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REVIEW

The Immune Modulatory Role of Surfactants in *Mycoplasma pneumoniae* Infection

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Abstract: *Mycoplasma pneumoniae* is a prevalent respiratory microbe that causes acute inflammation in the respiratory system. Surfactant proteins (SP), particularly SP-A and SP-D, are essential for the immunological protection against *M. pneumoniae* infection. Variant SP-A2 may lead to immune reactions, which could account for the variability in clinical manifestations among individuals. Mechanistically, these surfactant proteins may act as candidate receptors, facilitating both the adhesion of *M. pneumoniae* and internalization of community-acquired respiratory distress syndrome toxin. They also exhibit a high affinity for lipid ligands on the surface of *M. pneumoniae* membranes via their carbohydrate recognition domains, which aid in the direct clearing of the bacteria. In addition, SP-A and SP-D demonstrated synergistic effects in augmenting the intake and elimination of *M. pneumoniae* by alveolar macrophages. Furthermore, these surfactant proteins negatively regulate pulmonary inflammation by influencing lymphocyte and dendritic cell activities, reducing airway eosinophilic infiltration, and managing asthma-related inflammatory responses. A thorough understanding of the immunomodulatory roles of surfactant proteins in *M. pneumoniae* infection will shed light on how homeostasis is preserved during mycoplasma pneumonia and may guide the development of novel therapeutic strategies against this organism.

Keywords: *Mycoplasma pneumoniae*, surfactant protein-A, surfactant protein-D, immune modulation

Introduction

Mycoplasma pneumoniae is the principal causative agent of pneumonia and many respiratory disorders, including tracheobronchitis, bronchiolitis, laryngitis, and pharyngitis, and often worsens many respiratory diseases, such as asthma, emphysema, and chronic obstructive pulmonary disease.[1–4] *M. pneumoniae* also acts as an undesirable contaminant in advanced therapy medicinal products (ATMPs), posing significant challenges for detection, particularly in immunosuppressed patients.[5,6] In the normal population, approximately 30% of pneumonia cases are associated with *M. pneumoniae*. However, in vivo experiments have revealed significant differences in bacterial load following *M. pneumoniae* infection across various mouse strains,[7] highlighting the importance of host genetic background over the innate immune status. The pronounced difference in susceptibility between C57BL/6NCr and C3H/HeNCr mice represents different immune responses to *M. pneumoniae* infection.[8] Early resistance to *M. pneumoniae* in C57BL mice may stem from innate defense mechanisms associated with alveolar macrophages (AMs) enriched with pulmonary surfactants, whereas impairment of these mechanisms is observed in C3H/HeNCr mice.[9] Although *M. pneumoniae* develops multiple strategies to escape the host immune system and even cause persistent infection, it can still be cleared

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by the host immune immunity,[10] in which surfactants play a pivotal role prior to the activation of antibody-mediated adaptive immunity.[11]

Pulmonary surfactants are vital components of the bronchoalveolar microenvironment and are composed of proteins, neutral lipids, and phospholipids.[12] Surfactants are predominantly produced by alveolar type II cells and disseminate across the molecular layer of the alveolar fluid.[13] Their role is essential in alleviating tension at the interface between gas and liquid in the alveoli, preventing atelectasis upon exhalation, and facilitating effective gas exchange.[14] In addition to their biomechanical roles, the protein constituents of pulmonary surfactants serve as distinctive elements of the natural immune system.[15] Surfactant proteins can be classified into four categories: surfactant protein A (SP-A), SP-B, SP-C, and SP-D.[16,17] Among them, SP-A and SP-D belong to the C-type lectin family and are particularly important for their interactions with bacteria, fungi, and viruses, thereby playing crucial roles in immune defense against inhalation pathogens.[18] For instance, SP-A can facilitate pathogen recognition and modulate the activity of immune cells, including AMs, neutrophils, and dendritic cells (DCs), thereby promoting or suppressing the overall inflammatory response, depending on the infection environment.[19] Similarly, SP-D is crucial for adaptive and innate immunity, as it aggregates pathogens and promotes their uptake by AMs, while also regulating T and B cell activity to enhance the efficiency of the immune response.[20]

While pulmonary surfactants are not selective for the bacteria with which they interact, they exhibit a notable capacity to facilitate the internalization of community-acquired respiratory distress syndrome toxin (CARDS TX), which is a major virulence factor of *M. pneumoniae*.[21] Given that pulmonary surfactants serve as frontline defenders against infections, exploring their role in the immunological response to mycoplasma pneumonia is important. Since the 1990s, extensive literature has shown that surfactants contribute to various aspects of *M. pneumoniae* infections, including receptor recognition, bactericidal activity, and immune modulation (Figure 1). This study aimed to elucidate the functions of SP-A and SP-D in *M. pneumoniae* infections and immune responses. Understanding the relationship between surfactant proteins and *M. pneumoniae* is crucial for the development of targeted therapies aimed at restoring normal lung function and improving patient outcomes.

