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Pulmonary surfactant: a front line of lung host defense

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The lung is a uniquely vulnerable organ. Residing at the interface of the body and the environment, the lung is optimized for gas exchange, having a very thin, delicate epithelium, abundant blood flow, and a vast surface area. Inherent in this structure is an enormous immunological burden from pathogens, allergens, and pollutants resident in the 11,000 liters of air inhaled daily. Fortunately, protective immune mechanisms act locally in the lung to facilitate clearance of inhaled pathogens and to modulate inflammatory responses. These defensive mechanisms include both innate (nonantibody-mediated) and adaptive (antibody-mediated) systems. The purpose of this commentary is to review briefly the functions of one unique lung innate immune system, pulmonary surfactant, and to highlight the recent findings of Wu et al. (1) described in this issue of the *JCI*. Wu and colleagues report a new and intriguing innate immune function of surfactant: direct antimicrobial activity.

Pulmonary surfactant and lung host defense

Pulmonary surfactant is a lipoprotein complex that is synthesized and secret-

ed by the alveolar type II epithelial cell and the airway Clara cell into the thin liquid layer that lines the epithelium (reviewed in ref. 2). Once in the extracellular space, surfactant carries out two distinct functions. First, it reduces surface tension at the air-liquid interface of the lung, a function that requires an appropriate mix of surfactant lipids and the hydrophobic proteins, surfactant protein B (SP-B) and SP-C (3). Second, surfactant plays a role in host defense against infection and inflammation (4). Two of the surfactant proteins,

SP-A and SP-D, are members of the collectin protein family (5, 6), which includes the liver-derived serum manose binding lectin. Collectins have in common an N-terminal collagen-like region and a C-terminal lectin domain that binds carbohydrates in a calcium-dependent manner (Figure 1). The C-type lectin domains are arrayed with spatial orientation (7) that confers unique carbohydrate specificities, and their preferential binding sites are nonhost oligosaccharides, such as those found on bacterial and viral surfaces (8).

The most well-defined function of the collectins is their ability to opsonize pathogens, including bacteria and viruses, and to facilitate phagocytosis by innate immune cells such as macrophages and monocytes. SP-A and SP-D also regulate production of inflammatory mediators (reviewed in ref. 4). Mice made deficient in SP-A or SP-D by homologous recombination have an enhanced susceptibility to infection and inflammation induced by intratracheal

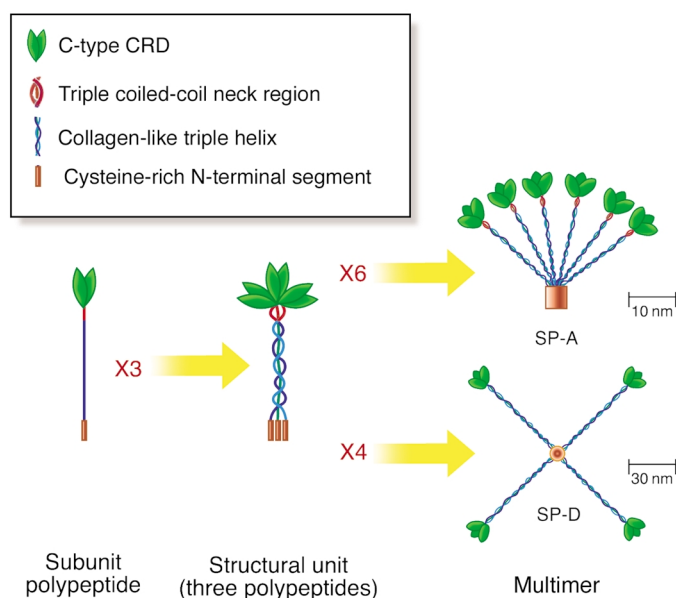


Figure 1

SP-A and SP-D are members of the collectin family of oligomeric proteins, which have collagen-like N-terminal regions and C-type carbohydrate recognition domains (CRDs). The CRDs bind carbohydrates such as those found on pathogen surfaces. SP-A consists of 6 structural units, which are assembled into a “flower bouquet” formation. SP-D consists of the tetrameric structural units assembled into an X-like structure. Figure adapted from an online article by N. Kawasaki that appears on Glycoforum (<http://www.glycoforum.gr.jp/science/word/lectin/LEA06E.html>).

