Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in
Colloid & Interface Science

Pulmonary surfactant-derived antiviral actions at the respiratory surface

Miriam Isasi-Campillo[a], Paula Losada-Oliva[a], Jesús Pérez-Gil, Bárbara Olmeda and Lucía García-Ortega

Abstract

Lung surfactant (LS) is a membrane-based lipid-protein complex that lines the alveoli, reducing the surface tension at the air-liquid interface and thus minimizing the work of breathing. Besides this function, LS is also the first physical barrier between the outside air and the systemic circulation, therefore playing a key role in the defense against harmful particles and microorganisms.

Viral respiratory tract infections (RTIs), and especially acute lower RTIs, are one of the leading causes of morbidity and mortality worldwide. LS participates in the network of interactions between viruses and the immune system to prevent or lessen the effects of the infection, but it is also altered by these pathogens, which can potentially impair its function.

The aim of this review is to provide an integrated multidisciplinary overview toward understanding the interplay between respiratory viruses and LS and its health impact on the respiratory system. The review is centered on the antiviral mechanisms of both LS proteins and lipids, and their different interactions that lead to varying outcomes. Finally, a summary of the clinical application of surfactant in the scene of lung viral infection is disclosed, including state-of-the-art approaches of the therapeutic use of surfactant components.

Addresses

Department of Biochemistry and Molecular Biology, Faculty of Biology, Research Institute "Hospital 12 de Octubre (imas12)", Complutense University, 28040 Madrid, Spain

Corresponding authors: Olmeda, Bárbara (bolmeda@ucm.es); García-Ortega, Lucía (luciagar@ucm.es)

[a] These authors contributed equally to this work.

Current Opinion in Colloid & Interface Science 2023, **66**:101711

This review comes from a themed issue on **Biological (bio-inspired) Colloids and Interfaces (2023)**

Edited by **Martin Malmsten** and **Stefan Zauscher**

For complete overview about the section, refer Biological (bio-inspired) Colloids and Interfaces (2023)

https://doi.org/10.1016/j.cocis.2023.101711

1359-0294/© 2023 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Keywords

Lung surfactant, Air-liquid interface, Lipid-protein interactions, Antiviral drugs, Inhaled drugs, Respiratory tract infections, Influenza, SARS-CoV-2, Respiratory syncytial virus.

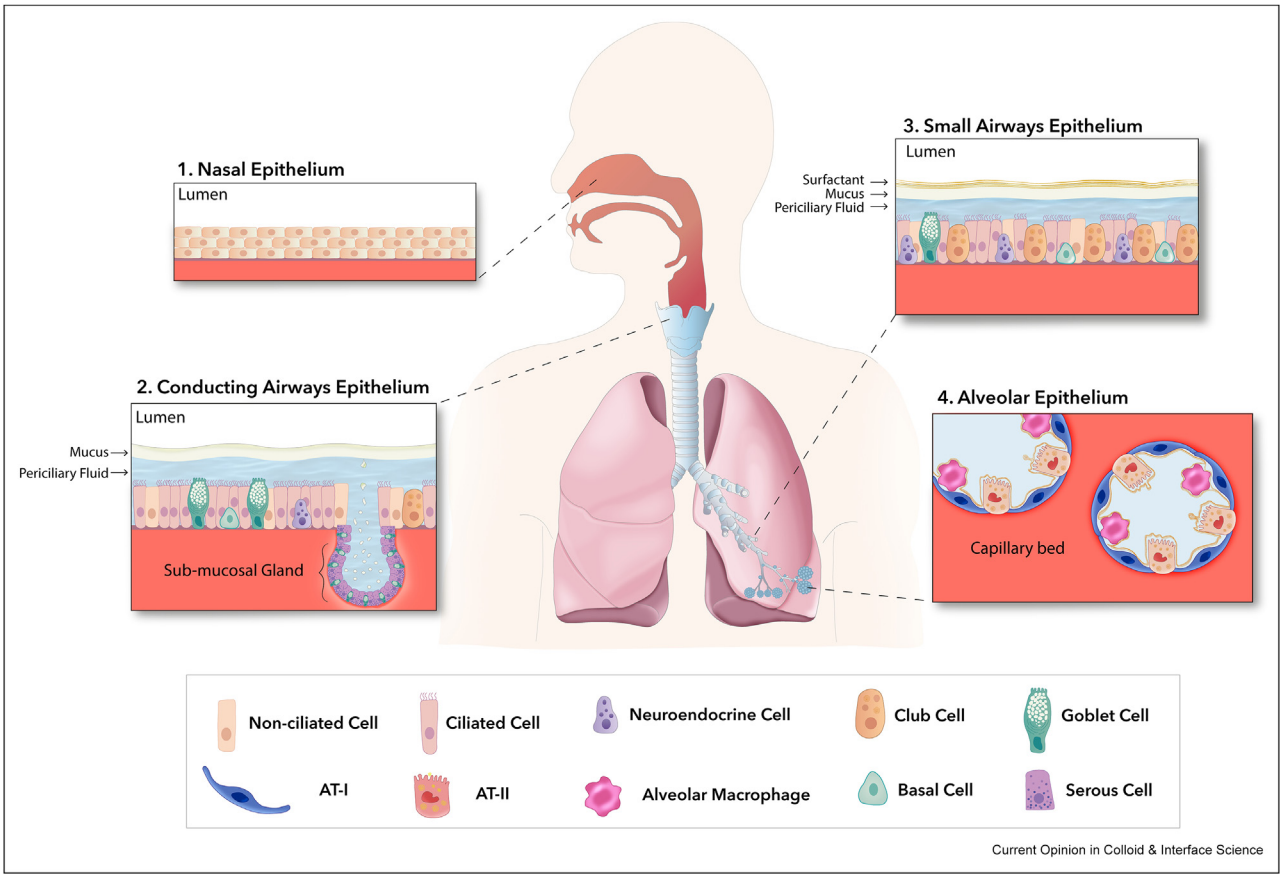
The respiratory system

The respiratory system is composed of the organs of the respiratory tract that allow airflow during breathing. It is divided into the upper respiratory tract (nose, mouth, sinuses, pharynx, and larynx), and the lower respiratory tract, which comprises the conducting airways (trachea and bronchi), the small airways (bronchioles) and the breathing part (the alveoli) [1]. In addition, this system is the first barrier between the body and the environment. Its epithelium is constantly exposed to organic matter, vapors, aerosols, and microbial pathogens and therefore requires effective local defense [2].

This epithelium is comprised of several cells types (Figure 1): (1) the nasal cavity is lined by stratified squamous epithelium [3]; (2) conducting airways are mainly coated with a pseudostratified cylindric mucociliated epithelium [4]; (3) in the small airways, the epithelium becomes simple cuboidal; (4) in the alveoli there are two main epithelial cell types: thin, elongated type I pneumocytes (AT-I cells), which cover 95% of the alveolar surface, and cuboidal type II pneumocytes (AT-II cells) [5,6]. Epithelial cell−cell junctions through tight junctions (TJs), adherent junctions (AJs), gap junctions, and desmosomes constitute the main physical barrier of the respiratory system [7,8]. In addition to epithelial cells, alveolar macrophages (AMs) constitute the first line of defense against pathogens and produce important chemical signals for immune response [9−12].

The respiratory epithelium is lined by layers of three different substances: mucus, periciliary fluid, and surfactant. The mucus layer contains 93−95% (w/w) water and 5−7% (w/v) solid material principally composed of mucins (large glycoproteins) together with phospholipids, proteoglycans, cellular debris, and various proteins, some of which possess antimicrobial, antiprotease, and antioxidant properties. This layer is secreted by the pseudostratified ciliated epithelium, mainly goblet cells, and, among its functions, it captures inhaled particles and microbial pathogens, neutralizes soluble gases, and is cleared by airflow and cilia activity. The periciliary fluid layer is a watery layer produced by the active transport of ions by the ciliated pseudostratified epithelium and is necessary for efficient ciliary beating

Figure 1



Structure and composition of healthy respiratory airways. During the inhalation phase, air flows into the upper airways of the respiratory tract, through the nose or mouth to the sinuses, pharynx, and larynx, until it reaches the lower airways, where the trachea carries the inhaled air to the bronchi. These are divided into smaller branches, or bronchioles, which are connected by thin ducts to distal bunches of microscopic air sacs also known as alveoli. The epithelial structure changes throughout the tract, from a more stratified conformation at the nasal cavity and pseudostratified epithelium at the trachea and bronchi, to a more simple and non-ciliated epithelium at the alveoli, which is mainly composed of AT-I and AT-II cells, and alveolar macrophages. The three layers that line the epithelium (mucus, periciliary fluid and surfactant) are also represented in the figure. Abbreviations: AT-I, type I pneumocytes; AT-II, type II pneumocytes.