Chemical Composition and Classification of Surfactant Proteins

The isolated surfactant contains approximately 80% lipids, 10% neutral lipids (predominantly cholesterol), and 10% proteins. The lipids of surfactants are mainly phospholipids (95%), of which phosphatidylcholine (PC) is the most common, accounting for 70–80%.[22] The main component of PC is saturated dipalmitoylphosphatidylcholine (DPPC), which can accumulate at very high densities at the air-liquid interface, allowing for the lung exchange of gases by reducing alveolar surface tension.[23] In addition, about half of the phospholipid components are desaturated, with approximately 15% acidic phospholipids such as phosphatidylglycerol (PG), phosphatidylinositol (PI), and bis(monoacylglycerol) phosphate, alongside minor quantities of phosphatidylethanolamine (PE), sphingomyelin (SM), and lysophosphatidylcholine (LPC).[14] These lipids are believed to be involved in selected interactions with cationic hydrophobic proteins.[24]

In contrast, the protein content of surfactants is significantly lower than that of lipids and surfactants are synthesized and secreted mainly by bronchiolar Clara cells and alveolar type II cells.[25] Currently, four common surfactant proteins are directly involved in reducing alveolar surface tension and regulating the host immune defense. These proteins can be categorized into macromolecular hydrophilic proteins, SP-A and SP-D, and smaller hydrophobic proteins, SP-B and SP-C, based on their hydrophilic properties and molecular weight.[17] SP-B and SP-C are tiny molecules that react with surfactant lipids to lower the surface tension between the gas and liquid and enhance lung compliance, thereby maintaining normal alveolar expansion.[26] Conversely, SP-A (26–36 kDa) and SP-D (39 kDa) tend to be large soluble hydrophilic proteins present on the majority of mucosal surfaces, with critical overlap and multiple functions in the natural defense and immune homeostasis of the lung.[27] Intriguingly, despite the fact that SP-A and SP-D are equally crucial in immune responses, the level of SP-D in the surfactant of the alveolar epithelium is significantly lower than that of SP-A (approximately 10 times).[28] SP-A and SP-D, along with mannose-binding lectins (MBL) and serum proteins conglutinin and collectin-43 (CL-43), belong to the C-type lectin family,[29] an archaic class of carbohydrate-binding proteins that perform diverse biological functions, including immune defense, blood coagulation, and cellular interaction mediation.[30]