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Nonstandard abbreviations used: surfactant protein (SP); acute respiratory distress syndrome (ARDS).

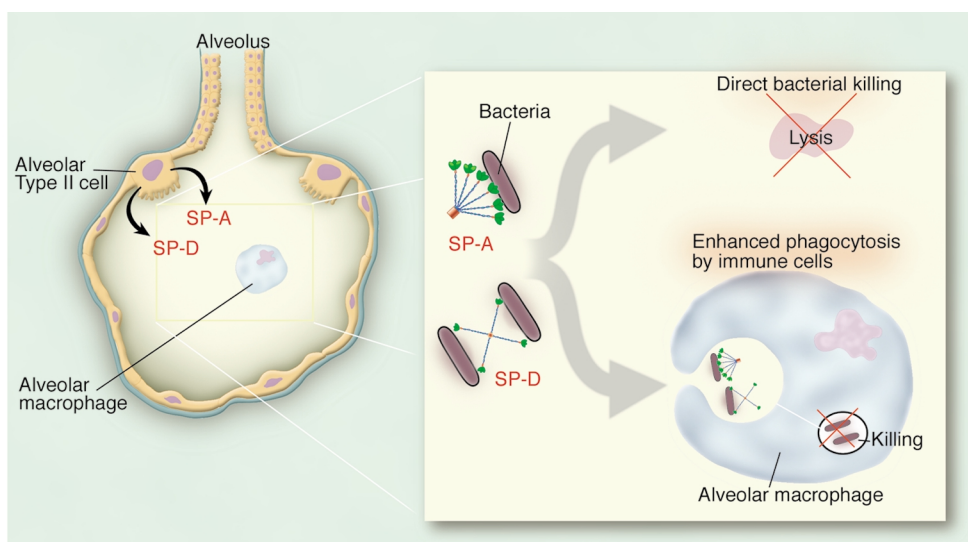


Figure 2

SP-A and SP-D enhance bacterial clearance and inhibit bacterial growth. SP-A and SP-D are oligomeric proteins synthesized by type II pulmonary epithelial cells and secreted in the liquid that covers the lung epithelium. Both SP-A and SP-D opsonize pathogens and enhance their phagocytosis by innate immune cells such as alveolar macrophages and neutrophils. In this issue of the *JCI*, Wu and coworkers provide compelling evidence that SP-A and SP-D also are directly bactericidal (1); they damage the bacterial cell membrane and inhibit bacterial growth. Thus, SP-A and SP-D enhance bacterial clearance via enhancing phagocytosis and via direct antimicrobial effects on bacteria. Images are not to scale.

administration of pathogens, including Group B *Streptococcus*, *Pseudomonas aeruginosa*, respiratory syncytial virus, *Haemophilus influenzae*, and inflammatory agents such as LPS (reviewed in ref. 9). Deficiencies in mannose-binding lectin have been characterized in humans and are associated with increased susceptibility to infection and autoimmune disease (10).

Newly defined antibacterial functions of surfactant

Data presented in the article by Wu and coworkers in this issue of the *JCI* (1) show convincingly that, in addition to facilitating pathogen uptake and killing by immune cells, SP-A and SP-D are directly antimicrobial, that is, they kill bacteria in the absence of immune effector cells (Figure 2). This conclusion is greatly strengthened by the multiple experimental approaches employed in the study. For example, Wu et al. demonstrate that exposure of *Escherichia coli* to SP-A and SP-D enhanced nuclear staining with propidium iodide, increased permeability to the antibiotic actinomycin D, and augmented release of proteins from the bacteria. Interestingly, inhibition of microbial

growth was at least partly independent of collectin-mediated aggregation of bacteria and appears to involve damage to the bacterial cell membrane by the C-type lectin domains. Although SP-A and SP-D inhibited bacterial growth of a number of laboratory and clinical isolates, the factors that determine their specificity and the mechanism by which they increase membrane permeability are not known and will be important future avenues of investigation.

Future directions: surfactant therapy for infectious and inflammatory lung diseases?

Deficiencies of surfactant have been associated with a variety of lung diseases in both adults and infants. For example, infants born before their lungs have matured sufficiently suffer from respiratory distress syndrome due to the inability of their immature type II cells to synthesize adequate amounts of functional surfactant. Treatment with surfactant replacement therapies that include lipids and SP-B and/or SP-C have been highly efficacious in improving lung function in preterm newborns (11).

