The potential of recombinant surfactant protein D therapy to reduce inflammation in neonatal chronic lung disease, cystic fibrosis, and emphysema

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By lowering surface tension at the air-water interface in the surfactant deficient premature lung, exogenous surfactant replacement therapy for neonatal respiratory distress syndrome has been highly successful in decreasing mortality after preterm birth. It has emerged in recent years that surfactant components not present in current surfactant formulations—particularly surfactant associated proteins A and D (SP-A and SP-D)—have additional roles in host defence distinct from the surface tension lowering effects of surfactant. SP-A and SP-D are calcium dependent carbohydrate binding proteins of the innate immune system important in the first line defence of the lung against microorganisms and in the control of lung inflammation. This review addresses the possibility that recently developed recombinant forms of SP-D could be useful therapeutically in attenuating inflammatory processes in neonatal chronic lung disease, cystic fibrosis, and emphysema.

Pulmonary surfactant is 90% lipid and 10% protein. There are four surfactant associated proteins: SP-A, SP-B, SP-C, and SP-D. The hydrophobic and lipophilic SP-B and SP-C are important for the surface tension lowering properties of surfactant, and congenital SP-B deficiency is incompatible with life. Artificial surfactants (for example, Exosurf, ALEC) do not contain SP-B and SP-C, but are present in the more effective natural surfactants such as Survanta. By contrast, the water soluble surfactant proteins, SP-A and SP-D, are lost in the process of surfactant extraction from animal lungs (bovine in the case of Survanta, porcine in the case of Curosurf) so that current surfactants in clinical use do not contain SP-A or SP-D. It has emerged recently that these proteins have important functions in pulmonary host defence and the control of lung inflammation, which raises the question of whether current surfactant therapies could be improved by supplementation with these natural surfactant components. Traditionally human SP-A and SP-D have been isolated from bronchoalveolar lavage of patients with alveolar proteinosis or from amniotic fluid, but yields (especially of SP-D) are not high from these sources and the protein exists in variable states of oligomerisation. Proteins from these natural sources would therefore not be very suitable as pharmaceutical agents because of their non-uniformity and difficulty of isolation, but artificial generation of recombinant forms of the proteins may allow in the future for large scale production of well defined and uniform therapeutic formulations.

THE LUNG COLLECTINS, SURFACTANT PROTEINS A AND D

SP-A and SP-D are glycoprotein belonging to the collectin family of innate immune molecules, so called because they have collagenous and lectin binding domains (CRDs) (Fig 1). Evidence has accumulated over the past 10–15 years that SP-A and SP-D act in the first line immune defence of the lung, by binding to pathogens and promoting their phagocytosis and killing by phagocytes. SP-A knock-out mice have normal lung histology, but lack of SP-A resulted, as predicted, in an increased susceptibility to pulmonary infection to bacteria and viruses. By contrast, murine SP-D deficiency unexpectedly led to spontaneous emphysematous change and the development of pulmonary fibrosis, revealing a critically important role for SP-D, in particular, in the control of lung inflammation. This short review focuses on how advances in our understanding of the complex roles played by SP-D in the lung may be relevant to the pathogenesis of a range of paediatric and adult lung disease and how the recent development of functional recombinant forms of SP-D has significantly raised the prospects of novel therapeutics using artificially generated lung collectins.

FUNCTIONS OF SP-D

First line defence against pathogens

SP-D recognised the pattern of carbohydrate structures on the surface of a wide range of bacteria, viruses, and fungi and binds to them through multiple low affinity calcium dependent interactions. Table 1 lists examples of the broad range of pathogens with which SP-D is known to interact. SP-D also binds to macrophages and neutrophils and promotes phagocytosis and killing of bound bacteria, fungi, and viruses. SP-D is chemotactic for alveolar macrophages.

Abbreviations: BPD, bronchopulmonary dysplasia; CLD, chronic lung disease; COPD, chronic obstructive pulmonary disease; LPS, lipopolysaccharide; MBL, mannann bindin lectin; RDS, respiratory distress syndrome; SP-A, surfactant protein A; SP-D, surfactant protein D
neutrophils, and monocytes, and acts as a rapid scavenger molecule for clearance of potentially proinflammatory bacterial components such as lipopolysaccharide (LPS). These properties suggest that SP-D acts as a soluble opsonin promoting rapid removal of pathogens and other noxious agents from the airways. Consistent with this, mice lacking SP-D show an exaggerated inflammatory response after infectious challenge.