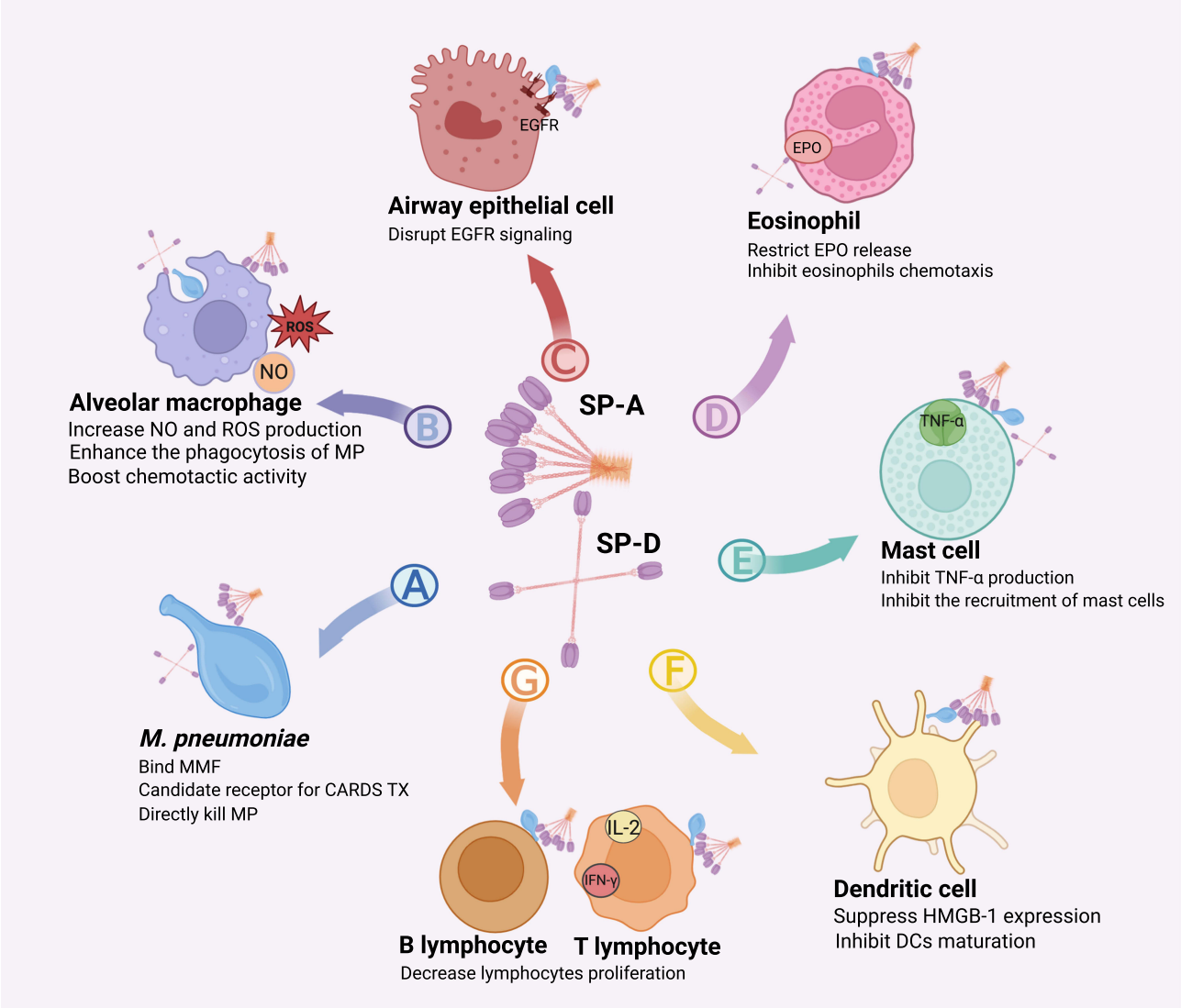

Figure 1 Overview of the immune modulation by pulmonary surfactant proteins upon *Mycoplasma pneumoniae* infection. Pulmonary surfactants contribute to various aspects of *M. pneumoniae* infection. SP-A and SP-D exhibit high affinity for live *M. pneumoniae* and MMF, enabling them to directly kill *M. pneumoniae*. In contrast, SP-A acts as a co-receptor for CARDS TX, resulting in ADP ribosylation and vacuolation in host cells after internalization (**A**). SP-A and SP-D attach to alveolar macrophages (AMs) with high specificity, release ROS and nitric oxide metabolites, increase chemotactic activity, and augment the phagocytic capacity of AMs to eliminate *M. pneumoniae* (**B**). The prominent function of surfactant proteins is the negative regulation of the immune system, including interaction with epidermal growth factor receptor to suppress epithelial cell secretion of mucin (**C**), alleviation of airway eosinophil infiltration and inflammation by inhibiting the release of EPO, or inhibition of eosinophil chemotaxis (**D**). In addition, SP-A is suggested to inhibit mast cells recruitment and the subsequent release of TNF-α to regulate the exacerbation of airway hyperresponsiveness and the associated influx of inflamed cells in reaction to *M. pneumoniae* infection (**E**). Other candidate functions include inhibiting dendritic cells maturation and suppressing the expression of HMGB-1 (**F**) or attenuating cytokine production by T lymphocytes, thereby directly or indirectly influencing T cell proliferation and increasing the number of activated B cells during *M. pneumoniae* infection (**G**). These factors enable the host immune system to fine-tune homeostasis during *M. pneumoniae* infection. Created in BioRender. You, X. (2025) https://BioRender.com/g33c697.

Structure of SP-A and SP-D

SP-A and SP-D are classified as collectins, because their lectin domains are associated with collagen-like regions. Like other types of lectins, both possess four distinct domains: the cysteine-rich N-terminal domain, the original triple helix collagen domain characterized by repeating Gly-X-Y triplets, the neck region featuring short hydrophobic amino acid segments as well amphiphilic helices, and the C-terminal carbohydrate recognition domain (CRD).[28] The triple-helical collagen-like regions of SP-A and SP-D consist of 23 and 59 repeated Gly-X-Y motifs, respectively, where X and Y may represent any amino acid, usually proline or hydroxyproline.[17,28] Similar to other Ca^{2+}-dependent lectins, the CRD of both SP-A and SP-D contain four conserved cysteine residues

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that serve as Ca^{2+}-dependent specific carbohydrate-binding sites.[31] Both SP-A and SP-D spatially construct effective trimers that are maintained by disulfide bonds in the N-terminal region, triple-helix collagen-like segments, and hydrophobic interactions that generate coiled helical regions by strong engagement of the three-helix neck segments.[27] These functional trimers can subsequently oligomerize into an octadecameric-like structure for SP-A (similar to a bunch of tulips) and a dodecameric cruciform-like structure, which may further reassemble into star multimers for SP-D.[19,27] The affinity and specificity of SP-A and SP-D for bacterial surfaces are derived from their distinctive carbohydrate-binding motifs and the spatial arrangement of their multiple CRDs, positioned at the C-terminus of every trimer unit at certain angles and intervals, ultimately augmenting the overall affinity for binding to carbohydrate targets on microorganisms and facilitating bacterial phagocytosis.[32] Therefore, SP-A and SP-D, also known as "carbohydrate pattern recognition molecules", predominantly interact with glycoconjugates and lipids on microbial surfaces through their CRDs.[27]

Functions of Surfactant Proteins in Immune System

The host lung defense system comprises AMs, antimicrobial peptides, surfactant lipids, and protein constituents.[14] Pulmonary proteins, specifically SP-A and SP-D, engage with diverse cell surface ligands on leukocytes, thereby modulating cellular functions related to phagocytosis and immune responses. Specifically, SP-A demonstrates anti-inflammatory effects in vitro by lowering the generation of cytokines, notably TNF-α and IL-1β, from AMs induced by LPS,[33] as well as inhibiting mitogen-induced T cell proliferation.[34] Furthermore, SP-A modulates the creation of major mediators, such as TNF and IL-2, in lung lymphocytes, which subsequently affects AM function within the innate pulmonary immune system.[35] Besides their functions as opsonins, SP-A and SP-D moonlight towards driving factors for apoptotic cell clearance and bacterial killing[11] (Table 1).

