

# Chorioamnionitis, Postnatal Factors and Proinflammatory Response in the Pathogenetic Sequence of Bronchopulmonary Dysplasia

Christian P. Speer

University Children's Hospital, Würzburg, Germany

## Key Words

Preterm infants · Bronchopulmonary dysplasia · Respiratory distress syndrome · Pathogenesis · Inflammation

## Abstract

Pulmonary inflammation has a central role in the multifactorial and complex pathogenesis of bronchopulmonary dysplasia. Pre- and postnatal factors such as chorioamnionitis, oxygen toxicity, mechanical ventilation and postnatal infections can induce and perpetuate an injurious and complex inflammatory response in the airways and lung tissue of very immature infants. This inflammatory process is characterized by the accumulation of inflammatory cells and an arsenal of proinflammatory mediators such as cytokines, toxic oxygen radicals, lipid mediators and potent proteases. An imbalance between pro- and anti-inflammatory factors favoring the proinflammatory process can be considered as a hallmark of lung injury. In addition, impaired mechanisms of tissue remodeling and repair together with a subnormal generation of growth factors could affect alveolarization and vascular development with lifelong consequences for the infants with bronchopulmonary dysplasia.

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## Introduction

Very immature infants who initially have minimal or absent signs of respiratory distress syndrome (RDS) may subsequently develop oxygen dependency and ventilatory needs within the first 10 days of life and remain oxygen-dependent for weeks and months [1]. More than half of these very immature infants with so-called 'new' bronchopulmonary dysplasia (BPD) may have been exposed to chorioamnionitis and a considerable number of them are born with inflamed lungs and signs of fetal inflammatory response. In addition, various postnatal factors such as resuscitation, high airway concentrations of inspired oxygen, mechanical ventilation, pulmonary as well as systemic infections and persistent ductus arteriosus may perpetuate or even amplify an injurious inflammatory response in the airways and interstitium which may subsequently affect normal alveolarization and pulmonary vascular development. The etiology of BPD is certainly multifactorial and the multiple-hit theory offers a plausible concept which helps to explain the complex pathogenetic mechanisms involved in this chronic lung disease of very immature preterm infants [2, 3]. This paper expands on previously published articles and summarizes, in a condensed form, the current pathogenetic concepts of the possible role of inflammation in the evolution of BPD [4–7].

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Prof. Christian P. Speer, MD, FRCPE  
University Children's Hospital, University of Würzburg, Josef-Schneider-Strasse 2  
DE-97080 Würzburg (Germany)  
Tel. +49 931 201 27831, Fax +49 931 201 27833  
E-Mail [speer\\_c@kinderklinik.uni-wuerzburg.de](mailto:speer_c@kinderklinik.uni-wuerzburg.de)

## Pre- and Postnatal Factors Inducing Pulmonary Inflammation

### *Chorioamnionitis*

Epidemiological data suggest a strong association between chorioamnionitis, funisitis and the development of BPD, and increased concentrations of proinflammatory cytokines in human amniotic fluid and fetal cord blood, indicating a systemic inflammatory response during chorioamnionitis, are independent risk factors of BPD [1, 5, 7]. A pronounced infiltration of inflammatory cells, an increased expression of cytokines and markers of endothelial activation as well as a large number of apoptotic airway cells have been observed in lung tissues of human fetuses with funisitis that have been exposed to chorioamnionitis [8–10]. In addition, the presence of proteomic biomarkers characteristic of inflammation in the amniotic fluid was associated with an increased fetal inflammatory response at birth [11]. In an animal model, maternal exposure to endotoxin resulted in a prolonged pulmonary inflammatory response, altered gene expression and delayed maturation of the lung [12]. Congenital infections, which induce pulmonary or systemic fetal inflammation, seem to be a more frequent event than previously realized [13].

