

RESEARCH ARTICLE

Acute lung injury in preterm fetuses and neonates: mechanisms and molecular pathways

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Abstract

Acute lung injury (ALI) results in high morbidity and mortality among preterm neonates and efforts have therefore been devoted to both antenatal and postnatal prevention of the disease. ALI is the result of an inflammatory response which is triggered by a variety of different mechanisms. It mostly affects the fetal lung and, in particular, causes damage to the integrity of the lung's alveolar-capillary unit while weakening its cellular linings. Chemotactic activity and inflammatory products, such as proinflammatory cytokines TNF- α , IL-1, IL-6, IL-11, VEGF, TGF- α and TGF- β , provoke serious damage to the capillary endothelium and the alveolar epithelium, resulting in hyaline membrane formation and leakage of protein-rich edema fluid into the alveoli. Chorioamnionitis plays a major part in triggering fetal lung inflammation, while mechanical ventilation, the application of which is frequently necessary in preterm neonates, also causes ALI by inducing proinflammatory cytokines. Many different ventilation-strategies have been developed in order to reduce potential lung injury. Furthermore, tissue injury may occur as a result of injurious oxygen by-products (Reactive Oxygen Species, ROS), secondary to hyperoxia. Knowledge of the inflammatory pathways that connect intra-amniotic inflammation and ALI can lead to the formulation of novel interventional procedures. Future research should concentrate on the pathophysiology of ALI in preterm neonates and on possible pharmaceutical interventions targeting prevention and/or resolution of ALI.

Introduction

The current most widely accepted definition of Acute Lung Injury (ALI) is acute onset of bilateral infiltrates, as detected via chest radiograph, with no evidence of left atrial hypertension and with a partial pressure of oxygen (PaO₂) fraction of inspired (FiO₂) ratio estimated at less than 300. A life-threatening disorder, ALI is characterized by severe lung inflammation as well as often by the coincidence of multiple organ failure [1].

In pediatric patients ALI carries an especially high mortality rate (22%) by comparison with overall mortality of pediatric patients in intensive care units, even though the total mortality rate for pediatric patients with ALI is considerably lower than that as reported in adults (22% versus 35–45%) [1–3]. The better pediatric prognosis as

Keywords

Acute lung injury, inflammation, preterm neonates, reactive nitrogen species, reactive oxygen species

History

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compared to that of adult patients clearly demonstrates the respective difference in the pathogenesis of ALI as well as the impact of inflammation on the developing lung.

As is well known, the number of surviving preterm neonates is nowadays increasing [2]. It is meanwhile also recognized that respiratory distress syndrome (RDS) is the prototypical lung condition of preterm newborns. Although the use of antenatal gluco-corticosteroids and more gentle ventilation techniques in neonates with RDS has significantly reduced the incidence of severe bronchopulmonary dysplasia (BPD) [4], nevertheless, some immature neonates who have minimal or no signs of RDS develop within the first 2 weeks of life a “new” BPD [5]. Almost 60% of the neonates manifesting new BPD have been exposed to chorioamnionitis and thus develop an inflammatory response prior to delivery [6]. This inflammatory response can be either systematic or local in the fetal lung. Increasing insight into the inflammatory cells, cytokines and apoptotic cells which take part in this procedure will play a key role in our understanding of the sequence of events that lead from chorioamnionitis to lung injury [7].

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Furthermore, it has been established that high concentrations of oxygen are capable of inflicting significant ALI in the developing lung, this being secondary to hyperoxia and also being attended by an inflammatory response [8]. Because ALI results in high morbidity and mortality in preterm neonates, medical care primarily consists in utilization of respiratory therapies aimed at minimizing further lung injury, while efforts are additionally being devoted to antenatal and postnatal prevention of the disease.

The purpose of this article is to report on and evaluate the recent bibliography dealing with antenatal-neonatal ALI, while discussing the mechanisms of ALI in preterm neonates, the molecular pathways that are common in inflammation as well as approaches to lung injury and maturation.