Augment Phagocytic Activity of Phagocytes

Surfactant proteins facilitate the absorption of germs, viruses, and allergens by AMs, monocytes, neutrophils, and DCs; delayed microbial clearance has been observed in surfactant-deficient mice.[45] Nevertheless, under specific circumstances, pulmonary surfactants can exert opposing effects, such as inhibiting immune cell phagocytosis of *Pneumocystis yersoni*.[11]

Under normal conditions, SP-A and SP-D are believed to impede phagocytosis in macrophages by engaging their CRDs interacting with the transmembrane receptor signal inhibition regulator-α (SIRP-α) and result in the activation of their downstream effectors like SHP-1 and RhoA.[46] After infection, the functions of SP-A and SP-D undergo a notable inversion, markedly enhancing the phagocytic abilities of macrophages. The mechanisms driving this shift include, but are not limited to ①Function as opsonins by interacting with membrane receptors such as SP-R210, Toll-like receptor 2 (TLR2), TLR4, CD14, SIRP-α, and CD91-calreticulin,[19] thereby augmenting the phagocytic capacity of macrophages against invading pathogens, including *Aspergillus fumigatus (conidia)*,[47] *Cryptococcus neoformans*,[48,49] *Candida*

Table 1 Potential Functions of SP-A and SP-D in *M. pneumoniae* Infection

Surfactant	Action Target	Function	Reference
SP-A	Function as receptor for CARDS TX	Lung epithelial cells vacuolization	[36]
	Binding to phosphatidylglycerol on *M. pneumoniae*	Aggregate *M. pneumoniae*	[37, 38]
	Binding to TNF receptors on Mast cells	Inhibit Mast cells release of TNF-α	[39]
	AMs	Enhance the mycoplasmacidal activity	[40]
	Binding to eosinophils	Limit eosinophils release of EPO	[41]
	Binding to *M. pneumoniae*	Inhibition of DCs maturation	[42]
	Block the EGFR signaling pathway	Limit mucin production by MMF	[43]
SP-D	Binding to phosphatidylinositol on *M. pneumoniae*	Aggregate *M. pneumoniae*	[38]
	Behave as C1q to bind AMs	Enhance the mycoplasmacidal activity	[44]

albicans,[50] and *Pneumocystis jirovecii*,[51,52] or directly interact with the phagocytosis receptor, scavenger receptor A, and mannose-receptor on macrophages to increase phagocytosis of microbes and apoptotic cells.[53] ②Acting as an activation ligand to sense pathogens through their CRDs region to stimulate the phagocytic function of macrophages and monocytes.[45] ③Behave as C1q to drive phagocytosis absorption of apoptotic cells through its interaction with CD91-calreticulin (C1qR),[54] a receptor complex common to the collagen lectin family[55] present on the surface of AMs owing to their homology with C1q,[56] although SP-D has a stronger mediating phagocytosis compared to SP-A.[55] ④Enhance Fc receptor- (FcR) and C3b/C4b receptor (CR1)-facilitated phagocytosis in monocytes and macrophages in vitro.[57,58] ⑤Binding to viscous DNA released by dead microbes or necrotic cells to facilitate the absorption of DNA or apoptotic cells by macrophages. SP-D-deficient mice exhibit impaired clearance of free DNA from the lungs.[59] Recently, a newly discovered secretory surfactant candidate, surfactant-associated protein 3 (SFTA3), was shown to enhance phagocytic efficiency in the lungs and may contribute to the clearance of particles and pathogenic microbes.[60]

Negatively Regulate the Inflammatory Response

Accumulating evidence shows that phosphatidylglycerol and phosphatidylinositol, the main components of pulmonary surfactants, exert anti-inflammatory action by inhibiting the activation of a variety of TLRs (such as TLR2/1, TLR3, TLR4, and TLR2/6), resulting in the secretion of anti-inflammatory mediators that weaken and destroy various respiratory RNA viruses.[61] Additionally, SP-A and SP-D are reported to be associated with CD14[62] or TLR2[63] and to block inflammatory cellular responses induced by stimuli such as smooth LPS, peptidoglycan, or yeast glycan. Borron et al reported that intratracheal administration of LPS leads to the production of TNF-α and nitric oxide (NO) in bronchoalveolar lavage fluid (BALF) in SP-A-deficient mice, which were significantly higher compared to those of wild mice.[64] Moreover, SP-A knockout models exhibit heightened vulnerability to various bacterial and viral infections, such as *Pseudomonas aeruginosa*,[65] *Group B streptococcus*,[66] *Influenza A virus*,[67] *Haemophilus influenzae indissoluble*,[36] and *Respiratory syncytial virus* (RSV),[68] and an enhanced inflammatory response to pathogen attack. From a mechanistic perspective, SP-A and SP-D may communicate with membrane receptors, including TLRs, SIRPa, and CD91-calreticulin, on leukocytes, thereby inhibiting microbial recognition and influencing the generation of cytokines and inflammatory agents in a microbial ligand-specific form.[45,69] This process then alters the activity of innate immune cells, such as AMs, that produce IFN-γ and inducible nitric oxide synthase (iNOS)[70] to negatively regulate the inflammatory balance during infection.