### *Infection*

Early-onset sepsis and systemic nosocomial infections have clearly been identified as individual risk factors for BPD [1, 5, 7, 14–17]. Besides direct adverse effects of microorganisms, a variety of inflammatory cells and mediators may affect the integrity of endothelial and bronchoalveolar cells. In addition, hemodynamic changes in the vascular bed associated with persistent ductus arteriosus seem to play an essential role in the development of BPD [18]. Vasoactive prostaglandin mediators released during septicemia probably prevent ductal closure or induce reopening of the duct [19]. The potential role of *Ureaplasma urealyticum* (Uu) in the evolution of BPD is still controversial. Uu is the microorganism most frequently isolated from the amniotic fluid in preterm births and a predominant pathogen detected in airway secretions immediately after birth [15, 20]. In the Alabama preterm birth study, Uu and *Mycoplasma hominis* were detected in cord blood cultures of 23% of very immature preterm infants. These infants were more likely to have neonatal systemic inflammatory response syndrome and probably BPD [21]. Animal experiments strongly indicate that antenatal Uu infection contributes to a prolonged proinflammatory response, early fibrosis, an altered de-

velopmental signaling and changes in morphology and lung function in the immature lung [20, 22]. The presence of Uu in the respiratory tract of preterm infants, even without clinical or laboratory signs of infection, was correlated with elevated cellular and molecular markers of inflammation and has been associated with an increased risk of BPD [23–25]. The inherent responses of the maternal and fetal immune system to antenatal Uu infection are still not understood; however, they probably determine the pulmonary outcome of preterm infants with Uu colonization [20]. In addition, the role of fetal and neonatal innate immunity such as surfactant proteins SP-A, SP-D and Toll-like receptors is far from clear [26].

### *Mechanical Ventilation*

Many in vitro studies and animal experiments clearly show that any ventilatory trauma of the immature lung may be injurious to airways and lung tissue. An excessive tidal volume (volutrauma), rather than high inspiratory pressure (barotrauma), is the primary determinant of lung injury. Overdistension of the lungs or cyclic opening and closing of lung units causes disruption of structural elements and a release of proinflammatory mediators with subsequent leukocyte influx [1, 5, 7, 27–33]. The strongest inflammatory reaction was observed in those ventilatory strategies with high peak pressure and no positive end-expiratory pressure. If animals were pretreated with lipopolysaccharide (LPS), bronchoalveolar lavage fluid levels of proinflammatory cytokines were impressively increased even with a 'less injurious' ventilation strategy [34]. 'Priming' of the fetal lung by LPS is a considerable pathogenetic factor in the initiation of the inflammatory reaction, and basically every form of mechanical ventilation may act as a 'second strike' that can amplify or aggravate the inflammatory response [7].

### *Hyperoxia and Hypoxia*

Very immature preterm infants with their reduced antioxidant defense system are at high risk of suffering from potential detrimental effects of hyperoxia and hyperoxemia [35, 36]. In preterm and full-term animals, hyperoxia has clearly been shown to be a strong and independent inducer of various mediators involved in pulmonary inflammation [37, 38]. Recently, differential gene expression with DNA microarray analysis in premature rat lungs exposed to prolonged hyperoxia during the sacular stage has been studied. Oxidative stress affected a complex orchestra of genes involved in inflammation, extracellular matrix turnover, coagulation and other events, and the majority of proinflammatory genes were

considerably upregulated [39]. These findings were associated with an increased influx of inflammatory cells, especially macrophages in pulmonary tissue. Moreover, hyperoxia resulted in progressive lung disease which strongly resembled BPD. Current knowledge about the role of hypoxia in pulmonary inflammation is limited. A recent animal study demonstrated that hypoxia had a substantial effect on LPS-induced pulmonary inflammation by increasing the magnitude of lung injury reflected by increased expression of inflammatory mediators, excessive neutrophil accumulation and increased vascular permeability [40]. The role of a genetic predisposition to BPD is currently under investigation but has not provided conclusive data to date [41–43].