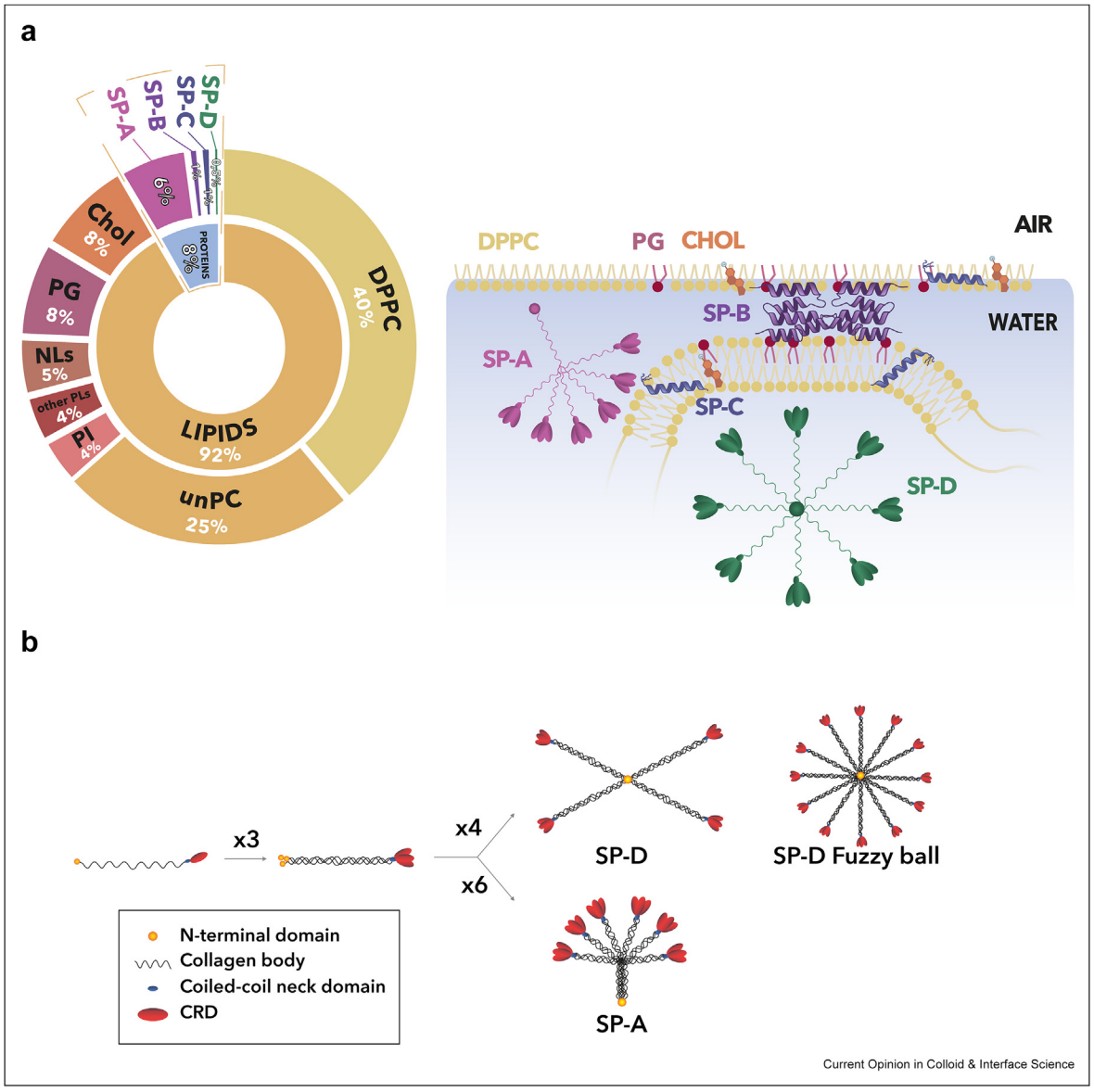
and mucin hydration. Finally, pulmonary surfactant, more widely known as lung surfactant (LS), which is synthesized and secreted by the Club cells (in the small airways) and AT-II cells, mainly coats the alveolar epithelium, although it is also present in the rest of the airways, and it's composed mainly of lipids, although with a small but essential protein portion. Its main function is to reduce surface tension during the gas exchange that takes place during respiratory cycles, thus preventing lung collapse [5,13,14]. Club and AT-II cells also produce other molecules, such as cytokines, growth factors [15], and endogenous antimicrobial peptides [16,17].

Lung surfactant

LS is a membrane-based system composed of a mixture of lipids (90%, w/w) and proteins (10%, w/w), which is adsorbed into the air-liquid interface of alveoli, optimizing the breathing mechanics by lowering the surface tension and thus preventing the alveolar collapse. It also prevents lung edema, relaxes the airway smooth muscle, and possesses multiple immune functions [18].

LS performance depends to a large extent on its composition. The major components of the system are lipids, mostly saturated phosphatidylcholine (DPPC) (~40%, w/w) (Figure 2). This phospholipid has saturated acyl chains that minimize surface tension by tightly packing at the air-liquid interface. The next more abundant lipids in the complex are unsaturated phosphatidylcholines (PC), which can account for 25% of the total mass, whereas negatively charged phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS) are less abundant (between 8% and 4% of LS total mass, each). In the case of humans, the major anionic molecular species are palmitoyl-oleoyl-phosphatidylglycerol (POPG) and

Figure 2



Composition and organization of LS in healthy human lungs. **a**) Lipid and protein composition of LS by mass and organization of the different components into LS membranes and films at the air-liquid interface. (**b**) Structure and oligomerization of surfactant collectins SP-A and SP-D. Both proteins are composed of monomers, each of which containing a N-terminal domain, a collagen-like domain, a neck region and a carbohydrate recognition domain (CRD). SP-A is formed by 6 trimers and SP-D can be found in a cruciform-like structure, that can be combined to form larger oligomeric structures known as "fuzzy balls". Proteins are not scaled. Abbreviations: CRD, carbohydrate recognition domain; Chol, cholesterol; DPPC, Dipalmitoylphosphatidylcholine; NLs, neutral lipids; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLs, phospholipids; SP-A, surfactant protein A; SP-B, surfactant protein B; SP-C, surfactant protein C; SP-D, surfactant protein D; unPC, unsaturated phosphatidylcholine.

dioleoyl-phosphatidylinositol (DOPI). Another important component is cholesterol, with ~8% of LS total mass. These components improve lipid adsorption, spreading, and fluidity of the surfactant film [19]. Finally, a residual 4% and 5% of surfactant accounts for other phospholipids (PL) and neutral lipids (NLs) respectively [19–25]. Although lipids are mainly involved in biophysical functions, they also take part in regulating innate immunity and modify the host response to pathogens [14,20,21,26].

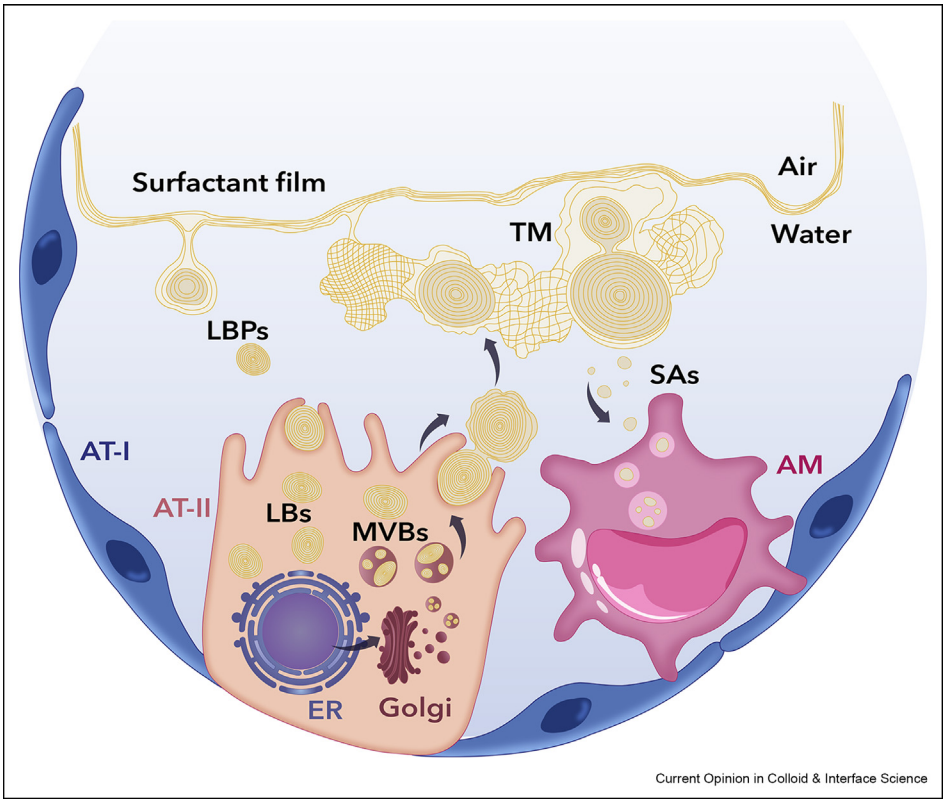
Regarding surfactant proteins, two of them are highly hydrophobic in nature (SP-B and SP-C) whereas SP-A and SP-D are hydrophilic [22–24,27]. SP-B and SP-C are key for the surface–active properties of LS, whereas the main role of collectins SP-A and SP-D is to mediate multiple

host defense functions [5,19,24,27–30], although they are also involved in the absorption and spreading of surfactant and participate in surfactant metabolism and recycling [11].

