Review

Innate Immunity

16(3) (2010) 138–142

ISSN 1753-4259 (print)
10.1177/1753425910366879

The intersection of surfactant homeostasis and innate host defense of the lung: lessons from newborn infants

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The study of pulmonary surfactant, directed towards prevention and treatment of respiratory distress syndrome in preterm infants, led to the identification of novel proteins/genes that determine the synthesis, packaging, secretion, function, and catabolism of alveolar surfactant. The surfactant proteins, SP-A, SP-B, SP-C, and SP-D, and the surfactant lipid associated transporter, ABCA3, play critical roles in surfactant homeostasis. The study of their structure and function provided insight into a system that integrates the biophysical need to reduce surface tension in the alveoli and the innate host defenses required to maintain pulmonary structure and function after birth. Alveolar homeostasis depends on the intrinsic, multifunctional structures of the surfactant-associated proteins and the shared transcriptional regulatory modules that determine both the expression of genes involved in surfactant production as well as those critical for host defense. Identification of the surfactant proteins and the elucidation of the genetic networks regulating alveolar homeostasis have provided the basis for understanding and diagnosing rare and common pulmonary disorders, including respiratory distress syndrome, inherited disorders of surfactant homeostasis, and pulmonary alveolar proteinosis.

Keywords: pulmonary surfactant, surfactant structure and function, pathogen-associated molecular pattern molecules, surfactant proteins, respiratory distress syndrome

INTRODUCTION

The technical and conceptual revolution in biology occurring during the last 30 years profoundly impacts our present understanding of cell and organ function. Training in the pre-/perigenetic phase of 20th century medicine, I could not have anticipated the scientific advances that provide the framework for contemporary advances in biology and medicine. My personal journey began with two initial steps. The first was taken during clinical training in newborn medicine where I experienced our inability to treat respiratory distress syndrome (RDS) in preterm infants, *circa* 1970. The second, a transformative step, was in recognizing and embracing the opportunities provided by advances in genetics, cellular and molecular biology that were to change science and medicine profoundly during the 1980s. The exponential growth of scientific knowledge and technology has made this a unique time to be engaged in both medicine and research related to lung function and neonatal medicine. In newborn intensive care units, advances in resuscitation, obstetrics, neonatal physiology, and nutrition made urgent the need to provide safe and effective ventilation for the treatment complications of prematurity related to respiratory distress syndrome. In parallel, the molecular dissection of the surfactant lipids, proteins, and genes that occurred during this time provided much of the conceptual framework upon which our present understanding of lung maturation and alveolar function is based.

Received 26 January 2010; Revised 17 February 2010; Accepted 23 February 2010

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Co-evolution of the surfactant system and innate defense

Transition to air-breathing at birth presents fundamental physical challenges required for postnatal survival, including the need: (i) for pulmonary surfactant as a biophysical solution for the physical requirement to reduce surface tension at the air–liquid interface; (ii) to protect the lung from environmental exposures to microbial pathogens, particles, and toxicants; and (iii) to minimize inflammation and remodeling to maintain lung structure upon which ventilation is dependent. Now, with hindsight, it is perhaps not surprising that many of the genes and proteins that have evolved to mediate surfactant homeostasis also have important roles in innate host defense of the lung. The interactions between surfactant proteins and lipids create diverse structures within alveolar cells and in the alveoli (*e.g.* lamellar bodies, tubular myelin and multilayers) that maintain surfactant function. The surfactant associated proteins, SP-A, SP-B, SP-C, and SP-D, known to regulate either the structure/function or metabolism of surfactant lipids, consist of molecules with structural domains that provide discrete functions that also serve distinct roles in innate host defense.[1–3] Surfactant protein-A and SP-D act as both pathogen-associated molecular pattern recognition molecules (PAMPs) and as signaling molecules that interact with various constituents of the innate host defense system, *e.g.* CD14, Toll-like receptors, calreticulin, SIRP1α, SP210, and others on the surface of cells, as reviewed by Kuroki *et al.*[3] While playing a critical role for spreading and stabilization of surfactant lipids,[4] both proSP-B and SP-C also have potential host defense functions. The amino-terminal domain of proSP-B binds bacterial pathogens and enhances their killing within the phagolysosome of alveolar macrophages.[5] Surfactant protein-C directly binds lipopolysaccharide (LPS) and enhances *Pseudomonas* clearance from the lung in experimental mice.[6,7] Each surfactant protein interacts with a broad spectrum of pathogens or pathogen-associated cell-surface molecules (for example, endotoxins, viral and fungal surfaces) via distinct domains that bind and/or aggregate particles, enhancing the opsonization and killing of microbial pathogens by the alveolar macrophages or other inflammatory cells. Each surfactant protein has distinct roles in surfactant homeostasis and innate defense. The surfactant proteins generally enhance antimicrobial functions of alveolar macrophages, facilitating clearance of pathogens, and/or minimizing inflammation and the subsequent alveolar remodeling and fibrosis that would impair lung function. In general, the actions of the innate host defense system in the lung serve to facilitate resolution of lung injury without resultant disruption of lung structure and function.

