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

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Introduction and Thesis Outline
I. HISTORICAL PERSPECTIVE

This dissertation describes studies on the role of pro-apoptotic pathways in acute lung injury in children, specifically focusing on respiratory syncytial virus infection and mechanical ventilation. To introduce these terms I will start with a historical perspective.

1.1. Acute lung injury

In 1967, Dave G. Ashbaugh and co-workers wrote up a case series on their experiences with twelve patients with massive lung damage initiated by a wide variety of unrelated insults including trauma, shock and hemorrhagic pancreatitis. They describe they literally felt ‘something’ was different in these patients who required mechanical ventilation with high inflation pressures to restore oxygenation, and showed heavy edemic lungs with hyaline membrane formation and defective surfactant function at autopsy. In addition, they were surprised to see that application of positive end-expiratory pressure (PEEP) improved the oxygenation in most patients, although in the end seven of the twelve patients died. Their case series, published in the Lancet (after it was rejected three times), became a landmark article on what later was called the adult respiratory distress syndrome (1971). However, it was soon recognized that patients of all age, including children and newborns, could develop this disease, and as such, its name was changed into acute respiratory distress syndrome (ARDS), recognizing its relevance for pediatric medicine as well. In retrospect, WWII military surgeons, including Mayor Lyman A. Brewer of the Second Auxiliary Surgery Group, had already found that lungs develop increased fluid content in traumatic war casualties and they had called this the ‘wet lung’ (1946). Later, during the Vietnam War, ‘DaNang lung’ or ‘shock lung’ described similar lung pathology after trauma.

The early clinical descriptions of ARDS showed that the acute hypoxemic respiratory failure may develop following a number of different disorders including sepsis, pneumonia, aspiration and trauma. However, in the 80s and early 90s, autopsy examinations of patients with such different etiologies of ARDS revealed that these patients had highly similar lung tissue alterations, which we now consider to be the hallmarks of ARDS: diffuse alveolar damage with neutrophilic alveolitis, vascular congestion, hemorrhage, microthrombi, and intra-alveolar serum protein precipitations, such as hyaline membranes, or protein rich edema fluid. Interestingly, the characteristic eosinophilic hyaline membranes, composed of fibrin depositions and cellular debris against denuded basement membrane, had long before already been described in the respiratory distress syndrome (RDS) (or hyaline membrane disease) in preterm infants.

In 1994 the first American-European Consensus Conference on ARDS took the task to create a clear definition of ARDS, ultimately to improve knowledge and treatment of this severe lung disease. Acute respiratory distress syndrome was designated a severe form of acute lung injury (ALI), with ALI/ARDS being a syndrome of severe lung inflammation.
and increased lung permeability leading to acute hypoxemic respiratory failure. A criteria system entirely based on clinical signs for ALI/ARDS was proposed:

- acute onset
- bilateral infiltration on chest radiograph
- pulmonary-artery wedge pressure of $< 19$ mmHg or the absence of clinical evidence of left atrial hypertension, and
- oxygenation anomaly: $\frac{\text{PaO}_2}{\text{FiO}_2} \leq 300$ mmHg (ALI) or $\frac{\text{PaO}_2}{\text{FiO}_2} \leq 200$ mmHg (ARDS).

Since 1994 there have been several large prospective, randomized intervention trials by the National Heart, Lung, and Blood Institute, National Institutes of Health ARDS Network (www.ardsnet.org). However, so far these trials have resulted in very limited progress in treatment options, and at the present time ALI/ARDS remains one of the biggest challenges in adult and pediatric critical care medicine\textsuperscript{14,15}.

