,298/ month
	4,39,938/ week
	62,848/ day
	2,618/ hour

[http://www.medindia.net/health_statistics/diseases/acute-respiratory-infection-2007.asp]

Globally India is in 13th place by its incident levels; however it is in the 1st place when compared to other developing countries (Table 2).

Table.2. Acute respiratory distress syndrome by country (Extrapolated Statistics)

Country/Region	Extrapolated Incidence	Population Estimated
USA	161,942	293,655,405
Canada	17,927	32,507,874
United Kingdom	33,237	60,270,708
France	33,322	60,424,213
Greece	5,871	10,647,529
Germany	45,454	82,424,609
Ireland	2,189	3,969,558
Italy	32,016	58,057,477
Netherlands	8,999	16,318,199
Poland	21,301	38,626,349
Spain	22,213	40,280,780
China	716,276	1,298,847,624
India	**587,355**	**1,065,070,607**
Japan	70,220	127,270,708

[http://www.ardsusa.org/acute-respiratory-distress-syndrome.htm]

1.1. Lipopolysaccharide is a major cause of ALI/ARDS

Lipopolysaccharide (LPS), is an endotoxin, derived from gram negative bacteria, is a major cause of ALI and ARDS. LPS is a component of the outer cell wall of gram-negative bacteria. The general architecture of LPS has three building blocks

a. Lipid A
b. an inner core region
c. *O*-specific side chain

1.1.1. Lipid A: Lipid A contains unusual fatty acids ('e.g.' hydroxy-myristic acid) and is embedded into the outer membrane while the rest of the LPS projects from the surface.

Lipid A is a disaccharide with multiple fatty acid tails penetrating into the membrane. This is the key for toxicity. When bacterial cells are lysed by the immune system, fragments of membrane containing lipid A are released into the circulation, causing fever, diarrhea and possible fatal endotoxic shock (also called septic shock).

1.1.2. Core oligosaccharide: Core oligosaccharide contains unusual sugars (*e.g.* KDO, keto-deoxyoctulosonate and heptose). The core oligosaccharide is attached to lipid A, which is also in part responsible for the toxicity of gram-negative bacteria.

1.1.3. O-antigen: The polysaccharide side chain is referred to as the O-antigen of the bacteria. O side chain (O-antigen) is a polysaccharide chain that extends from the core polysaccharide. The composition of the O-side chain varies between different gram-negative bacterial strains. The presence or absence of O-chains determine whether LPS is rough or smooth. Full length O-chains would render the LPS smooth while the absence or reduction of O-chains would make LPS rough. Bacteria with rough LPS usually have more penetrable to cell membranes [Tsujimoto *et al.,* 2003].

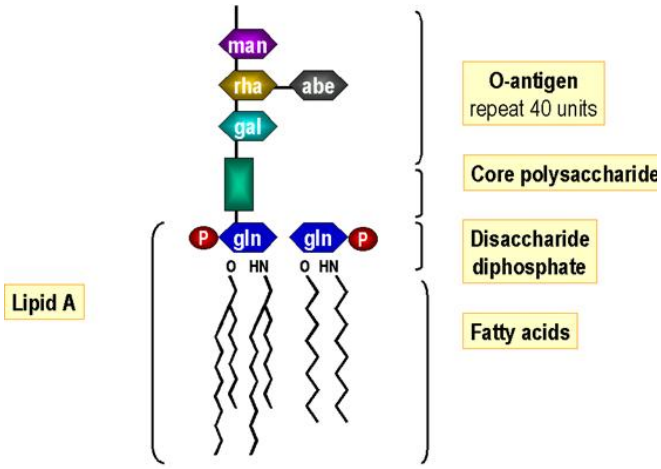

Fig.1. General Architecture of Lipopolysaccharide

The lipid A structure imparts the biological activity of LPS whereas the polysaccharide tail imparts antigenic characteristics, which vary among bacterial species. In a clinical situation, endotoxin (LPS) shock or endotoxin induced acute respiratory distress syndrome (ARDS) is frequently encountered. Severe lung injury caused by endotoxin is hard to control or treat and often causes death. It is a major complication in the control of infections in many patients, especially when immune suppression is present such as leukemia, AIDS, transplantation, steroid treatment and diabetes mellitus. Therefore, it is important to understand the mechanism of pathogenesis in LPS-induced lung injury [Brigham and Meyrick, 1986].

Recognition of LPS by host receptor(s) is the first step in a multi-step sequence, leading to activation of a number of signal transduction cascades in lung cells. The downstream effectors of these pathways result in production of a variety of inflammatory mediators, including proinflammatory cytokines, chemokines, adhesion molecules, ROS and nitric oxide by various cell types in the lung [Chabot *et al.,* 1998; Kubo *et al.,* 1999].

LPS binds to LPS binding protein (LBP) in plasma [Wright *et al.,* 1990] and is transferred to CD14, a glycosylphosphatidylinositol (GPI)-anchored protein, abundantly expressed on macrophages. LPS responses are dependent on the peripherally associated plasma membrane protein MD-2 [Nagai *et al.,* 2002] and the membrane-spanning complex formed by toll-like receptor (TLR) 4 [Poltorak *et al.,* 1998], through which signaling is propagated. TLRs activate four intracellular protein kinase cascades, the IB kinase (IKK)/NF-kB transcription factor cascade, the extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK) and p38 mitogen activated

protein kinase (MAPK) cascades, leading to the induction of many key cytokine genes that are involved in the innate immune response [Takeda *et al.,* 2003; Medzhitov, 2001; Barton and Medzhitov, 2003].

1.2. Acute Respiratory Distress Syndrome

ARDS was recognized in 1967 when the clinical, physiological, radiographic, and pathological abnormalities that were unique to a group of 12 patients were described, distinguishing them from other cases in a series of 272 patients treated for respiratory failure [Ashbaugh *et al.,* 1967]. Before 1992, the acronym ARDS represented "adult respiratory distress syndrome". The American-European Consensus Committee on ARDS standardized the definition in 1994 and renamed it "acute" rather than "adult respiratory distress syndrome" because it occurs at all ages. The distinction between ALI and ARDS is dependent on the degree of hypoxemia [Bernard *et al.,* 1994].

1.2.1. ARDS clinical course

ARDS is characterized by

- Acute onset.
- Bilateral infiltrates on chest radiograph sparing costophrenic angles.
- Pulmonary artery wedge pressure < 18 mm Hg (obtained by pulmonary artery catheterization), then lack of clinical evidence of left ventricular failure suffices.
- if PaO_2:FiO_2 < 300 mm Hg (40 kPa) acute lung injury (ALI) is considered to be present.
- if PaO_2:FiO_2 < 200 mm Hg (26.7 kPa) acute respiratory distress syndrome (ARDS) is considered to be present.

1.2.2. Pathophysiology of ARDS

Based on pathophysiological mechanisms ARDS is divided into two categories either direct (pulmonary) or indirect (extrapulmonary) injury [Fein and Colucci, 2000] leading to pulmonary inflammation. Direct injury includes those conditions in which a toxic substance directly injures the lung epithelium such as diffuse pulmonary infection (e.g., bacterial, viral, fungal, pneumocystis), toxic gas/smoke inhalation, pulmonary contusion, and aspiration of gastric contents [Fein and Colucci, 2000, Tasaka *et al.,* 2002].

