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Review

Surfactant protein A (SP-A) gene targeted mice

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Abstract

Mice lacking surfactant protein A (SP-A) mRNA and protein in vivo were generated using gene targeting techniques. SP-A (−/−) mice have normal levels of SP-B, SP-C and SP-D mRNA and protein and survive and breed normally in vivarium conditions. Phospholipid composition, secretion and clearance, and incorporation of phospholipid precursors are normal in the SP-A (−/−) mice. Lungs of SP-A (−/−) mice have markedly decreased tubular myelin figures and clear *Group B streptococci* and *Pseudomonas aeruginosa* less efficiently than SP-A wild type mice. These studies of SP-A (−/−) mice demonstrate that SP-A has an important role in the innate immune system of the lung in vivo. © 1998 Elsevier Science B.V. All rights reserved.

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1. SP-A structure and synthesis

SP-A is an abundant pulmonary surfactant associated protein encoded by two genes in the human and a single gene in the mouse [1,2]. The primary translation product generated from the SP-A mRNAs encodes a leader sequence which is cleaved during processing, an N-terminal collagen-like domain, a hydrophobic neck domain, and a C-terminal C-type lectin domain [3]. The mature peptide forms trimers that assemble to form an octadecamer that is the predominant airway form [4]. SP-A binds various lipids and glycolipids including dipalmitoylphosphatidylcholine (DPPC) and binds to surface receptors on type II cells and macrophages [5–9]. SP-A synthesis is initiated in the distal developing epithelium and remains in the type II cells, Clara cells, and subsets of cells in tracheobronchial glands in the adult [10]. SP-A levels are reduced during oxygen injury, viral injury, and in pneumonia caused by viruses and bacteria [11–13].

2. SP-A and surfactant homeostasis

2.1. In vitro studies

The structure, function and regulation of SP-A have been extensively studied in vitro (see also the chapter by McCormack). SP-A enhanced the uptake of phospholipids and decreased phospholipid secretion in type II cells in vitro [14,15]. SP-A binds and aggregates lipids in a calcium dependent manner, enhances surface adsorption of phospholipids, protects surface activity of phospholipids from protein inhibition [16–18] and enhances formation of tubular myelin in vitro [19]. Recent fluorescence studies demonstrated that SP-A, in the presence of calcium, aggregated surfactant lipids and proteins on the cell surface and inhibited intracellular uptake of surfactant [20]. Since phospholipid metabolism is only minimally altered in the SP-A (−/−) mouse model, the role of SP-A in regulating phospholipid levels in vivo is unclear.

3. SP-A and host defense

3.1. SP-A is a collectin

SP-A is a member of a larger family of polypeptides termed collectins. The collectins are a family of multimeric proteins that form larger oligomeric structures via NH_2-terminal and collagen-like domains [21]. SP-A and other members of the collectin family, including mannose binding protein (MBP) and surfactant protein D (SP-D) map to the long arm of human chromosome 10 and share similar intron-exon organization, suggesting that they arose by duplications of a primordial gene [22,23]. MBP is produced by the liver and secreted into the serum and children carrying mutations in MBP are more susceptible to recurrent infections [24], supporting the concept that the collectins are part of the innate immunity against microbial pathogens.

3.2. SP-A enhances bacterial phagocytosis in vitro

In vitro studies support a role for SP-A in enhancing microbial phagocytosis by macrophages by (1) acting as an opsonin or (2) directly stimulating macrophages (Table 1) (see also the chapter by Haagsman). SP-A binds some but not all bacteria. SP-A binds some strains of *Streptococcus pneumoniae* and *Staphylococcus aureus*; *Haemophilus influenzae* type a and non-typed; and *Escherichia coli* J5 [25–30]. It does not bind *E. coli* 0111 or K12; or some strains of *S. aureus* or *P. aeruguinosa* [29–32]. It weakly binds *H. influenzae* type b [28]. SP-A opsonization of *S. aureus*, *E. coli* J5 and *S. pneumoniae* enhances their phagocytosis by macrophages [25–27,30,31]. SP-A also stimulates phagocytosis of *E. coli* K12 and *P. aeruginosa* independently of opsonization [31,32]. SP-A enhances phagocytosis of serum opsonized *S. aureus* by macrophages [33]. SP-A enhances

