Acute lung injury in children: from viral infection and mechanical ventilation to inflammation and apoptosis

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Lung Epithelial Cell Apoptosis in Acute Lung Injury in Infants and Young Children

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ABSTRACT

Apoptosis of lung epithelial cells is implicated in the pathogenesis of acute lung injury. Most research on this subject has focused on adults. Up to date, very little is known about a potential interaction of this process with lung development in children. In the present study we aimed to summarize the current literature on lung epithelial cell apoptosis and common causes of acute lung injury in infants and young children and to identify new areas of research. Overall, few studies have specifically focused on lung epithelial cell apoptosis in acute lung injury in children. Nevertheless, the limited literature suggests that this may be an important pathogenic mechanism in respiratory distress syndrome of infants (IRDS) and viral respiratory tract infection. Apoptosis is an essential process during lung development and maturation. Insufficient attention has been paid to potential consequences of this for the short and long term outcome of acute lung injury in infants and young children.
INTRODUCTION

Characteristic and common causes of acute lung injury in infants and young children include respiratory distress syndrome of preterm infants (IRDS), viral lower respiratory tract infection and ventilator-induced lung injury. Although it is generally believed that lung injury during early life may have negative consequences for long term lung function, the exact effects of acute lung injury on the developing lung are incompletely understood. Likewise, to what extent the stage of lung maturation contributes to the susceptibility to acute lung injury remains controversial. Both acute lung injury incidence and mortality rates appear to be lower in infants and young children as compared to adolescents and adults, and therefore further insight into the mechanisms that may be involved herein are of great interest.

Apoptosis, a form of programmed cell death, is a highly regulated series of events leading to the elimination of cells. It is an essential process for normal organogenesis in the child, including the development and maturation of the lungs. Lung epithelial and mesenchymal cell apoptosis has been observed throughout different stages of lung development before and after birth, and is believed to greatly contribute to gas-exchange surface formation and airway branching. At the same time, apoptosis of lung epithelium has been associated with the pathogenesis of acute lung injury. When exposed to injurious events, airway and alveolar epithelial cells can react with a broad arsenal of protective measures such as the production of inflammatory mediators and anti-oxidants, depending on the cause of injury. Alternatively, the lung cell may lose its ability to survive and may die either by necrosis or apoptosis. In this light, the process of apoptosis has originally been viewed of as controlling injury and inflammation. Although indeed accumulating evidence suggests that apoptosis of the lung epithelium is an important cellular mechanism during acute lung injury, it remains a matter of debate under which conditions lung epithelial cell apoptosis is beneficial (e.g. controlling infection) or ‘out of balance’ and detrimental (e.g. acute respiratory distress syndrome, ARDS) to the patient. For example, a patient suffering from viral pneumonia could benefit from enhanced viral clearance by apoptosis of infected lung cells, but may experience severe oxygenation anomalies when widespread lung epithelium apoptosis occurs. An overshoot of lung epithelial cell apoptosis contributes to the loss of integrity of the alveolar capillary barrier function, resulting in pulmonary edema and surfactant abnormalities. During the past few years, lung epithelial cell apoptosis has been extensively studied in the context of acute lung injury. Because apoptosis is a regulated process that potentially can be intervened, unraveling the precise role and mechanisms may help find anchor points for new treatment strategies.

Up to date, research on lung epithelial cell apoptosis and acute lung injury has mainly focused on adult patients. Because one could easily argue that resting levels of apoptosis as part of the developmental stage in the lungs of young children and infants may influence both the susceptibility and consequences of acute lung injury, the role of lung epithelial cell apoptosis during acute lung injury in children deserves further exploration. In the present
study we aim to summarize the current literature on this subject to identify new areas of research in this specific age group.

MECHANISMS OF APOPTOSIS

Before going into more detail on lung epithelial cell apoptosis and acute lung injury in pediatric patients, we will present a brief conceptual framework of the cellular mechanisms of apoptosis especially focusing on acute lung injury.

Cell death in multicellular organisms occurs either by necrosis or apoptosis. Each of these two processes have distinct morphological and biochemical characteristics. Apoptosis is an active process and follows signaling through specific death pathways, as described below. In contrast, necrosis is a process that results from the inability of a cell to sustain its homeostatic mechanisms. However, a strict distinction between the two types of cell death is difficult because certain forms of apoptosis also show necrotic features, and conversely, it has been found that cells exposed to pro-apoptotic stimuli can also die due to necrosis. Apoptosis is associated with membrane blebbing, disruption of the cell into apoptotic bodies, and fragmentation of the DNA. In ‘classical’ apoptosis activation of a family of intracellular substrate specific proteases called caspases precedes this fragmentation. However, several caspase-independent signals of (non-‘classical’) apoptosis have also been discovered.

