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S-nitrosylation of Surfactant Protein D as a modulator of pulmonary inflammation

Elena N. Atochina-Vasserman, M.D., Ph.D.

Pulmonary, Allergy and Critical Care Division, Department of Medicine University of Pennsylvania, Vernon and Shirley Hill Pavilion, # H410C, 380 S. University Ave., Philadelphia, PA, USA, 19104-4539, Voice: (215) 260-0569, Fax: (215) 746-5985

Elena N. Atochina-Vasserman: atochina@mail.med.upenn.edu

Abstract

Background—Surfactant protein D (SP-D) is a member of the family of proteins termed collagen-like lectins “collectins” that play a role in non-antibody-mediated innate immune responses [1]. The primary function of SP-D is the modulation of host defense and inflammation [2].

Scope of Review—This review will discuss recent findings on the physiological importance of SP-D S-nitrosylation in biological systems and potential mechanisms that govern SP-D mediated signaling.

Major Conclusions—SP-D appears to have both pro- and anti-inflammatory signaling functions. SP-D multimerization is a critical feature of its function and plays an important role in efficient innate host defense. Under baseline conditions, SP-D forms a multimer in which the N-termini are hidden in the center and the C-termini are on the surface. This multimeric form of SP-D is limited in its ability to activate inflammation. However, NO can modify key cysteine residues in the hydrophobic tail domain of SP-D resulting in a dissociation of SP-D multimers into trimers, exposing the S-nitrosylated N-termini. The exposed S-nitrosylated tail domain binds to the calreticulin/CD91 receptor complex and initiates a pro-inflammatory response through phosphorylation of p38 and NF- κ B activation [3,4]. In addition, the disassembled SP-D loses its ability to block TLR4, which also results in activation of NF- κ B.

General Significance—Recent studies have highlighted the capability of NO to modify SP-D through S-nitrosylation, causing the activation of a pro-inflammatory role for SP-D [3]. This represents a novel mechanism both for the regulation of SP-D function and NO's role in innate immunity, but also demonstrates the S-nitrosylation can control protein function by regulating quaternary structure.

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Keywords

SP-D; surfactant protein D; TLR; Toll like receptors; NO; nitric oxide; SNO; S-nitrosothiol; pulmonary inflammation; macrophages polarization

1.1. S-nitrosylation as a regulator of lung inflammation through TLR4 signaling pathway

Toll-like receptors (TLRs) are family of pattern recognition receptors that represent the first line of defense against many pathogens. Remarkably, TLRs are capable of discriminating between pathogenic and commensal microorganisms, and inducing appropriate and distinct antimicrobial response [5]. As regulators of the inflammatory response against pathogens, TLRs help to strengthen the processes of innate and adaptive immunity [6].

In mammals, the recognition of lipopolysaccharide (LPS) requires at least three proteins: TLR4, CD14 and myeloid differentiation factor 2 (MD-2) [7] (Figure 1). LPS is opsonized by LPS-binding protein (LBP), and the complex is recognized by the opsonic receptor CD14 on the macrophage surface and forms the ternary complex LPS–LBP–CD14. Transfer of LPS from CD14 to MD-2 is tied to binding to TLR4 [8]. Lipid chains of LPS shift into the hydrophobic pocket of MD-2 to maximize hydrophobic contact. This structural shift allows the phosphate groups of LPS to form ionic interactions with a cluster of positively charged residues in TLR4 and MD-2 and this, in turn, results in dimerization of the two LPS-MD-2-TLR4 complexes arranged symmetrically [9]. TLR dimerization results in the recruitment of the adaptor protein, MyD88. The intracellular of TLR4 (TIR) binds to a homologous domain in MyD88 to constitute the so-called “death domain”. This death domain undergoes homophilic interaction with a similar domain of the serine/threonine protein kinase, IRAK, resulting in IRAK phosphorylation. Auto-phosphorylated IRAK forms a complex with TRAF6, which initiates oligomerization of TRAF6 [10]. This results in association with the heterotrimer of TAK1-TAB1-TAB2 to form a multi-component complex. Upon phosphorylation of TAK1 and TAB2, IRAK dissociated from the complex, and the TRAF6–TAK1–TAB1–TAB2 complex is translocated to the cytosol where it activates IKK [10]. IKK phosphorylates I κ B, leading to its proteolytic degradation and the translocation of NF- κ B to the nucleus with the consequent transcription of immune response genes [11].

There is considerable experimental evidence indicating that TLR signaling cascade contains a number of proteins, including MyD88, IKK β and NF- κ B, whose activity is regulated via S-nitrosylation [12]. It has been shown that S-nitrosylation of the p50–p65 heterodimer inhibits its binding to iNOS promoter DNA at the NF- κ B–DNA interface [13,14]. S-nitrosylation of the I κ B kinase β , a major regulator of NF- κ B, results in reduction of its kinase function, which in turn leads to a reduction in I κ B ubiquitinylation and subsequent degradation [15]. S-nitrosylation of the adaptor protein MyD88, located upstream from the IKK complex, results in inhibition of its binding to the sorting adaptor TIRAP, and therefore decreases LPS-induced NF- κ B activation [16]. All these studies demonstrate that S-nitrosylation inhibits TLR signaling through modification of components of the intracellular

signaling cascade. Therefore, one can see that the generation of intracellular SNO is an important mechanism in the control of acute-phase inflammatory responses.

1.2. SP-D as a target for S-nitrosylation

SP-D is a Ca^{2+} -binding lectin that is produced primarily as a multimer by alveolar type II cells and nonciliated bronchiolar cells in the lung [17]. SP-D shares considerable structural homology with other proteins of this type, including surfactant protein A (SP-A), conglutinin, bovine collectin-43, and mannose binding protein [18,19]. SP-D through its C-type carbohydrate-recognition domain (CRD) binds to carbohydrate structures present on a range of viruses, bacteria, yeasts and fungi [20,21]. Upon recognition of the infectious agents, SP-D initiates direct opsonization, neutralization, agglutination and phagocytosis [22]. In addition, it can interact with receptor molecules present on immune cells leading to enhanced microbial clearance and modulation of inflammation [23,24]. Targeted ablation of the SP-D gene results in chronic inflammation and an emphysema-like phenotype in the absence of infection [25,26]. The lungs of SP-D ($-/-$) mice are characterized by an inappropriate activation of alveolar macrophages with significant alterations in nitric oxide (NO) metabolism [27–30], indicating an important role for SP-D in regulating immune homeostasis and the function of the innate immune cells. Furthermore, selective inhibition of iNOS was shown to decrease inflammatory markers [31] and the additional ablation of the iNOS-gene in SP-D ($-/-$) mice attenuated the degree of pulmonary emphysema, indicating an important role of iNOS in the pathogenesis of this model [32].