1.2. Respiratory syncytial virus

A specific and frequent cause of ALI/ARDS in young children is lower respiratory tract infection by respiratory syncytial virus (RSV)\textsuperscript{16,17}. RSV is a negative-sense, enveloped RNA virus of the family \textit{Paramyxoviridae}, subfamily \textit{Pneumovirinae} and genus \textit{Pneumovirus}. It was first isolated from a group of sneezing, coughing chimpanzees at the Walter Reed Army Institute for Research in Washington DC in 1956, and was appropriately named: chimpanzee coryza agent (CCA)\textsuperscript{18}. Original CCA cultures induced similar upper respiratory symptoms in other chimpanzees, but not in guinea pigs or rodents. Interestingly, CCA appeared to be able to spread to laboratory workers who handled infected chimpanzees. A year later, Chanock \textit{et al.} isolated a viral agent highly similar to CCA from two infants with lower respiratory tract infection\textsuperscript{19,20}. Because of the tendency of this ‘new’ CCA-like virus to form syncytia in HeLa cells \textit{in vitro}, the virus was then renamed into respiratory syncytial virus. A study by Beem \textit{et al.} with nasopharyngeal cultures from 41 young children suffering from clinical respiratory symptoms ranging from mild to severe cases determined RSV as the principal etiological agent, and formed the beginning of recognition of RSV as the leading pathogen in respiratory tract infections in children\textsuperscript{21}.

1.3. Mechanical ventilation: the beginning of (pediatric) intensive care

Breathing or ventilation is essential to human life. It involves flow of air into the lungs (inspiration) initiated by negative intrapulmonary pressure upon active expansion of the thoracic cavity, and ends with airflow out of the lungs (expiration) due to respiratory muscle relaxation and elastic recoil. The oldest references to the act of taking over ventilation by blowing air mouth-to-mouth, and thus creating positive pressure driven airflow into the lungs, date from Egyptian mythology, Bible texts and the Greek physician Galen\textsuperscript{22}. In the first printed book on pediatric diseases \textit{Libellus de egritudinibus infantium} (1472) the Italian

\textsuperscript{1} human cell line from cervix carcinoma from Henrietta Lacks, † 1951.
physician Paulus Bagellardus describes neonatal resuscitation: ‘If she find it warm, not black, she should blow into its mouth, if it has no respiration, or into its anus’, although the latter suggestion appears somewhat dubious in the context of artificial ventilation. In the sixteenth century several descriptions of experimental thoracic procedures in animals refer to tracheotomy, such as by Vesalius in de *Humani Corporis Fabrica* in 1543: ‘But that life may be restored to the animal, an opening must be attempted in the trunk of the trachea, into which a tube of reed or cane should be put; you will then blow into this, so that the lung may rise again and take air.’ In the 1700s and 1800s around the time of the discovery of carbon dioxide and oxygen resuscitation after drowning or mining accidents occurred by positive pressure ventilation with a bellow, or later with pistons, and first descriptions of endotracheal intubation appear.

Major progress in the application of prolonged mechanical ventilation was made at the time of poliomyelitis epidemics in Europe and the United States in the 20th century. For example, from the late 20s physicians treated children suffering from polio paralysis with Drinker’s iron lung, a tank in which a patient lay from neck to toe and in which a negative pressure environment around the chest created airflow into the lungs. In 1952 Copenhagen’s Blegdams Hospital was overwhelmed by patients with acute respiratory failure during a major polio epidemic. It was there that tracheotomy followed by intermittent manual ventilation with a rubber bag (handled by medical students) was performed on a wide scale. The organization of wards specialized in prolonged artificial ventilation during which patients could recover from this disease formed the beginning of intensive care units (ICUs). Although initially adult ICUs focused primarily on the respiratory system, pediatric ICUs (first established in 1955) followed the model of neonatal ICUs in which specialized generalists managed patients of a specific age, suffering from a wide variety of critically ill diseases, including different organ systems. The clustering of critically ill patients in such specialized wards was later also recognized to be highly suitable for post-surgery patients, extending the field of intensive care medicine. Today, machines have taken over the ventilation of ICU patients and modern positive pressure mechanical ventilator devices come in many types, designed to control volume, pressure and frequency adapted to the patient’s breathing activity.

1.4. Apoptosis

In the organogenesis and development of living multicellular organisms the elimination of cells by cell death is as important as cell proliferation. One of the most popular examples highlighting the importance of cell death in the development of embryonic tissues is the ‘sculpting’ of our hands and fingers by the elimination of cells located in between digits. Detailed work derived from the 2002 Nobel Prize winners Sydney Brenner, H. Robert Horvitz and John E. Sulston showed that of the 1090 somatic cells of the nematode *Caenorhabditis elegans* hermaphrodite 131 are fated to die during development into adulthood, underlining the extent of cell death in this early stage of life. In addition, cell death counterbalances...
cell proliferation and thereby prevents excessive growth of tissues or tumor development, which is essential for normal homeostasis.