### **Cellular and Soluble Mediators of Pulmonary Inflammation, Mechanisms of Lung Injury and Repair**

#### *Inflammatory Cells*

Neutrophils and macrophages have an essential role in acute and chronic stages of pulmonary inflammation. Much higher and persisting numbers of inflammatory cells were detected in bronchoalveolar lavage fluid of preterm infants with BPD when compared with infants who recovered from RDS [44–48]. Immediately after initiation of mechanical ventilation, a neutrophil influx into the airways was observed in animals as well as in preterm infants, and this inflammatory reaction was associated with a decrease in the number of circulating neutrophils [49, 50], and it was shown to correlate with the extent of pulmonary edema formation and an increased risk of developing BPD [3, 51, 52]. In addition, circulating neutrophils and monocytes became activated within 1–3 h after initiation of mechanical ventilation as reflected by CD11b expression [53]. Following their activation, alveolar and pulmonary tissue macrophages secrete numerous cytokines and proinflammatory mediators which orchestrate the inflammatory response, particularly neutrophil recruitment. In lung tissues of preterm infants who had died during the early stages of RDS, the interstitial density of CD68-positive macrophages and neutrophils was at least 10- to 15-fold higher than in stillborn infants of equivalent age [54]. Since apoptosis of inflammatory neutrophils and their timely removal by resident macrophages are critical to the resolution of inflammation, neonatal neutrophils which seem to have a prolonged survival may have the functional capacity to perpetuate inflammation [55].

#### *Chemotactic Factors and Endothelial Interactions*

Airway secretions of infants with BPD contain high concentrations of well-defined chemotactic factors which are responsible for the recruitment of neutrophils: C5a, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1, IL-8, IL-16, lipoxygenase products, leukotriene B<sub>4</sub>, elastin fragments, metalloproteases, fibronectin and others [1, 5, 7, 44]. IL-8 is involved in the initiation of cellular endothelial interactions and is probably the most important chemotactic factor in the lung. In addition, potent  $\beta$ -chemokines that induce chemotaxis of monocytes and macrophages are present in the airway secretions of infants with RDS and BPD. These include monocyte chemotactic protein, macrophage inflammatory protein and growth-related protein [1, 5, 7]. Chemotactic activity and concentrations of numerous chemotactic and chemokinetic factors were considerably higher in infants with BPD when compared with babies who recovered from RDS and preceded the marked neutrophil influx in infants with BPD [44]. Application of a selective chemokine receptor antagonist clearly inhibited neutrophil influx into the rat lung, suppressed pulmonary inflammation and enhanced lung growth [56]. Similarly, inhibition of phosphodiesterase 4 decreased influx of monocytes and macrophages in the airways of preterm infants and positively affected the extent of lung injury [57, 58].

Neutrophils and monocytes leave the circulation via a one-way exit into the extra-alveolar space. The complex mechanisms of cellular attachment to endothelial cells are mediated through an interaction with adhesion molecules and their ligands. Various data indicate that recruitment of circulating neutrophils and monocytes into the airways and pulmonary tissue of preterm infants is well functioning [7, 59].

#### *Pro- and Anti-Inflammatory Cytokines*

The proinflammatory cytokines TNF- $\alpha$ , IL-1, IL-6 and IL-8 are synthesized by various inflammatory and pulmonary cells upon stimulation by hyperoxia, microorganisms, endotoxin (LPS), other bacterial cell wall constituents and biophysical factors such as volutrauma and barotrauma [1]. They play a crucial role in the initiation and in the evolution of the inflammatory response [41]. IL-1 present in the airway secretions of ventilated preterm infants induces IL-8 expression of epithelial cells via a nuclear transcription factor (NF- $\kappa$ B)-dependent pathway. NF- $\kappa$ B activation has been detected in airway neutrophils and macrophages as well as in tracheobronchial secretions from infants with RDS [60, 61]. Increased protein levels and high mRNA expression of proinflamma-

tory cytokines have been identified in airway secretions, bronchoalveolar and pulmonary cells and, moreover, in the systemic circulation of infants with evolving BPD by numerous investigators [1, 5, 8, 62]. A transient overexpression of IL-1 in rat lungs by adenoviral gene transfer was accompanied by a local increase in TNF- $\alpha$  and IL-6 expression and a vigorous acute inflammatory reaction with profound tissue injury and fibrotic changes [63]. Most recently, perinatal expression of IL-1 $\beta$  in pulmonary epithelial cells of a bitransgenic mouse model was shown to induce a lung disease which was clinically and histologically similar to BPD [64].