The SP-D primary translation product is composed of three polypeptide chains that are held together by disulphide bonds and further assembled into a large cruciform dodecamer [33] that resembles a “dandelion ball”. Critical to this oligomerization are two cysteine residues at positions 15 and 20 in the hydrophobic N-terminus [34,35] (Figure 2). Mutation of these residues results in the secretion of trimers, which represent a single arm of the dodecamer [34]. SP-D possesses seven cysteine residues [36] but only two of them ($\text{cys}^{15}/\text{cys}^{20}$) exist in S-nitrosylation-consensus sequences [37]. Therefore, the positioning of cysteines 15 and 20, within the most hydrophobic region of the N-terminal domain of the SP-D protein suggests that NO, through a covalent modification of these thiol groups, may control oligomerization of SP-D by S-nitrosylation [3] (Figure 2).

The dandelion ball structure of multimeric SP-D is too large (>1 MDa) to have any significant mobility on native gel electrophoresis. However, a mutant form of SP-D, termed the single-arm form due to its inability to form the multimeric dandelion ball structure, is easily separated by native gel. The two critical cysteine residues of the tail-domain, Cys 15 & 20, are mutated to Serine in this single-arm form of SP-D [34]. Treatment of rat recombinant SP-D (rrSP-D) with S-nitrosocysteine or free reduced cysteine results in disruption of its multimeric organization as shown by native gel electrophoresis (Figure 3A). That both of these treatments effectively disrupt SP-D’s dandelion ball structure indicates that reduced cysteine interaction, rather than disulfide bond formation, is critical in maintaining this multimer. The reduced nature of these cysteines is confirmed by their ability to be alkylated [3]. However, rrSP-D, but not the single arm mutant, was effectively nitrosylated by S-nitrosocysteine as determined by the biotin-switch assay (Figure 3, B).

Therefore, one can see that under lung injury and/or inflammatory conditions, increased NO production could lead to modification of these cysteine residues resulting in a dissociation of SP-D multimers into trimers (similar to exhaling on a “dandelion ball”), exposing S-nitrosylated tail domains.

SP-D, a modulator of immune cell responses to pathogens, binds to LPS, which is the major cell-wall component of gram-negative bacteria [38–40]. As a result of this binding, pathogens can be aggregated and/or opsonized, and this leads, in many cases, to enhanced killing and clearance by phagocytic cells; thereby preventing uncontrolled inflammation in the lung. Critical evidence for the significance of SP-D mediated phagocytosis of inhaled pathogens was provided by studies on bacterial infection. Recently Ikegami *et al.* demonstrated that SP-D deficient mice were more susceptible to intratracheal LPS than WT mice and that intratracheal administration of recombinant SP-D inhibited LPS-mediated lung inflammation in both SP-D deficient and WT mice [41]. Inhaled LPS activates the Toll-like receptor 4 (TLR4) signaling pathway, resulting in increased production of inflammatory cytokines and reactive species such as NO [42,43]. In order to understand the role that SP-D plays within immune signaling it is necessary to examine the mechanisms involved in innate immune activation.

Recently the native multimeric form of SP-D has been demonstrated to bind to TLR4 [44] CD14 [45] and sMD-2 [46], via its CRD domain inhibiting TLR4-mediated pro-inflammatory responses caused by both smooth and rough serotypes of LPS [47] (Figure 1). Since MD-2 is critical for triggering LPS signaling [48], the binding of SP-D to MD-2 could prevent TLR4 dimerization/activation and, therefore, inhibit LPS-induced inflammatory cell responses. Experiments with trimeric cys¹⁵/cys²⁰ mutant [49], SP-D/MBL chimera [50], SPA/SP-D chimera, and a collagenase-resistant fragment [47] demonstrated that the oligomeric structure of SP-D is a critical feature of its immunomodulatory function. It is worth noting that both SP-A and SP-D have been proposed to interact via their CRD domains with the inflammation inhibitory receptor, SIRP-1α [51]. This would provide another immunomodulatory mechanism for SP-D by activating SHP-1 and thus inhibiting NF-κB activation. Under baseline conditions, the hydrophobic N-terminal tail of SP-D exists in a reduced state hidden in the center of the dandelion ball multimer with the CRD domains exposed on the surface [3,52]. Pathogen recognition and binding by the SP-D dandelion ball leads to basal phagocytosis and a regulated release of inflammatory mediators, maintaining lung homeostasis in an infection and inflammation free state. In this way the degree of basal inflammation observed within a lung is dependent on the quantity of LPS that is found ubiquitously in the environment.

S-nitrosylation of SP-D to form SNO-SP-D trimers initiates a pro-inflammatory response (Figure 1) through calreticulin/CD91 binding and p38 activation [3,53]. Importantly, SNO-SP-D, but not trimeric SP-D, is chemoattractive for macrophages and induces p38 MAPK phosphorylation. In this context it is important that while SNO-SPD initiates macrophage chemotaxis and thus has a functional consequence, trimeric SP-D, either cysteine-treated rrSP-D or the single arm mutant, is not chemoattractive; despite the fact that the tail region of these molecules is exposed (Figure 3C). *Therefore, S-nitrosylation not only results in structural disruption of SP-D but is also critical to the signaling function within*

macrophages. The concept of both structural disruption and NO-mediated post-translational modification provides an explanation of the many prior conflicting studies reporting either pro- or anti-inflammatory effects of SP-D depending on the model system or stimulus used.

1.3. SNO-SP-D in animal models of pulmonary inflammation

The mechanism of macrophage activation through p38 phosphorylation and NF- κ B activation by SNO-SP-D has been observed in a variety of animal models. Using both mouse and rat models of bleomycin-induced lung injury, it has been shown that macrophage driven pulmonary inflammation is associated with formation of SNO-SP-D. Lung lavage fluid (BAL) from bleomycin-injured mice is a potent chemoattractant for RAW cells, however, treatment with either anti-SP-D or ascorbic acid, which selectively reduces S-nitrosylated proteins to release NO, blocked macrophage chemotaxis *in vitro* [3]. In addition, it has been shown that BAL from bleomycin-treated mice increases p38 phosphorylation within RAW264.7 cells in a SP-D dependent manner. In another mouse model of lung injury using LPS, post-translational modification of SP-D has also been observed [54]. In this study aerosolized LPS induced increases in airway NO levels, airway neutrophil numbers, lung neutrophil and CD8⁺ cell numbers, and BAL SP-D protein levels. Furthermore, SP-D recovered from the BAL of LPS-treated mice was covalently cross-linked and S-nitrosylated with concomitant disruption of its multimeric structure. Disruption of SP-D oligomeric structure was associated with worsened lung compliance and cytokine/chemokine production (KC, TNF α , IL-6) [54].