Normal lung growth

Lung development starts at 3 weeks of embryonic life and is traditionally divided into five stages (Figure 1). First is the Embryonic stage (0–7 weeks) at which point abnormalities are often incompatible with life or may lead to significant respiratory morbidity. There then follows the Pseudoglandular stage (8–17 weeks) during which the failure of normal lung growth may result in malformations such as cystic adenomatoid malformation, pulmonary sequestration and lung hypoplasia or lung cysts. Next comes the Canalicular stage (18–27 weeks): a possible premature delivery at this stage causes inevitable RDS. Abnormalities during the Saccular stage (28–36 weeks) can lead to pulmonary hypoplasia, acinar dysplasia and RDS. Finally, there is the Alveolar stage (37 weeks–2 years), during which formation of the secondary septa in the

terminal airway take place. It is during this stage that cup-shaped alveoli are formed and the alveolar walls undergo thinning and remodeling from a double to a single capillary loop [9]. This process continues until the age of 2 years and results in increase of the gas exchange surface and in maturation of the lung cells.

Pathophysiology

ALI is the result of an inflammatory response which is triggered by a variety of different mechanisms (Figure 2). It mostly affects the fetal lung and especially the integrity and function of the cellular linings of the alveolar-capillary unit. Studies have also demonstrated increased rates of neurological and gastrointestinal morbidity in neonates afflicted with this disorder [10,11]. The imbalance between pro- and anti-inflammatory cytokines determines the degree of inflammation in the fetal lung. Inflammatory cytokines, activated alveolar macrophages, erythrocytes, neutrophils and platelet activating factor have a major role in the lung alveolar inflammatory response. The apoptosis of neutrophils, which in this condition is inappropriately suppressed, has been described as a prolonged survival of neonatal neutrophils [12–14]. The chemotactic activity and inflammatory products cause serious damage to the capillary endothelium and the alveolar epithelium, resulting in hyaline membrane formation and leakage of protein-rich edema fluid into the alveoli. Surfactant function is inactivated by plasma protein leakage and the damage of type II pneumocytes causes further decrease in the surfactant production [15]. The final result is airspace collapse and ventilator-perfusion disturbance.