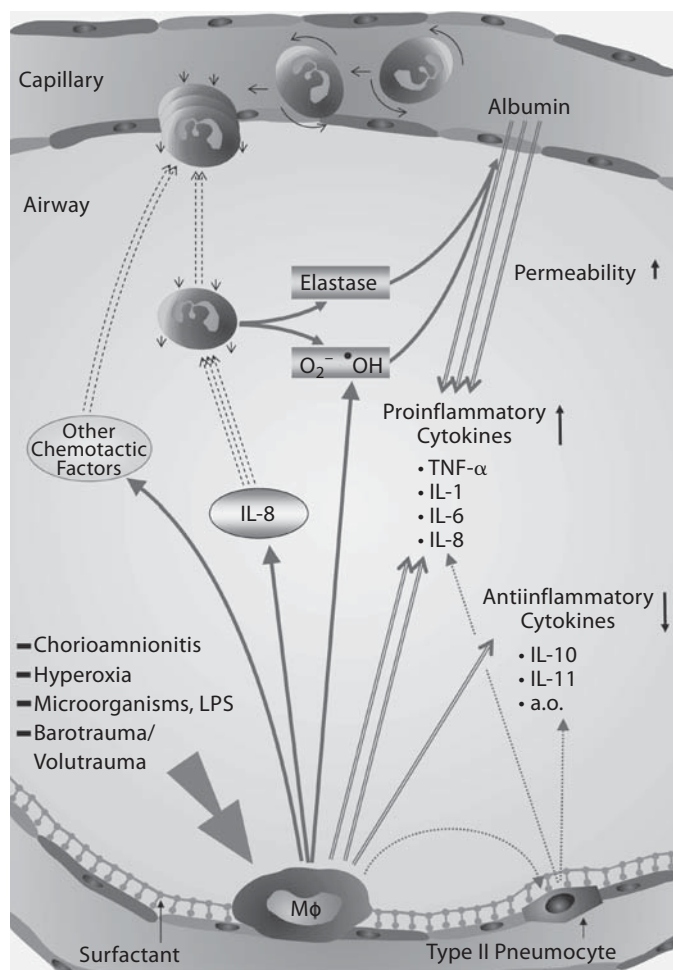
The influx of TNF- $\alpha$ -positive macrophages in pulmonary tissue of preterm infants who had died of severe RDS was found to be associated with a loss of endothelial basement membrane and a destruction of interstitial glycosaminoglycans [65]. Proinflammatory cytokines do not cross the placenta [66]. There is convincing experimental and clinical data which indicate that the profound proinflammatory cytokine response present in the airways and pulmonary tissue of preterm infants may reflect an inability to regulate inflammation through an adequate expression of the anti-inflammatory cytokines IL-4, IL-10, IL-11, IL-12, IL-13, IL-18 or IL-1 receptor antagonist [1, 5, 67–69]. Cellular IL-10 mRNA was undetectable in most airway samples of preterm infants with BPD, but it was expressed in all term infants with respiratory failure [67]. In general, monocytes of newborns seem to produce IL-10 far below the level needed to inhibit a submaximal release of IL-8 from mononuclear cells [70]. Interestingly, lung inflammatory cells of preterm infants exposed to IL-10 *in vitro* responded with a reduced expression of proinflammatory cytokines [71]. An imbalance between proinflammatory and anti-inflammatory cytokines, favoring the former, can be considered as an important feature of lung injury. In infants exposed to chorioamnionitis, insufficient inhibition of high fetal proinflammatory cytokine response shortly after birth may increase the risk of BPD [62].