In an infection based murine model of lung inflammation we have demonstrated that CD4⁺ T cell immune-reconstituted (IRD) mice infected with *Pneumocystis pneumonia* (*Pc*) exhibit impaired pulmonary function with substantial increases in the BAL SP-D level [4]. S-nitrosylation of SP-D and therefore alteration in SP-D higher order structure were markedly enhanced by immune-reconstitution during *Pc* infection. BAL fluid of *Pneumocystis* infected mice during IRD exhibit enhanced chemotaxis in a macrophage cell line *in vitro*. Removal of the SNO moiety from SNO-SP-D abolished the enhancement of chemotaxis. Doubly homozygous mice with mutations in both pale ear and pearl (EPPE) provides a model of Hermansky-Pudlak Syndrome (HPS), a rare form of oculocutaneous albinism associated with progressive lung inflammation [55]. EPPE mice exhibit a dramatic age dependent increase in BAL SP-D levels when compared with WT mice. Furthermore, EPPE mice demonstrated a shift in alveolar nitrite/nitrate ratio favoring the formation of higher oxides of nitrogen in association with the presence of S-nitrosylated and cross-linked forms of SP-D coincident with the onset of macrophage predominant inflammation [55].

All these studies provide general mechanistic evidence that SNO-SP-D plays a role in recruitment of effector cells *in vivo*. Thus, lung inflammation in a variety of model systems represents a feed forward system in which additional inflammation leads to further modification of SP-D by NO and subsequent pro-inflammatory effects mediated by SNO-SP-D. These studies emphasize the delicate balance that exists between SP-D, the innate immune system, and pulmonary inflammation. In summary, these *in vivo* models demonstrate that NO is capable of controlling the dichotomous nature of SP-D and that post-

translational modification by *S*-nitrosylation causing quaternary structural alterations, may be the key in switching its inflammatory signaling role [3].

1.4. SP-D in human studies

Human studies have found conflicting results for SP-D levels and lung disease. Several studies have demonstrated decreased SP-D levels in the BAL of children with cystic fibrosis (CF) [56–58]. Decreased SP-D levels are found in the BAL of children with RSV infection [59] and in patients with ARDS [60,61]. Several studies have assessed the value of plasma SP-D levels as a marker for human lung diseases. Eisner *et al.* reported significant increases in plasma SP-D during ALI/ARDS [62]. Conversely, Determann *et al.* have shown that SP-D level in the plasma of ALI/ARDS patients was not significantly different when compared to patients without lung injury [63]. Attempts have been made to correlate serum and BAL SP-D levels with human disease, including community-acquired pneumonia in adults, recurrent bronchitis and asthma in pediatric patients, COPD, and smoking [64–65], as well as suggesting the use of low serum SP-D levels as a biomarker for the development of bronchiolitis obliterans in hematopoietic stem cell transplant recipients [66].

However, the structural composition of the SP-D molecule may vary between healthy conditions and inflammatory lung diseases. During the inflammatory state post-translational modification of SP-D can result in alteration of their structure and function [53]. As in animal models, within human disease the native structure of SP-D has been found to be altered through nitration, *S*-nitrosylation, oxidation and/or crosslinking [3,4,55,67–71] and therefore may confound the measurement of total SP-D in BAL or plasma by commercially available ELISA kits.

Testing of a range of antibodies (in house and from different sources) against SP-D showed that all recognized a single band of approximately 43–50 kD under reduced conditions but some antibodies also recognized lower molecular weight proteins (~25–30 kD). Antibodies against SP-D from different sources demonstrated variability in the species binding affinity. For example, an antibody that did not react with mouse native SP-D demonstrated strong reactivity with mouse denatured isoforms of SP-D and human native SP-D. In addition, there was variability in how well tested antibodies bound to modified SP-D either through oxidation and/or crosslinking (unpublished data). Additionally, variations within the SP-D gene also result in alteration of its multimeric structure [72–76] and therefore may also contribute to conflicting results by measuring total SP-D level by ELISA. Finally, acknowledging the fact that different ELISAs use differing antibodies raised against various portions of the SP-D molecule suggesting some complexity in distinguishing between various structural forms of SP-D. Therefore, all these observations demonstrate that SP-D antibodies (in house and commercially available) should be used carefully and critically. In conclusion, the precise quantitation of SP-D and its reliability as a biomarker of various pulmonary diseases requires comprehension of the pro- or anti-inflammatory structure/function of the SP-D molecule.

Recently Ware *et al.* reported that two plasma biomarkers, SP-D and IL-8 are significantly increased during ALI [77]. In other cross-sectional cohort study, Todd *et al.* also have

shown that SP-D is increased in both BAL and plasma during ALI and that there was significant increase in SP-D breakdown products in the lungs of these patients [78]. The elevated BAL SP-D level was also associated with respiratory dysfunction, inflammation and increase in plasma SP-D and IL-8 levels during ALI [78]. However, an enhanced level of inflammatory markers, together with detection of significant amounts of SP-D breakdown products, during ALI raises the question about which of the pro- or anti-inflammatory forms of SP-D are elevated.

The ability to predict patients at risk for these outcomes and effectively modify their treatment may lead to improvement in patient care. Asthma, Hermansky Pudlak Syndrome type 1 (HPS1) and COPD are all associated with amplification of inflammatory signals in the distal lung. Recently, has been reported that BAL from HPS1 patients [55], asthmatic patients after segmental challenge with allergen [68], and patients with COPD [70], demonstrated the presence of SNO-SP-D and dissociation of SP-D multimeric structure. Furthermore, half of the asthmatic patients developed a covalently cross-linked SP-D after segmental challenge with allergen which correlated positively both with BAL eosinophil counts and with total levels of nitrate suggesting a strong relationship between cross-linked SP-D and severity of allergic inflammation [68]. Clearly, within human disease, SP-D is a target for post-translational modifications such as *S*-nitrosylation, oxidant-mediated cross-linking, and thus altered quaternary structure. The present data suggests that SP-D isoforms, in addition to contributing to local immunomodulation in the lung, are potential candidates as biomarkers for pulmonary inflammation.

1.5. SP-D directly influences alveolar macrophage polarization – a mechanism to modulate inflammatory responses?

Alveolar macrophages (AM) are the most abundant antigen-presenting cells in the airways and alveolar spaces, where they play a critical role in regulating immune responses and inflammation within the lung [79,80]. AM perform a number of important functions, including phagocytosis of particulate matter, secretion of cytokines and enzymes, and control of microbes. They are the first cells to contact many inhaled antigens, including infectious agents, allergens and small particulate debris and therefore, play a key role in initiating, progression and/or resolution of local pulmonary immune responses.