Modulation of Lymphocytes and DCs

SP-A may activate host immunological responses during the early stages of infection. When the anti-infective response is sufficient, SP-A subsequently inhibits the production of inflammatory mediators through AMs and ultimately influences T-lymphocyte activity, thereby safeguarding fragile lung tissue from T cell-mediated damage in the alveolar space.[70] Both SP-A and SP-D can directly regulate lymphocyte functions at various stages, such as inducing a $CD4^+CD25^+Foxp3^+$ suppressor regulatory T cell population through a TGF-β-dependent mechanism[71] and inhibiting the generation of cytokines by T lymphocytes, such as IL-2 and IFN-γ, thereby directly or indirectly influencing T cell proliferation, lymphokine-activated killer cell activation, and adhesion molecule expression,[72–74] ultimately reducing tissue damage caused by overactive immune responses. Furthermore, SP-A and SP-D inhibit DC maturation. For instance, SP-A attaches to DCs and inhibits their maturation in culture, consequently diminishing their capacity to stimulate allogeneic T cells.[75] However, surfactant proteins seem to demonstrate different effects in augmenting particle antigen presentation, as SP-A inhibits DCs maturation, whereas SP-D promotes bone marrow-derived DCs maturation and enhances its antigen-presenting capacity by binding to specific receptors, such as the SP-D receptor and CD91.[76] The diversity of SP-A and SP-D ensures the accurate regulation of immunity under various infection conditions.

Functions of Surfactants During *M. pneumoniae* Infection

Functions as Receptor for *M. pneumoniae*

Common virulence factors of *M. pneumoniae* include membrane lipoproteins, metabolites, and secretory toxins.[77] The newly discovered CARDS TX is a 68 kDa membrane-binding protein, referred to as MPN372, which is thought to have

a subunit similar to the S1 component of the pertussis toxin.[78,79] The expression level of CARDS TX can be markedly elevated once *M. pneumoniae* infects host cells and demonstrates a strong affinity for SP-A in AMs, alveolar epithelial cells, and various other tissue cells.[80] Recombinant CARDS TX is a Ca^{2+}-dependent protein that interacts with SP-A in a concentration-dependent manner. The CARDS TX antiserum has been shown to block the adhesion of *M. pneumoniae* to SP-A,[21] suggesting that SP-A may serve as a candidate receptor that facilitates both *M. pneumoniae* adhesion and CARDS TX internalization. Furthermore, CARDS TX binds to SP-A in airway epithelial cells through the AnxA2 receptor-mediated pathway, leading to its internalization and transport, which results in ADP ribosylation and vacuolation activity in mammalian cells.[81] Furthermore, the inhibition of AnxA2 and SP-A diminished the binding of CARDS TX and the ensuing vacuolation of cells.[21] Krishnan et al revealed that SP-A-deficient cells did not appear to influence CARDS TX internalization,[82] suggesting the existence of other receptors that could substitute for the absence of SP-A. Conversely, the suppression of AnxA2 significantly diminishes CARDS TX adherence, internalization, and vacuolation toxicity.[83] This indicates that SP-A may be merely one of the multiple receptors for CARDS TX, and its precise physiological function remains ambiguous, perhaps reflecting a larger dimension of the host's antimicrobial immunological response.

Direct Antibacterial Action by High Affinity Bind *M. pneumoniae*

In vitro investigations have indicated that SP-A and SP-D exert direct antimicrobial properties on various pathogenic organisms, including *Escherichia coli, Klebsiella pneumoniae,* and *Histoplasma capsulatum* by increasing their membrane permeability.[11,30] SP-A and SP-D exhibit opsonic activity within their immunological activities and bind to and aggregate with pathogens through their CRDs.[20] This process enhances the uptake of microorganisms by immune cells including macrophages and neutrophils, thus facilitating efficient pathogen clearance.[53] The membrane components of *M. pneumoniae* could promote eicosanoid synthesis in macrophages via TLR2 receptors. This pathogen-induced reaction can be eliminated by the anionic surfactant palmitoyl-oleoyl-phosphatidylglycerol,[84] which has generated great interest owing to the significance of surfactant proteins in *M. pneumoniae* infection. Specifically, the CRDs of SP-A and SP-D exhibit a strong affinity for lipid ligands on the membrane of *M. pneumoniae*, particularly a subset of unsaturated phosphatidylglycerols.[37,38] This is a crucial determinant in the relationship between SP (SP-A and SP-D) and ligands present on the membrane of complete *M. pneumoniae*[85] and is strictly dependent on Ca.[2+86] This interaction can be completely inhibited by the divalent cation-chelating agents EGTA and dipalmitoyl phosphatidylglycerol[87] and is unaffected by other portion of the surfactant proteins (ie, lipid or hydrophobic surfactant proteins).[14] This interaction not only impedes the growth of *M. pneumoniae*, as evidenced by decreased colony formation, metabolism, and DNA replication, but also significantly contributes to the establishment of antibody-independent immunity against the bacteria,[86] thus enabling SP-A and SP-D to facilitate the direct elimination of *M. pneumoniae*. Meanwhile, Kannan et al found that mannose did not impede the binding of *M. pneumoniae* to hSP-A,[21] revealing that CRDs and mannose-related components may not be directly involved in this interaction, but rather serve to enhance the clearance of pathogens by macrophages. Interestingly, the lipid profile recognized by SP-D differs from that of SP-A, although both share a certain degree of overlap.[38] Moreover, bacterial recognition by SP-D involves single nucleotide polymorphisms (SNPs), since tandem mutants (E321Q/N323D) with altered SP-D carbohydrate content were unable to bind the lipid ligands of *M. pneumoniae*.[38]