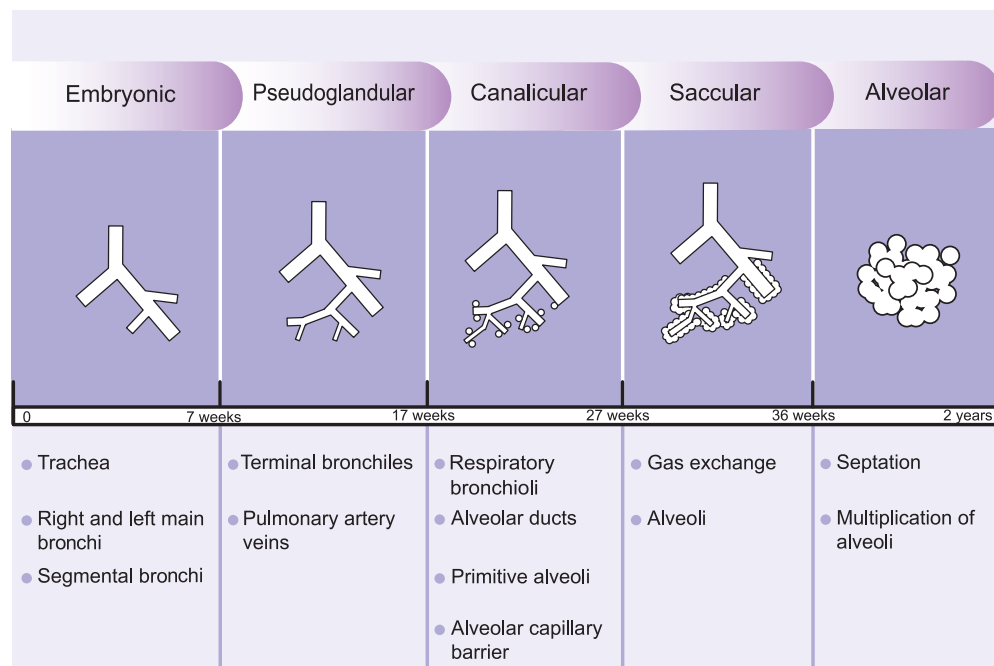


Figure 1. Stages of normal human lung development: During the first stage (0–7 weeks, embryonic stage), the lung arises as a ventral diverticulum of the primitive foregut endoderm with the lobar and segmental bronchi appearing at the 5th week and arteries and veins developing as avascular buds. There then follows the pseudoglandular stage (8–17 weeks) when branching of the airways and vessels takes place. Next is the canalicular stage (18–27 weeks) during which the differentiation in type II pneumocytes takes place and an alveolar-capillary barrier is developed. Subsequent stages are the saccular stage (28–36 weeks), which is characterized by enlargement of the peripheral airways with dilatation of the acinar tubules and thinning of the airway walls, and the alveolar stage (37 weeks–2 years) during which shaved alveoli are formed and the alveolar walls are thinned and remodeled from a double to a single capillary loop.

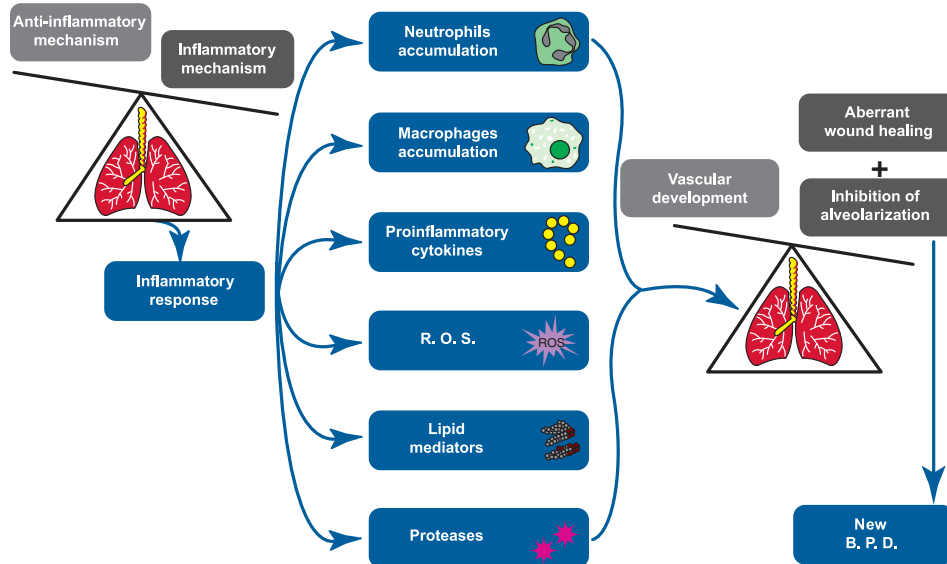


Figure 2. Pathophysiology of ALI: The imbalance between the inflammatory and anti-inflammatory mechanisms activates an inflammatory response in the fetal lung. This response activates proteases and proinflammatory cytokines and also causes accumulation of neutrophils and tissue macrophages and production of ROS, resulting in better vascular development in the fetal lung. On the other hand, alveolarization is inhibited and the lung trauma is not healed, leading to the “new” BPD and severe respiratory morbidity.

The lung injury develops heterogeneously and may affect different independent lung areas.

Crucial for the neonate's prognosis is the clearance of pulmonary edema fluid and transcapillary water transport [1]. There is a critical balance between inflammation, coagulation, apoptosis and restoration of water transport, re-establishment of which is anticipated to lead to resolution of the lung injury and production of surfactant.

Chorioamnionitis

Chorioamnionitis plays a major part in triggering preterm labor [16,17]. Infection/inflammation of the uterus is initiated by bacteria most often ascending from the upper vagina [18]. Inflammation involving the fetus is frequently caused by factors released antenatally which especially target the fetal lung. In fetuses with inflammation a higher incidence of sepsis has also been found. Although fetuses exposed to chorioamnionitis exhibit a decreased incidence of RDS, they are at heightened risk of developing Chronic Lung Disease (CLD) on account of the elevated levels of pulmonary inflammatory markers (Figure 3) [19]. Chorioamnionitis induces lung maturation but also causes BPD by decreased alveolar septation and decreased microvascular development with limited fibrosis [20]. Lung injury can occur after exposure to elevated proinflammatory cytokines in amniotic fluid or as a systematic inflammatory response [21,22]. The association of intrauterine infection with neonatal morbidity has recently been reviewed [16,23].