The intersection between pulmonary mechanics, surfactant and the innate host defense system of the lung provides insight into the integration of seemly diverse biological processes that are critical for pulmonary homeostasis. Mechanisms by which biomechanical or structural stimuli are transduced into cellular signals to regulate surfactant homeostasis under physiological conditions remain unknown. Excessive stretch as in 'volutrauma', and likewise atelectasis-associated trauma, cause cellular injury,[8] inflammation, and alveolar capillary leak that inhibits surfactant activity (Fig. 1). The close relationships among surfactant homeostasis, inflammation, and innate defense are also evident in the intrinsic structures of the surfactant proteins that interact with lipids, pulmonary pathogens, and inflammatory cells. The finding that genes mediating surfactant homeostasis (SP-A, SP-B, SP-C, SP-D, and ABCA3) and innate host defense proteins (*e.g.* lysozymes, paraoxanase, and Clara secretory protein) share common *cis*-acting transcription factor binding sites indicates the conservation of genetic networks regulating both processes in the lung.

Surfactant and innate defense systems in the lung share common transcriptional regulators

The identification of the genes encoding the surfactant proteins provided the molecular tools that began to open the gates required to enter into the study of a number of fascinating biological questions regarding the evolution of the vertebrate lung and the complex processes involved in lung organogenesis and maturation. How is the lung different from other organs such as other

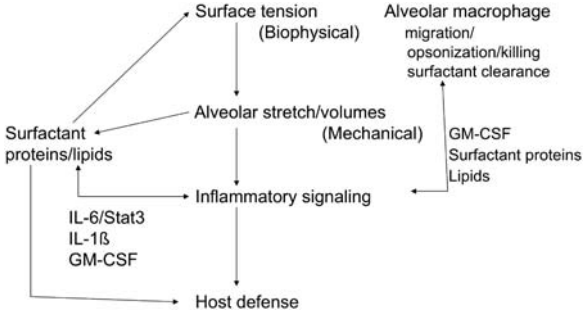

Fig. 1. The integration of surfactant function and innate immunity. Surfactant lipids and proteins interact to reduce surface tension at the air–liquid interface thereby influencing lung mechanics. Atelectasis and volutrauma are sensed by pulmonary cells with resultant production of inflammatory mediators that, in turn, regulate surfactant production, clearance, and function. The surfactant proteins modulate the activities of alveolar macrophages, influencing innate host defense and surfactant catabolism. Surfactant proteins themselves contain discrete domains that interact with pathogens as pathogen-associated pattern recognition molecules (PAMPs) to enhance antimicrobial activity in the lung. How these multiple regulatory modules are integrated to control surfactant homeostasis, host defense, and inflammation precisely, upon which lung function depends, remains a mystery.

endodermally derived tissues like thyroid, pancreas or gastrointestinal tract? What processes underlie branching morphogenesis, pulmonary vasculogenesis, mucociliary clearance or fluid homeostasis before and after birth? What processes determine sacculation and alveolarization, and differentiation of the peripheral lung required for ventilation at birth? Remarkably, the 'upstream' transcriptional regulators of lung morphogenesis, for example, thyroid transcription factor-1 (TTF-1 or Nkx2.1), the forkhead ortholog A2 (FOXA2), glucocorticoid receptor (NR3C1), and GATA6), are transcription factors regulating both lung formation, perinatal lung maturation and the expression of genes critical for surfactant homeostasis and innate host defense (for reviews, see Maeda *et al*.[9] and Morrisey and Hogan;[10] Fig. 2). Surfactant content, structure and function are maintained throughout life, implying the need to have in place regulatory mechanisms/systems, both at the transcriptional and post-transcriptional levels, capable of integrating changes in ventilation/stretch with: (i) surface pressure; (ii) surfactant lipid and protein production; and (iii) inflammation. In hindsight, these sensing systems must be regulated at multiple levels to maintain surfactant function at birth and thereafter. The molecular and functional 'sensors' that integrate transcriptional or post-transcriptional regulation of the production of surfactant lipids and proteins, their processing, packaging, secretion, clearance, and catabolism remain only partially understood. At the most naive level, we have not yet identified 'a man with an oil dipstick' measuring surfactant content or function in the alveolus, that, in turn, instructs all of the components of the homeostatic machinery to precisely regulate surfactant levels before and after birth or after lung injury.