The synthesis of surfactant lipids and proteins takes place in the endoplasmic reticulum and the Golgi apparatus of the AT-II cells (Figure 3) [31]. SP-A, SP-B, and SP-D are also synthesized and secreted by Club cells [30,32]. Surfactant lipids and proteins are assembled into lamellar bodies (LBs), that are exoticized to the alveolar space [14]. Upon secretion by AT-II cells, the surface-active components of LS quickly adsorb into the air-liquid interface, forming the surfactant film. Once adsorbed at the interface, surfactant reduces surface tension during expiration, thus preventing alveolar collapse [33–37]. Finally, clearing and recycling of surfactant is carried out mainly by AT-II cells, with a minor fraction being phagocytosed and degraded by the AMs [38–40]. More specifically, SP-C has been shown to be involved in this process, favoring surfactant uptake by pneumocytes and macrophages [41]. SP-D also contributes to the fragmentation of phospholipid reservoirs and LS homeostasis [29] while SP-A has been described to regulate surfactant levels through signaling pathways involving AT-II cells [42].

SP-B is the most crucial protein of the surfactant system, as its deficiency is lethal. It is synthesized as a precursor of 40 kDa which is processed into a mature 18 kDa homodimer, that can be oligomerized into higher-order structures [43]. In the case of SP-C, processing occurs from a precursor form of 21 kDa to the final mature protein monomer of 4.2 kDa, which can also be found in dimers [44]. Both hydrophobic proteins, SP-B and SP-C, are cationic, and part of their functions rely on their ability to interact with the anionic lipids in surfactant layers, especially PG. SP-B and SP-C are important for the formation and structural organization of the LS film, ensuring its recycling and maintenance [23,24,27,45]. In the case of SP-B, apart from its biophysical functions, it may have an anti-inflammatory role, as it has been shown to reduce NO production by AMs under LPS stimulation [46]. Moreover, the N-

Figure 3



Metabolism of LS in the alveoli. Surfactant is synthesized by AT-II cells and packed into LBs, which are secreted into the aqueous subphase and quickly adsorbed into the air-liquid interface, resulting in a surfactant film that lines the alveoli. Other extracellular structures formed by surfactant, such as LBPs and tubular myelin, are also represented in the aqueous subphase. Finally, surfactant is recycled or degraded by AT-II cells and alveolar macrophages. Abbreviations: AM, alveolar macrophage; AT-I, type I pneumocytes; AT-II, type II pneumocytes; ER, endoplasmic reticulum; LBPs, lamellar body-like particles; LBs, lamellar bodies; MVBs, multivesicular bodies; SAs, small aggregates; TM, tubular myelin.

terminal module of the SP-B precursor ($SP\text{-}B^{N}$) has been proposed to exhibit antimicrobial functions in combination with SP-A [47,48]. On the other hand, it is known that SP-C activity is associated with cholesterol of surfactant membranes, and interestingly it might also be related to a potential anti-inflammatory activity of the protein [49–52].

Surfactant hydrophilic proteins SP-A and SP-D belong to a family of collagenous carbohydrate-binding proteins, known as collectins [30,53,54]. The primary structure of most of these proteins is characterized by the presence of several structural traits. Each monomer holds four regions: (1) a short cysteine-rich N-terminal domain involved in interchain disulfide bond formation; (2) a collagen-like sequence consisting of Gly-X-Y repeats; (3) a coiled-coil helical domain; (4) and a carbohydrate recognition domain (CRD) that binds Ca^{2+} (Figure 2) [55,56]. This last domain defines the function of the collectins, mediating the innate immune response, as they recognize the carbohydrate epitope moieties at the surface of different microorganisms [57]. The basic structural trimer of collectins is formed by folding the collagenous domains into triple helices and by winding a coiled–coiled domain [30,53,54,58–60]. Nevertheless, the quaternary structure differs across collectins, so that trimers multimerize to varying degrees. In the case of human SP-A, two protein variants exist, SP-A1 and SP-A2, as a result of gene duplication. In both cases, the protein structure consists in 6 trimeric assembled units, which looks like a "bouquet of tulips", composed of 35-kDa-monomers held together by disulfide bonds at the N-terminus [56]. On the other hand, mature SP-D can be assembled as several oligomeric forms including trimers, hexamers, dodecamers, or even larger oligomers (called "fuzzy balls") composed of 43-kDa-monomers, again stabilized by the N-terminal domain [61,62] (Figure 2b).

Besides some role in surfactant homeostasis, surfactant collectins display important roles in host defense against multiple pathogens [19,63,64] and regulate interaction with allergens [23,24,64]. The ability of these proteins to recognize and neutralize pathogens relies on the presence of sugar moieties at their surface, and it is achieved through a variety of mechanisms, including aggregation, apoptosis, opsonization, activation of phagocytosis, inhibition of microbial growth, or modulation of inflammatory response [65,66]. Importantly, the defense function of surfactant collectins is enhanced by their N-terminal domain-mediated oligomerization [65,66].

In this review, we will focus on the importance of the different components of LS on the innate immune system, particularly their role in protection against viral infections. Also, we will discuss their potential use as therapeutical tools for viral infections.

Viral respiratory tract infections

Viral respiratory tract infections (vRTIs) are the result of virus particles being inhaled or directly contacting the mucosal surface of the nose, mouth, or eyes [67]. They are one of the leading causes of morbidity and mortality worldwide and a significant factor in asthma and chronic obstructive pulmonary disease (COPD) exacerbations [68]. According to WHO (World Health Organization), they are the world's most deadly communicable diseases, and the fourth leading cause of death. These infections can affect people across all age groups, but they require special consideration in young children and the elderly [69]. Among all viruses that can cause RTIs, in this review we will focus on the main ones, belonging to the Ortho and Paramyxoviridae, Picornaviridae, Coronaviridae, and Adenoviridae families (Figure 4) [70].

Influenza A virus

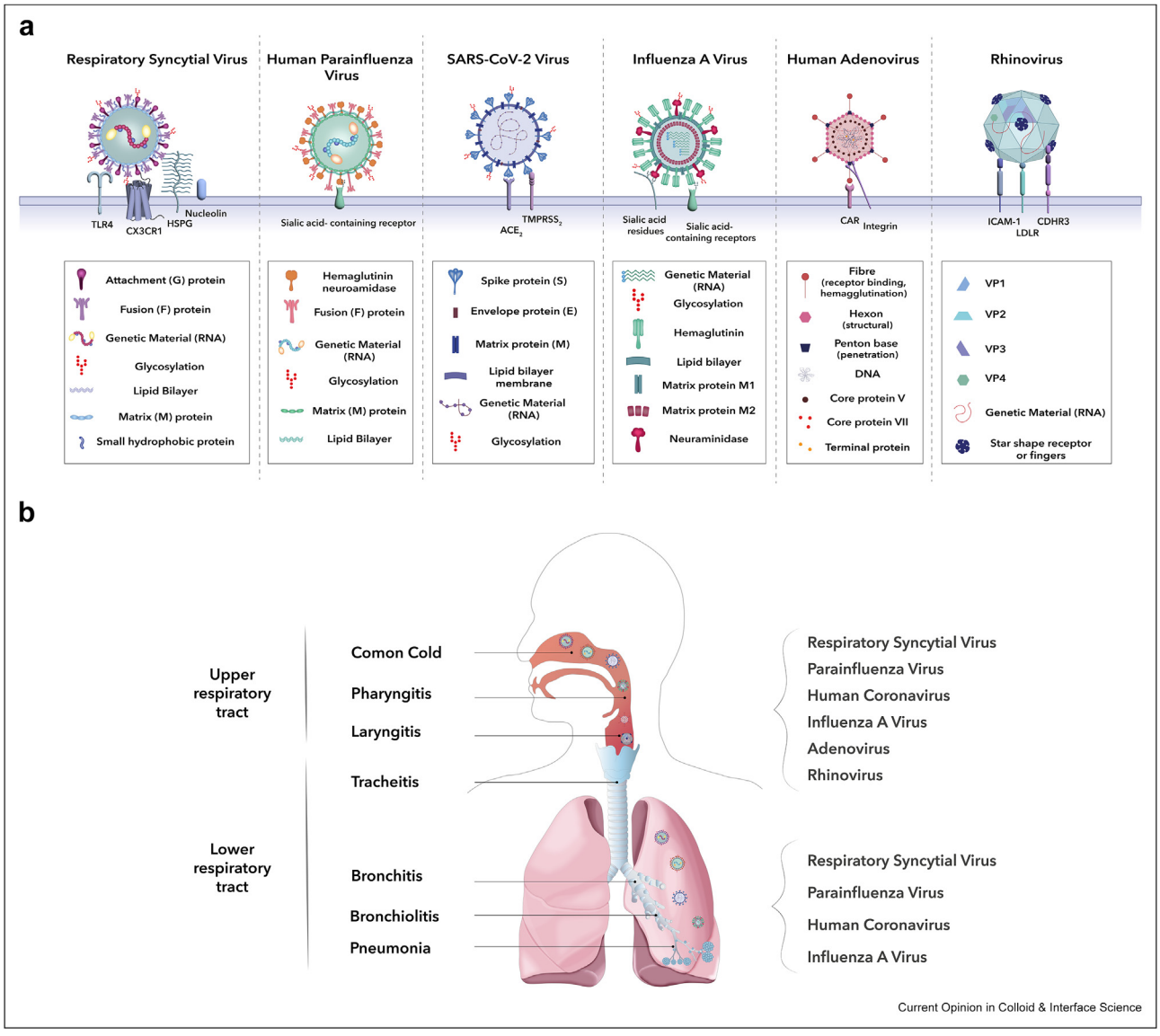
Influenza A viruses (IAV) remain a serious global health concern, resulting in up to half a million deaths annually [71,72]. These viruses cause respiratory infections ranging from mild manifestations in the upper respiratory tract, which are characterized by fever, sore throat, runny nose, cough, headache, muscle pain, and fatigue, to more severe cases when reaching the lower respiratory tract, which may develop lung inflammation and acute respiratory distress syndrome (ARDS) [73,74].