**Immune modulation**

In addition to acting rapidly in clearance of pathogens by close interaction and modulation of phagocytic cell function, SP-D is also known to modulate the function of other immune cells. In common with other components of the innate immune system, SP-D plays a role in instructing the secondary immune response after challenge with infectious or allergic agents. For example, SP-D enhances the presentation of *Escherichia coli* antigens to dendritic cells, has been shown to inhibit IL-2 dependent T lymphocyte proliferation, and has an inhibitory effect on allergen induced lymphocyte proliferation and histamine release in children with asthma. Thus SP-D not only acts in first line host defence but also affects secondary immune responses.

**Control of inflammation**

Rapid removal of apoptotic cells is recognised as a centrally important mechanism for maintenance of immune homeostasis and the resolution of inflammation. As apoptosis progresses, the integrity of the plasma membrane is lost with consequent leakage of potentially toxic intracellular contents and triggering of an inflammatory response in bystander cells. We have recently reported that SP-D deficiency in the mouse leads to an accumulation of apoptotic and necrotic alveolar macrophages in the airways. Subsequent activation of healthy bystander macrophages and the resultant increased production of reactive oxygen species and matrix metalloproteinases provides a mechanism whereby SP-D deficiency per se leads to the development of emphysema. The studies reveal a critical role for SP-D in immune homeostasis and in the regulation of inflammation in the lung by controlling apoptotic cell numbers.

**Antioxidant properties**

The enhanced production of reactive oxygen species in SP-D deficient mice may be particularly damaging because it has been shown in vitro that SP-D has potent protective properties as an antioxidant. The collectins appear to directly interfere with lipid oxidation by inhibiting the formation of lipid radicals or by acting as free radical chain terminators. The loss of these protective properties would likely contribute to oxidative lung injury and contribute to chronic inflammation in SP-D deficiency. SP-D deficiency in the mouse causes aberrant alveolar development such that emphysematous change is apparent by 4–6 weeks of life. SP-D’s antioxidant properties and its importance in modulating apoptotic cell numbers in the lung suggests a protective function for SP-D in preventing abnormal alveolar remodelling after oxidative lung injury, and perhaps also a role in immune and lung developmental processes.

Table 2 lists multiple functions of surfactant protein D.

### SP-D BASED THERAPY FOR LUNG DISEASE—GENERATION OF RECOMBINANT SP-D

Considerable effort has been made in recent years to develop recombinant forms of human collectins to assess their efficacy in alleviating disease in models of human respiratory infection and inflammation. However, generating adequate amounts of whole length protein is problematic. The delivery of large oligomerised proteins to the lung also presents difficulties because they may be denatured by nebulisation. However, while whole length surfactant protein D may be essential for the preservation of certain functions, smaller fragments of the protein do remain biologically active in vitro and in vivo. These smaller fragments are more robust in resisting denaturation by nebulisation or aerosolisation, so that administration to non-intubated patients may become feasible. We have recently been successful in generating a truncated recombinant fragment of SP-D which maintains important biological function in vivo. This fragment lacks the greater part of the collagenous region existing as a trimer of carbohydrate binding domains which can be readily expressed in *E coli* in large amounts and refolded correctly into an active form. In mice 30–40% of an intranasally administered dose of the fragment reaches the lungs and ameliorates the chronic lung inflammation seen in SP-D deficiency. Treatment of SP-D deficient mice with the recombinant fragment of human SP-D reduced the excessive numbers of alveolar macrophages in the alveolar space, partially corrected the disturbance of