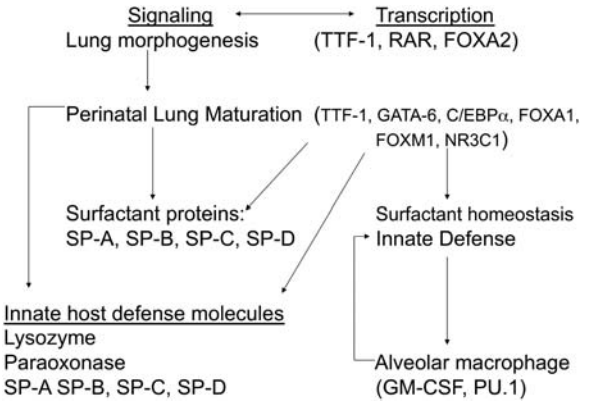

Fig. 2. Regulation of lung morphogenesis, differentiation, surfactant homeostasis, and innate defense. Shown is a simplified schematic showing the co-regulation of lung formation, surfactant homeostasis. and innate host defense. Transcriptional regulators of lung morphogenesis and differentiation (*e.g*. TTF-1, RAR, FOXA2, *etc*.) play critical roles in endoderm patterning and lung-branching morphogenesis, the perinatal differentiation of the alveolar epithelium (C/EBPα, NR3C1 (glucocorticoid receptor), FOXA2, and GATA6), as well as in the control of expression of genes critical for surfactant homeostasis and innate defense. Surfactant proteins interact with microbial pathogens and alveolar macrophages to enhance their clearance from the lung. Alveolar macrophages also play critical roles in surfactant catabolism in a process requiring GM-CSF that regulates alveolar macrophage differentiation and function via the transcription factor PU.1. Alveolar macrophages and other pulmonary cells release various cytokines that may regulate surfactant production in the alveolar epithelium (*e.g*. TNF-α, IL-1β, IL-6/Stat3), and also recruit and activate inflammatory cells.[30–32]

Mutations in genes controlling surfactant homeostasis cause lung disease

The study of pulmonary surfactant in newborn infants has provided the reagents needed to identify previously unexplained 'idiopathic' lung disorders, as reviewed elsewhere.[11–13] A number of human diseases related to mutations in genes encoding the surfactant proteins or involved in surfactant homeostasis have been identified during the last two decades. Mutations in *SFTPB*, *SFTPC*, *ABCA3*, and *SFTPA* cause severe alveolar disease in newborn infants and in older individuals.[12–15] Mutations in *SFTPB* and *ABCA3* profoundly disrupt surfactant production and packaging, usually presenting as respiratory failure in term newborn infants.[13,14] In contrast, mutations in *SFTPC* and *SFTPA* generally present later in infancy or in adulthood.[12,15] Disruption of surfactant homeostasis related to the lack of expression of critical proteins (SFTPB and ABCA3) or to cell injury, in some cases related to the misfolding of surfactant protein (*e.g*. proSP-C) may contribute to lung injury, atelectasis, inflammation and tissue remodeling. Mutations in TTF-1, a critical transcriptional regulator of surfactant protein expression and respiratory epithelial cell maturation, cause pulmonary disease of varying severity often associated with central nervous system and thyroid dysfunction.[16] Together, these inborn errors of surfactant metabolism present as rare disorders previously termed chronic pneumonitis of infancy (CPI), non-specific interstitial pneumonitis (NSIP), and idiopathic pulmonary fibrosis (IPF), the clinical findings being dependent on age at presentation and clinical setting.[12,15] In these disorders, surfactant dysfunction and alveolar type II cell injury are associated with inflammation and the activation of alveolar macrophages with resultant tissue remodeling, contributing to respiratory failure.