Indirect injury is a more common predisposition and occurs by means of blood-borne systemic inflammatory processes such as sepsis, septic shock [Bhatia *et al.,* 2000; Borish and Steinke, 2003; Fein and Colucci, 2000; Tasaka *et al.,* 2002; Goodman *et al.,* 1998; Pan *et al.,* 2000; Shokuhi *et al.,* 2002; Strieter *et al.,* 1999; Udobi *et al.,* 2003], acute pancreatitis [Aho *et al.,* 1983; Ashbaugh *et al.,* 1967; Bhatia *et al.,* 2000; Bhatia *et al.,* 2000a; Brady *et al.,* 2002; Leme *et al.,* 2002; Napolitano, 2002], and other clinical events including major surgery, trauma, multiple transfusions, dyspnea, ischemia-reperfusion injury, and decreased lung compliance [Borish and Steinke, 2003; Rabinovici *et al.,* 2002].

1.2.3. Phases in ARDS

In ARDS, the injured lung is believed to go through three phases: exudative, proliferative and fibrotic, but the course of each phase and the overall disease progression is variable. The pathological features of the lung in ARDS is due to severe injury in the alveolocapillary unit. The morphologic picture of the lung in ARDS has been labeled as diffused alveolar damage [Rinaldo and Rogers, 1982] and extravasation of intravascular fluid that dominates the onset of the disease.

1.2.3.1. The exudative phase occurs in the first week after the onset of the respiratory failure. The histological features are dense eosinophilic hyaline membranes and alveolar collapse. The endothelial cells swell, the intercellular junctions widen and pinocytic vesicles increase, causing the capillary membrane to be disrupted and resulting in capillary leak and edema formation. Type I pneumocytes also become swollen with cytoplasmic vacuoles, which eventually detach from the basement membrane [Dematte *et al.,* 1997].

1.2.3.2. The proliferatory phase begins as early as the third day but is more prominent in the second and third week after symptom onset. Type II cells begin to proliferate and reline the denuded basement membrane [Dematte *et al.,* 1997]. Fibrosis becomes pronounced in this phase. Fibroblasts and myofibroblasts migrate through breaks in the alveolar membrane into the fibrinous intra-alveolar exudate, forming a cellular granulation tissue. Sparsely cellular, dense fibrous tissue forms collagen and gets deposited. Epithelial cells migrate over the surface of the organizing granulation tissue and transform the intra-alveolar exudate into the interstitial tissue [Tomashefski, 1990]. Surfactant abnormalities are thought to occur because of damage to type II pneumocytes and because of the alveolar flooding, leading to destabilization of surfactant monolayer in the air spaces.

1.2.3.3. The fibrotic phase can start as early as 36 h after the onset of injury; extensive remodeling of the lung by sparsely cellular collagenous tissue occuring by the third or fourth week of respiratory failure [Dematte *et al.,* 1997]. Air spaces are irregularly enlarged, and there is alveolar duct fibrosis. Type III elastic collagen is replaced by type

I rigid collagen over time, leading to a stiff lung. The extent of fibrosis correlates with mortality [Meduri *et al.,* 1994]. Early in ARDS, pulmonary vasoconstriction, thromboembolism and interstitial edema, all of which are potentially reversible, raise the pulmonary artery pressure. After several weeks, fibrous obliteration of the microcirculation and arterial muscularization contribute to irreversible pulmonary hypertension. Patients with ARDS also are at risk of pulmonary emboli because of immobilization and the presence of indwelling vascular catheters [Sylvester *et al.,* 1990].

1.2.4. Polymorphonuclear leukocytes (PMNs) role in ARDS

PMNs have been recognized as important contributors to the pathogenesis of ARDS. As a result of an exaggerated systemic inflammatory response syndrome, leukocytes become activated within the general circulation, and some of them lodge within the pulmonary microcirculation. As the condition worsens, leukocytes migrate into the pulmonary interstitium, and increased endothelial permeability leads to tissue edema [Murakami *et al.,* 1995]. Disruption in the epithelial and endothelial barriers of the lungs is associated with a massive increase in epithelial and endothelial permeability with accumulation of high-molecular-weight proteins that are normally excluded from the air spaces [Haskell *et al.,* 1999]. This occurs together with a marked influx of PMNs, so that PMNs become the predominant leukocytes in the alveolar spaces. Normally, 90% or more of the air space cells are alveolar macrophages (AMs), < 10% are lymphocytes, and only 1–2% are PMNs. In patients with ARDS, up to 90% air space cells are PMNs. When ARDS is sustained, PMNs persist in the airspaces, and the number of macrophages is reduced. When ARDS is resolved, the number of macrophages

increases, but lymphocyte accumulation seldom occurs [Steinberg *et al.,* 1994; Weiland *et al.,* 1986].

1.2.5. Differences between normal and injured alveolus during ALI/ARDS.

In the acute phase of the syndrome, there is sloughing of both the bronchial and alveolar epithelial cells; protein-rich hyaline membranes form on the denuded basement membrane. Neutrophils adhere to the injured capillary endothelium and marginate through the interstitium into the air space, which is filled with protein-rich edema fluid. In the air space, alveolar macrophages secrete cytokines; interleukin (IL)-1, -6, -8, and -10; and tumor necrosis factor α (TNF-α), which act locally to stimulate chemotaxis and activate neutrophils. IL-1 can also stimulate the production of extracellular matrix by fibroblasts. Neutrophils can release oxidants, proteases, leukotrienes, and other proinflammatory molecules such as platelet-activating factor (PAF).

A number of anti-inflammatory mediators also present in the alveolar milieu include IL-1 receptor antagonist, soluble TNF receptor, autoantibodies against IL-8, and cytokines such as IL-10 and -11. The influx of protein-rich edema fluid into the alveolus leads to the inactivation of surfactant (Matthay, and Zemans, 2011) and destabilizes the surfactant monolayer in the air spaces. This dysfunction promotes alveolar collapse and worsens gas-exchange abnormalities [Martin and Goodman, 1999]. It is now widely accepted that the formation of inflammatory mediators play an important role in the pathophysiology of ARDS. These mediators include tumor necrosis factor (TNF)-α; interleukin (IL)-1, -4, -6, -8, -10, and -13; substance P; platelet activating factor (PAF); complement component (C5a); adhesion molecules (e.g., vascular adhesion molecule-1, intercellular adhesion molecule-1); E- and P-selectins; L-selectin; and vasoactive

mediators (e.g., nitric oxide). The transcription factor NF-$_{K}$B plays a central role in the regulation of many genes responsible for the generation of mediators in inflammation. Investigations into the interactions between various cell populations have led to the concept of cytokine networking with chemokines playing a central role.

1.2.6. Role of oxidant and *antioxidant in the pathogenesis of lung inflammation*

The great external surface area (1-2 m^2) of the human airway epithelium plus its direct contact with the environment, makes the respiratory tract a major target for oxidative injury from inhaled oxidants (LPS, cigarette smoke, ozone, hyperoxia, nitrogen and sulphur oxides and other airborne pollutants) [Halliwell and Gutteridge, 1989]. In fact, epidemiological studies have shown associations between air pollution and respiratory disease [Prescott *et al.,* 1998; Sheppard, *et al.,* 1999]. In addition, pulmonary cells are also exposed to locally generated endogenous oxidants.

Biological systems are capable of forming highly reactive moieties, both free radicals and non-radicals, named reactive oxygen (ROS) and nitrogen (RNS) species (see Table 2) [Roberfroid and Calderon, 1995; Gaston, *et al.,* 1994]. These biologically active species serve in cell signaling as messenger molecules of the autocrine or paracrine system [Saran and Bors, 1989; Suzuki *et al.,* 1997] and also in host defence (biocidal effects against microbial and tumour cells) [Notter, 2000] but their excess production may result in tissue injury and inflammation [Halliwell and Gutteridge, 1989; Halliwell *et al.,* 1992; Gutteridge and Halliwell, 1994; Clement and Housset, 1996].