Enhancing the Eradication of *M. pneumoniae* by AMs

AMs, situated within a phospholipid-rich milieu alongside SP-A and SP-D, act as key effectors that facilitate early clearance of *M. pneumoniae* both in vivo and in vitro, significantly contributing to the ensuing acquired immune response.[19] SP-A and SP-D have been documented to attach to AMs with high specificity,[38,44] affect the release of reactive oxygen species (ROS)[88] and NO metabolites,[69] boost chemotactic activity,[89] and augment the phagocytic capacity of AMs to eliminate *M. pneumoniae*. The mechanism by which SP-A and SP-D eliminate *M. pneumoniae* may be linked to a temperature-sensitive, NO-dependent pathway,[90] as pharmacological inhibition of iNOS markedly diminished SP-A-induced eradication of *M. pneumoniae*.[40] Hickman-Davis et al further revealed the SP-A-mediated death of *M. pneumoniae* with AMs via the generation of peroxynitrite.[40] In vivo tests also demonstrated that, following

M. pneumoniae infection, the concentrations of NO in the BALF within C57BL/6 mice were significantly higher than those in SP-A-deficient mice,[91] and the *M. pneumoniae* load and inflammation severity in iNOS-deficient mice were considerably greater than those in wild-type mice.[92] However, the absence of iNOS expression cannot be offset by alternative NO sources.[40] Furthermore, as the synthesis of NO is facilitated by TNF-α, SP-A may also impede NO production by inhibiting TNF-α secretion in Ams.[93] Interestingly, the function of SP-A in the eradication of *M. pneumoniae* appears to be restricted to lower bacterial doses and the initial phases of infection. SP-A regulates NO production in a stimulus-specific manner; it inhibits NO production in the uninfected state but promotes NO formation during *M. pneumoniae* infection.[91] Furthermore, SP-A and SP-D modulated ROS generation in AMs. ROS, including hydrogen peroxide, hydroxyl radicals, and superoxide, enhanced the antibacterial efficacy of SP-A and SP-D against *M. pneumoniae*.[94] Simultaneously, superoxide anions and hydrogen peroxide may kill *M. pneumoniae* directly or indirectly by generating extremely reactive oxygen-nitrogen intermediates, including hydroxyl radicals and peroxynitrite.[40]

Negative Modulation of *M. pneumoniae*-Induced Inflammation

Polymorphisms of SP-A and *M. pneumoniae* Pneumonia

Allelic variations in SP-A significantly influence its immunomodulatory functions. Unlike rodents, human SP-A is encoded by two functional genes, SFTPA1 (SP-A1) and SFTPA2 (SP-A2), which are transcribed in opposite directions.[95] Each gene exhibits considerable genetic and epigenetic complexity, which variably influences alveolar cell activity and the composition of surfactants.[96] The distinction between the gene products of SP-A1 and SP-A2, along with their corresponding coding variations, was determined at four specific amino acid positions: 66, 73, 81, and 85.[96] Notably, a particular gene allelic variant in SP-A2 (Gln 223 Lys) is prevalent in the population, where the allelic variation corresponds to the substitution of Gln (Q) with Lys (K) at site 223 in the lectin domain.[97] Notwithstanding the structural similarities between SP-A1 and SP-A2, their functional mechanisms diverge.[98] Ledford et al established that the recombinant human isoform of SP-A2 with a lysine substitution at position 223 (rhSP-A2 Lys223) binds to *M. pneumoniae* membrane components (MMF) with high affinity, whereas the rhSP-A2 variant with glutamine at position 223 (rhSP-A2 Gln223) shows markedly reduced affinity for MMF.[43] Furthermore, mice expressing SP-A2 Gln223 exhibited enhanced neutrophil chemotaxis in response to MMF challenge compared to those expressing SP-A2 Lys223.[43] Additionally, SP-A2 displays superior biological activity to SP-A1 in several key areas; it binds more effectively to neutrophils, promotes bacterial engulfment by AMs, enhances the production of proinflammatory factors by macrophage-like cell lines, and improves survival rates in lung transplantation patients.[99–102] In summary, SP-A2 polymorphisms may lead to differential immune responses following *M. pneumoniae* infection, potentially contributing to the variability in clinical manifestations among individuals.