Table 1
Role of SP-A in bacterial clearance in vitro

Bacteria	Binds SPA in vitro	SPA enhanced attachment and/or phagocytosis by macrophages, in vitro	SPA enhanced killing in vitro
Group B Streptococcus	Yes	Yes	–
P. aeruginosa	No [25,31,32]	Yes [31,32]	–
S. pneumonia, not typed	Yes [25,27]	Yes [25]	–
S. pneumonia, type 25	Yes [27]	No [27]	–
H. influenza, type a	Yes [28]	Yes [28]	Yes [28]
H. influenza, type b	Weakly [28]	No [28]	No [28]
H. influenza, not typed	Yes [25]	Yes [25]	–
S. aureus	Yes [26,27] No [31,32]	Yes [26,27,31,32,34] –	No [26,27]
E. coli J5	Yes [29,30]	Yes [30]	Yes [30]
E. coli 0111	No [29,30]	–	–
E. coli K12	No [31,32]	Yes [31,32]	–

macrophage phagocytosis of *Klebsiella pneumoniae* and *Mycobacterium* by increasing activity of the mannose receptor thereby enhancing bacterial binding and uptake [34,35]. SP-A also binds Herpes simplex type I infected cells [36], influenza A [37] and *Pneumocystis carinii* [38,39]. The structure of SP-A and activity of SP-A in vitro strongly supports a role of SP-A in clearance of microbial pathogens from the lung as part of the innate immunity.

4. SP-A gene-targeted mice

4.1. SP-A gene targeting

Homologous recombination in embryonic stem (ES) cells was used to generate a null mutation of the SP-A locus [40]. The SP-A gene was cloned from strain 129J, its sequence determined, and a targeting construct generated by replacement of a portion of exon 3, intron 3 and exon 4 with pGKneoBpA (Fig. 1). The targeting construct was electroporated into ES14.1 cells and correctly targeted cells were selected by double selection with G418 and gancyclovir. The correctly targeted allelic mutation contained a strong alternative promoter (pGK), a strong polyadenylation signal, and lacked sequences encoding a major portion of the collagen-like domain. Chimeric mice were bred to generate heterozygotic mice (+/−) carrying one copy of the targeted allele. Homozygote offspring lacking SP-A (SP-A −/−) were produced from mating the heterozygotes. The distribution of genotypes followed a Mendelian inheritance pattern [40], indicating that the targeted SP-A allele did not cause unanticipated fetal losses. Homozygote (SP-A −/−) targeted mice lacked SP-A mRNA and protein but survived normally in the vivarium. Body weight, lung weight, and lung DNA concentration did not differ between SP-A (+/+) and SP-A (−/−) mice studied at 8 weeks and 8 months of age [41].

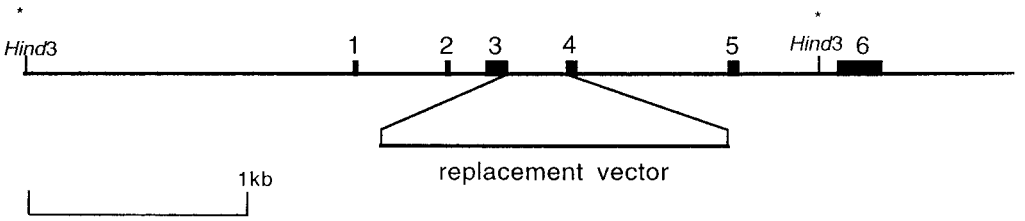

Fig. 1. SP-A targeting construct. The mouse SP-A gene locus is diagrammed on the top line with positions of exons marked and numbered as closed boxes. The position of *Hin*dIII sites used to generate the targeting vector are depicted with an asterisk. Portions of exon 3 and exon 4 and all of intron 3 including splice junctions were replaced by the replacement vector, pGKneoBpA [40].