Surfactant inactivation and deficiencies have also been associated with a

variety of adult lung diseases including pneumonia, asthma, and acute respiratory distress syndrome (ARDS) (reviewed in refs. 12, 13). Clinical trials have been undertaken for treatment of ARDS with surfactant replacement therapies containing either lipids or lipids plus SP-B and/or SP-C (14). However, these treatments have not been as effective in treating adult ARDS compared to infant respiratory distress syndrome. The new data presented by Wu and coworkers showing that SP-A and SP-D have direct bactericidal activity, in addition to their well-described opsonic activity and ability to regulate inflammatory mediator production, suggest that supplementation of the lipid-based therapies with SP-A and/or SP-D would further enhance their ability to treat infectious and inflammatory lung diseases (1). Importantly, recent studies have demonstrated that these proteins are expressed at extrapulmonary sites (15–17), raising the intriguing possibility that they may be efficacious for treatment of inflammatory and infectious diseases in other organs as well.

Acknowledgments

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The mitochondriotoxic domain of Vpr determines HIV-1 virulence

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Most patients infected with HIV-1 develop AIDS unless they receive antiretroviral medication. However, a small number of HIV-infected individuals with high viral titers remain disease free and do not experience progressive immunosuppression, even in the absence of therapy. Such individuals are labeled long-term nonprogressors (LTNPs) and are characterized by a series of laboratory parameters that are usually compromised in HIV-1 carriers who ultimately develop AIDS. In particular, LTNPs possess a high frequency of peripheral CD4⁺

T cells, as well as a low level of spontaneous apoptosis, correlating with a normal mitochondrial transmembrane potential ($\Delta\Psi_m$) among circulating T cells. It has long been assumed that, as an *experimentum naturae*, LTNPs might furnish valuable clues for the identification of molecular determinants of HIV-1 pathogenesis. A study by Badley and colleagues involving LTNPs, reported in this issue of the *JCI* (1), strongly suggests that viral protein R (Vpr) is a major HIV-1 virulence factor.

Vpr – a cytotoxic protein encoded by HIV-1

The HIV-1 genome encodes structural and enzymatic proteins common to all retroviruses, but also some accessory proteins that are not always required for the replication of HIV-1. One of these accessory proteins, Vpr, is found in virions, HIV-1-infected cells, and in the serum and cerebrospinal fluid of HIV-1 carriers. Vpr is a small (96 amino acids) soluble protein composed of three α -helical domains (Figure 1a). Vpr is dispensable for viral replication

in T lymphocytes, but not in monocytes, and thus is rapidly lost among laboratory HIV-1 isolates. In contrast, Vpr is maintained in most HIV-1-infected patients, indicating that this protein may be important for the *in vivo* biology of HIV-1. *In vitro*, Vpr reduces the proliferation of CD4⁺ lymphocytes and of various other cell types via a G² cell cycle arrest. Moreover, Vpr induces cell death through the intrinsic pathway of apoptosis, which involves mitochondrial membrane permeabilization (MMP), the release of cytochrome *c* from mitochondria, and the cytochrome *c*-dependent activation of caspases (2, 3).

A fraction of Vpr transfected into cells can be found in the mitochondrial compartment (2). When added to purified mitochondria, recombinant or synthetic Vpr crosses the outer membrane through the voltage-dependent anion channel. It then interacts with the adenine nucleotide translocator (ANT) in the inner mitochondrial membrane to form a composite ion channel. This channel dissipates the $\Delta\Psi_m$ and thus favors MMP and subsequent apoptosis (3). The physical interaction between Vpr and ANT has been determined by coimmunoprecipitation, electrophysiological measurements, and surface plasmon resonance (3). Vpr fails to kill ANT-deficient cells (3), which suggests that the Vpr/ANT interaction is central to the apoptosis-inducing properties of Vpr.

Vpr is mutated in LTNPs

In this issue of the *JCI*, Badley and colleagues (1) report that 80% of LTNPs manifest a point mutation in Vpr

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Nonstandard abbreviations used: long-term nonprogressive/nonprogressor (LTNP); mitochondrial transmembrane potential ($\Delta\Psi_m$); viral protein R (Vpr); mitochondrial membrane permeabilization (MMP); adenine nucleotide translocator (ANT).