Restricts Mucin Production Induced by *M. pneumoniae* Components

MMF are predominantly identified via TLR1, TLR2, and TLR6.[103] MMF can bind to these receptors and activate downstream signaling pathways including NF-κB,[104] mitogen-activated protein kinases (MAPKs),[105] and the epidermal growth factor receptor (EGFR).[106] EGFR has long been recognized as a prominent receptor that regulates mucin production through endogenous and exogenous ligand signaling cascades, including the Ras/ERK or PI3K/Akt pathways.[43] Previous reports indicated that SP-A exhibits a high affinity for live *M. pneumoniae* and MMF.[107] In the absence or downregulation of SP-A, MMF induces increased activation of the EGFR signaling pathway, resulting in enhanced mucin production.[43] In vitro experiments have indicated that SP-A can inhibit EGF-induced phosphorylation of EGFR, ERK, and Akt in a dose-dependent way, thereby inhibiting cell proliferation and motility.[43,108] Additionally, SP-A-deficient mice show heightened sensitivity to MMF exposure, and the pharmacological inhibition of EGFR before MMF stimulation significantly decreases mucin production and neutrophil infiltration in SP-A-deficient mice.[109] Mechanistically, SP-A may interact with EGFR via the neck region of the CRD, effectively blocking the binding of EGF to EGFR and suppressing mucin production.[107] These findings indicate that SP-A may contribute to the negative regulation of pulmonary inflammation to a certain degree.

Alleviates Airway Eosinophilic Infiltration and Inflammation

The differences between non-allergic and allergic inflammation largely revolve around eosinophils, which are frequently observed in the airways of patients with asthma and interact with invading *M. pneumoniae*.[110] In vivo investigations have demonstrated that local allergen exposure in patients with asthma leads to a substantial influx of eosinophils, accompanied by elevated SP-D and reduced SP-A levels in BALF.[111] This shift in surfactant protein dynamics underscores their potential role in regulating the immune reactions during allergic inflammation.

The dysregulation of SP-A and SP-D metabolism appears to be a critical underlying illness characterized by eosinophil dominance.[112] Studies have shown that both SP-A and SP-D modulate eosinophil chemotaxis in inflammatory airway diseases, thereby mitigating the inflammatory response and pathological injury associated with *M. pneumoniae* infection.[113] Consequently, SP-A appeared to disrupt the host's natural mechanism of clearing *M. pneumoniae*. One proposed mechanism involves SP-A interacting to eosinophils, limiting the release of eosinophil peroxidase (EPO) upon encountering *M. pneumoniae*.[41] SP-A polymorphisms may influence eosinophil regulation. The variant SP-A Lys223, stemming from the substitution of glutamine (Q) with lysine (K), significantly enhances eosinophilic granuloma formation and offers a nuanced rationale for the capacity of SP-A to limit EPO release,[114] highlighting the genotypic-phenotypic correlation in airway diseases.[115] Additionally, the CRD of SP-A binds to eosinophils to modulate their degranulation and apoptosis, promoting phagocytosis by macrophages, which inhibits extracellular trap formation and reduces airway inflammation.[116] In contrast, SP-D plays a relatively minor role, although it can bind directly to the eosinophil surface and inhibit chemotaxis.[116,117] Certain dysfunctions in SP-A and SP-D are hypothesized to worsen *M. pneumoniae* infection, as elevated reactive nitrogen species in eosinophilic diseases caused by *M. pneumoniae* disrupt the normal oligomerization of these proteins.[112] In summary, SP-A and SP-D promote homeostasis during *M. pneumoniae* infection while mitigating excessive responses that may lead to harm.

Regulation of Asthmatic Inflammation

Asthma is a persistent respiratory condition that can be exacerbated by *M. pneumoniae* infection.[118] In patients with asthma, a significant link exists between the number of airway mast cells (MCs) in the airway smooth muscle and airway hyperresponsiveness (AHR).[119] MCs serve a crucial role as reservoirs of TNF-α,[120] amplifying local inflammation and worsening conditions such as asthma following *M. pneumoniae* infection. SP-A is suggested to significantly influence immunological responses by inhibiting the recruitment of MCs and the subsequent generation of TNF-α following *M. pneumoniae* infection. Meanwhile, in SP-A-deficient mice infected with *M. pneumoniae*, MCs further exacerbate AHR and the recruitment of inflammatory cells, which is accompanied by a substantial increase in TNF-α production.[39] These findings highlight the protective role of SP-A against asthma. In patients with asthma with SP-A dysfunction or deficiency, *M. pneumoniae* infection can lead to overproduction of TNF-α by epithelial cells, AMs, and MCs, contributing to increased inflammation and tissue damage.[121] In contrast, SNPs in the SP-A and SP-D genes, particularly the SP-A2 SNP rs1965708[122] and SP-D SNP rs721917,[123] correlate with a heightened risk of asthma. Individuals with asthma harboring these SNPs exhibited reduced lung function and poor asthma control.[124] This suggests that these genetic variants influence the expression or function of surfactant proteins, thereby altering the immune response and potentially contributing to the development or severity of asthma.[116]