4.2. Surfactant proteins and phospholipids in SP-A (−/−) mice

Lung SP-A mRNA and protein content were reduced approx. 50% in heterozygote SP-A (+/−) mice, demonstrating the critical role of SP-A gene dosage in the regulation of SP-A synthesis. SP-B, C, and D mRNAs and protein levels were assessed in SP-A (−/−) mice by Northern and S1 nuclease analysis and Western blots. No detectable differences in mRNA or protein levels were observed in SP-A (+/+), (+/−) and (−/−) mice, indicating that there are no compensatory changes in other surfactant proteins in the absence of SP-A [40].

Surfactant phospholipid pool sizes were compared in SP-A (+/+) and (−/−) mice. Saturated phosphatidylcholine levels were slightly higher in alveolar washes and tissues of SP-A (−/−) mice at 8 weeks and 8 months of age [41]. There were no differences in phospholipid composition [40]. The small changes in phospholipid pool sizes had no appreciable effect on survival of SP-A (−/−) mice housed in pathogen free containment.

4.3. Surfactant metabolism in SP-A (−/−) mice

The incorporation of radiolabeled palmitic acid and choline into saturated phosphatidylcholine was assessed following intratracheal instillation. Incorporation of radiolabeled palmitic acid and choline into saturated phosphatidylcholine was virtually identical in SP-A (−/−) and SP-A (+/+) mice. Incorporation of radiolabeled phospholipid precursors were slightly greater in SP-A (−/−) mice 48 h after instillation. This time period reflects synthesis, secretion and reuptake suggesting that SP-A may have a small effect on reuptake in vivo. The rate of phosphatidylcholine synthesis, assessed by incorporation of radiolabeled precursors into saturated phosphatidylcholine in lung slices of SP-A (+/+) and (−/−) mice, were identical. Secretion of radiolabeled phosphatidylcholine precursors also did not differ between genotypes. Clearance of radiolabeled dipalmitoyl phosphatidylcholine (DPPC) was similar from lungs of SP-A (+/+) and (−/−) mice, ranging from 9.4 to 11.2 h. Likewise, clearance of radiolabeled SP-B from lungs of SP-A (+/+) and (−/−) mice was nearly identical [41]. The clearance rate of radiolabeled SP-A was significantly lower in SP-A (−/−) mice (approx. 10.2 h in +/+ compared to 14.5 h in −/− mice) [42].

4.4. Properties of surfactant from SP-A (−/−) mice

Surface tension lowering properties of isolated surfactant from SP-A (−/−) and SP-A (+/+) mice were assessed at various phosphatidylcholine concentra-

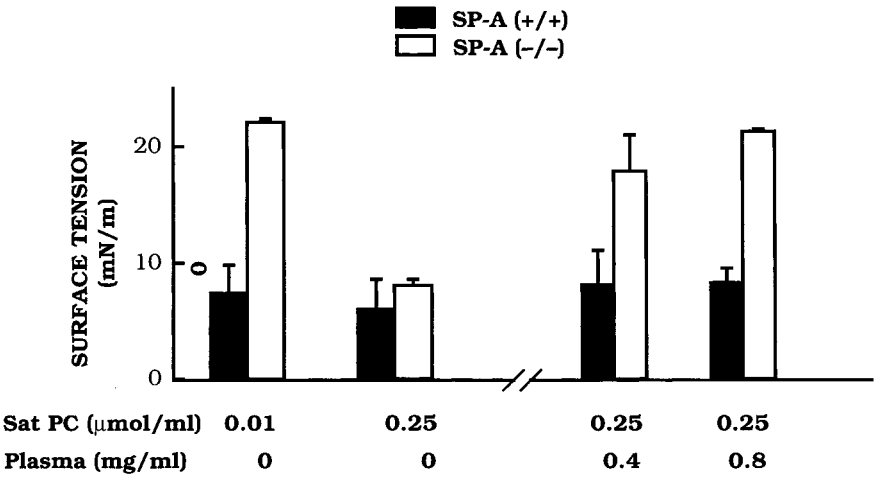

Fig. 2. Minimal surface tension of isolated surfactant. Surfactant was isolated from 8–12 week old mice as previously described [40]. Minimal surface tension was measured on a Wilhelmy balance at 37°C and is reported from the fourth cycle. At 0.01 μmol/ml of saturated PC but not at 0.25 μmol/ml SP-A (−/−) produced a higher surface tension than SP-A (+/+) surfactant. In the presence of 0.4 or 0.8 mg/ml of plasma protein, minimum surface tension produced by SP-A (−/−) surfactant was significantly greater than produced by SP-A (+/+) surfactant [42].