Several studies have reported that alveolar macrophages recovered from the lung lavage of normal mice have a unique phenotype when compared to typical tissue macrophages [81–83]. The unique features of AM could reflect the unusual environment in which they are found. Thus macrophages have the ability to modify/reprogramme their secretory activity, and acquire different phenotypes depending on the tissue environment and the concentration of pathogenic product [84]. The development of these studies has led to the identification of multiple macrophage phenotypes. Often these phenotypes are categorized into two alternative profiles: M1 and M2 (Figure 1).

Classic M1 phenotype is formed by the interaction of macrophages with bacteria, viruses, stimulation of IFN- γ or LPS. M1 phenotype is characterized by increased production of pro-inflammatory cytokines (Th1) such as TNF- α , IL-1 β , IL-6, IL-12, macrophage

inflammatory protein 1 α (MIP-1 α), as well as increased generation of nitric oxide (NO) and reactive oxygen intermediates [85–86]. These cells operate to kill microorganisms and tumor cells and produce pro-inflammatory cytokines [86,87]. The M1 phenotype exhibit enhanced antigen presentation, intracellular killing, and have increased levels of nitric oxide.

M2 macrophages, or “alternatively activated” macrophages are formed by the interaction with extracellular parasites or stimulation of IL-4, IL-13, TGF- β , or glucocorticoids [88]. M2 is characterized by the production of anti-inflammatory cytokines (Th2) such as IL-4; IL-13 and IL-10. M2 phenotype regulates the inflammatory response, and is also involved in angiogenesis, remodeling and repair tissue damaged by inflammation.

Although it has been shown that there are tissue specific signals that cause monocytes to differentiate into their appropriate macrophages [89] the signals that control switching between M1, “classically” activated or pro-inflammatory, and M2 “alternatively” activated or anti-inflammatory are less well-defined. SP-D has emerged as a potential regulator of macrophage phenotype and function [30,90]. Both increased relative SNO-SP-D content [68] and SP-D deficiency [25] predisposes the lung to excessive pro-inflammatory responses while over expression of native multimeric SP-D is protective [91]. Mice that are constitutively deficient in SP-D develop progressive lung inflammation and time-dependent airspace remodeling [25] with increased expression of iNOS [29] and reduced expression of Arg1, PPAR γ , Dectin1 and FIZZ1, suggesting that within these animals the macrophages phenotype is M1 favored [92]. Over expression of SP-D is protective against the excess inflammation that occurs as a result of acute lung injury either via intratracheal bleomycin administration [91] or from hyperoxia [93]. *In vitro*, using primary murine macrophages, preliminary observations have shown that SNO-SP-D can induce expression of M1 related genes, such as IL-1 α , NOS2, and Ptg2, while native SP-D inhibits the expression of these genes in the presence of LPS [94].

In conclusion, one can see that SP-D, a critical regulator of innate immune function within the lung, is regulated by S-nitrosylation of two key target cysteines within the tail domain. SNO-SP-D is altered in its multimeric state and the “dandelion ball” structure of SP-D is lost, resulting in exposure of tail domains. This exposure allows for activation of the p38 MAPK pathway via interaction with CD91, however, it may also alter the interaction of SP-D with both pathogens and other surface receptors. In this review I have proposed that SP-D can alter the interaction between LPS and TLR4 and that this signaling pathway is dependent upon SP-D multimerization. Therefore, S-nitrosylation of SP-D will switch this regulatory collectin from a reparative signaling molecule to an acute inflammatory mediator by favoring M1 over M2 macrophage differentiation. This represents a novel mechanism of SNO signaling by controlling quaternary structure, and demonstrates a role for S-nitrosylation in the regulation of innate immunity.

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Abbreviations

TLR	Toll-like receptor
TIR	Toll/IL-1 receptor domain
LPS	lipopolyssacharides
LBP	LPS-binding protein
MD-2	myeloid differentiation factor 2
MyD88	myeloid differentiation protein 88
IRAK	IL-1 receptor associated kinase
TRAF6	TNF receptor associated factor
MAPK	mitogen-activated protein kinase
TAK1	transforming growth factor b-associated kinase 1
TAB1	TAK1-binding protein 1
TAB2	TAK1-binding protein 2
IKK	I κ B kinase
IκB	inhibitor of NF- κ B
NF-κB	nuclear factor kappa B
SIRP-1α	signal inhibitory regulatory protein -1 α
SHP-1	tyrosine-protein phosphatase-1
MBL	mannose-binding lectin
ARDS	Acute respiratory distress syndrome
ALI	Acute lung injury
COPD	Chronic obstructive pulmonary disease

Reference List

1. Crouch E, Wright JR. Surfactant proteins A and D and pulmonary host defense. *Annual Review of Physiology*. 2001; 63:521–554.
2. Wright JR. Immunoregulatory functions of surfactant proteins. *Nature Reviews Immunology*. 2005; 5:58–68.
3. Guo CJ, Atochina-Vasserman EN, Abramova E, Foley JP, Zaman A, Crouch E, Beers MF, Savani RC, Gow AJ. S-Nitrosylation of Surfactant Protein-D Controls Inflammatory Function. *Plos Biology*. 2008; 6:2414–2423.
4. Atochina-Vasserman EN, Gow AJ, Abramova H, Guo CJ, Tomer Y, Preston AM, Beck JM, Beers MF. Immune reconstitution during *Pneumocystis* lung infection: disruption of surfactant component expression and function by S-nitrosylation. *J Immunol*. 2009; 182:2277–2287. [PubMed: 19201882]
5. Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nature Reviews Immunology*. 2008; 8:411–420.
6. Beutler B, Hoebe K, Du X, Ulevitch RJ. How we detect microbes and respond to them: the Toll-like receptors and their transducers. *Journal of Leukocyte Biology*. 2003; 74:479–485. [PubMed: 12960260]

7. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature*. 2000; 406:782–787. [PubMed: 10963608]
8. Han JH, Ulevitch RJ. Limiting inflammatory responses during activation of innate immunity. *Nature Immunology*. 2005; 6:1198–1205. [PubMed: 16369559]
9. Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature*. 2009; 458:1191–1195. [PubMed: 19252480]
10. Wu H, Arron JR. TRAF6, a molecular bridge spanning adaptive immunity, innate immunity and osteoimmunology. *Bioessays*. 2003; 25:1096–1105. [PubMed: 14579250]
11. Stancovski I, Baltimore D. NF-kappaB activation: the I kappaB kinase revealed? *Cell*. 1997; 91:299–302. [PubMed: 9363938]
12. Foster MW, Hess DT, Stamler JS. Protein S-nitrosylation in health and disease: a current perspective. *Trends in Molecular Medicine*. 2009; 15:391–404. [PubMed: 19726230]
13. Marshall HE, Stamler JS. Inhibition of NF-kappa B by S-nitrosylation. *Biochemistry*. 2001; 40:1688–1693. [PubMed: 11327828]
14. Kelleher ZT, Matsumoto A, Stamler JS, Marshall HE. NOS2 regulation of NF-kappa B by S-nitrosylation of p65. *Journal of Biological Chemistry*. 2007; 282:30667–30672. [PubMed: 17720813]
15. Reynaert NL, Ckless K, Korn SH, Vos N, Guala AS, Wouters EFM, van der Vliet A, Janssen-Heininger YMW. Nitric oxide represses inhibitory kB kinase through S-nitrosylation. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:8945–8950. [PubMed: 15184672]
16. Into T, Inomata M, Nakashirna M, Shibata KI, Hacker H, Matsushita K. Regulation of MyD88-dependent signaling events by S nitrosylation retards toll-like receptor signal transduction and initiation of acute-phase immune responses. *Molecular and Cellular Biology*. 2008; 28:1338–1347. [PubMed: 18086890]
17. Crouch EC. Structure, biologic properties, and expression of surfactant protein D (SP-D). *Biochimica et Biophysica Acta-Molecular Basis of Disease*. 1998; 1408:278–289.
18. Hansen S, Holmskov U. Lung surfactant protein D (SP-D) and the molecular diverted descendants: Conglutinin, CL-43 and CL-46. *Immunobiology*. 2002; 205:498–517. [PubMed: 12396011]
19. Hickling TP, Clark H, Malhotra R, Sim RB. Collectins and their role in lung immunity. *J Leukoc Biol*. 2004; 75:27–33. [PubMed: 12972515]
20. Seaton BA, Crouch EC, McCormack FX, Head JF, Hartshorn KL, Mendelsohn R. Review: Structural determinants of pattern recognition by lung collectins. *Innate Immun*. 2010; 16:143–150. [PubMed: 20423923]
21. Chroneos ZC, Sever-Chroneos Z, Shepherd VL. Pulmonary surfactant: an immunological perspective. *Cell Physiol Biochem*. 2010; 25:13–26. [PubMed: 20054141]
22. Hartshorn KL, Crouch EC, White MR, Eggleton P, Tauber AI, Chang D, Sastry K. Evidence for A Protective Role of Pulmonary Surfactant Protein-D (Sp-D) Against Influenza-A Viruses. *Journal of Clinical Investigation*. 1994; 94:311–319. [PubMed: 8040272]
23. Kingma PS, Whitsett JA. In defense of the lung: surfactant protein A and surfactant protein D. *Current Opinion in Pharmacology*. 2006; 6:277–283. [PubMed: 16580255]
24. Kuroki Y, Takahashi M, Nishitani C. Pulmonary collectins in innate immunity of the lung. *Cellular Microbiology*. 2007; 9:1871–1879. [PubMed: 17490408]
25. Botas C, Poulain F, Akiyama J, Brown C, Allen L, Goerke J, Clements J, Carlson E, Gillespie AM, Epstein C, Hawgood S. Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:11869–11874. [PubMed: 9751757]
26. Ochs M, Knudsen L, Allen L, Stumbaugh A, Levitt S, Nyengaard JR, Hawgood S. GM-CSF mediates alveolar epithelial type II cell changes, but not emphysema-like pathology, in SP-D-deficient mice. *Am J Physiol Lung Cell Mol Physiol*. 2004; 287:L1333–L1341. [PubMed: 15310555]
27. Wert SE, Yoshida M, Levine AM, Ikegami M, Jones T, Ross GF, Fisher JH, Korfhagen TR, Whitsett JA. Increased metalloproteinase activity, oxidant production, and emphysema in

- surfactant protein D gene-inactivated mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97:5972–5977. [PubMed: 10801980]
28. Hawgood S, Poulain FR. The pulmonary collectins and surfactant metabolism. *Annual Review of Physiology*. 2001; 63:495–519.
 29. Atochina EN, Beers MF, Hawgood S, Poulain F, Davis C, Fusaro T, Gow AJ. Surfactant protein-D, a mediator of innate lung immunity, alters the products of nitric oxide metabolism. *Am J Respir Cell Mol Biol*. 2004; 30:271–279. [PubMed: 12871850]
 30. Douda DN, Palaniyar N. Pulmonary collectins, arginases and inducible NOS regulate nitric oxide-mediated antibacterial defense and macrophage polarization. *The Open Nitric Oxide Journal*. 2010; 2:69–76.
 31. Atochina-Vasserman EN, Beers MF, Kadire H, Tomer Y, Inch A, Scott P, Guo CJ, Gow AJ. Selective inhibition of inducible NO synthase activity in vivo reverses inflammatory abnormalities in surfactant protein D-deficient mice. *Journal of Immunology*. 2007; 179:8090–8097.
 32. Knudsen L, Atochina-Vasserman EN, Gow AJ, Beers MF, Ochs M. The increased iNOS-activity in the SP-D knockout mice mediates the development of pulmonary emphysema but not changes related to intracellular surfactant homeostasis. *Am J Respir Crit Care Med*. 2010; 181:A2457.
 33. Crouch E, Persson A, Chang D, Heuser J. Molecular-structure of Pulmonary Surfactant Protein-D (SP-D). *Journal of Biological Chemistry*. 1994; 269:17311–17319. [PubMed: 8006040]
 34. Brown-Augsburger P, Chang D, Rust K, Crouch EC. Biosynthesis of surfactant protein D. Contributions of conserved NH₂-terminal cysteine residues and collagen helix formation to assembly and secretion. *J Biol Chem*. 1996; 271:18912–18919. [PubMed: 8756121]
 35. Zhang L, Ikegami M, Crouch EC, Korfhagen TR, Whitsett JA. Activity of pulmonary surfactant protein-D (SP-D) in vivo is dependent on oligomeric structure. *J Biol Chem*. 2001; 276:19214–19219. [PubMed: 11278637]
 36. van Eijk M, Haagsman HP, Skinner T, Archibold A, Reid KBM, Lawson PR. Porcine lung surfactant protein D: Complementary DNA cloning, chromosomal localization, and tissue distribution. *Journal of Immunology*. 2000; 164:1442–1450.
 37. Stamler JS, Lamas S, Fang FC. Nitrosylation: The prototypic redox-based signaling mechanism. *Cell*. 2001; 106:675–683. [PubMed: 11572774]
 38. Kuan SF, Rust K, Crouch E. Interactions of Surfactant Protein-D with Bacterial Lipopolysaccharides - Surfactant Protein-D Is An Escherichia-Coli Binding-Protein in Bronchoalveolar Lavage. *Journal of Clinical Investigation*. 1992; 90:97–106. [PubMed: 1634623]
 39. Wang H, Head J, Kosma P, Brade H, Muller-Loennies S, Sheikh S, McDonald B, Smith K, Cafarella T, Seaton B, Crouch E. Recognition of heptoses and the inner core of bacterial lipopolysaccharides by surfactant protein D. *Biochemistry*. 2008; 47:710–720. [PubMed: 18092821]
 40. Wang L, Brauner JW, Mao GR, Crouch E, Seaton B, Head J, Smith K, Flach CR, Mendelsohn R. Interaction of recombinant surfactant protein D with lipopolysaccharide: Conformation and orientation of bound protein by IRRAS and simulations. *Biochemistry*. 2008; 47:8103–8113. [PubMed: 18620419]
 41. Ikegami M, Scoville EA, Grant S, Korfhagen T, Brondyk W, Scheule RK, Whitsett JA. Surfactant protein-d and surfactant inhibit endotoxin-induced pulmonary inflammation. *Chest*. 2007; 132:1447–1454. [PubMed: 17925426]
 42. Stuehr DJ, Marletta MA. Mammalian Nitrate Biosynthesis - Mouse Macrophages Produce Nitrite and Nitrate in Response to Escherichia-Coli Lipopolysaccharide. *Proceedings of the National Academy of Sciences of the United States of America*. 1985; 82:7738–7742. [PubMed: 3906650]
 43. Balamayooran T, Balamayooran G, Jeyaseelan S. Review: Toll-like receptors and NOD-like receptors in pulmonary antibacterial immunity. *Innate Immun*. 2010; 16:201–210. [PubMed: 20418253]
 44. Ohya M, Nishitani C, Sano H, Yamada C, Mitsuzawa H, Shimizu T, Saito T, Smith K, Crouch E, Kuroki Y. Human pulmonary surfactant protein D binds the extracellular domains of toll-like receptors 2 and 4 through the carbohydrate recognition domain by a mechanism different from its binding to phosphatidylinositol and lipopolysaccharide. *Biochemistry*. 2006; 45:8657–8664. [PubMed: 16834340]