A deficit in the precise balance between exposure to oxidants and endogenous antioxidants results in 'oxidative stress' which appears to be involved in the pathogenesis of a number of growing diseases, including lung pathologies such as ARDS, chronic obstructive pulmonary disease (COPD), asthma, idiopathic and iatrogenic

pulmonary fibrosis, cystic fibrosis, HIV-associated lung disease, lung cancer and other pulmonary diseases and conditions [Clement and Housset, 1996; Barnes, 1990; Cross *et al.,* 1994; Barnes, 1995].

Table.3. Reactive oxygen and nitrogen species of biological interest [Gaston *et al.,* 1994; Chabot *et al.,* 1998]

	Free radicals	***Non-radicals***
Reactive oxygen species	Superoxide ($O_2^{-\bullet}$)	Hydrogen peroxide (H_2O_2)
	Hydroxyl ($OH^{-\bullet}$)	Hypochlorous acid (HOCl)
	Peroxyl (R-O$^\bullet$)	Ozone (O_3)
	Alkoxy (R-O$^\bullet$)	Singlet oxygen ($^1O_2$)
	Hydroperoxyl ($HO_2^\bullet$)	Hydroperoxide (R-OOH)
	Hydroperoxide (R-OO$^\bullet$)	
Reactive nitrogen species	Nitric oxide (NO$^\bullet$)	Peroxynitrite anion ($ONOO^-$)
	Nitrogen dioxide ($NO_2^\bullet$)	Nitronium cation (NO_2^+)
		Nitrosyl or nitrosonium cation (NO^+)
		Alkyl peroxynitrite (R-OONO)
		Nitroxyl anion (NO^-)

Many ambient air pollutants may induce oxidative stress in the lung that arises when ROS overwhelms antioxidant defenses. After this imbalance ROS readily reacts with proteins, lipids and DNA resulting in a number of pathological consequences.

Oxidant pollutants may elicit their effects through (1) the production of secondary mediators generated by reaction of pollutants or pollutant-induced ROSs or free radicals with lipids in the epithelial lining fluid (ELF) or cell membrane as well as proteins and antioxidants; (2) activation of signaling pathways by ROSs or secondary mediators; (3) oxidation of cellular proteins; (4) damage to DNA. In addition to pollutant-induced generation of ROSs, endogenous sources of ROSs such as inflammatory cells, phagocytes recruited to the site of injury, and other cellular processes may contribute to the oxidative stress state caused by pollutant exposure.

Antioxidant molecules and enzymes mitigate the effects of ROSs in the body [Ciencewicki *et al.,* 2008].

1.2.7. Oxidant interaction with molecules

A primary consequence of oxidative stress is lipid peroxidation or oxidative degeneration of lipids. Lipid peroxidation is caused by a free radical chain reaction mainly involving membrane polyunsaturated fatty acids. If not quenched, this reaction can permanently damage cell membranes, ultimately leading to cell death. Exposures to oxidant air pollutants cause lipid peroxidation in human beings and rodents. [Chen *et al.,* 2007; De Burbure *et al.,* 2007; Elsayed *et al.,* 2002; Ergonul *et al.,* 2007; Liu and Meng, 2005; Yargicoglu *et al.,* 2007] Furthermore, the end products of lipid peroxidation can lead to subsequent pathological consequences. One of these end products, 4-hydroxy-2-nonenal (HNE), has numerous downstream effects. *In vitro* treatment of cells with HNE can cause lipid peroxidation [Keller *et al.,* 1997] and may potentiate oxidative stress through depletion of intracellular glutathione and induction of peroxide production [Uchida *et al.,* 1999].

1.2.8. Possible precipitating causes of ARDS

- Bacterial lung infection (BLI)
- Shock
- Severe blood loss (SBL)
- Bone fractures - if they cause shock (BF).
- Severe infections
- Viral lung infection
- Fungal lung infection
- Lung trauma

- Pneumonia
- Sepsis
- Poliomyelitis
- Blood transfusion adverse reaction (type of adverse reaction)
- Heart bypass surgery adverse reaction (type of adverse reaction)
- Smoke inhalation
- Toxic fume inhalation
- Asthma
- Emphysema
- Muscular dystrophy
- Pancreatitis
- Guillaine-Barre syndrome

1.3. Pulmonary System

The lung alveolar system is the largest surface of our body that is exposed to the environment, covering up to ~120 m^2 [Schmitz and Muller, 1991]. The lung is exposed continuously to inhaled pathogens, pollutants, particles and cigarette smoke. Therefore, the pulmonary immune system needs to provide defence against harmful substances and to prevent inappropriate inflammatory response. Several defence mechanisms contribute to the innate immunity of the lung, including filtration in the naso-oropharynx, sneezing, coughing, mucociliary clearance, opsonins (Ig), innate immune cells (Alveolar macrophage, Neutrophil), and surfactants [Wright *et al.,* 2000].

1.3.1. Role of pulmonary cells in respiration

The lung contains ~40 different cell types and among those the surface is covered by two major types of epithelial cells, alveolar epithelial type 1 cells (AEC1) and AEC2, respectively [Figure B]. AEC1 cells cover ~95% of the alveolar surface while comprising only 8% of the total cells in the normal adult human lung. AEC2 cells constitute ~15% of total cells but only covers about 5% of the alveolar surface area in the healthy human lung [Crapo *et al.,* 1982]. These are flattened cells, contain just a few organelles and serve as a thin barrier between blood and the air. AEC1 cells are

involved in cation transport [Johnson *et al.,* 2006] and also function as signaling partners of AEC2 cells. More than 600 genes have been found to be differentially expressed in AEC1 and AEC2 cells [Gonzalez *et al.,* 2005]. Another cell type present in alveoli is macrophages, which removes particles and microorganisms coming with the air [Schmitz and Muller, 1991].

AEC2 cells have many important functions in normal respiratory physiology. They participate in clearance and repair by proliferating and migrating to damaged areas. They participate in defense response by expressing various receptors (in particular, Toll-like receptors). It is involved in the lung cytokine/chemokine network by secreting and responding to an array of cytokines and chemokines [Homer *et al.,* 2002, Thorley *et al.,* 2007, Vanderbilt *et al.,* 2003 and Witherden *et al.,* 2004]. They regulate in the transmigration of monocytes across epithelial layer and possibly participate in T cell activation. However, histochemical, electron microscopic and autoradiographic studies strongly suggest that "pulmonary surfactant" is produced by the AEC2 from 24 weeks of gestation, and in adequate amount from 35 weeks of gestation [Batenburg *et al.,* 1979; Van Golde *et al.,* 1988].

1.3.2. Pulmonary surfactant

The possible role of surface forces in the lung was first reported in 1929 by the Swiss scientist Kurt von Neergaard. Two and a half decades later, Pattle (1955) and Clements (1956) independently demonstrated the existence of surface-active material in the alveoli of animal lungs. Soon after, Avery and Mead (1959) established the relationship between this surface-active material, lung surfactant, and hyaline membrane disease of human neonates.

Pulmonary surfactant is composed of ~80% glycerophospholipids, 5 - 10% neutral lipids and 8 - 10% proteins with 5 - 6% total surfactant mass being constituted by four specific surfactant proteins (SP) [Goerke, 1998]. The phospholipid fraction of

surfactant is mainly responsible for forming surface active films at the respiratory air–liquid interface [Perez-Gil and Keough, 1998, Veldhuizen, 1998], but it also provides the scaffold or matrix on which different surfactant structures are assembled.

1.3.3. Biophysical properties of surfactants

- Prevents alveolar collapse during respiration.
- Supports lung expansion during inspiration.
- Prevents pulmonary oedema by balancing hydrostatic force.
- Stabilizes and maintain small airway structures.
- Improves mucociliary function.
- Facilitates the transport of small particles (<6 μm) into the epithelial lining fluid.
- Reduces surface tension , facilitates the removal of particles and cellular debris from the alveoli into the large airways.