#### *Oxidative and Proteolytic Damage*

High inspiratory oxygen concentrations can induce direct oxidative cell damage through increased production of reactive oxygen species which are released by neutrophils and macrophages at sites of inflammation. In addition, reactive oxygen species are generated under hyperoxic conditions, during reoxygenation injury by the cell-bound xanthine oxidase system and in the presence of free iron [35, 72]. Free iron was detected in the vast majority of ventilated preterm infants with RDS [72]. Oxygen rad-

icals exert direct toxic effects on bronchoalveolar structures by induction of inflammation, lipid peroxidation, oxidative inactivation of protective antiproteases and up-regulation of metalloproteinases [35, 72]. Animal experiments indicate that oxidative stress is a very early and crucial event in the initiation of pulmonary inflammation [73], and the activity of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase may not be sufficient to combat oxidative injury [35]. Very immature preterm infants are particularly susceptible to hyperoxia and oxygen radicals since the activity of the antioxidant system is much lower compared to term newborns and the system has yet to mature. As a consequence, very immature preterm infants have a profound deficiency in antioxidant enzyme activity at the time when they are receiving high inspiratory oxygen concentrations and are most likely to be exposed to hyperoxemia [35].

At sites of inflammation neutrophils and macrophages release various potent proteases which play an essential role in the destruction of the alveolar-capillary unit and the extracellular matrix (fig. 1). These include elastase,  $\beta$ -glucuronidase, myeloperoxidase, cathepsin, metalloproteinase and others. There is an imbalance between elastase, a powerful neutral protease, and  $\alpha_1$ -proteinase inhibitor within the airway of preterm infants with RDS and BPD [44, 74].  $\alpha_1$ -Proteinase inhibitor is presumably functionally inactivated by oxygen intermediates. Various markers of tissue destruction probably caused by free elastase have been identified in airway secretions and urine of preterm infants and, moreover, alveolar septation was markedly reduced in lungs of infants with increased elastase expression [3]. In addition, an imbalance between cysteine proteases and their inhibitors has recently been described [75]. Similarly, high concentrations of different matrix metalloproteinases that are involved in remodeling throughout all stages of lung development have been identified in airway secretions of infants with BPD and when overexpressed might cause disruption of the extracellular matrix. Protective levels of tissue inhibitors of metalloproteinases were rather low in these infants, also suggesting an imbalance within the metalloproteinase system [76, 77]. Blocking matrix metalloproteinase-9 (MMP-9) activity has recently been shown to reduce oxidative injury-mediated lung damage in mice [78]. In contrast, a deficiency of MMP-9 in transgenic mice increased pulmonary cell death suggesting that MMP-9 activity in the inflamed lung has a protective role against tissue injury [79]. Elastase and other neutral proteases were shown to prime macrophages for an increased release of toxic oxygen metabolites [80].



**Fig. 1.** Schematic representation of pulmonary inflammatory events in immature preterm infants at risk of BPD. Pre- and postnatal risk factors induce the generation and secretion of proinflammatory cytokines by alveolar macrophages and other cells. These proinflammatory mediators which have chemotactic and chemokinetic properties are responsible, in part, for the upregulation of endothelial and cellular adhesion molecules and for the recruitment and influx of neutrophils (PMN) and monocytes into the airways. Various inflammatory mediators such as free elastase, oxygen radicals ( $O_2^-$ ,  $\cdot OH$ ) and proinflammatory cytokines induce an increased alveolar-capillary permeability. As a consequence, plasma proteins such as albumin entering the airways contribute to edema formation, the inactivation of the surfactant system and deterioration of lung function. An imbalance between pro- and anti-inflammatory mediators seems to favor proinflammatory mechanisms.

#### Increased Alveolar Capillary Permeability

An increased alveolar-capillary permeability is pathognomonic for the early and later stages of inflammation and it is clearly associated with a deterioration of lung function [1, 5]. Numerous inflammatory mediators

and cells have detrimental effects on the microvascular integrity, among them a variety of lipid mediators including leukotrienes, prostacyclin, platelet-derived factor and endothelin-1. Protein leakage into the alveoli and airways of preterm infants takes place within 1 h after initiation of mechanical ventilation [51, 81]. At a postnatal age of 10–14 days, preterm infants who later developed BPD had a large increase of albumin concentrations in their airway secretions; albumin and other serum proteins profoundly contribute to alveolar edema, to the inactivation of the surfactant system and a deterioration of lung function [44, 82]. Magnetic resonance imaging in infants with BPD showed an increased lung water content and a gravity-induced collapse of the lung [83]. In mechanically ventilated infants with RDS, a simultaneous activation of clotting, fibrinolysis, kinin-kallikrein and the complement system has been observed [1]. These findings indicate that injury to the pulmonary vascular endothelium may subsequently promote neutrophil and platelet activation, and may induce pulmonary as well as systemic inflammation and activation of the clotting system [84].