Since the initial concept of cell death, as is discussed in Rudolph Virchow’s lectures on cellular pathology in 1858, numerous morphologic descriptions using a broad terminology have been proposed in an attempt to define the complex process of a dying cell. ‘Apoptosis’, derived from Greek (apó meaning away or from, and ptosis meaning falling down), refers to a distinct type of cell death morphologically characterized by cell shrinkage (as opposed to swelling, oncosis), and enhanced cell density. Other prominent features of an apoptotic cell include the pyknotic condensation of the chromatin, fragmentation of the nucleus (karyorhexis) and budding, which describes the emission of cellular processes containing intact organelles and nuclear fragments. The term ‘apoptosis’ was introduced by J.F. Kerr in 1972 after having studied cell death in toxic liver injury by electron microscopy for almost 10 years 26. However, throughout history, terms such as ‘karyolysis’ (1879), ‘chromatolysis’ (1885), ‘shrinkage necrosis’ (1965) and ‘programmed cell death’ (1965) have preceded the term ‘apoptosis’ and described more or less similar morphologic observations 27;28.

After the early morphological definition of apoptosis there has been a surge of research focusing on the actual biochemical and genetic mechanisms implemented in this type of cell death. At the beginning of this, in 1986, the first genes recognized to be involved in the apoptotic process were bcl-2 and ced-3 and -4 25;29;30. Bcl-2, cloned from a follicular lymphoma cell line, was found to inhibit apoptosis upon overexpression and therefore for the first time linked the development of cancer to inhibition of cell death (in contrast to stimulation of cell proliferation). After bcl-2, many genes, such as p53, ced and bim as well as other important components such as Fas, cytochrome c and the family of caspases were implicated in the control of apoptosis. In fact, the aspect of control, embodied by the underlying genetic program and protein machinery, is now considered to be a main characteristic of apoptosis. It provides the means for rapid cell death triggered by both external and internal stimuli at any point during life and this may differ from ‘programmed cell death’, which technically refers to cell death occurring at a specific (programmed) time point. Furthermore, the suicidal program of apoptosis contrasts with ischemic or accidental cell death for which the term necrosis is widely used, although in another paradigm necrosis refers to the final morphologic appearance of a dead cell, rather than to a mechanism or process of cell death 27.

Currently, the field of apoptotic research is broad and one of the most popular in modern biology. Besides its pivotal role in organogenesis and development, apoptosis is now implicated in a wide variety of diseases, including cancer, autoimmune diseases, neurodegenerative disorders and numerous infectious diseases 31. Data accumulating over the past years has shown that unbalanced apoptosis, in regard to the extent and/ or timing, forms the basis of many pathogenic processes. Intervention in the genetic
mechanism and regulatory proteins of apoptosis may therefore provide new treatments for disease. 

2. ALI/ARDS

2.1. Incidence and mortality
Approximately 6% of adult patients admitted to the ICU in Western countries develops ARDS, and this is associated with a mortality rate as high as 40-55% 15;33. To grasp the impact of such numbers: it is estimated that in the United States annually more than 190,000 patients develop ALI/ARDS, of whom approximately 75,000 die 34. In comparison, studies have reported less than 3% of all children admitted to the pediatric ICU (PICU) develops ARDS, being fatal in up to 35% of the cases 16;17;35. Interestingly, studies among both adult 34 and pediatric 17 ALI/ARDS patients seem to confirm this positive correlation between an adverse outcome and age.

2.2. Clinical disorders
ALI/ARDS can develop following a wide variety of clinical disorders with direct or indirect lung injury, including sepsis, pneumonia, aspiration, trauma, transfusion, drowning and burns. Bacterial pneumonia and sepsis are among the most common etiologies of ALI/ARDS in both adults and children, and form an important risk factor for death 6;35. However, specific viral pathogens, such as RSV, induce ALI/ARDS in young children in recurrent seasonal outbreaks, but are associated with low mortality rates upon supportive treatment 16;17;36;37. Other risk factors for death in pediatric patients include multiple organ failure, dysfunction of the central nervous system and pre-existing immune disorders 16;17;35.