Table 2
Decreased clearance of Group B beta hemolytic *Streptococcus* in SP-A (−/−) mice

Time (h)	SP-A (−/−)	SP-A (+/+)	*n*
6	$4.5\times10^5\pm2.1\times10^5$*	$6.2\times10^3\pm5.4\times10^3$	10
24	$6.3\times10^4\pm3.8\times10^4$*	$1.1\times10^3\pm9.8\times10^2$	10
48	$9.9\times10^4\pm3.3\times10^4$*	$4.6\times10^4\pm4.1\times10^4$	10

Quantitative cultures of lung homogenates were performed 6, 24 and 48 h after trans-tracheal inoculation of the animals with 10^4 colony forming units of GBS. Data are reported as bacterial colonies/g of lung and are means ± S.E.M. for *n* animals, $*P<0.05$ compared to SP-A (+/+) mice using the median scores non-parametric test.

tions with the Wilhelmy balance. In the presence of calcium, surface tension lowering properties of isolated surfactant from SP-A (−/−) and SP-A (+/+) surfactant was similar. In contrast, in the absence of calcium, reduction of surface tension by SP-A −/− surfactant, at low phosphatidylcholine concentrations was reduced. Significant differences were only detected at phospholipid concentrations approx. 3 orders of magnitude lower than that present in the lung [40]. This finding supports the concept that SP-A may improve surfactant function in conditions associated with reduced phosphatidylcholine levels (Fig. 2).

Conversion of surfactant from large to small aggregate forms differed between SP-A (+/+) and SP-A (−/−) mice. During mechanical cycling, conversion from large to small aggregates was 7.3 ± 5.0% for SP-A (−/−) mice compared to 37.0 ± 3.0% for wild type mice [42]. Surfactant from SP-A (+/+) mice contained 48 ± 1% large aggregates compared to 15 ± 2% for SP-A (−/−) mice. Large aggregate surfactant from SP-A (−/−) and SP-A (+/+) mice increased thoracic compliance when delivered to ventilated preterm rabbits to the same extent and resulted in similar maximal lung volume at 30 cm H_2O. Despite detectable differences in conversion rates of large aggregate surfactant fractions between (−/−) and (+/+) mice, both were equally effective in restoring lung function in neonatal preterm rabbits. Adsorption rates of isolated surfactant from (+/+) and (−/−) mice were similar. In the presence of plasma protein concentrations greater than 0.4 mg/ml, surface tension reducing properties of SP-A (−/−) surfactant were markedly reduced compared to surfactant from SP-A (+/+) mice (Fig. 2), confirming previous studies demonstrating that SP-A protects surfactant from inactivation by serum proteins [42].

4.5. Lack of tubular myelin from SP-A (−/−) mice

To assess the structure and abundance of tubular myelin, lung sections were examined by electron microscopy. Forty fields from SP-A (+/+) contained 15 large tubular myelin figures whereas 70 fields from SP-A (−/−) lungs contained only two small tubular myelin figures [40]. Although abundant tubular myelin figures were readily detected in large aggregate surfactant isolated from SP-A (+/+) mice, tubular myelin was not detected in pellets from SP-A (−/−) mice, supporting previous in vitro findings that SP-A contributed to tubular myelin formation. The markedly decreased tubular myelin figures in SP-A (−/−) mice strongly suggest that tubular myelin is not required for pulmonary surfactant homeostasis or function in vivo.