45. Sano H, Chiba H, Iwaki D, Sohma H, Voelker DR, Kuroki Y. Surfactant proteins A and D bind CD14 by different mechanisms. *J Biol Chem.* 2000; 275:22442–22451. [PubMed: 10801802]
46. Nie XM, Nishitani C, Yamazoe M, Arika S, Takahashi M, Shimizu T, Mitsuzawa H, Sawada K, Smith K, Crouch E, Nagae H, Takahashi H, Kuroki Y. Pulmonary Surfactant Protein D Binds MD-2 through the Carbohydrate Recognition Domain. *Biochemistry.* 2008; 47:12878–12885. [PubMed: 18991397]
47. Yamazoe M, Nishitani C, Takahashi M, Katoh T, Arika S, Shimizu T, Mitsuzawa H, Sawada K, Voelker DR, Takahashi H, Kuroki Y. Pulmonary Surfactant Protein D Inhibits Lipopolysaccharide (LPS)-induced Inflammatory Cell Responses by Altering LPS Binding to Its Receptors. *Journal of Biological Chemistry.* 2008; 283:35878–35888. [PubMed: 18990700]
48. Tissieres P, Pugin J. The role of MD-2 in the opsonophagocytosis of Gram-negative bacteria. *Curr Opin Infect Dis.* 2009; 22:286–291. [PubMed: 19395959]
49. Brown-Augsburger P, Hartshorn K, Chang D, Rust K, Fliszar C, Welgus HG, Crouch EC. Site-directed mutagenesis of Cys-15 and Cys-20 of pulmonary surfactant protein D - Expression of a trimeric protein with altered anti-viral properties. *Journal of Biological Chemistry.* 1996; 271:13724–13730. [PubMed: 8662732]
50. Hartshorn KL, White MR, Crouch EC. Contributions of the N- and C-terminal domains of surfactant protein D to the binding, aggregation, and phagocytic uptake of bacteria. *Infection and Immunity.* 2002; 70:6129–6139. [PubMed: 12379690]
51. Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, Henson PM. By binding SIRPalpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell.* 2003; 115:13–23. [PubMed: 14531999]
52. Crouch E, Chang D, Rust K, Persson A, Heuser J. Recombinant pulmonary surfactant protein D. Post-translational modification and molecular assembly. *J Biol Chem.* 1994; 269:15808–15813. [PubMed: 8195236]
53. Atochina-Vasserman EN, Beers MF, Gow AJ. Review: Chemical and structural modifications of pulmonary collectins and their functional consequences. *Innate Immun.* 2010; 16:175–182. [PubMed: 20423921]
54. Atochina-Vasserman EN, Abramova H, Wright JR, Beers MF, Gow AJ, Pastva AM. Lipopolysaccharide (LPS)-induced lung injury is associated with post-translational modifications of Surfactant Protein D (SP-D). *Am J Respir Crit Care Med.* 2010; 181:A4972.
55. Atochina-Vasserman EN, Bates SR, Zhang P, Abramova H, Zhang ZG, Gonzales L, Tao JQ, Gochuico BR, Gahl W, Guo CJ, Gow AJ, Beers MF, Guttentag S. Early alveolar epithelial dysfunction promotes lung inflammation in a mouse model of Hermansky-Pudlak Syndrome. *Am J Respir Crit Care Med.* 2011; 184:449–458. [PubMed: 21616998]
56. Postle AD, Mander A, Reid KBM, Wang JY, Wright SM, Moustaki M, Warner JO. Deficient hydrophilic lung surfactant proteins A and D with normal surfactant phospholipid molecular species in cystic fibrosis. *American Journal of Respiratory Cell and Molecular Biology.* 1999; 20:90–98. [PubMed: 9870921]
57. Noah TL, Murphy PC, Alink JJ, Leigh MW, Hull WM, Stahlman MT, Whitsett JA. Bronchoalveolar lavage fluid surfactant protein-A and surfactant protein-D are inversely related to inflammation in early cystic fibrosis. *Am J Respir Crit Care Med.* 2003; 168:685–691. [PubMed: 12829455]
58. Griese M, Wiesener A, Lottspeich F, von Bredow C. Limited proteolysis of surfactant protein D causes a loss of its calcium-dependent lectin functions. *Biochimica et Biophysica Acta-Molecular Basis of Disease.* 2003; 1638:157–163.
59. Kerr MH, Paton JY. Surfactant protein levels in severe respiratory syncytial virus infection. *Am J Respir Crit Care Med.* 1999; 159:1115–1118. [PubMed: 10194154]
60. Greene KE, Wright JR, Steinberg KP, Ruzinski JT, Caldwell E, Wong WB, Hull W, Whitsett JA, Akino T, Kuroki Y, Nagae H, Hudson LD, Martin TR. Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. *Am J Respir Crit Care Med.* 1999; 160:1843–1850. [PubMed: 10588595]