Ventilator-induced lung injury

During the last three decades, mechanical ventilation has become an indispensable therapeutic modality for the treatment of respiratory failure. On the other hand, mechanical ventilation can injure the lung either directly or by inducing

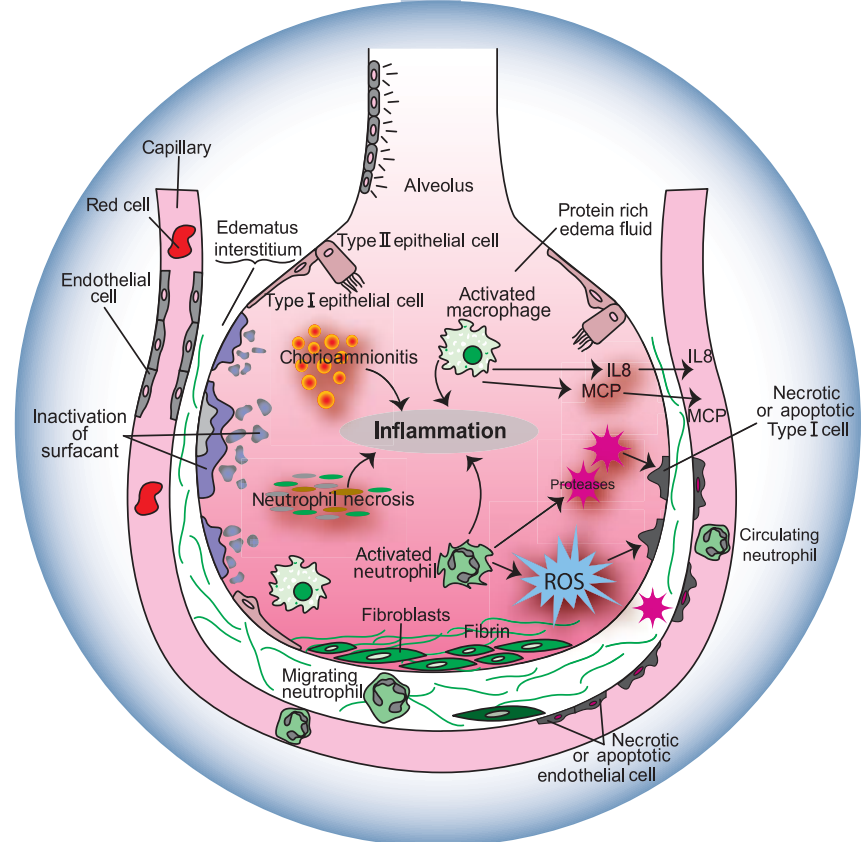
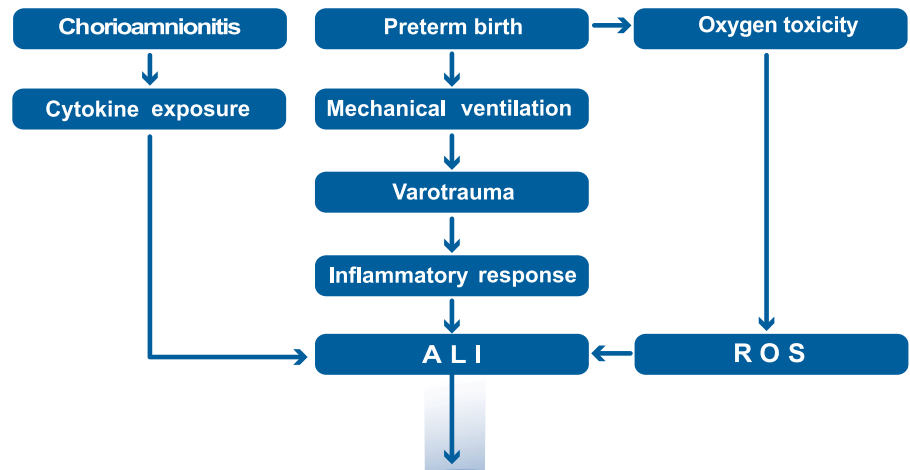
an inflammatory response that then injures the lung [24–26]. In addition, the use of mechanical ventilation to establish functional residual capacity (FRC) can increase endothelial and epithelial permeability, causing severe tissue damage [27,28].