Influenza A is an enveloped, segmented and negative sense RNA virus, belonging to the Orthomyxoviridae family. The genome of this virus contains eight RNA segments, which encode as many as 18 proteins [74]. Proteins involved in cell infection are hemagglutinin (HA) and neuraminidase (NA) [75]. IAV binds to sialic acid (SA) residues as receptors on the surface of the host cell through its trimeric HA. This receptor-mediated interaction results in attachment, entry, and fusion of the viral envelope with the endosomal membrane. Once at the cytosol, IAV begins to replicate its RNA genome until finally new viral particles are assembled at the membrane. At this point, NA inactivates SA residues from the infected cell, avoiding their interaction with HA and providing the spreading of the virus [53,76]. The primary targets of IAV are airway epithelial cells (AECs), resulting in loss of epithelial integrity and alteration of the respiratory process [77].

Respiratory syncytial virus

Respiratory syncytial virus (RSV) is the main cause of bronchiolitis and pneumonia in infants in developed countries. This virus is an enveloped, negative sense, single-stranded RNA virus of the *Pneumoviridae* subfamily (within the Paramyxoviridae family). Its genome contains ten genes, which encode different proteins, including two important envelope glycoproteins: G glycoprotein

Figure 4



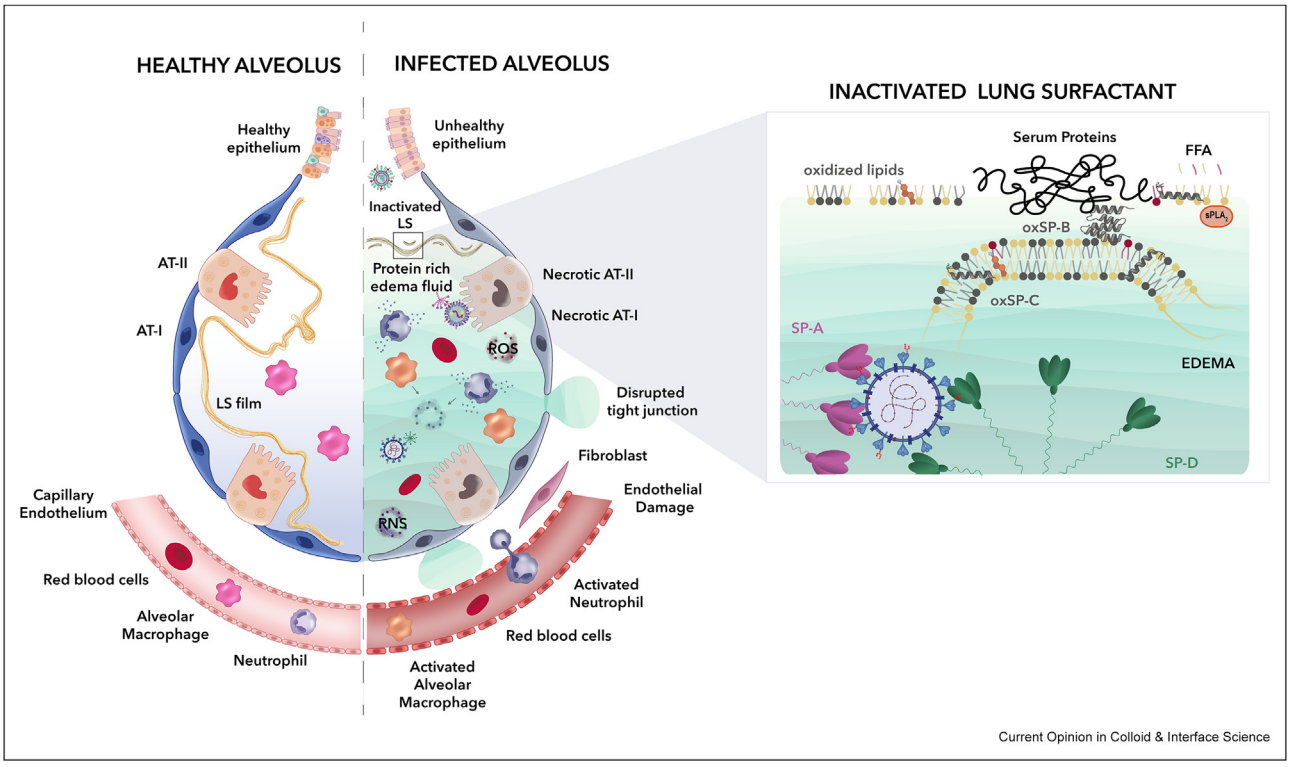
Viral types responsible for respiratory tract infections. (**a**) Schematic representation of respiratory syncytial virus, human parainfluenza virus, SARS-CoV-2 virus, influenza A virus, adenovirus and rhinovirus, and their interaction with cell receptors. (**b**) Schematic representation of the distribution and clinical presentation of different respiratory viruses in the upper and lower respiratory tract. Abbreviations: ACE2, angiotensin converting enzyme 2; CAR, Coxsackievirus and adenovirus receptor; CDHR3, cadherin-related family member 3; CoV, human coronavirus; CXC3R1, C-X-C Motif Chemokine Receptor 1; HPIV, human parainfluenza virus; HSPG, Heparan sulfate proteoglycans; IAV, influenza A virus; ICAM-1, intercellular adhesion molecule 1; LDLR, low-density lipoprotein receptor; RN, rhinovirus; RSV, respiratory syncytial virus; TLR4, Toll-like receptor 4; TMPRSS2, Transmembrane serine protease 2.

that mediates cell attachment and F glycoprotein that mediates fusion of the virion with the cell membrane [78,79]. RSV infections occur via nostrils or mouth and once inside, they begin to infect AECs of the upper respiratory system, moving to the lower airways. It has been determined that ciliated cells in the bronchial epithelia and AT-I cells in the alveoli are the main cells targeted by RSV infection [80]. Once infected, the process of transcription and replication of RSV occurs very swiftly, producing an alteration in the integrity of the epithelial tissue and consequent inflammation. This inflammation can generate irreversible alterations in the respiratory system [81].

Coronavirus

Coronaviruses (CoVs) are a varied group of enveloped viruses with positive-sense, non-segmented, single-stranded RNA genomes [82]. There are numerous types

Figure 5



Inflammatory mechanisms, alveolar epithelial and endothelial damage, and surfactant inactivation of an infected, compared to a healthy alveolus. The entry of a virus in the alveolus leads to necrosis of alveolar cells, disruption of the tight junction between them, which causes lung flooding with edema, producing leakage of serum proteins that interfere with the adsorption capability of the LS, impairing its function. Furthermore, a viral infection triggers an exacerbated immune response that causes the recruitment and infiltration of immune cells, such as activated neutrophils and activated macrophages, boosting the production of pro-inflammatory and pro-fibrotic mediators (green nebula) (TFG-β, TNF-α, MMPs, TIMPs, IL-1, IL-4, IL-5, IL-6, IL-13, IL-17) and ultimately leads to the buildup of reactive oxygen and nitrogen species (red and violet nebula), that can oxidize or hydrolyze surfactant lipids and proteins (black proteins and lipids), or proteases and phospholipases ($sPLA_2$) that can hydrolyze surfactant lipids, further inactivating surfactant. Abbreviations: AT-I, type I pneumocytes; AT-II, type II pneumocytes; Chol, cholesterol; DPPC, Dipalmitoylphosphatidylcholine; FFA, free fatty acids; oxSP-B, oxidized surfactant protein B; oxSP-C, oxidized surfactant protein C; PG, phosphatidylglycerol; RNS, reactive nitrogen species; ROS, reactive oxygen species; SP-A, surfactant protein A; SP-D, surfactant protein D; $sPLA_2$, Secretory phospholipase A2.

of human coronaviruses that circulate in the population causing a "common cold", such as HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1. In contrast, severe acute respiratory syndrome-associated coronavirus (SARS-CoV), middle east respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) cause severe viral pneumonia by infecting alveolar pneumocytes, bronchial epithelial cells, and upper respiratory tract cells in humans [83]. This kind of virus is characterized by the presence of a trimerized surface protein called Spike glycoprotein or S-protein, which is critical for invasion of the host cell [84]. Specific binding of the S-protein to different cells receptors like Angiotensin-converting enzyme 2 receptor (ACE2), human aminopeptidase N (APN), and dipeptidyl peptidase 4 (DPP4) is responsible for the entry of coronaviruses into host cells [82,85].