Table 3
Decreased clearance of *P. aeruginosa* in SP-A (−/−) mice

Time (h)	SP-A (−/−)	SP-A (+/+)	*n*
6	$2.5\times10^8\pm7.8\times10^7$*	$8.3\times10^7\pm3.6\times10^7$	10
24	$1.3\times10^7\pm7.4\times10^6$*	$3.7\times10^5\pm2.4\times10^5$	10
48	$1.9\times10^5\pm1.0\times10^5$	$1.1\times10^6\pm9.3\times10^5$	10

Quantitative cultures of lung homogenates were performed 6, 24 and 48 h after trans-tracheal inoculation of the animals with 10^8 colony forming units of *P. aeruginosa*. Data are reported as bacterial colonies/g of lung and are means ± S.E.M. for *n* animals, $*P<0.05$ compared to SP-A (+/+) mice using the median scores non-parametric test.

5. SP-A (−/−) mice are susceptible to lung infection

5.1. Clearance of Group B beta hemolytic streptococci

Group B beta hemolytic streptococci (GBS) are common pathogens in premature and term neonates. To determine whether SP-A protects the lung from GBS in vivo, SP-A (−/−) and (+/+) mice were infected with GBS by tracheal injection. Clearance of GBS from the lungs of SP-A (−/−) mice was significantly reduced compared to SP-A (+/+) mice (Table 2). Moreover GBS proliferated in the lungs of SP-A (−/−) mice and was detectable in the spleen at 24 and 48 h. Lack of SP-A increased pulmonary bacterial infection and permitted septic spread in the GBS mouse model [43]. When GBS was premixed with SP-A (100 or 150 μg/10^4 bacteria), clearance was as efficient as in SP-A (+/+) mice [44]. Recently, SP-A was found to bind to GBS in the presence of calcium suggesting that SP-A acts as an opsonin for GBS (J.R. Wright, personal communication).

Decreased GBS clearance was associated with decreased binding and uptake of the organisms by alveolar macrophages. Fewer macrophages contained ingested bacteria from the SP-A (−/−) than from the SP-A (+/+) mice 6 h post infection. Oxygen radical production by the alveolar macrophages was markedly reduced in SP-A (−/−) mice [44]. SP-A increases Group B strep clearance in vivo by enhancing macrophage phagocytosis and by enhancing production of microbiocidal free radicals.

5.2. Pseudomonas

To determine whether SP-A also enhances clearance of Gram-negative bacteria, we determined clearance rates of mucoid *P. aeruginosa*, a common pulmonary pathogen in cystic fibrosis and hospital acquired pulmonary infection. Clearance of trachealy instilled *P. aeruginosa* was significantly reduced in SP-A (−/−) compared to SP-A (+/+) mice in vivo at 6 and 24 h post infection (Table 3). Macrophages isolated 1–2 h post infection contained significantly less *Pseudomonas* from SP-A (−/−) than SP-A (+/+) mice. Free radical synthesis by polymorphonuclear leukocytes did not differ in infected SP-A (+/+) and (−/−) mice, demonstrating that SP-A enhanced free radical synthesis by these cells is not critical to clearance of mucoid *Pseudomonas* from the lung [45]. SP-A does not bind to this *P. aeruginosa* in vitro so it is likely that SP-A enhances phagocytosis of *P. aeruginosa* in vivo by stimulating macrophage receptor activity by a process independent of opsonization.

6. Concluding comments and perspectives

We have generated mice lacking SP-A to decipher the role of pulmonary SP-A in vivo. Unlike a null mutation of SP-B where mice die of an RDS syndrome immediately after birth [46], SP-A deficient mice survive and breed normally. Phospholipid composition, incorporation of phospholipid precursors, secretion of phosphatidylcholine, and clearance of dipalmitoylphosphatidylcholine did not differ significantly between SP-A (+/+) and SP-A (−/−) mice. Small increases in phospholipid pools and decreased clearance of SP-A from SP-A (−/−) mice did not significantly alter lung function in vivo. Lack of tubular myelin figures and susceptibility of isolated SP-A (−/−) surfactant to protein inhibition in vitro confirmed previous conclusions that SP-A is involved in tubular myelin formation and in protection of surfactant from protein inhibition. Reduced clearance of *Group B streptococci* and *P. aeruginosa* supports the concept that SP-A plays an important role in innate immunity, protecting the lung from microbial infection and injury.

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