After birth the potential gas volumes are small, surfactant may be deficient, the lung matrix is not fully developed and the airspaces contain residual lung fluid. The use of high tidal volumes to expand alveoli can cause volutrauma, while ventilation in surfactant deficient lungs with collapsed alveoli causes atelectrauma. These lead to lung inflammation causing tissue injury locally and possibly systematically [29] and, critically, lung inflammation due to ventilation potentially leads to long-term respiratory morbidity. Ventilation of preterm lungs causes pulmonary inflammation resulting in leukocyte infiltration and the production of inflammatory mediators [30]. The highest levels of inflammatory cytokine are seen in ventilator strategies with pressure and no positive end expiratory pressure (PEEP) [31]. Inhaled nitric oxide (NO) has been shown to play a major role in lung growth and development as a selective pulmonary vasodilator. It is also thought to exert anti-inflammatory action due to the effect on vessels' permeability and neutrophil trafficking [32].

Injurious products of oxygen (reactive oxygen species, ROS)

ALI secondary to hyperoxia is characterized by an inflammatory response bringing about destruction of the alveolar-capillary barrier, followed by cell death [33]. Tissue injury occurs as a result of injurious products of oxygen (ROS) [34]. Focal endothelial cell injury is caused through toxic concentrations of ROS, while long-lasting exposure provokes necrosis of epithelial cells, as shown in Figure 4 [8,35]. Cell death brings about disruption of the alveolar-capillary membrane that leads to flooding of the alveoli, provoking

Figure 3. Mechanisms of injury in preterm neonates: Chorioamnionitis is the prime factor that triggers events contributing to the pathogenesis of ALI during prenatal and postnatal development. It can cause prenatal exposure to inflammatory cytokines either in utero or in preterm birth. Preterm infants very often need mechanical ventilation, which can lead to varotrauma and further inflammatory response in the alveoli. Furthermore, the oxygen toxicity results in excessive production of reactive oxygen species (ROS), thus triggering an inflammatory response in the premature lung.



significant perturbations in pulmonary mechanics and impairment of gas exchange [36].

Preterm infants are often exposed to high concentrations of oxygen as part of their respiratory support and are thus at increased risk of oxidative stress as their anti-oxidant defenses are as yet immature. Moreover, in preterm neonates intracellular defenses are not completely developed with changes occurring late into pregnancy, while, additionally, vitamin C levels decline during the first few days of life [37].

Molecular and cellular pathways

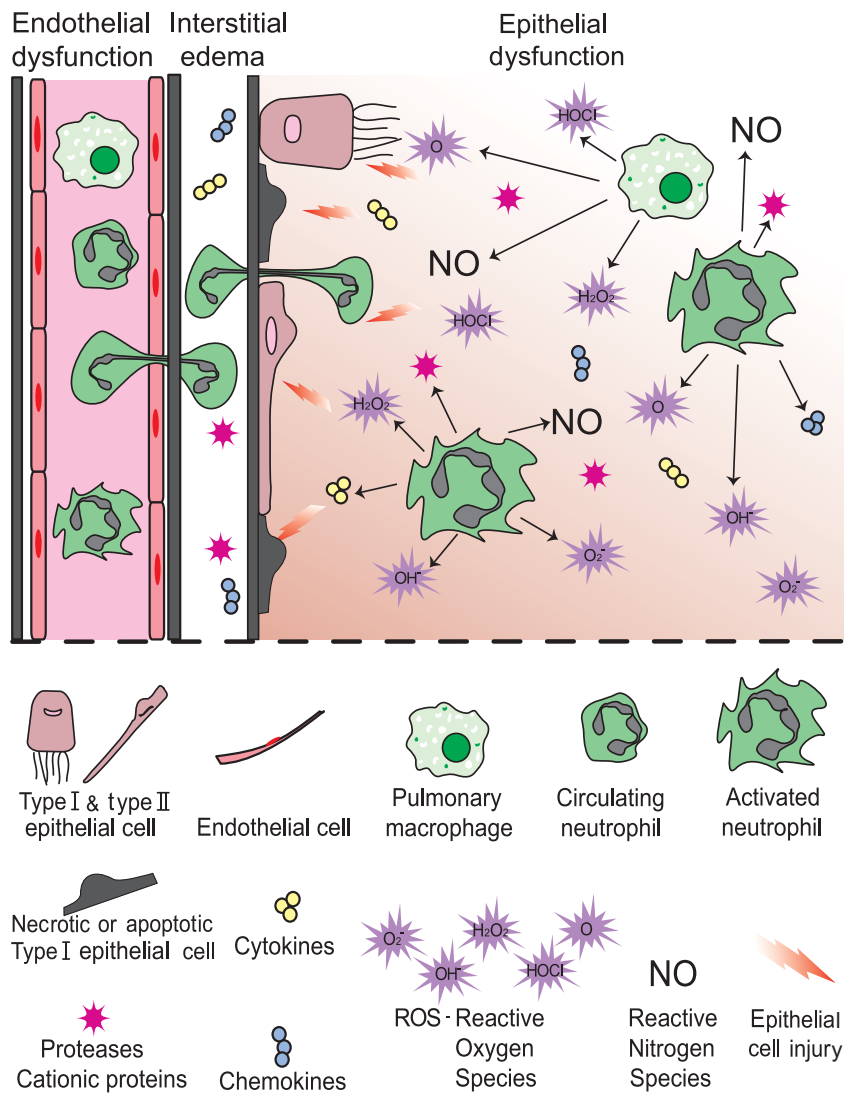
Over the last few years there have been an increasing number of studies on the molecular mechanisms of ALI. Animal models that focus on understanding the cellular mediators of