Other respiratory viruses

Rhinoviruses (RVs) are non-enveloped positive-strand RNA viruses from the Picornaviridae family. RVs are the main cause of common cold in humans, but may also have lower respiratory tract involvement, with a clinical presentation of bronchitis or pneumonia [86,87]. Human Adenovirus (AdV) is a non-enveloped DNA virus that causes a wide range of illnesses, such as conjunctivitis, gastroenteritis, and respiratory infections [88]. Human Parainfluenza (HPIV) are single-stranded, enveloped RNA viruses that cause a broad spectrum of respiratory clinical manifestations, including colds, bronchiolitis, and pneumonia [89]. Human immunodeficiency virus (HIV) is a genetically related member of the *Lentivirus* genus of the Retroviridae family. This virus is characterized by its ability to induce failure of the immune system, which allows the emergence of opportunist infections and

cancer [90]. Even though the respiratory route is not a mode of transmission of HIV, HIV replication does occur in the lung, becoming an important reservoir of virus [91]. This virus contains envelope glycoproteins gp120 and gp41, which recognize cell surface receptors. Concretely, gp120 is essential for virus entry into T cells upon interaction with CD4 protein and is the primary target for binding HIV by various C-type lectins. Human cytomegalovirus (HCMV), also known as human herpesvirus 5, has a large double-stranded DNA genome. It is a highly widespread virus in the population but typically in a latent state. However, in immunocompromised patients, the clinical picture is complicated and may lead, although rarely, to a respiratory disease [92,93].

Transmission, replication, and clinical manifestations of vRTIs

The first step of a vRTI is the transmission and entry of the virus. There are four main modes of transmission from the infected to the new host: direct contact, indirect contact, droplets, and aerosol (or airborne). However, it is important to highlight that some viruses, like influenza, coronaviruses, and rhinoviruses can also infect gastrointestinal tract cells, so fecal transmission is also possible [94]. There are viral determinants for transmission or survival such as composition and structure of the envelope and capsid, and the internal proteins and genomes, as well as the ability to form viral aggregates. Environmental determinants also play an essential role, such as temperature, humidity, salinity, pH, ventilation, airflow, and ultraviolet radiation. Finally, host determinants of contagiousness, susceptibility, and transmission at individual and population levels must be also considered [94].

Once inside the new organism, viruses, as opportunistic agents, look for target cells to deploy their replication and propagation machinery. As a result, they bind to specific receptors located on the surface of respiratory tract cells [67]. As described above, most respiratory viruses are enveloped particles, so after receptor binding, the virus enters the target cell either through fusion of its envelope with the plasma membrane (RSV, HIV, and CMV) or through receptor-mediated endocytosis (IAV, HPIV, CoV, and AdV) [95]. This initiates the production and replication of viral proteins, which leads to the release of new viral particles into the respiratory tract [67].

Regarding the clinical presentation, it depends on the location of the target cells in the respiratory tract, although often several symptoms can overlap (Figure 4b). If the receptor is in the upper respiratory tract, clinical manifestations are more likely to be mild and not incapacitating, including cough, rhinitis, or pharyngitis (RV, AdV, HPIV, IAV). On the contrary, if the infection reaches the lower respiratory tract, symptoms might be more severe and patients are more likely to develop bronchitis, bronchiolitis, pneumonia (HPIV, IAV, RSV, and SARS viruses) and to suffer dyspnea, hypoxia interstitial inflammation, pulmonary infiltrates, massive cytokine storm, and, consequently, ARDS, leading to respiratory failure [67,70,96]. Lower RTI can occur by different mechanisms: 1) Direct infection of lung cells without prior replication in the upper respiratory tract, due to the selective tropism of some viruses such as IAV; 2) Spreading of the infection from the upper to the lower respiratory tract, for example by coronavirus; 3) Hematogenous spreading, for example in cytomegalovirus infection [97].

What happens when the virus reaches the mucosa at the respiratory surface?

The respiratory epithelium and its different coating layers described above constitute the first line of defense against microorganisms, specifically against the respiratory viruses. It participates in the innate and adaptive immune response through the release of proinflammatory molecules and activation of immune system cells [7]. Once viruses enter the respiratory tract, they are confronted by a large defense machinery located in the upper and lower airways, which includes mucociliary escalators, intercellular apical junctional complexes (AJCs), immune mediators secreted by the AECs, and LS, among others [8,98].

At the upper airways the mucus, along with the periciliary fluid lining the epithelium, forms a semipermeable barrier, allowing the exchange of nutrients, water, and gases and preventing the entry of pathogens such as viruses. Together, they constitute the mucociliary clearance system. This system allows the elimination of inhaled particles and is the result of the coordinated activity between the submucosal glands and goblet cells, producers of this viscous layer, as well as the ciliated cells, responsible for the coordinated movement of the fluid film into the pharynx. One of the most important components of this system are the mucins, specifically the mucins MUC5AC and MUC5B [4,99]. These mucins play a key role in antimicrobial responses, and it has been shown that in the presence of certain respiratory viruses, such as IAV, their expression is induced allowing optimal virus elimination [100,101]. Regarding AJCs, which contribute to the maintenance of the epithelial barrier integrity, they include TJs and AJs located at the apicolateral membranes of AECs. These junctions prevent the disruption of epithelium homeostasis and thus the entry of pathogens, such as viruses, which have not been eliminated by the mucociliary system [98,102]. In fact, the importance of this barrier has been determined in infections by different types of IAV, in which despite epithelial cell apoptosis, transepithelial resistance and protein expression of AJCs was maintained [103].

In addition, to prevent viral infection, respiratory cells of the upper and lower tract have on their surface several pattern recognition receptors (PRRs) that recognize specific pathogen-associated molecular patterns (PAMPs) on the surface of pathogens and initiate signaling cascades that lead to an innate immune response [104]. The best-characterized PRRs are the TLRs. These receptors are integral type I membrane glycoproteins that recognize a variety of PAMPs, activating signaling pathways that induce proinflammatory cytokines and interferons (IFNs) expression [105]. TLR3 has been found to be expressed in intracellular endosomes and recognizes viral replication products such as viral ssRNAs from RV, RSV, and IAV [106–108]. Other TLRs (TLR7/8 and TLR9) detect ssRNAs and dsRNAs of IAV and AdV respectively [7]. TLR4, expressed on the cell surface, interacts with the RSV fusion protein [109]. On the other hand, two RNA helicases RIG-1 and MDA5 target viral replication products in the cytosol [7].

Once the epithelium recognizes the specific virus, it secretes different cytokines, chemokines, and antimicrobial peptides. Cytokines (TNF-α, IL-6, IL-8, IL-1b, etc.) regulate immune responses, cell functions, proliferation, differentiation, and viral propagation. Chemokines (type I (IFN-α/β) and type III (IFN-l)) play a key role in the regulation of the immune system by stimulating the recruitment of immune cells (neutrophils, macrophages, T cells, NK cells, etc.) in the lungs. In addition, antimicrobial determinants, such as RNS and ROS, lactoferrin, inflammatory pro-resolving lipids (lipoxins), etc., are also secreted to inhibit viral activity [110,111].

Finally, the defense of lower airways counts with additional mechanisms involving LS. Besides its biophysical role in breathing mechanics, LS is an integral component of the host defense system, protecting the alveolus from microorganisms and exogenous substances [111]. In this sense, the most relevant defense machinery against viruses is constituted by LS collectins (SP-A and SP-D). These proteins, integrated into the lipoprotein surfactant complexes, have an immunomodulatory capacity and trigger a cytochemical response through their binding to different receptors located in cells of the innate immune system (AMs, monocytes, neutrophils, among others) [112–114]. In addition, they are capable of binding to pro-inflammatory mediators such as IFN and lipopolysaccharide (LPS), thus limiting apoptosis, cytotoxicity, and inflammation in the alveolus [115,116]. Finally, these proteins act as opsonins, binding to antigenic determinants located on the surface of pathogens promoting their phagocytosis by cells of the immune system [54,117]. Specifically, SP-A even has direct antibiotic activities against Gram-negative respiratory bacteria [118]. On the other hand, although the hydrophobic proteins of LS, together with the lipids, are mainly responsible for surfactant biophysical function, some studies have determined that they may also play a role as mediators of the anti-inflammatory response and against respiratory pathogens like viruses or bacteria [26,119,120].

Role of surfactant collectins against respiratory viruses

Several studies have determined that collectins of the surfactant system act as antiviral proteins [14]. As detailed below, it has been described how they directly interact with different viruses through their CRDs by engaging terminal monosaccharide residues or via recognition of PAMPs on viruses, serving as soluble pattern recognition receptors (PRRs). Different profiles in saccharide selectivity of the CRDs have been reported, so that SP-A shows priority binding for N-acetylmannosamine and L-fucose, while SP-D binds to inositol, maltose, and glucose [66]. This selectivity could be important to understand their different capacity to bind glycosylated proteins on the surface of viral capsids. Moreover, other modes of interaction with viral particles apart from glycosylation recognition of the viral protein are also possible.

