Surfactant phospholipids and proteins in lung defence

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COLLECTINS AND INNATE IMMUNITY

Pulmonary surfactant comprises two hydrophobic proteins SP-B and SP-C, which are important for the adsorption and spreading of the surfactant film at the air-liquid interface[1]. Besides the hydrophobic proteins two other surfactant proteins have been described: SP-A and SP-D. These proteins are members of a family of collagenous carbohydrate binding proteins (collagenous C-type lectins), now commonly known as collectins. Collectins consist of oligomers of trimeric subunits. Each monomer consists of a carboxy-terminal C-type lectin domain (CLD), bound via a short neck region to a collagen-like domain, and a cysteine-containing amino terminal cross-linking domain. Collectins are examples of pattern recognition molecules of the innate immune system.

The adaptive immune system is most effective when the host has had prior exposure to the pathogen. However, the clonal expansion of B and T lymphocytes necessary for full scale cell mediating humoral immune defences generally require 24-72 h to develop. Accruing evidence suggests that this temporal gap in host immunity is filled by cells and molecules of the innate immune system, such as macrophages and the collectins SP-A and SP-D. These collectins may also have an important protective function during infancy when the adaptive immune system is still developing. SP-A, SP-D, and molecules derived from these collectins might be good candidates to be used as drugs to prevent or treat lung infections, because of their ability to interact with a wide variety of micro-organisms and to regulate the inflammatory response. Several functions of SP-A and SP-D and in particular those that we have studied in our laboratory will be discussed below.

PRO-INFLAMMATORY AND ANTI-INFLAMMATORY EFFECTS OF SP-A

SP-A may regulate the adaptive immune system by dampening the inflammatory response in the lungs in order to prevent damage of the delicate respiratory epithelium. Whether the effects of SP-A, that are observed experimentally *in vitro* and *in vivo*, are pro-inflammatory or anti-inflammatory may depend on the type of pathogen or insult, the number of pathogens that are used for inoculation, the type of target cells, activation state of these cells, lipids, and source or structure of SP-A. Small amounts of pathogens that are inhaled could be cleared without an inflammatory response whereas infection with vast amounts of pathogens would require an inflammatory response. The tuning of SP-A and the (lipids of the) surfactant system to an anti-inflammatory or pro-inflammatory mode may depend on the number of pathogens inhaled. Thus, small numbers of pathogens that are encountered in everyday life are cleared without eliciting an inflammatory response.

INTERACTIONS OF SP-D WITH PATHOGENS:

Candia albicans

Collectins may play a role in the first-line immunity against *Candida albicans.* SP-D was found to bind *C albicans*, resulting in agglutination of the microorganisms. Incubation of *C albicans* with SP-D resulted in profound fungal growth inhibition and decreased hyphal outgrowth[2]. This observation was the first indication that surfactant proteins could affect microbial growth directly. In addition, it was found that SP-D inhibited phagocytosis of *C albicans* by alveolar

macrophages, probably due to the formation of large aggregates of the fungus. These data suggest that SP-D has an important role in the first-line defence against *C albicans* in the lung, agglutinating *C albicans* and limiting their growth, without the need for macrophage activation. In view of the presence of SP-D at extrapulmonary sites, SP-D may also have an important role in the protection of other tissues that may be colonised by this or other pathogens. Additional support for a direct effect of collectins was provided by the recent study by Wu *et al*[3]. These investigators showed that SP-A and SP-D inhibited growth of Gram-negative bacteria by mechanisms that led to an increased permeability of the microbial cell membrane.

INTERACTIONS OF SP-D WITH PATHOGENS: INFLUENZA A VIRUS

Influenza A virus (IAV) infections are a major cause of respiratory disease of humans and animals. Pigs can serve as important intermediate hosts for transmission of avian IAV strains to humans, and for the generation of reassortant strains which may result in the appearance of new pandemic IAV strains in humans. Binding of SP-D to influenza A virus (IAV) involves the interaction of the SP-D lectin domain with glycosylated proteins present in the viral envelope: haemagglutinin and neuraminidase. However, the interaction between SP-A and IAV involves the binding of the conserved sialylated N-linked oligosaccharide present in the CLD of SP-A to the sialic acid receptor present on the haemagglutinin of IAV. Recent investigations concerning the structure of SP-D from pig lung showed that, in contrast to SP-D from other species studied so far, porcine SP-D (pSP-D) attached to its CLD, a highly heterogeneous complex type oligosaccharide moiety that is sialylated[4,5].