the disease are also widely used, these studies promising valuable insights that could substantially aid future medical intervention.

Chorioamnionitis

Inflammation in the lung involves the expression of acute phase cytokines, this leading to neonatal lung injury. However, the mediators that interfere with lung development are not as yet well known, the only available information being from transgenic animal models. Such proinflammatory cytokines as TNF- α , IL-1 and IL-6 are crucial mediators in the early stages of inflammatory response: they are synthesized by fibroblasts, type II pneumocytes, alveolar macrophages, and endothelial cells stimulated by inflammatory factors [38].

Figure 4. Hyperoxia-induced acute lung injury: hyperoxia in the fetal lung causes a major production of reactive oxygen species (ROS) and Reactive Nitrogen Species. Circulating neutrophils and pulmonary macrophages are activated and the produced superoxide anion and nitric oxide destroy epithelial cells. The high concentration of proteases, chemokines and cytokines destroys the alveolar-capillary barrier and leads to flooding of the alveoli and impairment of gas exchange. Continuing exposure without regression can cause tissue necrosis and chronic respiratory dysfunction.



Neutrophils and the monocyte chemoattractant protein (MCP) are increased in bronchoalveolar lavages of fetal lung of neonates exposed to chorioamnionitis. This reflects an injury response as a result of an epithelial permeability defect, and apoptosis and its resolution are accompanied by increased cell proliferation. It is also reported that ventilated preterm neonates exposed to chorioamnionitis had less RDS, while high cord plasma levels of IL-6 predicted less RDS. It has furthermore been shown that fetal exposure to inflammation conduces to the low prevalence of RDS among very preterm neonates [39]. TNF- α , TGF- β , IL-11 and IL-6 are also inflammatory products that are over-expressed in the lungs of developing mice impair alveolarization [40]. With regard to elevated levels of IL-1 β , IL-8, TNF and other promoters of inflammation in the airways of neonates soon after birth, these markers of inflammation typically persist, putting the infants at risk of subsequently developing BPD. Also of note, IL-10, an anti-inflammatory cytokine, was found to be untraceable in airways samples of preterms, this additionally corroborating the finding that these patients are frequently predisposed to persistent chronic inflammation [41].

A correlation has additionally been demonstrated between the elevated IL-6 in cord plasma with the subsequent development of BPD in preterm neonates [42]. The elevated

levels of IL-6, IL-1 β , IL-8 and TNF- α in amniotic fluid in preterm births also predicted the subsequent development of BPD [22]. A recent study showed that the IL-6 and IL-8 levels of plasma and bronchoalveolar lavage were strongly associated with increased 28-day mortality and decreased ventilator-free days [43]. Furthermore, it was reported that inflammation of the cord by white blood cells predicted BPD in neonates [44] and raised levels of amniotic fluid TNF- α , this moreover correlated with an increased incidence of RDS [45]. In another study, histologic chorioamnionitis was associated with BPD and the neonates had elevated cytokine levels in tracheal aspirates [46].