Antiviral activity of collectins through virus binding may follow different mechanisms like inhibition of virus interaction with the target cell, or opsonization thanks to their oligomerization ability and subsequent clearance or phagocytosis by macrophages or neutrophils. Besides virus interaction, collectins are also involved in immunomodulation of processes like inflammation. They have been shown to bind several receptors at the surface of immune cells causing different and even opposite effects depending on the physiological situation [121]. Part of this duality could be related to the occupancy of the CRD and alternative interaction modes with immune cell receptors [122]. However, this aspect, in the context of viral infections, has been still poorly explored.

In this section, we briefly summarize what is known about collectin interactions with different respiratory viruses (Table 1). For more information, specific reviews on lung collectins are recommended [54,79,121,123].

Collectins and influenza A virus

The most extensive data set available regarding viral neutralization by collectins relates to IAV. In fact, different interaction modes with the virus have been described for SP-D and SP-A. SP-D recognizes, through its CRD, specific surface patterns of high mannose oligosaccharides expressed on the HA or NA of IAV, preventing their interaction with the host cell receptors by steric impairment, and thus reducing viral uptake into epithelial cells. Moreover, oligomerization of SP-D into supratrimeric structures allows multivalent high-avidity interactions between the collectin and IAV [54,124]. These multiple binding sites also promote IAV aggregation facilitating viral clearance through the gradient of

Table 1

Antiviral properties and interactions involved of lung surfactant collectins.

Virus	Collectin	Virus-specific interactions	Antiviral-related activities
IAVs	SP-D	Mannose carbohydrates in the HA and NA (glycosylation-dependent binding) [124,125]	Viral aggregation [125,126]. Potentiates IAV-neutrophils responses [76]. Limits IAV replication and blunts inflammatory responses to the virus [127]. Inhibits hemagglutinin binding activity and reduces viral uptake into epithelial cells [125]. Decreases neuraminidase activity [128]. SP-D deficient-mice decreases IAV clearance [129] SP-D levels increase in IAV infection [129].
	SP-A	HA cell attachment site through sialic acids in SP-A (calcium-dependent) [130]	Inhibits hemagglutinin binding activity of IAV and induces modest viral aggregation [131]. Enhances neutrophil and alveolar macrophage uptake of IAV in vitro [132].
RSV	SP-D	F and G glycoproteins (calcium-dependent) [133,134].	SP-D deficient mice increase inflammatory markers and reduce phagocytosis by alveolar macrophages [133]. SP-D levels were not detected in one-third of the infants infected with RSV [135]. Inhibits viral infectivity by blocking the entry of RSV to cell [134]. Enhances the phagocytosis and pulmonary clearance of RSV in mice [133].
	SP-A	F and G glycoproteins [136].	SP-A deficient mice shows more susceptibility to RSV infection with high levels of proinflammatory cytokines, viral load, and infiltration [137]. Neutralizes virus infectivity [136]. Infants ventilated for severe RSV infection present reduced levels of SP-A [135]. Enhances uptake of RSV by peripheral blood mononuclear cells (PBMCs) and alveolar macrophages [138]. Modulates the cellular cytokine release on RSV infection [138]. Enhances RSV clearance in vivo [137]. SP-A2 polymorphisms are associated with the severity of RSV infection in infants [139].
AdV	SP-A		Decreased inflammation and increased clearance of Adenovirus [140]
Parainfluenza	SP-D		SP-D inhibits hemagglutination activity of Sendai virus, the related murine parainfluenza virus [72]. Increased SP-D mRNA levels in parainfluenza type 3-infected lambs [141].
	SP-A		Increased SP-A mRNA levels in parainfluenza type 3-infected lambs [141].
Coronavirus	SP-D	SARS-CoV S-protein [142].	Binds to HCoV-229E virions in a dose-dependent manner [143]. Prevents infection of bronchial epithelial cell line more efficiently than SP-A [143]. Aggregates the virus through direct interaction with S-protein [142]. Plasma levels of SP-D were elevated in SARS-type pneumonia [144], also, serum levels of SP-D were elevated in patients with COVID and macrophage activation syndrome [145]. High serum levels of SP-D are correlated with more severe COVID-19 disease [146]. rfhSP-D acts as an entry inhibitor of SARS-CoV-2 infection into overexpressing ACE2 receptor cell line [147,148].
	SP-A		Binds to HCoV-229E virions in a dose-dependent manner [143]. Prevents infection of bronchial epithelial cell line and reduces infection of alveolar macrophages [143].

surfactant from the lower to the upper airways and the mucocillary escalator, or via phagocytic cells such as macrophages and neutrophils [76]. Accordingly, several studies have demonstrated that high-level HA glycosylation is involved in increased IAV susceptibility to SP-D-mediated inhibition. Therefore, the infection with a SP-D insensitive (low glycosylated HA) IAV strain entails an increase in both virus replication and clinical symptoms whereas infection with a SP-D sensitive strain (high glycosylated HA) leads to a milder clinical course, due to the effect of SP-D on virus neutralization and spreading reduction [72]. In fact, HA proteins of pandemic strains (1918, 1957, 1968, and 2009) causing high lung infection, were poorly glycosylated and showed a limited SP-D binding, in contrast to a seasonal H1N1 strain that induced only mild disease in infected mice and a higher binding to the collectin [76].

On the other hand, glycosylation has also an effect on the structural stability and integrity of HA and NA and provides the virus with an effective mechanism to skip immune checkpoints by hiding neutralization epitopes and allowing evasion from recognition by antiviral antibodies [76]. All this suggests that glycosylation confers adaptive advantages to IAV, while it also makes the virus more sensitive to SP-D.

Regarding SP-A interaction with IAV, it has been shown that HA binds to sialic-acid residues on SP-A in a calcium-dependent manner, in contrast to the HA glycosylation-dependent binding of SP-D, limiting the ability of the virus to reach and attach to cellular sialylated receptors in the host cells [53,127]. Also, SP-A causes aggregation of IAV, preventing its spreading and favoring its elimination by the immune system, although to a lesser extent than SP-D [131].

Despite SP-A is present at higher concentrations than SP-D in the respiratory lining fluid, its antiviral activity is lower [125]. Several studies with SP-A knockout mice showed an increased inflammatory response and viral load after infection with IAV, although these changes were much less pronounced than in SP-D knockout mice. In addition, double SP-A and SP-D knockout mice had a pattern of infection nearly identical to SP-D knockout mice [130]. On the other hand, in response to IAV infection in mice increased levels of SP-D but not SP-A were detected [127]. Altogether, these results indicate that SP-D plays a more important role than SP-A in the innate response to IAV infection [72]. However, the role of both collectins may be complementary in many cases since the mechanism of SP-A interaction with IAV does not depend on HA glycosylation [149]. In this regard, some studies have described that strains resistant to SP-D are not to SP-A [72].

Collectins and respiratory syncytial virus

LS collectins play a key role in the defense against RSV infection. Both proteins are capable of binding to the RSV glycosylated G and F proteins and neutralizing RSV in vitro and in vivo, however, mechanisms are not well understood, neither their dependence on viral protein glycosylation. Binding to G protein seems to be calcium-dependent, but it is not clear for SP-A whether it occurs through its N-terminal [54,121,133,134]. Moreover, recombinant fragments of both proteins, consisting of the CRD, neck, and a shorter collagenous tail (rfhSP-A and rfhSP-D), have been proven to trimerize and neutralize RSV. Regarding the effect of collectin genetic variations, it has been shown that SP-A2 polymorphisms are related to severity of RSV infection [139].

Several studies with collectin-deficient mice have been performed for RSV infection. SP-A and SP-D deficient mice developed a reduced viral clearance and a more aggressive immune response increasing inflammatory markers and recruitment of inflammatory cells like neutrophils together with defects in the generation of ROS species by AMs. Administration of native SP-A or SP-D to these deficient mice infected with RSV prevented lung inflammation and enhanced RSV clearance. SP-D was also found to partially reverse RSV-induced inhibition of oxygen radical production by macrophages and neutrophils [133,137]. On the other hand, RSV was shown to modify mRNA expression levels of SP-D by reducing translation efficiency [150].

Coronavirus and collectins

Within the surfactant, collectins SP-A and SP-D are also important to modulate coronavirus infection [121]. Previously to SARS-CoV-2 pandemic, elevated serum levels of SP-D were reported in patients with (SARS)-related pneumonia [144]. With this precedent, the study of collectin levels in patients with COVID-19 was performed. Lung-specific SP-A and SP-D serum levels were elevated in patients with SARS-CoV-2, pointing them as potential biomarkers of COVID-19 pneumonia severity [144,146]. However, SP-D levels in BAL of patients with COVID-19 were lower [151]. In order to determine the relevance of collectins levels and their potential implication in viral infectivity, the ability of SP-D and SP-A to interact with HCoV-229E virions was characterized, showing that this interaction inhibits infection of human bronchial epithelial cells and AMs [143]. However, low levels of binding of SP-A to glycosylated S-protein have been reported, so that the mechanism underlying the inhibitory effect of this protein upon coronavirus infection remains to be solved [142]. On the other hand, a different study has revealed that SP-D binds to glycosylated S-protein in a calcium-dependent manner and inhibits SARS-CoV-2 replication in Caco-2 cells [151]. In this sense, high therapeutic

potential is predicted for the recombinant fragment of SP-D (rfhSP-D) as it has been shown to inhibit infection and replication of SARS-CoV-2 in clinical samples. This inhibition seems to be mediated by interaction of rfhSP-D with the receptor-binding domain of the S1 subunit of SARS-CoV-2 spike protein [147,148].