2.3. Treatment
Up to date, mechanical ventilation and supplemental oxygen remain the cornerstone treatments for ALI/ARDS in adults and children. Experimental treatments include the use of corticosteroids 38, prone positioning 39;40, high PEEP 41, high frequency ventilation 42, exogenous surfactant 43;44, nitric oxide inhalation 45, recruitment manoeuvres ('open lung ventilation') 46, and restrictive fluid regimes 47. Although several of these studies identified patient subgroups that may benefit from such strategies, so far, these have failed to show consistent improvement in primary end points such as mortality in randomized controlled trials 48-50. An exception to this was the 1996-1999 NIH ARDS Network ‘ARMA’ study which showed that mechanical ventilation with low tidal volume (6 ml/kg) as compared with high tidal volume (12 ml/kg) increased the survival of ALI/ARDS patients: 31% vs. 40% mortality respectively 51. This study provided a 'simple', beneficial and clinically relevant treatment strategy for ALI/ARDS patients which could be generally applicable by physicians...
at ICUs worldwide. However, at the same time it also highlighted the potential detrimental (iatrogenic) effects of ICU treatment on the course of ALI/ARDS, as it further proved the clinical relevance of the ventilator induced lung injury (VILI) concept, which refers to lung damage by direct physical forces as well as biological (pro-inflammatory) mediators caused by mechanical ventilation 52-54.

Remarkably, despite the ‘success story’ of the ARMA trial, Phua et al. recently reported a systematic review showing no decrease in mortality in ALI/ARDS patients over the last 10 years 15. One of the potential problems in finding effective treatment may be the relative high heterogeneity of ALI/ARDS patients with respect to etiology and underlying disorders. Furthermore, because the diagnosis of ALI/ARDS is entirely based on a set of non-specific clinical parameters, there may be a poor correlation with histopathological findings which is a potential confounding factor in treatment trials 55.

2.4. Pathogenesis

Given the lack of specific treatment and high mortality in ALI/ARDS, more insight into pathogenesis is urgently needed. Here, I will present two main pathogenic theories, the ‘neutrophil hypothesis’ and the ‘epithelial cell hypothesis’. Although the central events in these hypotheses may show considerable overlap and interaction in vivo, they help to form a conceptual framework for this thesis. It is important to note that there may be several other mechanisms that are important in ALI/ARDS pathophysiology 6, but their discussion is beyond the scope of this thesis.

In the ‘neutrophil hypothesis’ it is proposed that lung tissue damage occurs secondary to the extensive influx and activation of polymorphonuclear (PMN) neutrophils releasing a number of potentially harmful reactive oxygen- and nitrogen species and proteolytic enzymes, such as elastase, cathepsin G, and metalloproteinases 56-58. In the acute phase of ALI/ARDS neutrophils adhere to the lung capillary endothelium and migrate through the interstitium into the alveolar spaces. Studies in humans with ALI/ARDS have shown high PMN counts in bronchoalveolar lavage fluid (BALF), and marked neutrophilic accumulation in lung tissue potentially due to delayed apoptosis 8,9,59. The recruitment and activation of neutrophils is in part mediated by a number of (chemotactic) cytokines such as interleukin (IL)-1, IL-6, IL-8 and TNFα, which are released locally by macrophages and epithelial cells 6,60. Although a well orchestrated neutrophil response in the lungs is critical in the host defense against microorganisms, it is thought that dysregulation leading to enhanced neutrophil influx and activation and delayed apoptosis contributes to the tissue injury in ALI/ARDS 61. Animal models for endotoxemia- and aspiration-induced ALI/ARDS, have shown that PMN depletion or inhibition of chemotactic signaling diminishes lung inflammation and permeability 62-67. Yet in humans with normal lungs recruitment of PMNs by leukotriene B4 does not alter the lung permeability, and ALI/ARDS can develop in adults and children with profound neutropenia 68-70. Together these data suggest that
the neutrophilic response plays an important role in the development of ALI/ARDS, but that at the same time other pathogenic mechanisms can be involved as well.