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**Table 1** Surfactant protein D interacts with a broad range of pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Bacteria</td>
<td>Kuan et al, 1992</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Tino et al, 1992</td>
</tr>
<tr>
<td><em>Salmonella minnesota</em></td>
<td>Tino et al, 1992</td>
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<tr>
<td><em>Haemophilus influenzae</em></td>
<td>Tino et al, 1992</td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Tino et al, 1992</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Tino et al, 1992</td>
</tr>
<tr>
<td>Viruses</td>
<td>Levine et al, 2001</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Hickling et al, 1999</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td></td>
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<tr>
<td>Fungi</td>
<td>O’ Riordan et al, 1995</td>
</tr>
<tr>
<td><em>Pneumocystis carinii</em></td>
<td>Strong et al, 2002</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td></td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>Schild et al, 1993</td>
</tr>
</tbody>
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**Table 2** Multiple functions of surfactant protein D

<table>
<thead>
<tr>
<th>Function</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Binding and agglutination of pathogens</td>
<td>Kuan et al, 1992</td>
</tr>
<tr>
<td>Enhanced phagocytosis/killing of pathogens</td>
<td>Tino and Wright, 1996</td>
</tr>
<tr>
<td>Rapid clearance of bacterial endotoxin</td>
<td>van Rynendaal et al, 1999</td>
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<tr>
<td>Moderates inflammatory response to infection</td>
<td>LeVine et al, 2000</td>
</tr>
<tr>
<td>Moderates inflammatory response in allergy</td>
<td>Strong et al, 2002</td>
</tr>
<tr>
<td>Antioxidant properties</td>
<td>Bridges et al, 2001</td>
</tr>
<tr>
<td>Clearance of apoptotic cells</td>
<td>Clark et al, 2002</td>
</tr>
<tr>
<td>Antigen presentation</td>
<td>Brinker et al, 2001</td>
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</tbody>
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surfactant lipid homeostasis, and reduced macrophage activation and the number of apoptotic macrophages persisting in the airways. The SP-D recombinant fragment preferentially binds to apoptotic and necrotic cells in vitro, consistent with an opsonic role in promoting removal of dead and dying cells to limit inflammation.18

RELEVANCE OF SP-D TO THE PATHOGENESIS OF HUMAN LUNG DISEASE

Table 3 lists potential disease targets for SP-D based therapy.

**Neonatal chronic lung disease (CLD)**

Surfactant replacement therapy has been very successful in reducing mortality from respiratory distress syndrome (RDS), but up to 40% of infants surviving after birth at less than 28 weeks gestation develop CLD. Both synthetic (for example, ALEC, Exosurf, Ventacure) and animal extracted surfactant preparations (for example, Survanta, Curosurf) have been used to combat RDS and contain neither SP-A nor SP-D.

The lungs of premature infants are known to be deficient in all surfactant components, including SP-D. Miyamura et al reported low levels of SP-D in amniotic fluid from preterm births.21 SP-D levels in tracheal lavage samples from premature infants are low, and do not correlate with gestational age, but are related to infection status (Clark et al, unpublished data). Dexamethasone induces SP-D expression in vitro,22 and in a study by Wang et al, infants receiving postnatal dexamethasone treatment showed increased levels of SP-D from days 3 to 14, improved pulmonary status, and decreased number of days on a ventilator.23 Unfortunately the numbers in this study were too small to assess any specific association of SP-D levels with the development of chronic lung disease. However, in a more recent study, Beresford and Shaw reported a specific association between low levels of SP-D and the development of CLD. Infants developing CLD by day 28 had significantly lower SP-D levels on day 2 and day 3, whereas SP-A or surfactant protein B levels did not differ significantly between these groups over the first four days.24