1.3.4. Immunological properties

- Surfactant phospholipids inhibit proliferation, immunoglobulin production and cytotoxicity of lymphocytes.
- Surfactant phospholipids inhibit cytokine (TNF, IL-1, IL-6) release from activated macrophages.
- Collectins SP-A and SP-D regulate phagocytosis, chemotaxis and oxidative burst of alveolar macrophages.
- Collectins neutralize free radicals and reactive oxygen species.
- Collectins SP-A and SP-D facilitate opsonization of various microorganisms, particles and are also able to capture bacterial toxins.

1.3.5. Pulmonary Surfactant Lipids

Pulmonary surfactant consists of a unique and complex mixture of lipids (85-90%) and surfactant proteins (10%). Surfactant lipids are mostly phospholipids (90%) that are essential for reducing surface tension within the lungs, with 10% of neutral

lipids, such as cholesterol and trace amounts of TG [Orgeig and Daniels, 2001; Tolle *et al.,* 2002].

1.3.5.1. Phosphatidylcholine

PC is a major component of cellular membranes [Van Meer *et al.,* 2008] and most abundant in surfactant phospholipid [Veldhuizen *et al.,* 1998]. Among the surfactant phospholipids, PC constitutes ~ 70-80% [Harwood and Richards, 1985], mostly in the form of disaturated dipalmitoyl phosphatidylcholine (DPPC; constituting ~60%) [Creuwels *et al.,* 1997; Hamm *et al.,* 1992], which plays an essential role in decreasing surface tension [Perez-Gil and Weaver, 2010; Goerke, 1998]. The hydrophilic component of DPPC is oriented towards the liquid surface of the alveolar air-water interface, while the hydrophobic component, palmitic acid (C16:0), is oriented towards the air phase. Evolution has probably selected DPPC as the main phospholipid species in surfactant because at physiological temperature, the saturated chains of DPPC can be packed to a very high density at the air–water interface, providing large reduction of surface tension required to stabilize the lung at the end of expiration [Wustneck *et al.,* 2005; Hawco *et al.,* 1981]. Palmitoyloleoyl phosphatidylcholine (POPC), for instance, the main unsaturated phospholipid in surfactant [Davis *et al.,* 1981], increases the membrane fluidity at physiological temperatures and is important to improve their dynamic properties.

Two pathways contribute to the production of DPPC, (1) direct *de novo* synthesis and (2) remodeling of unsaturated PC. The direct biosynthesis pathway of DPPC occurs by means of conversion of phosphatidic acid (PA) to 1, 2-dipalmitoyl diacylglycerol (DAG) by phosphatidate phosphatase and further it is converted to DPPC by choline phosphotransferase. The acyl-chain composition of phospholipids is mainly determined by a deacylation-reacylation cycle named Lands' cycle [Lands, 1958], depicted in the remodeling pathway and accounts for 75% of DPPC in AEC2 cells. Deacylation of unsaturated PC at the sn-2 position by a calcium-dependent phospholipase A_2

[Filgueiras and Possmayer, 1990]; peroxiredoxin 6, a calcium-independent phospholipase A_2 may also contribute to deacylation of PC [Chen *et al.,* 2000, Fisher and Dodia, 2001] followed by reacylation of the resultant 1-palmitoyl-2-lysophosphatidylcholine with palmitoyl-CoA via a *lysophosphatidylcholine (lysoPC) acyltransferase (LPCAT)*. Identification of transcriptional networks that integrate expression of LPCAT1 and other lipogenic enzymes with surfactant proteins should provide important insight into the molecular pathways governing surfactant homeostasis. Understanding the regulation of lipid synthesis is potentially important for the development of new therapeutic agents to increase endogenous surfactant.

1.3.5.2. Phosphatidylglycerol

Phosphatidylglycerol (PG) is a second major phospholipid in adult surfactant which constitutes ~10% of the surfactant phospholipids. PG may interact with certain surfactant proteins (SP-B in particular) and may be related to selective adsorption of DSPC and reorganization of these phospholipids from the monomolecular film at the air-water interface of the alveolus [Rodriguez-Capote *et al.,* 2001; Ross *et al.,* 2002; Takamoto *et al.,* 2001]. A key step in PG synthesis is conversion of CDP-diacylglycerol to phosphatidylglycerolphosphate by phosphatidylglycerophosphate synthase [Batenburg *et al.,* 1994 and 1998] and it can also be synthesised by the remodeling pathway via a *lysophosphatidylglycerol (lysoPG) acyltransferase (LPGAT).* Transcription of the gene encoding this enzyme (PGS1) is increased three fold during maturation of human lung epithelial cells in *in vitro* condition [Wade *et al.,* 2006] and is similarly increased in AEC2 when cultured under conditions that promote differentiation [Mason *et al.,* 2003]. Targeted disruption of the Pgs1 locus in AEC2 could provide important new information regarding the role of PG in lamellar bodies (LB) maturation, surfactant function and the potential for PI to compensate for loss of PG. Recent studies evidenced that PG can suppress viral infection and inflammatory response in the lung [Kuronuma *et al.,* 2009, Numata *et al.,* 2010].

1.3.5.3. Minor phospholipids and neutral lipids

Minor surfactant phospholipids are phosphatidylinositol (PI), phosphatidylethanolamine (PE) and phosphatidylserine (PS). Immature mammalian surfactants contain a high percentage of PI instead of PG [Egberts and Noort, 1986, Hallman and Gluck, 1980; Hallman *et al.,* 1976]. Apart from phospholipids the remaining lipid fraction, is neutral lipid, mainly cholesterol which has an important function [Orgeig and Daniels, 2001; Tolle *et al.,* 2002] with trace amounts of triglycerides and fatty acids (FAs) [Goerke, 1998]. Most of the cholesterol in the surfactant is derived from serum lipoproteins [Guthmann *et al.,* 1997], whereas phospholipids are synthesized by AEC2. After birth, considerable proportion of surfactant is continuously recycled, and the demand for de novo synthesis is much lower.

1.3.5.4. Lamellar bodies

Lipid–protein surfactant complexes are assembled in AEC2 in the form of tightly packed membranes, which are stored in specialized organelles (0.1–2.4 μm in diameter) called LB [Schmitz and Muller, 1991]. Each AEC2 contains 120–180 LBs [Young *et al.,* 1991]. Upon secretion of LBs, surfactant develops a membrane-based network that spreads efficiently on the whole respiratory surface and permits an efficient exchange of molecules between the gas and the liquid regions in alveoli.

1.3.6. Synthesis, secretion and turnover of surfactant

Pulmonary surfactant is synthesized, assembled, transported and secreted into the alveolus. It is degraded and then recycled in a highly complex and regulated manner. This process is slower in newborns (especially those born prematurely) than in adults or those with lung injury. The rate of synthesis and the half-life of surfactant are influenced by many factors. Surfactant secretion can be stimulated by a number of mechanisms. AEC2 have beta-adrenergic receptors and respond to beta-agonists with increased surfactant secretion [Mason and Voelker, 1998; Dietl *et al.,* 2001; Jobe, 2002].

AEC2, macrophages and the alveolar lining play a major role in surfactant turnover. Cyclical changes in the alveolar surface appear to promote conversion of newly secreted, apoprotein-rich, active surfactant aggregates into protein-poor, inactive forms that are ready for clearance [Jobe, 2002]. Surfactant components are removed from air spaces by AEC2 and alveolar macrophages, with the bulk done by the AEC2. Surfactant is also transformed during the cyclic compression and expansion of alveoli from large, highly surface-active aggregates into smaller, less active subtypes [Ueda *et al.,* 1994].