61. Cheng IW, Ware LB, Greene KE, Nuckton TJ, Eisner MD, Matthay MA. Prognostic value of surfactant proteins A and D in patients with acute lung injury. *Critical Care Medicine*. 2003; 31:20–27. [PubMed: 12544988]
62. Eisner MD, Parsons P, Matthay MA, Ware L, Greene K. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. *Thorax*. 2003; 58:983–988. [PubMed: 14586055]
63. Determann RM, Royakkers AA, Haitsma JJ, Zhang H, Slutsky AS, Ranieri VM, Schultz MJ. Plasma levels of surfactant protein D and KL-6 for evaluation of lung injury in critically ill mechanically ventilated patients. *BMC Pulm Med*. 2010; 10:6. [PubMed: 20158912]
64. Griesse M, Steinecker M, Schumacher S, Braun A, Lohse P, Heinrich S. Children with absent surfactant protein D in bronchoalveolar lavage have more frequently pneumonia. *Pediatric Allergy and Immunology*. 2008; 19:639–647. [PubMed: 18266831]
65. Mutti A, Corradi M, Goldoni M, Vettori MV, Bernard A, Apostoli P. Exhaled metallic elements and serum pneumoproteins in asymptomatic smokers and patients with COPD or asthma. *Chest*. 2006; 129:1288–1297. [PubMed: 16685021]
66. Nakane T, Nakamae H, Kamoi H, Koh H, Takeoka Y, Sakamoto E, Kanashima H, Nakamae M, Ohta K, Terada Y, Koh KR, Yamane T, Hino M. Prognostic value of serum surfactant protein D level prior to transplant for the development of bronchiolitis obliterans syndrome and idiopathic pneumonia syndrome following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplantation*. 2008; 42:43–49. [PubMed: 18347569]
67. Matalon S, Shrestha K, Kirk M, Waldheuser S, McDonald B, Smith K, Gao Z, Belaaouaj A, Crouch EC. Modification of surfactant protein D by reactive oxygen-nitrogen intermediates is accompanied by loss of aggregating activity, in vitro and in vivo. *FASEB J*. 2009; 23:1415–1430. [PubMed: 19126597]
68. Atochina-Vasserman EN, Winkler C, Abramova H, Schaumann F, Krug N, Gow AJ, Beers MF, Hohlfeld JM. Segmental Allergen Challenge Alters Multimeric Structure and Function of Surfactant Protein D in Humans. *Am J Respir Crit Care Med*. 2011; 183:856–864. [PubMed: 21131470]
69. Starosta V, Rietschel E, Paul K, Baumann W, Griesse M. Oxidative changes of bronchoalveolar proteins in cystic fibrosis. *Chest*. 2006; 129:431–437. [PubMed: 16478863]
70. Winkler C, Atochina-Vasserman EN, Holz O, Beers MF, Erpenbeck VJ, Krug N, Roepke S, Lauer G, Elmlinger M, Hohlfeld JM. Comprehensive characterisation of pulmonary and serum surfactant protein D in COPD. *Respir Res*. 2011; 12:29. [PubMed: 21396106]
71. Crouch EC, Hirche TO, Shao B, Boxio R, Wartelle J, Benabid R, McDonald B, Heinecke J, Matalon S, Belaaouaj A. Myeloperoxidase-dependent inactivation of surfactant protein D in vitro and in vivo. *J Biol Chem*. 2010
72. Sorensen GL, Husby S, Holmskov U. Surfactant protein A and surfactant protein D variation in pulmonary disease. *Immunobiology*. 2007; 212:381–416. [PubMed: 17544823]
73. Leth-Larsen R, Garred P, Jensenius H, Meschi J, Hartshorn K, Madsen J, Tornøe I, Madsen HO, Sorensen G, Crouch E, Holmskov U. A common polymorphism in the SFTPD gene influences assembly, function, and concentration of surfactant protein D. *J Immunol*. 2005; 174:1532–1538. [PubMed: 15661913]
74. Heidinger K I, König R, Bohnert A, Kleinsteinber A, Hilgendorff A, Gortner L, Ziegler A, Chakraborty T, Bein G. Polymorphisms in the human surfactant protein-D (SFTPD) gene: strong evidence that serum levels of surfactant protein-D (SP-D) are genetically influenced. *Immunogenetics*. 2005; 57:1–7. [PubMed: 15700120]
75. Griesse M, Maderlechner N, Ahrens P, Kitz R. Surfactant proteins A and D in children with pulmonary disease due to gastroesophageal reflux. *Am J Respir Crit Care Med*. 2002; 165:1546–1550. [PubMed: 12045131]
76. Hartshorn KL, White MR, Tecle T, Tornøe I, Sorensen GL, Crouch EC, Holmskov U. Reduced influenza viral neutralizing activity of natural human trimers of surfactant protein D. *Respiratory Research*. 2007; 8. [PubMed: 17263874]
77. Ware LB, Koyama T, Billheimer D, Wu W, Bernard GR, Thompson BT, Brower RG, Standiford TJ, Martin TR, Matthay MA. Prognostic and Pathogenetic Value of Combining Clinical and