In preterm neonates exposed to chorioamnionitis who developed BPD, increased concentration of intercellular adhesion molecules (ICAM-1) has been detected in the airway secretions [47,48]. Endotoxin induced chorioamnionitis was also studied in fetal sheep lung. Cytokines that inhibit vascular development, such as interferon- γ -inducible protein (IP-10) and TGF- β increased, while endothelial NO synthetase decreased in the small vessels of the fetal lung [49–51]. Moreover, VEGF-mRNA, VEGF receptor-2 and PECAM-1 decreased and there was smooth muscle hypertrophy of the distal pulmonary arteriole. The number of alveoli decreased and alveolar size increased, indicating diminished septation

and alveolar simplification [52]. It is also known that neonates from mothers with chorioamnionitis had lower VEGF-C in tracheal aspirate fluid [53]. Moreover, indomethacin decreases VEGF-C concentrations, possibly through inhibition of Cox-2 [54,55]. Preterm neonates have a clear decrease in postpartum levels of VEGF-C and, as this induces lymphangiogenesis of the lung, it affects normal lung development. Impaired fibrinolysis was also shown to play also a major role the pathogenesis of ALI, as in a human study the Plasminogen activator inhibitor-1 (PAI-1) was found significantly higher in the cases with decreased ventilator-free days [56].

Ventilator-induced lung injury

Mechanical ventilation is unavoidable in a number of cases of preterm neonates in order to sustain life, even though it is liable to cause ALI by inducing proinflammatory cytokines. Different strategies of mechanical ventilation have been proposed with the aim of minimizing ventilator induced lung injury. Animal model studies have demonstrated that PEEP (positive end-expiratory pressure) diminishes edema formation and cell damage [29] while also lessening inflammatory cell recruitment during protracted ventilation [57]. High frequency oscillatory ventilation, a technique designed to prevent wide alterations in pressure and volume during ventilation, also decreases inflammation [58]. On the other hand, high tidal volumes without PEEP resulted in increased cytokine concentrations. Continuous positive airway pressure (CPAP) was also shown to induce inflammatory changes after application of intravenous endotoxin (LPS) in rat models [59]. In a randomized clinical trial, it was demonstrated that high-frequency oscillatory ventilation (HFOV) with an optimum volume strategy reduced serum levels of proinflammatory cytokines [60].

The effect of ventilation strategy on inflammatory mediators is studied in animal models. Tremblay et al found that zero PEEP and high volume ventilation resulted in a 56-fold increase of TNF- α lung lavage levels versus controls. Identical end inspiratory lung distention with PEEP resulted in only a 3-fold increase in TNF- α , whereas in the absence of positive end respiratory pressure, TNF- α was increased 6-fold [31]. In a sheep model the use of PEEP right after birth decreased the inflammatory mediators that initiate the fetal lung injury [61].

Lung injury stimulates Transforming Growth Factor-beta (TGF-beta), this tending to partially limit the inflammatory reaction, while it also plays a critical part in tissue remodeling and repair [62,63]. If the injury is not localized, this leads to increased levels of TGF- β and its receptors, resulting in severe pulmonary fibrosis [64]. Moreover, ventilator induced lung injury in preterm lambs resulted in increased protein and activated granulocytes in the alveolar fluid and in large increases in IL-1 β and IL-6 mRNA and more modest increases of IL-8 and TNF- α mRNA [65,66]. Studies carried out in premature baboons ventilated with HFOV demonstrated that the secretion of platelet activating factor, which is a potent chemotactic [67] and neutrophil activating mediator [68] was decreased by comparison with those animals that were ventilated with intermittent positive-pressure ventilation (IPPV) [69].