The role of recurrent microbial infection in exacerbating inflammation and increasing the risk of development of neonatal lung disease has been highlighted recently.25 There is also evidence from in vitro studies showing inhibition of neutrophil phagocytosis of neonatal pathogens in the presence of some surfactant preparations (for example, Survanta, Pumactant, Exosurf) that surfactant therapy might reduce the efficiency of the neonatal lung in clearing pathogens.26 Infection, inflammation, oxidative lung injury, barotraumas, and aberrant development are all processes considered to be important in the development of neonatal CLD,27,28 and these are all processes in which SP-D may have a protective role. In addition to promoting clearance of invading pathogens from the airways, and down regulating the proinflammatory response to pathogens, SP-D may be important in promoting normal alveolar repair after lung injury by reducing chronic inflammation, leading to broncho-pulmonary dysplasia (BPD) via its antioxidant properties and by promoting clearance of apoptotic cells in the airways. Grigg et al reported some years ago that there is an increased number of apoptotic neutrophils in tracheal aspirates from premature infants with RDS,29 and a recent histological study showed that increased apoptosis was a prominent feature in lungs from patients with BPD.30 Alveolar macrophages releasing reactive oxygen species are considered to have a role in the pathogenesis of BPD.31 Increased macrophage numbers32 and increased neutrophils33 in neonatal broncho-alveolar lavage have both been implicated as risk factors for the development of CLD. The phenotype of the SP-D deficient mouse shows the importance of SP-D in regulating high numbers of alveolar macrophages and in the clearance of apoptotic cells. By reducing numbers of inflammatory and apoptotic cells, early recombinant SP-D therapy in SP-D deficient preterm infants could potentially help prevent the chronic inflammatory changes characterising neonatal CLD. Since the recombinant fragment can be produced in large amounts, it could conceivably be added to current surfactant formulations or administered separately via the endotracheal tube to intubated infants.

**Cystic fibrosis**

The importance of the innate immune system in the lungs of patients with cystic fibrosis has recently come under intense scrutiny.34 Infection with Pseudomonas aeruginosa (especially with organisms in the Burkholderia cepacia complex) and with the fungus Aspergillus fumigatus which causes allergic responses to compound respiratory compromise, frequently heralds marked clinical deterioration. SP-D interacts with Pseudomonas35 and promotes phagocytosis and killing of spores of Aspergillus fumigatus in vitro.36 SP-A and SP-D levels are decreased in bronchoalveolar lavage from patients with cystic fibrosis,37 and there is increased proteolytic degradation of SP-A38 and SP-D (Dombrowsky H, Postle AD, Reid KB, Clark H, et al, unpublished). We have recently reported that administration of native full length SP-A, SP-D,39 and a recombinant truncated fragment of human SP-D40 have in vivo protective effects in murine models of allergic hypersensitivity to Aspergillus fumigatus. Clearance of apoptotic cells is of critical importance in the control of inflammation and is defective in cystic fibrosis.41 We have recently shown a specific role for SP-D in controlling the numbers of apoptotic inflammatory cells in the lungs, and shown that this number is reduced with partial resolution of inflammation after administration of recombinant SP-D.42 Thus recombinant SP-D may have a part to play in future therapeutic strategies for cystic fibrosis, by helping combat infection, by modulating allergic responses to fungal infection, and by limiting inflammation by promoting clearance of apoptotic inflammatory cells.

**Emphysema**

The single most important risk factor for the development of chronic obstructive pulmonary disease (COPD) and emphysema in adults is cigarette smoking.43 Cigarette smoke induces alveolar macrophage apoptosis in vitro44 and in vivo.45 Majo et al have recently reported that apoptosis in lung tissue samples from smokers showed a bilinear relation with the amount smoked, increasing sharply in smokers with emphysema; they concluded that apoptosis might be one of the mechanisms of lung destruction leading to the development of emphysema.46 It has recently been reported that SP-D levels are very low in bronchoalveolar lavage of smokers.47 Relative SP-D deficiency in smokers may result in increased numbers of apoptotic cells lingering in the airways and thus contribute to emphysema in this patient group. One study has described an SP-D polymorphism seen more frequently in sufferers of COPD compared to healthy controls48; of note in this respect is the observation that high serum levels of SP-A and SP-D were predictive of survival in a population of patients with idiopathic pulmonary fibrosis.49 Against this background, the findings that recombinant SP-D...
administration to SP-D deficient mice reduces apoptotic cell numbers, raises the possibility that recombinant SP-D therapy in humans could inhibit a mechanism contributing to emphysema provoked by cigarette smoking.

CONCLUSIONS
Surfactant proteins A and D have multiple functions in immune defence and regulation in the lung. Premature infants, cystic fibrosis patients, and smokers developing emphysema are known to be deficient in SP-D. It has recently become possible to generate biologically active fragments of SP-D in large amounts that could be useful therapeutically. There is a need for further investigation to assess the potential benefits of this drug in a clinical setting.

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