Other viruses and collectins

The role of collectins in the protection against the viruses described above has been extensively investigated. However, although superficially studied, their participation in other viral infections has been also suggested. SP-A reduces lung inflammation, decreasing the levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, and IL-1b in mice infected with adenovirus. In addition, SP-A deficient mice have a low rate of adenoviral clearance by AMs [140]. In neonatal lambs infected with Parainfluenza Virus Type 3, RNA levels of SP-A and SP-D were elevated compared with non-infected lambs. This increase was associated with less viral antigens in lung tissue, so it could be related to the antiviral activity of collectins [141]. Finally, recent studies have determined that SP-A and SP-D, also present in the female genito-urinary tract, can interact with envelope glycoprotein of HIV (gp120), inhibiting binding to CD4 and therefore infection of CD4+ T cells [54,72].

With all this, the importance of collectins in the defense against viruses is clear. However, more integrated studies are required for providing a global view of surfactant significance on virus protection, including the involvement of other surfactant proteins, lipids, and inflammatory markers.

Antiviral activity of lung surfactant lipids

As described above, lipids constitute the prevailing part of LS, with DPPC as the major molecular species and other minor lipids like PG, PI, and cholesterol also bearing essential functions. Although lipids are mainly related to biophysical surfactant functions, they are also involved in attenuating inflammation and in modifying the host response to pathogens showing a protective role [19]. The ability of anionic lipids to interact with certain respiratory viruses has been demonstrated [152]. In particular, POPG and PI have been found to prevent plasma membrane attachment and consequent infection of epithelial cells thanks to their high-affinity interaction with viruses like IAV and RSV [153–156]. Moreover, anionic surfactant lipids regulate inflammatory cascades through inhibition of TLRs by, for example, binding to specific interacting proteins like CD14 in macrophages [25]. This is the case of PG and PI treatment of bronchial epithelial cells infected with either IAV or RSV. They decrease the production of inflammatory mediators like IL-8 by TLR-mediated response inhibition [152,155,156]. Finally, virus infection and subsequent lung inflammation are prevented by intranasally inoculated POPG or PI to mice infected with either IAV or RSV [153,156].

Beyond these common effects observed for anionic lipids regarding IAV and RSV, antiviral activities of these surfactant components have been out of scope. The recent SARS-CoV-2 pandemic has prompted an in vitro study with VeroE6 cells highly expressing the ACE2 receptor and infected with SARS-CoV-2 in the presence of POPC, POPG, or PI. It was observed that treatment with anionic surfactant lipids significantly suppressed viral load and the cytopathic effects in the infected cells [26]. However, much research remains to be done to determine whether lipids could act as potent inhibitors of this coronavirus infection.

Overall, these studies place POPG, PI, and derivatives as outstanding candidates for viral infection prevention and treatment.

Antiviral activity of surfactant hydrophobic proteins

Little is known about the role of hydrophobic surfactant proteins in antiviral defense. Polymorphisms in the SP-B and SP-C genes have been shown to be associated with increased susceptibility to severe RSV infections [157,158]. In this line, mice deficient in SP-C had lower virus clearance, along with a more severe and prolonged inflammatory clinical presentation [157].

Alterations in the respiratory system in the presence of viruses

During vRTIs, damage is generated at different points of the respiratory network. Along the progression of the infection, specific and local effects of the virus turn to events affecting at a more general level. The importance of the presence of LS in the respiratory system goes further than the anti-viral properties shown by several of its components, as the complex all together is essential to return the lung to homeostasis.

Damage at the upper airways affects cell integrity, including alterations in AJCs and cell morphology or exacerbated apoptosis, among others [159–162]. Following epithelial disruption, interference with the epithelial repair mechanisms (involving matrix metalloproteases, cytokines, and growth factors) can also occur, leading for instance to pulmonary fibrosis, reduced lung function, and increased mortality [163–165]. Regarding mucociliary defense mechanism, overproduction of mucins together with ciliary alteration can lead to airway obstruction and exacerbation of viral infection [166,167].

If the infection reaches, or starts in, the lower respiratory tract, lung function may be compromised (Figure 5). Viruses begin to replicate in the alveolar cells, specifically infecting AT-II cells in the case of SARS-CoV-2 and IAV,

or both AT-II and AT-I cells in the case of AdV and RSV, the latter also affecting endothelial cells. As a result, infected cells can undergo lysis (AdV) [168] or apoptosis (IAV and SARS-CoV-2) [169], and sometimes lead to the formation of syncytium (RSV, SARS-CoV-2) and inclusion bodies (AdV and RSV) [111,170]. Viruses often take advantage of the presence of surfactant phospholipids to enter the cells. For example, DPPC improves adenoviral entry by direct interaction [171] and, interestingly, adenovirus induces the efflux of apical PC by the ABCA1 transporter, which may be related to an opportunistic use of the cellular lipid metabolism [172].

Infection induces the infiltration of inflammatory cells into the alveoli and an aberrant production of TNF-α that alters the functionality of sodium channels of AT-II cells. Sodium transport is coupled to their ability to keep the alveoli free of fluid, driving it from the alveolar space back into the interstitium and to the blood circulation, so that alteration of these channels by viral infection triggers lung edema [2,173,174]. Alveolar edema necessarily imposes negative consequences on the gas exchange, as it increases the tightly regulated thin aqueous layer below the air-liquid interface of alveoli. Furthermore, plasma proteins leak into the alveolus and act as surface-active molecules which are adsorbed into the interface in competition with surfactant components, resulting in the inactivation of the surfactant system [175–177]. Therefore, surface tension reduction fails, leading to the unbalance of physical forces that attempt to alveolar collapse, increasing epithelial injury and thus exacerbating edema, entering a vicious cycle of lung damage.

At the same time, as mentioned above, inflammatory cells are recruited into the alveoli, such as neutrophils or inflammatory macrophages [2]. For instance, increased levels of the neutrophil/lymphocyte ratio are related to severe forms of viral pneumonia and COVID-19 [178]. Inflammatory cells, which include macrophages, monocytes, T cells, mast cells, and neutrophils, secrete into the lower respiratory tract different isoforms of sPLA2s, which display antiviral and anti-inflammatory activities [179]. However, a dysregulated immune response, such as in SARS-CoV-2 or RSV infections [180–182], can cause an increase of sPLA2 in the alveoli, that hydrolyses surfactant lipids and degrades surfactant membranes, releasing free fatty acids (FFA) and lysophospholipids (LPL) [179,181–184]. The direct insertion of these membrane-perturbing molecules together with cholesterol into surfactant layers increases membrane fluidity, which impairs even more the biophysical activity of surfactant [175,185–189]. Hydrolysis of LS phospholipids by sPLA2 is therefore an additional contribution to exacerbation of ARDS [190,191]. On the other hand, the pathological response to viral infections of the lower respiratory system also includes, as aforementioned, the release of antiviral effectors such as ROS or RNS, which create an oxidative inflammatory environment that can cause the oxidation of surfactant lipids and proteins [37,192]. For example, IAV increases the formation of oxPAPC (oxidized 1-palmitoyl-2-arachidonoyl-phosphatidylcholine) in the airspaces [19].

Finally, lower RTIs lead to a dysregulated immune response, causing widespread lung damage and secondary complications like systemic inflammation, bacterial coinfection (tuberculosis bacterial pneumonia, etc.), exacerbation of asthma or preexisting COPD, development of fibrosis, pulmonary arterial hypertension, and autoimmune diseases [193–197].

Lung surfactant and antiviral therapies

Once a viral infection has been established in the lungs, antiviral therapies must enter the scene to control the infection. In recent years, mainly as a consequence of the emergence of COVID-19 pandemic, LS has been postulated as a potential actor in the development of antiviral therapies.

As previously mentioned, some surfactant components, particularly collectins and anionic lipids, have shown promising therapeutic potential due to their antiviral or immunoprotective effects. Some studies point to recombinant forms of SP-D as a potent inhibitor of the inflammatory process produced by SARS-CoV-2 infection. SP-D has been shown to decrease the number of apoptotic and necrotic alveolar cells, thus preventing depletion of surfactant synthesis by AMs [198,199].

Patients with COVID-19 usually have damage at the alveolar level, particularly in AT-II cells. This injury induces a decrease or complete depletion of LS synthesis [200] and, to date, there is no effective treatment for respiratory failure due to SARS-CoV-2 infection. Exogenous surfactants have been used in the treatment of ARDS and other lung diseases [201]. Their administration increases the surfactant pool in the lungs, inducing a decrease in alveolar inflammation and thus an improvement in pulmonary ventilation in children [202,203]. In contrast to the well-established exogenous supplementation in the surfactant-lacking lungs of premature infants, LS therapy in inflammatory and lung-injured scenarios, such as those generated by viral infections, has encountered important hindrances mainly related to the inactivation of the exogenous surfactant as well as to the high doses required to treat adult patients [175]. Until the burst of the 2019 pandemic, clinical trials had showed almost no benefits in the use of surfactant administration in adults with ARDS [204,205]. However, in the context of COVID-19 disease, the use of surfactant mixtures has been reevaluated, as in some cases an atypical form of ARDS seems to occur, which resembles neonatal respiratory distress, and that could be related to the pronounced decrease of LS synthesis consequent to

SARS-CoV-2 direct injury to the AT-II cells [206]. In this line, five clinical trials are nowadays ongoing to estimate whether the administration of surfactant preparations (extracted from natural sources [207–210] or synthetic [211]) can succeed as a therapeutic strategy in the treatment of COVID-19.