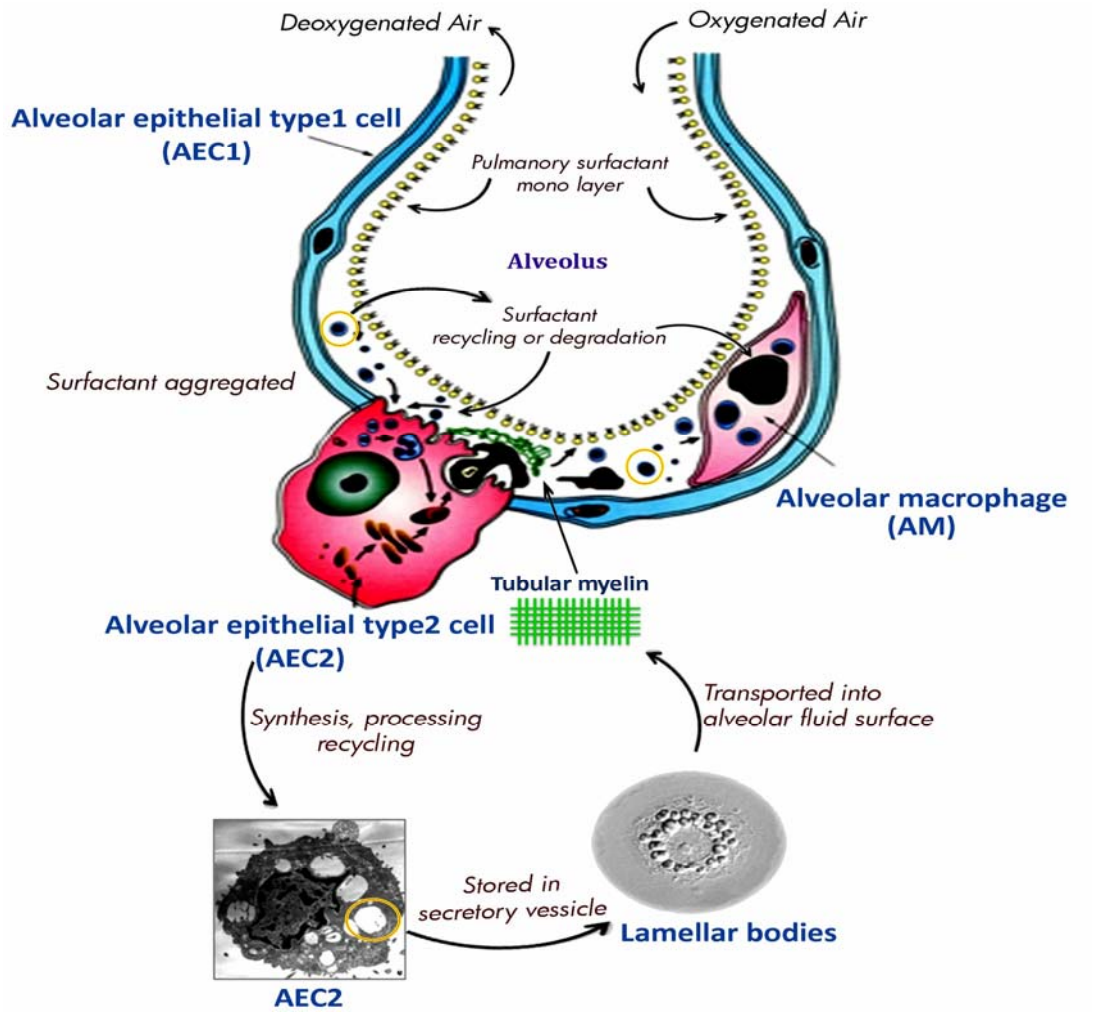

Fig.2. *Surfactant components are synthesized, secreted and recycled back in type II epithelial cells of the alveolus. The extracellular surfactant is uptaken by AEC2, catabolized and transported to lamellar bodies for recycling. Alveolar macrophages (AM) participate in the degradation of surfactant components.*

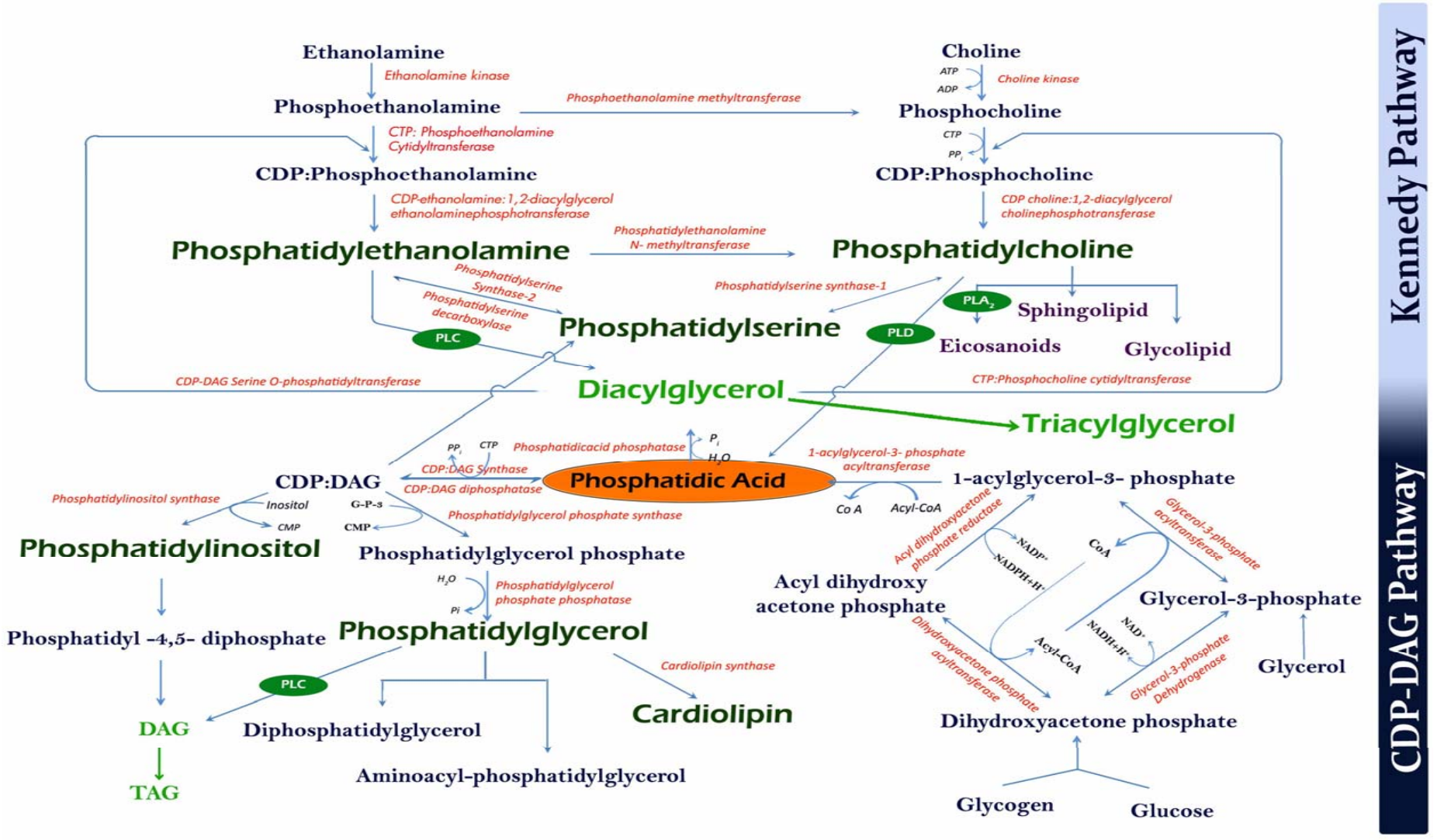

Fig.3. Eukaryotic mammalian phospholipid biosynthetic pathway.