#### Repair Mechanisms and Growth Factors

Lung injury and the associated inflammatory process lead to an induction of TGF- $\beta$  that limits some of the inflammatory reactions and plays a key role in mediating tissue remodeling and repair [85]. However, if reparative processes are exaggerated, as indicated by increased expression of TGF- $\beta$  in the lungs of preterm animals and in preterm infants with BPD, normal lung development may be inhibited and fibrosis may ensue [1, 86–89]. Recently, a reduced expression of connective tissue growth factor, which is responsible for various downstream actions of TGF- $\beta$  and which is a second important key mediator in the induction of pulmonary fibrosis, has been found in a sheep model [90]. Overexpression of TGF- $\beta$  and subsequent downregulation of connective tissue growth factor together with suboptimal levels of various growth factors may partially explain the pathogenesis of the ‘new BPD’ which is characterized by growth arrest of lung tissue and pulmonary vessels rather than by fibrosis. Low concentrations of hypoxic-inducible factor, keratinocyte, hepatocyte growth factors, angiopoietin and endostatin, which participate in normal lung and vascular development as well as tissue regeneration after lung injury have been detected in infants with BPD [91–96]. Similarly, an impaired expression of vascular endothelial growth factor and its angiogenic receptors in lungs from extremely preterm animals developing BPD were shown to contrib-

ute to dysmorphic microvasculature and disrupted alveolarization [97]. Postnatal intratracheal administration of adenovirus-mediated vascular endothelial growth factor gene therapy improved survival and promoted lung capillary formation; moreover, the alveolar development was preserved in this rat model of irreversible lung injury [97].

## Conclusion

During the past decade, it has become evident that there are multiple pre- and postnatal events which contribute, at least in part as a multiple-hit scenario, to the development of BPD in preterm infants. Chorioamnionitis, cytokine exposure in utero and a fetal inflammatory response plus various postnatal risk factors may induce a pulmonary inflammatory response that is probably associated with aberrant wound healing. As a devastating consequence, normal alveolarization as well as vascular development can be compromised with lifelong consequences for the infant. The inflammatory response is characterized by a rapid accumulation of inflammatory cells and, moreover, by an arsenal of injurious mediators that might directly affect the alveolar capillary unit and tissue integrity. Generally, an imbalance between pro- and anti-inflammatory mediators favoring proinflammatory mechanisms is a key feature in the pathogenesis of BPD. However, we have to realize

that the exact pathogenetic sequence of acute and chronic inflammation is mainly hypothetical and speculative since the possible interaction between inflammatory cells and humoral mediators as well as regulatory aspects of inflammation in tissue injury and repair are largely descriptive and the molecular basis of these events has only been partially defined to date. Moreover, most of the reported findings reflect associations of in vitro experiments, animal studies and clinical observations in preterm infants with RDS and BPD rather than causal relationships. Especially in preterm babies with the burden of numerous pre- and postnatal risk factors and a considerable heterogeneity in disease severity as well as the limitations in study design, it seems impossible to define the significance of an individual risk factor in the pathogenesis of pulmonary inflammation [5]. Many studies have examined mediators at single or very few time points and may have missed the phasic nature of the inflammatory process, others may have investigated an insufficient number of mediators to draw reasonable conclusions about the interaction between pro- and anti-inflammatory mechanisms [41]. Nevertheless, our current understanding of the inflammatory mechanisms has opened up avenues which will allow us to get a deeper insight into the pathogenesis of inflammatory events and thus allow strategies to be developed that will help to prevent or ameliorate BPD in high-risk infants [98].

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