At least three major pathways may be of importance for lung epithelial cell apoptosis during acute lung injury (Figure 1). A receptor-mediated (extrinsic) pathway can be triggered by ligation of transmembrane death receptors belonging to the family of tumor necrosis factor receptors (TNFR), such as Fas (CD95) and TNF-related apoptosis-inducing ligand (TRAIL) receptor. These receptors are activated by Fas ligand (FasL) or TRAIL, either in membrane-bound or soluble form, or by TNF. The extrinsic pathways are associated with intracellular caspase-8 activation leading to further downstream activation of other caspases, such as the main effector caspase-3, and execution of apoptosis. Elevated levels of both soluble FasL and TNFα has been found in bronchoalveolar lavage fluid (BALF) of humans with ARDS. In addition, blocking the Fas/FasL pathway has been found to attenuate lipopolysaccharide (LPS)- and shock/sepsis-induced acute lung injury in animal models. Studies have shown that both the TNFα and FasL death receptor pathways may modulate hyperoxia/oxidative stress-induced apoptosis during acute lung injury. However, it has also been demonstrated that mice deficient for TNFR or Fas are not protected against oxygen toxicity, suggesting that these death receptor pathways are not essential in hyperoxia-induced acute lung injury.

A second pathway of apoptosis can be triggered by injurious events such as DNA damage, oxidants, radiation and intracellular calcium overload. This (intrinsic) pathway involves the release of pro-apoptotic factors, like cytochrome c from the mitochondria
into the cytosol, and is regulated by the Bcl-2 family of proteins. Thereafter, apoptosis is triggered through both caspase-dependent and caspase-independent death pathways. There is evidence of cross-talk between the receptor and mitochondrial mediated pathway, suggesting that these two signaling routes to interact during apoptosis. Activation of the mitochondrial pathway appears to play an important role in hyperoxia-induced acute lung injury, and some studies suggest that reactive oxygen species directly induce cytochrome c release from the mitochondria into the cytosol.

Finally, cytotoxic T-cells and natural killer cells exploit a pro-apoptotic pathway that is thought to play an important role in the clearance of virus-infected cells. In the classical paradigm these effector lymphocytes elicit the formation of pores in the membrane of an infected target cell by means of a protein called perforin. Subsequent

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**Figure 1.** Pathways of caspase-(in)dependent apoptosis in lung epithelial cells mediated by mitochondrial disruption or death receptor stimulation initiated by recruited effector lymphocytes, neutrophils (PMN) or macrophages. Members of the Bcl-2 family including Bcl-2, Bax and Bcl-XL and p53 regulate cytochrome c release from the mitochondria in response to stimuli such as DNA damage, reactive oxygen species (ROS) or calcium overload. Cytochrome c in the cytosol assembles with apoptotic peptidase activating factor 1 (Apaf-1) to activate initiator caspase-9. Adaptor proteins interact through their death domain upon activation of the death receptor pathway by FasL or TRAIL, leading to activation of initiator caspase-8. The mitochondrial and death receptor pathway can interact through Bid. The activation of caspase-8 or caspase-9 leads to the downstream activation of the caspase cascade including effector caspase-3,-6 and -7, resulting in apoptosis. Granzymes delivered to the cytosol by effector lymphocytes can activate caspases and Bid to induce apoptosis. The inhibitor-of-apoptosis proteins (IAPs) can block several caspases thereby inhibiting cell death.
delivery through these pores of cytolytic granular proteases, known as granzymes, into the cytosol of the target cell leads to activation of both caspase dependent and independent apoptotic pathways. Upregulation of mRNAs of granzymes and perforin has been found in BALF of humans with ARDS 29.

APOPTOSIS AND PEDIATRIC ACUTE LUNG INJURY

We used a Medline database and references from identified articles to perform a literature search relating to epithelial cell apoptosis, acute lung injury and intensive care treatment in children. In this section we specifically focus on common causes of acute lung injury in infants and young children because of the importance of lung development in this age group.