- Biochemical Indices in Patients With Acute Lung Injury. *Chest*. 2010; 137:288–296. [PubMed: 19858233]
78. Todd DA, Marsh MJ, George A, Henderson NG, Barr H, Sebastian S, Clark GT, Koster G, Clark HW, Postle AD. Surfactant phospholipids, surfactant proteins, and inflammatory markers during acute lung injury in children. *Pediatric Critical Care Medicine*. 2010; 11:82–91. [PubMed: 19550365]
 79. Lambrecht BN. Alveolar macrophage in the driver's seat. *Immunity*. 2006; 24:366–368. [PubMed: 16618595]
 80. Thepen T, Kraal G, Holt PG. The Role of Alveolar Macrophages in Regulation of Lung Inflammation. *Cells and Cytokines in Lung Inflammation*. 1994; 725:200–206.
 81. Bosio CM, Dow SW. Francisella tularensis induces aberrant activation of pulmonary dendritic cells. *Journal of Immunology*. 2005; 175:6792–6801.
 82. Gonzalez-Juarrero MI, Orme M. Characterization of murine lung dendritic cells infected with Mycobacterium tuberculosis. *Infection and Immunity*. 2001; 69:1127–1133. [PubMed: 11160010]
 83. von Garnier C, Filgueira L, Wikstrom M, Smith M, Thomas JA, Strickland DH, Holt PG, Stumbles PA. Anatomical location determines the distribution and function of dendritic cells and other APCs in the respiratory tract. *Journal of Immunology*. 2005; 175:1609–1618.
 84. Zhang XK, Morrison DC. Lipopolysaccharide-Induced Selective Priming Effects on Tumor-Necrosis-Factor-Alpha and Nitric-Oxide Production in Mouse Peritoneal-Macrophages. *Journal of Experimental Medicine*. 1993; 177:511–516. [PubMed: 8426119]
 85. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends in Immunology*. 2004; 25:677–686. [PubMed: 15530839]
 86. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Frontiers in Bioscience*. 2008; 13:453–461. [PubMed: 17981560]
 87. Mantovani A, Sica A, Locati M. Macrophage polarization comes of age. *Immunity*. 2005; 23:344–346. [PubMed: 16226499]
 88. Gordon S. Alternative activation of macrophages. *Nature Reviews Immunology*. 2003; 3:23–35.
 89. Guth AM, Janssen WJ, Bosio CM, Crouch EC, Henson PM, Dow SW. Lung environment determines unique phenotype of alveolar macrophages. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2009; 296:L936–L946. [PubMed: 19304907]
 90. Atochina-Vasserman EN, Abramova H, Tomer Y, Scott P, Nazarov VA, Kruglov SV, Beers MF, Gow AJ, Malyshev IY. SP-D-Dependent Regulation of NO Metabolism in Lipopolysaccharide-Stimulated Peritoneal Macrophages. *Bulletin of Experimental Biology and Medicine*. 2009; 147:415–420. [PubMed: 19704937]
 91. Casey J, Kaplan J, Atochina-Vasserman EN, Gow AJ, Kadire H, Tomer Y, Fisher JH, Hawgood S, Savani RC, Beers MF. Alveolar surfactant protein D content modulates bleomycin-induced lung injury. *Am J Respir Crit Care Med*. 2005; 172:869–877. [PubMed: 15994463]
 92. Jain D, Tomer Y, Malur A, Thomassen MJ, Beers MF. SP-D Modulates Alveolar Macrophage Polarization and Activation. *Am J Respir Crit Care Med*. 2009; 179:A6270.
 93. Jain D, Atochina-Vasserman EN, Tomer Y, Kadire H, Beers MF. Surfactant protein D protects against acute hyperoxic lung injury. *Am J Respir Crit Care Med*. 2008; 178:805–813. [PubMed: 18635887]
 94. Guo, CJ.; Atochina-Vasserman, EN.; Abramova, H.; Manoj, VM.; Beers, MF.; Gow, AJ. Oxidative/Nitrosative Stress and Disease. New York Academy of Sciences; 2009. Surfactant protein-D structure is critical in determining macrophage phenotypic response to pathogens.

Highlights

- Multimerization of SP-D is a critical feature of its function
- NO is capable of modifying SP-D through *S*-nitrosylation resulting in a disruption of the SP-D quaternary structure
- The formation of SNO-SP-D initiates a pro-inflammatory response through NFκB activation
- Disruption of the native multimeric state of SP-D results in a loss of its anti-inflammatory capability

Figure 1. The “Dandelion Ball Model” of the immunomodulatory functions of SP-D in macrophages

Under baseline conditions the hydrophobic N-terminus of SP-D is hidden in the center of the “dandelion ball” with exposed carboxy terminal domains outside. Numerous studies have shown that CRD-specific interactions of SP-D with immune cells may be mediated through a number of receptors. It has been proposed that binding of SP-D “dandelion ball” via its CRD domains with SIRP-1 α induces the activation of tyrosine phosphatase SHP-1, resulting in blockage of the downstream signaling through p38 MAP kinase [51]. The interaction of CRD domain of SP-D “dandelion ball” with sCD14, sMD2 and TLR4 may blocks the binding of LPS to these receptors leading to inhibition of NF- κ B activation [44–46]. Potentially, this may be achieved by SP-D inhibiting TLR4 dimerization in the presence of LPS.

Under pro-inflammatory circumstances *S*-nitrosylation of cysteine residues in the hydrophobic tail domain of SP-D results in disruption of multimeric SP-D structure (disassembling of a “dandelion ball”) and exposure of *S*-nitrosylated tail domain [3]. The exposed *S*-nitrosylated tail domain is now available to bind with the calreticulin/CD91 receptor complex and initiate a pro-inflammatory response through phosphorylation of p38 and NF- κ B activation. [3,4].

In addition, within this model one can see that the disassembled “dandelion ball” of SP-D would have lost its ability to block LPS mediated activation of NF- κ B. In the absence of multimeric SP-D, or in its absence, LPS forms a CD14-MD2-TLR4 complex which triggers TLR4 dimerization and recruitment of the adaptor protein, MyD88. Dimerization results in IRAK phosphorylation and oligomerization of TRAF6. Assembled TRAF6–TAK1–TAB1–TAB2 complex is translocated to the cytosol where it phosphorylates I κ B through activation of IKK and therefore results in translocation of NF- κ B to the nucleus with the consequent transcription of immune response genes [8–11,45].

Figure 2. Schematic structure of the SP-D

(A) A Kyle Doolittle hydropathy plot for the SPD sequence was constructed using an averaging sliding window of 9 residues; a positive value that indicates a region of hydrophobicity. (B) The amino acid sequences of mature SP-D polypeptide chain consist of four structural domains: an N-terminal domain; a collagen domain; a α -helical neck region; and a globular CRD involved in Ca^{2+} -dependent binding of various groups of ligands. The three polypeptide chains combine to form a trimeric structure that is assembled into a large symmetrical cruciform dodecamer consisting of a tetramer of trimers. The position of reduced cysteines 15 and 20 buried within the most hydrophobic region of the N-terminal domain, suggest it as a motif for *S*-nitrosylation.

Figure 3. Signaling in SP-D is NO-dependent

NO can modify SP-D by nitrosylating two critical cysteines in its tail domain resulting in disruption of SP-D oligomeric structure and the release of *S*-nitrosothiol containing SP-D trimers (SNO-SP-D) that stimulate macrophage migration.

(A) Disruption multimeric structure of SP-D. Recombinant rat SP-D (rrSP-D) or the mutant (Ser^{15/20}) was incubated with 200 μ M saline, SNOC (*S*-nitrosocysteine) or L-cysteine for 30 min at room temperature and then the multimeric structure of SP-D was analyzed by native gel electrophoresis followed by western blotting with anti-SP-D antibody. **(B) *S*-nitrosylated SP-D** was analyzed by biotin switch assay followed by SDS-PAGE and Western blotting with anti-SP-D antibody. **(C) Chemotactic activity** of modified rrSP-D or the mutant Ser^{15/20} was analyzed using Boyden chamber with RAW 264.7 cells.