1.3.7. Catabolism of surfactant

Overall, maintenance of the balance between the intracellular storage pool and the functional alveolar pool require tight integration of synthesis, secretion, recycling and degradation. Pulmonary surfactant is mainly catabolised by AEC2 and alveolar macrophages. AEC2 and macrophage equally contribute to uptake of surfactant in the airways. The uptake of surfactant by AEC2 is either catabolised by lysosome or recycled back to lamellar body. Surfactant uptake by macrophage (20% was recovered from BAL fluid and 80% was associated with lung tissue) will be catabolised [Gurel *et al.,* 2001]. Increases in surfactant pool sizes were observed in defective surfactant catabolism by alveolar macrophages. The loss of surfactant from the airspaces is balanced by secretion of surfactant stored in lamellar bodies: regulation of surfactant secretion is therefore critical for homeostasis.

1.3.8. Pulmonary surfactant in lung disease

In 1959, Avery and Mead suggested that surfactant deficiency could cause hyaline membrane disease, currently called respiratory distress syndrome (RDS). Abnormal surfactant levels and composition in humans have been associated with respiratory dysfunction and inflammation in ALI/ARDS [Todd *et al.,* 2010], pulmonary fibrosis [Gunther *et al.,* 1999], emphysema [Wert *et al.,* 2000], cystic fibrosis [Griese *et al.,* 1997], COPD [Ohlmeier *et al.,* 2008], and RDS in newborns [Halliday, 2008]. Genetic variation or deletion in surfactant protein genes is associated with COPD [Guo *et al.,* 2001], interstitial lung diseases [Brasch *et al.,* 2004], cancer [Wang *et al.,* 2009; Jiang *et al.,* 2005], pulmonary infections [Puthothu *et al.,* 2007], congenital alveolar proteinosis [Tredano *et al.,* 2004], and enhanced development of broncho-pulmonary dysplasia [Pavlovic *et al.,* 2006]. In parallel, studies with SP-A, SP-B, SP-C and SP-D-deficient mice support the crucial roles for surfactant proteins in surfactant stability and monolayer formation [Ikegami *et al.,* 2005], metabolism [Korfhagen *et al.,* 1998], pathogenesis of acute and chronic inflammation, respiratory distress [Nesslein *et al.,* 2005], lung injury [Yang *et al.,* 2002] and susceptibility to infection [LeVine *et al.,* 1998]. Deficiencies in

surfactant protein components are associated with dysregulated inflammatory responses [Ikegami *et al.,* 2007] and presence of abnormal surfactant vesicles [Todd *et al.,* 2010].

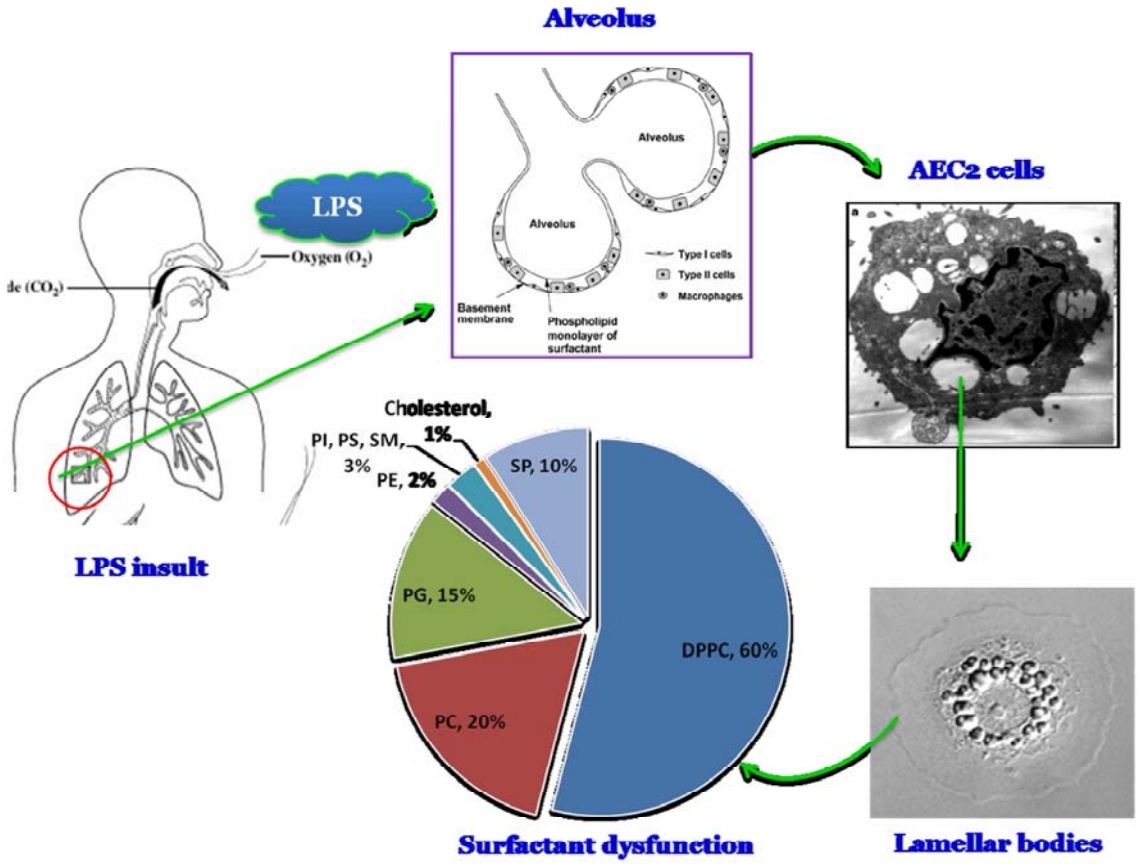

Fig.4. LPS insult leads to surfactant dysfunction. Recognition of LPS by a host receptor(s) is the first step in a multistep sequence leading to the activation of a plethora of signal transduction cascades in a variety of lung cells (AEC1, AEC2 cells). The downstream effectors of these pathways then result in the production of a variety of inflammatory mediators, including proinflammatory cytokines and chemokines, adhesion molecules, reactive oxygen species, and nitric oxide leading to damage the AEC2 cell thereby dysfunctioning the pulmonary surfactants.

Destruction of surfactant increases surface tension at the air–liquid interface, which results in alveolar collapse, culminating in acute lung injury, such as Acute Respiratory Distress Syndrome (ARDS) [Lewis and Jobe, 1993]. Hydrolysis of DPPC is an early pathophysiological event that leads to an accumulation of lyso-phosphatidylcholine (lyso-PC) [Gregory *et al.,* 1991]. The later plays a crucial role in acute lung injury since it damages the AEC1 cellular membranes [Niewoehner *et al.,* 1987] increases capillary permeability [Lindhal *et al.,* 1986], and markedly inactivates

the surfactant tensioactivity [Holm *et al.,* 1991]. Furthermore, among lyso-phospholipid subclasses, lyso-PC is the most effective in potentiating the diacylglycerol-induced activation of particular calcium-dependent protein kinase C isoforms, suggesting a role in cell activation [Sasaki *et al.,* 1993; Asaoka *et al.,* 1991]. Finally, lyso-PC exhibits chemotactic activities toward lymphoid [Hoffman *et al.,* 1982; Ryborg *et al.,* 1994] and mononuclear cells [Quinn *et al.,* 1988] and induces the expression of vascular cell adhesion molecules and intercellular adhesion molecules in endothelial cells [Kume *et al.,* 1992; Yokote *et al.,*1993], suggesting a role in cell recruitment during inflammation.