Haemagglutination inhibition assays revealed that both porcine SP-A (pSP-A) and pSP-D displayed substantially greater inhibitory activity against IAV strains isolated from human, swine and horse, than lung collectins from other species. The more potent activity of pSP-D results from the additional sialylated N-linked oligosaccharide present in its CLD. The enhanced anti-influenza activity of pSP-D, as demonstrated by assays of viral aggregation, inhibition of infectivity, and neutrophil response to IAV, are also due to the extra oligosaccharide moiety[6]. The greater haemagglutination inhibitory activity of pSP-A is due to porcine-specific structural features of the conserved asparagine-linked oligosaccharide in the CLD of SP-A.

It may be concluded that the N-linked carbohydrate attached to the CLD contributes to the activity of the pSP-D, probably by providing an additional mechanism of attachment of the virus in addition to the usual mode of attachment of the lectin domain to virus-associated carbohydrates. Pigs are thought to exchange IAV strains with birds and humans and to serve as vessel for the mixing that leads to new strains that can cause human IAV pandemics. It is speculated that the distinct structural and functional properties of pSP-D (and of pSP-A) are related to the emergence in pigs of such new IAV strains.

SP-D IS A LIPOPOLYSACCHARIDE SCAVENGER

LPS is a component of the outer membrane of Gram-negative bacteria that activates immune cells to release a wide range of inflammatory mediators, which contribute to the pathogenesis of sepsis and acute respiratory distress syndrome (ARDS). The respiratory tract is continuously exposed to LPS from the cell wall of inhaled Gram-negative bacteria. For this reason the epithelial surface of the lung must have an efficient defence system to protect the gas-exchange function of the alveoli. Collectins may be part of this system. SP-A binds to rough LPS and lipid A *in vitro*. Both Ca^{2+}-independent and Ca^{2+}-dependent binding of SP-A to LPS has been reported. SP-D binds to core polysaccharides and/or O-specific antigens of LPS in a Ca^{2+} dependent fashion. SP-A and SP-D may be involved, in concert with other factors, in the neutralisation and clearance of inhaled free endotoxin without eliciting an inflammatory response. An established model of ARDS, induced by intratracheal LPS aerosolisation in rats[7], was used to investigate whether the lung collectin SP-D was able to bind and clear LPS in an acute phase reaction to intratracheally aerosolized LPS[8]. SP-D binds free LPS in the lung immediately after aerosolized LPS is inhaled. Electron microscopic analysis of alveolar macrophages in the broncho-alveolar lavage shows that immediately after aerosolisation of LPS, large aggregates of SP-D with LPS are formed that are phagocytosed by the alveolar macrophages. These results suggest that SP-D is secreted within minutes upon LPS inhalation and contributes to the clearance and subsequent inactivation of LPS that is inhaled or that is released at sites of colonisation by Gram-negative bacteria.

PRESENCE OF SP-D IN THE GASTRO-INTESTINAL TRACT

The presence and importance of surfactant proteins A and D was well established at the respiratory tract where both molecules were first discovered. However, we found, by *in situ* hybridisation, that SP-D is also expressed at extrapulmonary sites, which are in close contact with numerous potentially harmful micro-organisms (Herías, MV *et al*, unpublished data). This supports a more general role for this collectin in local innate host defence. Recent work in our laboratory is aimed to study localisation, regulation and function of collectins and other proteins of the innate mucosal immune system in the gastrointestinal tract. The ultimate goal is to investigate whether intestinal health of infants and newborn farm animals can be improved by (dietary) modulation of their innate intestinal immune status.

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