Perinatal acute lung injury
The most common type of acute lung injury in newborns is the respiratory distress syndrome of preterm infants (IRDS). IRDS is primarily associated with surfactant deficiency, but other pathogenic mechanism such as an exaggerated inflammatory and pro-apoptotic response may play a role as well 30;31. Lukkarinen et al. found increased leukocyte infiltration and increased numbers of apoptotic epithelial cells in autopsy lung specimens of ventilated neonates who died from IRDS, as compared to ventilated control patients without obvious lung disease 32. Similarly, May et al. reported an increase in the apoptosis markers terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) and caspase-3 activity in alveolar epithelial cells of 27 ventilated infants with fatal IRDS, as compared to still borns 33. In addition, autopsy samples of ventilated infants with bronchopulmonary dysplasia following IRDS showed increased apoptosis of both alveolar and bronchial epithelial cells 34. However, these studies should be interpreted with caution because of their retrospective design and the very heterogeneous character of the cohorts studied. In particular, the contribution of mechanical ventilation or hyperoxia per se to epithelial cell apoptosis was not addressed in these studies. In the study of May et al. surfactant treatment seemed to reduce alveolar epithelial cell apoptosis 33, an observation in line with results described by White et al showing that surfactant protein A (SP-A) inhibits alveolar epithelial cell apoptosis through interaction with tyrosine phosphorylation pathways downstream of the SP-A receptor 35.

Another line of research relevant to apoptosis during acute pulmonary disease in newborns has focused on meconium-induced acute lung injury. This disorder involves an acute inflammatory reaction following perinatal meconium aspiration. Several recent animal studies have shown that increased apoptosis of particularly airway epithelial cells is an important feature of meconium instilled lungs 36;37. In addition, A549 cells (a human carcinomic, alveolar basal epithelial cell line) show caspase-3 dependent apoptosis following exposure to meconium 37.
Respiratory tract infection

Respiratory tract infections, in particular of viral origin, are a frequent cause of acute respiratory insufficiency in young children. Many in vitro studies have investigated lung epithelial cell apoptosis during infection with common pediatric respiratory viruses such as respiratory syncytial virus (RSV), influenza-, rhino- and adenovirus, some of which have been reviewed extensively elsewhere 38-42. During infections with these viruses, apoptosis is considered to be a major host defense mechanism, by limiting viral spread and replication. On the other hand, viruses often display strategies to evade apoptosis. Several in vitro studies using different lung epithelial cell lines have shown apoptosis and up-regulation of pro-apoptotic gene expression such as Fas in response to respiratory viral invasion 40;41;43;44. However, it remains unclear under which conditions excessive and uncontrolled lung epithelial cell apoptosis following viral infection may contribute to acute lung injury and organ dysfunction. It is likely that intrinsic host factors, as has been suggested for asthmatic adults and children with cystic fibrosis 42;45, as well as pathogen factors determine this balance between ‘good’ or ‘bad’ apoptosis.

Worldwide, RSV is the most common cause of lower respiratory tract infection in young children 46;47. Every winter a high number of children with severe RSV infection require mechanical ventilation and many of them fulfill the criteria of ARDS 48;49. Several animal studies have highlighted the importance of apoptosis during RSV infection in vivo. Viuff et al. demonstrated that apoptosis is an important way of clearance of infected cells in a model of bovine RSV infection in calves 50. A recent murine study suggested a detrimental role for the Fas/FasL system during severe RSV infection 51. In that study, RSV-induced clinical illness was strongly reduced in mice carrying a gene encoding for a nonfunctional form of FasL, while viral clearance was moderately delayed. However, no direct evidence for lung epithelial cell apoptosis in relation to disease severity was presented. Such cytopathological events may be difficult to demonstrate in these animal models because RSV is not a natural pathogen for mice and replicates to a limited extent in murine respiratory epithelial cells 52.

Bacterial infection, either as the primary cause or secondary to viral infection, often leads to acute lung injury in children, comparable with adults. In adult animal models LPS-induced acute lung injury appears to be associated with widespread apoptosis of the lung epithelium 17;53. However, to our knowledge, so far no studies have focused on the role of lung epithelial cell apoptosis secondary to bacteria or bacterial products in newborn mice or children.