Unexpected insight into the pathogenesis of pulmonary alveolar proteinosis

The remarkable intersection between innate immunity and the surfactant system is further exemplified by the

relationships between alveolar macrophages, GM-CSF signaling, and the catabolism of alveolar surfactant that are now known to underlie the pathogenesis of pulmonary alveolar proteinosis (PAP). These unexpected findings were derived from the serendipitous discovery that mice lacking GM-CSF (produced by gene targeting in transgenic mice) develop PAP in association with the accumulation of surfactant lipids and proteins that was found to be caused by a failure of alveolar macrophage maturation upon which surfactant clearance and catabolism are dependent, as reviewed elsewhere.[17–19] Defects in GM-CSF signaling caused by auto-antibodies underlie the pathogenesis of PAP in most adult patients.[20] Likewise, mutations in the GM-CSF signaling caused by mutations in the gene encoding the GM-CSF receptor (*CFS2RA*) have been associated with early-onset PAP in experimental animals and in patients.[21,22] Not surprisingly, the clearance and killing of certain microbial pathogens by alveolar macrophages are also dependent upon GM-CSF signaling, GM-CSF expression being induced by various microbial pathogens in turn, activating alveolar macrophage function.[23,24] The findings that treatment of the mice lacking GM-CSF with recombinant GM-CSF or treatment of mice lacking the common β-chain of the GM-CSF receptor with complementing bone marrow corrected PAP in the mouse models, provides the basis for the development of new therapies for patients with PAP that are currently being explored in the clinic.

Implications for the diagnosis and therapy of pulmonary disease

Understanding the relationships among biomechanics of ventilation, surfactant homeostasis, inflammation, and host defense offers a multitude of opportunities to diagnose and treat pulmonary disorders. Surfactant lipids and proteins have long provided biomarkers indicating pulmonary maturation before birth and susceptibility to lung diseases in both pediatric and adult patients. The recognition that volume-related trauma, occurring during mechanical ventilation, causes inflammation and tissue injury, provided the rationale for successful trials to minimize lung injury during the treatment of acute respiratory distress syndromes (ARDS) and the development of resuscitation and ventilatory strategies to protect the preterm lung from barotrauma.[25,26] Surfactant replacement therapies minimize alveolar injury caused by low and high volume ventilation, reducing alveolar capillary leak and surfactant inhibition.

Antibodies or ELISAs generated against the surfactant proteins have been useful in immunochemistry for the diagnosis of hereditary surfactant disorders and the assessment of prognosis and responses to therapy in diverse lung disorders. Quantitation of serum levels of surfactant proteins (*e.g.* SP-D) have also been used in the evaluation of PAP and interstitial lung diseases. Antibodies against TTF-1, an important transcriptional regulator of the surfactant protein genes, are routinely used to identify adenocarcinomas derived from the lung. Understanding the roles of surfactant lipids and proteins that contribute to surface tension reduction led to remarkable advances in the treatment of respiratory distress syndrome (RDS) in preterm infants, transforming the care of premature infants with lung disease. Surfactant-like peptides based on the structures of SP-B or SP-C have been used to produce various synthetic surfactants that are being tested in the treatment of ARDS.[27] Recent studies in preterm lambs also support the potential utility of adding surfactant protein D to surfactant replacement that may protect surfactant activity, inhibit ventilation-induced inflammation during resuscitation or enhance innate immunity.[28,29] The remarkable ability of surfactant to spread rapidly throughout the lung also provides a strong rationale for its use for the delivery of therapeutic molecules, genes, siRNAs, proteins, drugs for future therapies of both pulmonary and non-pulmonary disorders in the future.

Conclusions

In reflection, the most rewarding aspects of my past 30 years in clinical neonatology and the study of pulmonary biology has been witnessing and participating in: (i) the remarkable improvement in clinical outcomes for premature infants; (ii) the generation of new knowledge that has transformed our appreciation for the mysteries of lung formation, function, and pulmonary medicine as they rapidly unfold; and (iii) the daily interactions with students, trainees, co-workers, and colleagues here in Cincinnati, nationally and internationally. Finally, we are in the early days in understanding lung structure, function, surfactant homeostasis, innate host defense, and repair. The opportunities for new discoveries that will transform our understanding of lung biology and lead to improved clinical care are limitless.

Acknowledgements

The author acknowledges support from the National Institutes of Health throughout my career, presently HL085610, HL090156, HL095580, the March of Dimes, and interactions with co-workers in Neonatology, Perinatal and Pulmonary Biology at CCHMC.

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