The ‘epithelial cell hypothesis’ proposes that enhanced lung epithelial cell death is a key initial event in the development of ALI/ARDS. Throughout the lungs and airways a single layer of epithelial cells lines the surface between the human body and the outside environment. The epithelium acts as a physical barrier to the entry of foreign antigens or pathogenic microbes and viruses, and functions as a major source of inflammatory mediators essential for host defense. In addition, in the airways pseudostratified epithelium produces mucociliary transport which clears debris from the lumen, while in the alveoli the epithelium ensures gas-exchange (type I cell) together with surfactant production and ion transport (type II cell). Under normal conditions the permeability of the tight alveolar epithelium is low, in particular in comparison to the capillary endothelial barrier, thereby preventing plasma proteins and liquid from entering the alveolar and airspaces. Epithelial damage is a hallmark of ALI/ARDS, and given the above mentioned diverse functions of the lung epithelium it is not surprising that injury to this cell layer can sink the patient into a spiral of negative events: epithelial injury increases lung permeability resulting in the formation of protein-rich edema in the alveolar spaces leading to impaired gas-exchange; disrupted ion-fluid transport in injured epithelial cells may impair the re-absorption of the edema; both decreased production and edema-induced inactivation of surfactant proteins and lipids further alters lung physical properties such as enhanced surface tension leading to enhanced respiratory effort; at the same time, lung pathogens or locally produced inflammatory mediators may spill over to the circulation resulting in sepsis/systemic pro-inflammatory responses (decompartmentalization) and multiple organ failure.

3. THESIS

A key focus point of the above-mentioned ‘epithelial cell hypothesis’ is the activation of specific pro-apoptotic pathways in or directed against the lung epithelial cell. Early descriptive studies in humans with ALI/ARDS have shown characteristic apoptotic changes in alveolar epithelial cells, and this has led to the search of potential pro-apoptotic (soluble) mediators in the lungs, ultimately to find new therapeutic targets. The overall goal of the present dissertation is to investigate the role of several classical pro-apoptotic pathways in the development of lung epithelial injury, with special focus on pediatric ALI/ARDS, including severe RSV disease.

3.1. Outline

Chapter 1 reviews the current literature on the role of lung epithelial cell apoptosis in (pediatric) ALI/ARDS. We give an overview of several classical pro-apoptotic pathways...
implicated in the ‘epithelial cell hypothesis’. Furthermore, we discuss our hypothesis that the ongoing lung development and maturation in young children and infants may affect the susceptibility to apoptosis in ALI/ARDS and vice versa. In chapter 2 we investigate a classical apoptosis marker in lung tissues of pediatric patients who died with ARDS.

In chapter 3-6 we investigate the role of three classical extrinsic pro-apoptotic pathways:
- Chapter 3 describes the interplay between the ‘neutrophil hypothesis’ and the ‘epithelial cell hypothesis’ mediated by the Fas (CD95) death receptor/FasLigand (FasL) system in mice.
- Chapter 4 describes the role of the tumor necrosis factor related apoptosis-inducing ligand (TRAIL) death receptor pathway in the ‘epithelial cell hypothesis’ in children with severe RSV disease.
- Chapter 5 and 6 describe the role of the granzyme pathway in the ‘neutrophil hypothesis’ and the ‘epithelial cell hypothesis’ in severe RSV disease in children and a mouse model.

Finally, as discussed above, ICU treatment may be an important co-factor in the development of ALI/ARDS. Therefore, in chapter 7 we investigate the role of mechanical ventilation in the ‘neutrophil hypothesis’ and the ‘epithelial cell hypothesis’ in severe RSV disease in a mouse model.
REFERENCES

(2) Petty TL. In the cards was ARDS: how we discovered the acute respiratory distress syndrome. Am J Respir Crit Care Med 2001;163:602-3.
(54) Webb H, Tierney DF. Experimental pulmonary edema due to intermittent positive pressure ventilation with high