Intensive care treatment

Oxygen treatment and mechanical ventilation are the cornerstones of the management of critically ill patients with acute lung injury. However, it is widely accepted that both treatment modalities can cause additional injury to the lung.
Potential mechanisms of hyperoxia-induced lung injury involve the production of reactive oxygen species, release of pro-inflammatory cytokines and cell death. Several experimental studies have shown that besides necrosis, activation of pro-apoptotic pathways is a prominent feature in the cellular response to oxidative stress, although the exact contribution of lung epithelial cell apoptosis in hyperoxia-mediated lung injury in the clinical setting is unclear. Several studies have evaluated the influence of age on hyperoxia-induced lung injury and apoptosis. Auten et al. found DNA damage and oxidation, independent from Bcl-2 and Bax transcription or with caspase-6 activity, in lung parenchymal cells of newborn rats that were exposed to hyperoxia for 8 days. However, Mantell et al. found evidence for a delayed apoptotic response in lung parenchymal cells in newborn rabbits exposed to hyperoxia, as compared to their adult counterparts. Furthermore, in a recent study Mao et al. found that activation of Fas results in a protective proliferative response rather than a pro-apoptotic response in hyperoxia-treated newborn mice. Interestingly, newborns of rodents and rabbits exposed to hyperoxia have a delayed inflammatory reaction in their lungs and survive longer than adult animals. For example, treatment with 100% O\textsubscript{2} is lethal in adult rabbits at 72 hr exposure, while newborns live for several days thereafter with lung inflammation and edema not being evident until about 96 hr of exposure. Taken together, these findings suggest age-related differential pro-apoptotic responses may be involved in the lower susceptibility of newborns to hyperoxia-induced lung injury.

Mechanical stress leading to cellular stretch during mechanical ventilation may trigger responses that include pro-inflammatory mediator release and apoptosis by mechanotransduction in lung epithelial cells. The mechanisms responsible for ventilator-induced lung injury are being extensively investigated in order to find optimal ventilator strategies to minimize iatrogenic damage. As mentioned above, studies in mechanically ventilated preterm infants have reported increased numbers of apoptotic lung epithelial cells in vivo. However, in these studies the relative contribution of mechanical ventilation, hyperoxia and primary lung disease to the demonstrated apoptosis remains unclear. Recently, Smith et al. presented preliminary data on the role of age in lung injury induced by a combination of mechanical ventilation and LPS challenge: they found an upregulation of anti-apoptotic response genes in the juvenile mice, as compared to adult mice, suggesting a protective effect of age in this model.

**APOPTOSIS AND LUNG DEVELOPMENT**

To support our hypothesis that both outcome and susceptibility to acute lung injury might be affected by age, we will give a brief overview on the role of apoptosis in lung development.

The development of organs requires an orchestrated and complex interplay between proliferation, differentiation and apoptosis. During organ morphogenesis, apoptosis...
occurs either by direct stimulation or by lack of growth and/or differentiation factors, resulting in removal of unwanted or superfluous structures. Although most organogenesis occurs \textit{in utero}, several organ systems do not complete their development and maturation until after birth. In humans, alveolarization and microvascular maturation of the lungs continue up to at least a few years after birth \textsuperscript{71}.

Apoptosis has only recently been implicated in the process of lung development, probably related to cell stretching and under the influence of sex hormones \textsuperscript{7,72-74}. During gestation, mesenchymal and alveolar epithelial cell apoptosis coincides with airway branching and alveolar epithelium thinning, respectively \textsuperscript{73,74}. After birth, superfluous surfactant producing type 2 alveolar epithelial cells are removed by apoptosis and by differentiation into type 1 alveolar epithelial cells, which form most of the alveolar gas-exchange surface \textsuperscript{75}. In a series of elegant experiments, De Paepe \textit{et al}. provide evidence that regulation of the Fas/FasL system is critical for type 2 alveolar epithelial cell apoptosis in developing lungs of both rabbits and mice \textsuperscript{76-78}.

One of the major underlying mechanisms believed to be involved in regulation of apoptosis in the lungs is cellular stretch \textsuperscript{7,79}. Just as normal breathing causes lung cell stretch after birth, fetal breathing movements and growing lung buds may generate physical strain in the developing lung. Several possible events in lung cell mechanotransduction have been suggested, such as stretch activation of ion channels, conformational change of cytoskeleton proteins (e.g. cadherins and integrins) or direct stretch dependent transcriptional regulation (e.g. upregulation of FasL) \textsuperscript{7,80}. However, the precise mechanism by which lung cell stretch is linked to activation of apoptotic pathways and the potential impact of additional stress caused by mechanical ventilation is far from clear.