1.4. The immune system in health and disease—a brief overview

The immune system protects the host from environmental infectious agents such as pathogenic bacteria, viruses, fungi, and parasites and from other noxious insults. It also permits tolerance to self-antigens and to non-threatening environmental agents such as food proteins and commensal gut bacteria. The system has two functional divisions: the innate (or natural) immune system and the acquired (also termed specific or adaptive) immune system. Both components involve various blood-borne factors and cells. Immune cells originate in bonemarrow and are found circulating in the blood stream, organized into lymphoid organs such as the thymus, spleen, lymphnodes and gut-associated lymphoid tissue, or dispersed in other locations. Different immune cell types have highly specialised roles (e.g.phagocytosis, antigen presentation, antibody production, destruction of virally infected cells) and acting together they create a coordinated and regulated immune response. Certain cells of the immune system retain memory of previous immunologic encounters so ensuring a more rapid response upon re-infection. Thus, the four key roles of the immune system are:

- To act as an exclusion barrier.

- To discriminate ‘‘self’’ from ‘‘non-self’’ so assuring tolerance.
- To eliminate the source of ‘‘non-self’’ antigens.
- To retain memory of immunologic encounters.

Although mounting an effective immune response to pathogens is central to host defence and is essential to preventing infectious disease, a breakdown in the mechanisms that act to provide tolerance to self or to benign environmental allergens can lead to inappropriate immunologic activity and, in some cases, damage the host and cause disease. Adverse immunologic responses are:

- Auto immune reactions involving loss of tolerance and an immunologic reaction to self antigen.
- Allergic reactions involving loss of tolerance and an immunologic reaction to a normally benign environmental or food antigen.
- Inflammatory bowel reactions involving loss of tolerance and an immunologic reaction to commensal gut bacteria.
- Chronic inflammatory responses to local injury
- Over zealous inflammatory responses to external insults such as major surgery, injury, trauma and critical illness.

Thus, there is tremendous interest in finding means by which to modify or manipulate the responses of immune cells since this might enable either an improvement in host defence mechanisms therefore reducing the incidence and severity of infectious diseases or a reduction in adverse immunologic reactions thereby reducing the severity and burden of autoimmune, allergic and inflammatory conditions. Research over the last 30 years suggest changing the fatty acid composition by nutrition can be a means by which immune cell behaviour and the immune response, including its inflammatory component can be modified.

1.4.1. Lymphoid organs

The mammalian immune system, a cooperative venture between the innate and acquired arms, offers an optimal environment for defense against the invasion of pathogens at any site in the body. The sites of organized lymphoid cell accumulations are called primary and secondary lymphoid organs (SLOs) [Mebius and Kraal, 2005]. Diverse populations of functionally mature, but naive, lymphocytes are produced in the absence of foreign antigens in the primary lymphoid organs (thymus, fetal liver, bone marrow). These cells sow the SLOs to optimally take action to foreign invaders. Secondary or peripheral lymphoid organs maintain mature naive lymphocytes and initiate an adaptive immune response. The peripheral lymphoid organs are the sites of lymphocyte activation by antigen. Activation leads to clonal expansion and affinity maturation. Mature Lymphocytes recirculate in the blood until they encounter their specific antigen [Kuby *et al.,* 2003]

1.4.2. Spleen

The spleen (from Greek "*splen"*) found virtually in all vertebrate animals has an important role with red blood cells and the immune system. In humans, it is located in the left upper quadrant of the abdomen. It removes old red blood cells and holds a reserve of blood cells in case of hemorrhagic shock and also recycles iron [Mebius and Kraal, 2005].

The spleen is divided into two anatomically and functionally distinct areas: red pulp and white pulp. The red pulp, in its activities is a hematogenous organ, removes damaged red blood cells and acts as a site for iron storage and turn over. The white pulp is an organized lymphoid structure. The spleen is highly vascularized, but has no high endothelial venules (HEVs) or afferent lymphatic vessels. Rather, the splenic artery,

located immediately below the capsule is the source of cells and antigens. The marginal sinus and marginal zone (MZ) demarcate the red and white pulp. The MZ, which surrounds the white pulp, represents a important transition between the innate and acquired immune systems; it is the first region after the red pulp encountered by blood borne antigens and has an abundant supply of specialized phagocytic cells: the MZ macrophages and MZ metallophilic macrophages. The MZ also contains a specialized subset of B cells that differ phenotypically and functionally from follicular B cells and are considered a bridge between the innate and adaptive immune systems.

Spleen synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation. The spleen is purple and gray [Mebius and Kraal, 2005; Loscalzo *et al.,* 2008]. Recently, it has been found to contain in its reserve half of the body's monocytes within the red pulp. These monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cells and macrophages while promoting tissue healing [Swirski *et al.,* 2009; Jia and Pamer, 2009]. It is one of the centers of activity of the reticuloendothelial system and can be considered analogous to a large lymph node, as its absence leads to a predisposition toward certain infections [Jia and Pamer, 2009].

The spleen is a highly organized secondary lymphoid organ that plays a central role in the primary defense against all types of antigens that appear in the circulation, and is a major site of antibody production [Bohnsack and Brown, 1986; Koch et al. 1982]. Absence of the spleen causes an increased susceptibility to systemic infections by encapsulated bacteria [Hansen and Singer, 2001].

1.4.3. Thymus

The word *thymus* comes from the Latin derivation of the Greek word *thymos*, meaning "warty excrescence". Galen of Pergamum (130–200 ad), who first noted that thymus was proportionally large during infancy [May, 1968], referred thymus as an "organ of mystery".

1.4.3.1. Function of the Thymus

In ancient times, thymus was believed to be the seat of the soul or the organ of purification of the nervous system [Jacobs *et al.,* 1999; Haubrich, 1997; Skinner, 1961; May, 1968]. Thymus is one of the central lymphoid organs and plays an important role in cellular immunity by generating circulating T lymphocytes. Although the need for the thymus to generate a continuous supply of T cells decreases with advancing age, thymus continues to serve as the site of T-cell differentiation and maturation throughout life [Shimosato and Mukai, 1997; Hong, 2001]. One of the major functions of the thymus, the maturation of thymocytes, has been studied extensively with molecular and cellular biology. Various inductive, hormonal, and proliferative signals from epithelial cells contribute to maturation of thymocytes [Shimosato and Mukai, 1997]. T-cell antigen receptors of thymocytes interact with epithelial major histocompatibility complex antigens in the process of thymocyte maturation [Shimosato and Mukai, 1997].

1.5. Role of fatty acids in immune cells

Fatty acids fulfill a variety of roles within immune cells and act as:

- fuels for generation of energy.
- components of cell membrane phospholipids contribute to the physical and functional properties of the membranes.

- covalent modifiers of protein structure influencing the cellular location and function of protein regulators of gene expression either through effects on receptor activity/intracellular signaling processes/ transcription factor activation.
- precursors for synthesis of bioactive lipid mediators like prostaglandins(PGs), leukotrienes(LTs), lipoxins and resolvins.

Changes in membrane phospholipid fatty acid composition might influence immune cell function in a variety of ways. The range of possibilities includes:

- alterations in the physical properties of the membrane such as membrane order (‘‘fluidity’’) and raft structure.
- effects on cell signalling pathways, either through modifying the expression, activity or avidity of membrane receptors or modifying intracellular signal transduction mechanisms. As a result of these effects, transcription factor activation is altered and gene expression modified.
- alterations in the pattern of lipid mediators produced. The different mediators have different biological activities and potencies.