Inhibition of DCs Maturation and Lymphocytes Activation

Although SP-A has various immunomodulatory functions that influence the phenotype and activity of adaptive immune cells,[125] its role in DCs maturation and lymphocyte activation following *M. pneumoniae* infection remains unclear. The only available reports, based on research by Ledford, indicate no significant variation in the overall amount of myeloid cells discovered in lung digests of *M. pneumoniae* infected SP-A-deficient mice in relation to WT C57BL/6 mice. However, the composition of cells, such as antigen-presenting cells, inflammatory monocytes, and neutrophils in BALF, along with the number of DCs, was markedly elevated in SP-A-deficient mice. Concurrently, concentrations of chemotactic factors for immature DCs, eg MCP-1, MIP-1α, and GM-CSF, were elevated in the BAL of SP-A-deficient mice following *M. pneumoniae* infection. Further studies confirmed that SP-A-deficient mice exhibited more mature DCs and an elevation in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells during *M. pneumoniae* infection. Nonetheless,

the elevated burden of *M. pneumoniae* was not consistent with an increase in T lymphocytes. Additionally, the quantity of activated B cells ($B220^+IgM^+CD69^+$) extracted from the lungs increased significantly, along with increased levels of high mobility group box 1 (HMGB-1) in SP-A-deficient lungs and mediastinal lymph nodes. HMGB-1, typically linked to necrotic cells, which are indicative of tissue damage and inflammatory signaling responses, has been recognized as a potent pro-inflammatory cytokine that actively regulates DCs maturation.[125,126] Since the exogenous addition of SP-A could suppress HMGB-1 release from THP-1 cells activated by *M. pneumoniae*, it is speculated that SP-A contact with *M. pneumoniae* may similarly limit HMGB-1 secretion from DCs, which is consistent with the finding that the experimental group recovered with SP-A failed to induce HMGB-1 production from DCs.[42] Therefore, SP-A reduces lung inflammation following *M. pneumoniae* infection by inhibiting DCs and activating T and B cells through the regulation of HMGB-1 expression.

Perspective and Conclusion

Surfactant proteins, particularly SP-A and SP-D, are crucial for maintaining pulmonary homeostasis and resisting pathogenic infections. A wide range of their functions not only influence the immune response during pathogen encounters but also significantly impact the inflammatory response. Due to this complexity, investigators often find it challenging to identify the specific roles of surfactant proteins under various infectious conditions. For instance, while SP-A may exhibit protective effects in models of *M. pneumoniae* infection, it can also demonstrate pro-inflammatory effects when confronted with other pathogens such as *Influenza A virus, S. pneumoniae*, RSV, *P. aeruginosa*, and HIV.[19] This functional diversity complicates the interpretation of experimental results and the investigation of the underlying mechanisms.

Studies investigating the mechanisms of action of surfactant proteins often rely on cellular or animal models. However, cellular models struggle to fully replicate the complex host immune environment, and existing animal models such as mice exhibit large gaps in the genetic background of the human immune system. Notably, variations in the sequence and structure of mouse surfactant proteins compared to those of humans introduce uncertainties in research on *M. pneumoniae*, complicating the interpretation of the study results. With the advent of organoids and humanized cell models, it has become feasible to recreate the lung microenvironment and immune response in vitro,[127] address the limitations of animal models, and provide a reliable experimental platform for investigating the pathogenesis of *M. pneumoniae*.

SP-A exhibits high polymorphism and is unevenly distributed among different individuals and ethnic groups, making it challenging to draw universal conclusions regarding the investigation of surfactant proteins. This polymorphism results in varying reactivity across patients with *M. pneumoniae* infection, further increasing the complexity of studies. Future research should investigate how this genetic variation influences individual susceptibility to *M. pneumoniae* and the outcomes of infection. By conducting studies on individuals with different genotypes, we may uncover the relationship between genetic polymorphisms and susceptibility to *M. pneumoniae*. This knowledge could facilitate the creation of personalized treatment strategies for individual genetic profiles, thereby enhancing the precision of anti-infection therapies.

Future research should focus on the processes that allow SP-A and SP-D to recognize *M. pneumoniae*. Advanced structural biology techniques, such as cryo-electron microscopy, allow direct observation of the binding process between surfactant proteins and *M. pneumoniae* at the atomic level, offering new insights into the diverse processes of surfactant proteins activity. Additionally, by comparing surfactant proteins in the context of *M. pneumoniae* and other pathogens, we can identify specific immune responses further elucidated their roles in the infectious environment. Investigating the regulatory mechanisms and immunomodulatory effects of surfactant proteins will help to clarify their specific functions in *M. pneumoniae* infection. Understanding these mechanisms will be instrumental in developing customized anti-infection therapies.

In the future, surfactant proteins should be integrated with other pulmonary immune factors, such as chemokines and cytokines, to investigate their synergistic effects on the regulation of *M. pneumoniae* infection. By systematically studying the interactions among these factors, we aimed to construct a more comprehensive lung immune regulatory network to provide new insights into the treatment of *M. pneumoniae* pneumonia.

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Disclosure

The authors report no conflicts of interest in this work.

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