Injurious products of oxygen secondary to hyperoxia

Activation of key caspases and mitochondrial dysfunction underlie the molecular mechanisms of hyperoxia-induced lung injury and cell death. IL-1 α mRNA was increased in the lungs of newborn mice after hyperoxia [33] and IL-1 β mRNA was increased 5-fold [70]. Acute alveolitis and slight edema were detected and increased expression of IL-1 α preceded the neutrophil influx, possibly indicating the involvement of these in the inflammatory response. IL-6 is synthesized by almost all nucleated cells and acts as an autocrine, paracrine and exocrine inflammatory hormone [71]. In animal studies, IL-6 was increased in tracheal aspirate after hyperoxia exposure [70,72,73], while dexamethasone treatment reduced these levels [74]. IL-8 was also increased, about 10-fold, in newborn rats exposed to hyperoxia [75] and IL-8 seems to have a role in the influx of neutrophils in response to hyperoxia [76] though the influence of infections cannot be excluded [34]. On the other hand, mice [70] and baboons [72] exposed to hyperoxia did not demonstrate any changes in lung mRNA of IL-10 at any time point.

VEGF, which possesses a high level of expression in normal tissues in the lung [71], is apparently essential for the proper development of alveolar tissue. Exposure to hyperoxia leads to a biphasic VEGF response, with VEGF release initially being increased, this likely causing lung injury through its triggering of vascular permeability alterations [77]. A decrease in VEGF then follows, allowing for angiogenesis and alveolarization and, thereby, enabling recovery [78]. Finally, TNF- α was increased in the lungs of neonatal rats [79] and mice [70] exposed to hyperoxia, while TGF- β is upregulated on exposure to hyperoxia in neonatal rats [80] and mice [81,82] TGF- β modulates the differentiation, proliferation and migration of cells that contribute to normal pulmonary assembly and maturation. Hyperoxia during the final stage of lung development increased TGF- β , decreased alveologenesis and disrupted vasculogenesis in the preterm lung [82]. In mice models, improvement of pulmonary alveogenesis and vasculogenesis was achieved after treatment with TGF- β antibodies. Furthermore, in mouse models, exposure to anti-TGF- β antibodies improved somatic growth without any progression of lung inflammation [82]. In rats exposed to hyperoxia, treatment with antibodies against neutrophils chemokines resulted in normal alveolar development.

Conclusion

This review has focused on recent developments and findings in the ALI field specifically related to embryos and neonates, with special emphasis given to the immunological and coagulatory results obtained within the last few years. Modern ventilation techniques do not as yet have a major impact on decreasing CLD, even though they demonstrate theoretical advantages, as compared to current ventilator techniques, in decreasing ALI.

Neonates with ALI that received exogenous surfactant (calfactant) had decreased mortality and more rapid improvement in oxygenation index. In preterm neonates who need mechanical ventilation, steroids are widely used [83]. The anti-inflammatory activity of steroids improves lung function

and aids extubation from mechanical ventilation. On the other hand, steroids have adverse effects on neuro-development, while still unresolved is the optimal type, dose, duration and timing of postnatal use. Furthermore, though the antenatal use of antibiotics aims at reducing the chorioamnionitis-induced inflammation, this procedure demonstrates marginal benefits, while also to be taken into consideration is the possibility of worsening the neuro-developmental outcome. Given that IL-1, IL-6, IL-8, TGF- β , VEGF and TNF- α appear to play a crucial role in the sequence of inflammation, further experimental and clinical studies on these are necessary to establish new therapies capable of improving the perinatal outcome. Therefore, further research is essential to find that ventilation strategy that will strike the optimal balance between management of the inflammatory response and the need to sustain life. Cell transplantation is a novel strategy in the treatment of lung diseases; although it has not yet been established as an entirely safe intervention [84], it shows promise as a future option in the treatment of ALI. Additionally, an important challenge for future research is the formulation of a targeted therapeutic approach that inhibits lung inflammation, while treatment with antibodies offers promising potential for prevention and/or resolution of ALI. In a recent human study it was demonstrated that a conservative fluid management strategy improves the clinical outcome by increasing the ventilator-free days in children with ALI [85]. Apoptosis also plays a major role in lung injury resolution, as extensive apoptosis of type II pneumocytes is mainly responsible for the disappearance of these cells. However, further investigation is needed to gain deeper insight into the mechanisms of apoptosis in ALI before establishing clinical applications.

In summary, future research should concentrate on the pathophysiology of ALI in preterm neonates as well as on possible pharmaceutical interventions targeted at prevention or resolution of ALI.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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