It is generally accepted that biomembrane composition may be altered because of nutritional, environmental or xenobiotic exposure. Fatty acyl distributions appear to be most easily affected [Yorio and Frazier, 1990]. The FA species composition has a genetic component as evidenced by organ and tissue specific molecular species patterns [Ordway *et al.* 1991]. Biological tissues seem to have a degree of freedom within which a oscillation in molecular species composition is allowed. Beyond certain threshold values, the organ may be incapable to function in an optimal way. It has been reported that SFA and UFA obviously affect lymphocyte function both in *in vivo* and *in vitro* conditions [Meade and Mertin, 1978; Gurr, 1983; Erickson, 1986].

1.6. PL metabolism on spleen and thymus

At present, little is known about PL metabolism during endotoxemic condition in spleen and thymus. The magnitude of alterations of the cellular levels of certain PLs during mitogenic stimulation of lymphocytes has been highlighted by some authors [Maccecchini and Burger, 1977; Shier et al. 1976; Sugiura and Waku, 1984; Chaby et al. 1986; Shires et al. 1989; Brunswick et al. 1990; Tamir and Isakov, 1994]. The turnover of PC has been reported to increase instantly after the stimulation with lectins, a mitogen resulting in transient accumulation of LPC [Shier *et al.* 1976]. Mitogenic stimulation of lymphocytes is related with a decrease in the proportions of palmitic, stearic, linoleic and arachidonic acids and an increase in the proportion of oleic acid [Calder *et al.* 1994]. Conjugated linoleic acid (CLA) has also been reported to modulate the immune response, modify cytokine production and to increase lymphocyte proliferation [Hayek *et al.* 1999; Turek *et al.* 1998; Yang and Cook, 2003; Chew *et al.* 1997] in *in vitro* animal studies. Saturated fatty acids (SFA) and unsaturated fatty acids (UFA) markedly affect lymphocyte function both *in vivo* and *in vitro* [Meade and Mertin, 1978; Gurr, 1983; Erickson, 1986]. Polyunsaturated fatty acids (PUFA) are converted to various eicosanoids having powerful immunopharmacological properties [Lee and Austen, 1986; Smith *et al.* 1985]. Studies carried out in *in vivo* [Erickson, 1986; Erickson *et al.* 1980; Bennett *et al.* 1987] and *in vitro* [Buttke, 1984; Pourbohloul *et al.* 1985] depict that fatty acids have a greater effect on T cells and cell-mediated immunity than on B cells and humoral immunity.

The airway epithelium is the first site of contact for inhaled environmental stimuli, functions as a physical barrier to environmental insult, and is an essential part of innate immunity. Epithelial barrier disruption is caused by inhaled allergens, dust, and irritants, resulting in inflammation, bronchoconstriction and edema as seen in

asthma and other respiratory diseases [Budinger and Sznajder, 2006; Knight and Holgate, 2003; Matthay *et al.,* 2005; Zhao *et al.,* 2009]. Further, increased epithelial permeability also results in para-cellular leakage of large proteins, such as albumin, immunoglobulin G and polymeric immunoglobulin A, into the airway lumen [Gourgoulianis *et al.,* 1999; Hulsmann *et al.,* 1994].

LPS, which possesses powerful biological activities, can enter the bloodstream and elicit inflammatory responses that may lead to shock and ultimately death [Glauser *et al.,* 1991]. Lung is the most frequently involved organ, and ARDS induced by LPS is the major reason of death [Fenton and Golenbock, 1998]. Because of its grave clinical and economical consequences, ARDS has been the subject of much investigation in a variety of species. One widely used murine model of ARDS, is produced by the intraperitoneal administration of bacterial LPS [Kabir *et al.,* 2002]. Hence, we used Albino rats of Wistar strains to produce ALI/ARDS. The induction was confirmed by hall mark of ARDS such as pulmonary edema, leukocyte and neutrophil infiltration in lung tissue and profound lung inflammation and vascular permeability damage. Our present study shows significant impairment of phospholipids in lung, spleen and thymus during ARDS condition and it was corroborated by both *in vitro* and *in vivo* studies.

Aim and scope of the study

ARDS is mainly caused by dysfunctioning of surfactant phospholipids. LPS is a potent compound to produce ARDS. The vital process of mammalian breathing is dependent on an extensive gas exchange surface provided by the alveoli in the lung periphery. Surface tension on the epithelial side of the air-blood barrier exerts a pressure that is stabilized by spreading of a lipid-rich film (pulmonary surfactant) at the alveolar air-liquid interface. A change in surfactant composition leads to alveolar collapse. Thus the integrated regulation of surfactant synthesis, secretion and metabolism is essential for breathing and ultimately survival. To understand the pathogenic mechanism, alteration of phospholipid metabolism in pulmonary system is well studied. Previous reports have shown more about PC, especially DPPC but regulation of phospholipid during endotoxemia is yet to be elucidated. The immunological effect of LPS is well documented in spleen and thymus; however impact on lipids particularly phospholipid metabolism is yet to be studied. Hence understanding the role of phospholipid during endotoxemia may provide a better understanding between the relationship of phospholipids, their molecular species changes and immune impairment.

To study "**the impact LPS on phosphoglyceride metabolism**", we sought to address this by both in vitro and in vivo experiments with the following objectives:

- Initially developing respiratory distress syndrome in adult rats.
- Studying the impact of ARDS on lung, spleen and thymus phosphoglyceride metabolism and its molecular species composition.
- Studying the relationship between phosphoglyceride, their fatty acids changes and immune impairment.
- Elucidating the mRNA expression of PL remodeling enzymes during ARDS.

Understanding the mechanisms governing the PL alteration in the lung, spleen and thymus during ALI/ARDS may reveal versatile treatment options that could have a beneficial impact on multiple lung disorders.

Synopsis

Lipopolysaccharide (LPS) is a potent stimulator of immune response and acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). Despite advances made in understanding the pulmonary biology, the mortality associated with ALI/ARDS is still ~ 40%. Impact of LPS on lipid regulation and lipid remodeling enzymes during ARDS is remains enigmatic. Hence we were trying to understand the role of lung lipid metabolism during endotoxemia by both *in vitro* and *in vivo* experiments. Lung tissue slices and alveolar epithelial type II cells (AEC2) were exposed to different concentration of LPS (0-100 µg). *In vitro* metabolic labelling reveals significant alteration in major pulmonary surfactant, phosphatidylcholine (PC) and phosphatidylglycerol (PG). To further understand the PL metabolism we have developed an animal model for ALI/ARDS. Rats selected from colonies were randomized into 4 groups, comprising of six rats each and were overnight fasted. Group I, Control animals (24 h saline treated); Group II, III and IV were given LPS (10 mg/kg body wt.) intraperitoneally and sacrificed after 6, 12 and 24 h respectively under ketamine anaesthesia (75 mg/kg). The induction of ARDS was confirmed by neutrophil sequestration, vascular leakage and multiple organ failure (hallmark of ARDS). The antioxidants deficiency also attenuated the inflammation and tissue damage. LPS impairs lung phospholipid metabolism. The FA distribution in individual PL showed an overall increase in UFA content. This may be due to the sensitive response of the organs upon LPS treatment. The changes in the FA may directly or indirectly affect the membrane structure, fluidity. The mRNA expression of PC, and PG remodeling enzymes showed the reduction in PC was not due to PLD and may be due to the hydrolysis of PC-specific phospholipase C/defect in PC synthesizing enzyme during LPS endotoxemia. The reduction in PG was due to $sPLA_2$-IIA.

Key words: ARDS, LPS, pulmonary surfactant phospholipid, PLD, $sPLA_2$-IIA