Based on the above mentioned observations it is very likely that resting levels of apoptosis in the lungs of children differ from those in adults, depending on the developmental stage of the lungs. High or low resting levels of both extrinsic and intrinsic pro- and anti-apoptotic factors may affect the susceptibility of the lung to injurious events. Whether such an influence is advantageous or detrimental to the outcome cannot be known unless more research specifically focuses on this subject. Up to date, this has been given insufficient attention to in the literature. In addition, more insight is needed into the potential long-term effects of dysregulation of apoptosis during acute lung injury on lung function. Although research evaluating the long term consequences of acute lung injury in children is very scarce, some reports show both restrictive and obstructive disease in a substantial part of children who have suffered from ARDS \textsuperscript{81-84}. Long term effects of apoptotic imbalance during organ development mediated by injury have been suggested for the brain and kidneys \textsuperscript{85-87}. Interestingly, De Paepe \textit{et al}. showed that FasL expression induced by a transgenic tetracycline expression system in airway and alveolar epithelial cells in mice during perinatal lung development results in disrupted alveolarization associated with increased apoptosis \textsuperscript{88}. In addition, Dieperink \textit{et al}. showed that hyperoxia treatment of fetal mouse lung explants results in decreased
airway branching, possibly mediated by increased apoptosis. These findings support the hypothesis that disruption of the delicate interplay between apoptosis and proliferation during acute lung injury in early childhood may influence normal lung development.

**THERAPEUTIC RELEVANCE**

Specific targeting of pro-apoptotic pathways in the lungs has been shown to affect the outcome of acute lung injury in murine models. In vivo administration of caspase- and Fas inhibitors in adult mice attenuates LPS-induced acute lung injury, whereas Fas activating antibody causes increased lung permeability and histopathological alterations.

Recent clinical and experimental observations have suggested that the renin-angiotensin system is involved in the pathogenesis of acute lung injury. A high activity of angiotensin converting enzyme (ACE), leading to an increase of angiotensin II, is believed to have a detrimental effect on the susceptibility and outcome of ARDS, whereas high ACE2 activity, leading to a decrease of angiotensin II, was found protective. Interestingly, it has been shown in vitro that Fas-mediated apoptosis of alveolar epithelial cells require angiotensin II binding to its cell receptor. Lukkarinen et al reported that angiotensin II receptor inhibition reduces epithelial cell apoptosis in ventilated surfactant-deficient rats. However, a relationship with ACE gene polymorphism and outcome of acute lung injury, as has been found for adults, could not be demonstrated in ventilated very low birth weight infants. Finally, animal studies have shown that both ACE and angiotensin II inhibition led to a decrease of meconium-induced lung injury and lung epithelial cell apoptosis.

The influence of corticosteroids on lung epithelial cell apoptosis is controversial. Dorscheid et al have reported an increase of primary airway epithelial cell apoptosis with induced cytochrome c release and caspase activity following continuous exposure to corticosteroids in vitro. In contrast, Wen et al showed reduced apoptosis of A549 cultured cells after treatment with a high concentration of dexamethasone. In line with these results, dexamethasone was found to significantly diminish bleomycine-induced acute lung injury, lung epithelial cell apoptosis and Fas/FasL expression in mice. To our knowledge, there are no data on the effects of corticosteroids on lung epithelium apoptosis specifically focusing on age differences.

Future animal and human experiments should reveal the clinical relevance of these studies in the search of new treatment strategies for both adult and pediatric patients with acute lung injury.
CONCLUSIONS

Accumulating evidence shows that dysregulation of apoptosis of lung epithelial cells is a key event in the pathogenesis of acute lung injury. Although research specifically focusing on children is limited, there is considerable evidence showing that this mechanism is also very relevant for pediatric acute lung injury. The consequences of dysregulation of apoptosis in infants and young children, in whom a tight regulation of apoptosis is essential for normal development and maturation, are incompletely understood. One could hypothesize that both short- and long term outcome of acute lung injury in children is influenced by altered susceptibility to pro- and anti-apoptotic events as part of their developmental stage. Additional research into this subject is therefore highly needed. Increased insight into the mechanisms that modulate apoptosis in acute lung injury may lead to novel therapeutic strategies. Several promising anchor points, including manipulation of the renin-angiotensin system, have already been identified. In addition, knowledge of the mechanisms that link apoptosis with cell stretch and hyperoxia may help identify better respiratory support strategies for critically ill children with acute lung injury.
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