Beyond its potential use as part of antiviral therapies, LS has been also proposed to reinforce immune response when administered as mucosal adjuvant of viral vaccines. SF-10, a lipid-protein mixture containing DPPC, PG, palmitic acid, and a synthetic SP-C, has been used as adjuvant in influenza HA oral vaccination. This cocktail induces efficient systemic and local immunity with characteristically high levels of secretory HAv-specific IgA in various mucosal organs [212]. On the other hand, lipid components of LS were used to encapsulate cGAMP, a second messenger produced in response to viral infections and a potent activator of the stimulator of interferon genes (STING), an immune sensor in alveolar epithelial cells. Intranasal application of LS-GAMP nanoparticles together with an inactivated H1N1 influenza virus vaccine achieved immune protection against a broad spectrum of heterosubtypic influenza viruses [213,214].

Finally, LS has begun to be considered as a potential drug delivery tool in antiviral therapies. Antiviral drugs employed in the treatment of viral infections could be potentially incorporated into exogenous surfactant to improve their spreading through the airways towards their final targets, including the lower respiratory tract and the alveoli. In this sense, surfactant has been proven to be an effective vehiculization agent for different drugs, such as corticoids or other anti-inflammatory molecules [215,216]. Although specific studies of LS as vehiculization agent in the context of viral infections are still at dawn, a promising future can be hypothesized for this therapeutic role of surfactant, as it could potentially act both as a drug carrier and as a therapeutical element due to its own antiviral and anti-inflammatory properties.

Conclusion

In addition to its critical role in maintaining respiratory function, lung surfactant has been found to possess antiviral properties that help to protect the respiratory tract against viral infections. Several studies have demonstrated that LS components can directly inhibit viral entry and replication within the respiratory tract, reducing the severity of viral respiratory infections. Further research is needed to fully understand the mechanisms by which LS interacts with viruses and to develop new surfactant-based strategies for preventing and treating viral RTIs. Nonetheless, current evidence suggests that preserving LS production and function may be an effective therapeutic approach for mitigating the effects of viral infections on respiratory function while ensuring proper alveolar homeostasis. Overall, a deeper understanding of the relationship between LS and viral respiratory infections will pave the way for novel therapeutic interventions to combat respiratory diseases.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jesús Pérez-Gil reports a relationship with Chiesi Farmaceutici SpA that includes: consulting or advisory, funding grants, and speaking and lecture fees.

Data availability

No data was used for the research described in the article.

Acknowledgements

M I-C and P L-O are recipients of predoctoral fellowships from the Complutense University of Madrid. Work in the laboratory of the authors is currently funded by the Spanish Ministry of Science and Innovation (PID2021-124932OB-100) and the Regional Government of Madrid (P2018/NMT-4389).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Knudsen L, Ochs M: **The micromechanics of lung alveoli: structure and function of surfactant and tissue components**. *Histochem Cell Biol* 2018, **150**:661–676, https://doi.org/10.1007/s00418-018-1747-9.

2. Calkovska A, Kolomaznik M, Calkovsky V: **Alveolar type II cells and pulmonary surfactant in COVID-19 era**. *Physiol Res* 2021, **70**:S195–S218, https://doi.org/10.33549/PHYSIOLRES.934763. –S208.

3. Adivitiya, Kaushik MS, Chakraborty S, Veleri S, Kateriya S: **Mucociliary respiratory epithelium integrity in molecular defense and susceptibility to pulmonary viral infections**. *Biology* 2021, **10**:95, https://doi.org/10.3390/biology10020095.

4. Gohy S, Hupin C, Ladjemi MZ, Hox V, Pilette C: **Key role of the epithelium in chronic upper airways diseases**. *Clin Exp Allergy* 2020, **50**:135–146, https://doi.org/10.1111/cea.13539.

5. Grubor B, Meyerholz DK, Ackermann MR: **Collectins and cationic antimicrobial peptides of the respiratory epithelia**. *Vet Pathol* 2006, **43**:595–612, https://doi.org/10.1354/vp.43-5-595.

6. Laulajainen-Hongisto A, Toppila-Salmi SK, Luukkainen A, Kern R: **Airway epithelial dynamics in allergy and related chronic inflammatory airway diseases**. *Front Cell Dev Biol* 2020, **8**:204, https://doi.org/10.3389/fcell.2020.00204.

7. Vareille M, Kieninger E, Edwards MR, Regamey N: **The airway epithelium: soldier in the fight against respiratory viruses**. *Clin Microbiol Rev* 2011, **24**:210–229, https://doi.org/10.1128/CMR.00014-10.

8. Whitsett JA, Alenghat T: **Respiratory epithelial cells orchestrate pulmonary innate immunity**. *Nat Immunol* 2015, **16**:27–35, https://doi.org/10.1038/ni.3045.

9. Naeem A, Rai SN, Pierre L: *Histology, alveolar macrophages*. StatPearls; 2019. https://pubmed.ncbi.nlm.nih.gov/clipboard/. Accessed 27 December 2022.

10. Woo YD, Jeong D, Chung DH: **Development and functions of alveolar macrophages**. *Mol Cell* 2021, **44**:292–300, https://doi.org/10.14348/molcells.2021.0058.

11. Allard B, Panariti A, Martin JG: **Alveolar macrophages in the resolution of inflammation, tissue repair, and tolerance to infection**. *Front Immunol* 2018, **9**, https://doi.org/10.3389/fimmu.2018.01777.

12. Joshi N, Walter JM, Misharin AV: **Alveolar macrophages**. *Cell Immunol* 2018, **330**:86–90, https://doi.org/10.1016/j.cellimm.2018.01.005.

13. Nkadi PO, Merritt TA, Pillers DAM: **An overview of pulmonary surfactant in the neonate: genetics, metabolism, and the role of surfactant in health and disease**. *Mol Genet Metabol* 2009, **97**:95–101, https://doi.org/10.1016/j.ymgme.2009.01.015.

14. Olmeda B, Martínez-Calle M, Pérez-Gil J: **Pulmonary surfactant metabolism in the alveolar airspace: biogenesis, extracellular conversions, recycling**. *Ann Anat* 2017, **209**:78–92, https://doi.org/10.1016/j.aanat.2016.09.008.

15. Zissel G, Ernst M, Rabe K, Papadopoulos T, Magnussen H, Schlaak M, *et al.*: **Human alveolar epithelial cells type II are capable of regulating T-cell activity**. *J Invest Med* 2000, **48**:66–75.

16. Nova Z, Skovierova H, Calkovska A: **Alveolar-capillary membrane-related pulmonary cells as a target in endotoxin-induced acute lung injury**. *Int J Mol Sci* 2019, **20**:831, https://doi.org/10.3390/ijms20040831.

17. Hiemstra PS, Amatngalim GD, van der Does AM, Taube C: **Antimicrobial peptides and innate lung defenses**. *Chest* 2016, **149**:545–551, https://doi.org/10.1378/chest.15-1353.

18. Han S, Mallampalli RK: **The role of surfactant in lung disease and host defense against pulmonary infections**. *Ann Am Thorac Soc* 2015, **12**:765–774, https://doi.org/10.1513/AnnalsATS.201411-507FR.

19. Fessler MB, Summer RS: **Surfactant lipids at the host–environment interface. Metabolic sensors, suppressors, and effectors of inflammatory lung disease**. *Am J Respir Cell Mol Biol* 2016, **54**:624–635, https://doi.org/10.1165/rcmb.2016-0011PS.

20. Schmidt R, Meier U, Markart P, Grimminger F, Velcovsky HG, Morr H, *et al.*: **Altered fatty acid composition of surfactant phospholipids in interstitial lung disease**. *Am J Physiol Lung Cell Mol Physiol* 2002, **283**:L1079, https://doi.org/10.1152/ajplung.00484.2001. –L1085.

21. Goerke J, Clements JA: *Alveolar surface tension and lung surfactant*. Compr Physiol, Wiley; 1986:247–261, https://doi.org/10.1002/cphy.cp030316.

22. Goerke J: **Pulmonary surfactant: functions and molecular composition**. *Biochim Biophys Acta, Mol Basis Dis* 1998, **1408**:79–89, https://doi.org/10.1016/S0925-4439(98)00060-X.

23. Wright JR: **Immunoregulatory functions of surfactant proteins**. *Nat Rev Immunol* 2005, **5**:58–68, https://doi.org/10.1038/nri1528.

24. Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP: **Activation of airway epithelial cells by toll-like receptor agonists**. *Am J Respir Cell Mol Biol* 2004, **31**:358–364, https://doi.org/10.1165/rcmb.2003-0388OC.

25. Kuronuma K, Mitsuzawa H, Takeda K, Nishitani C, Chan ED, Kuroki Y, *et al.*: **Anionic pulmonary surfactant phospholipids inhibit inflammatory responses from alveolar macrophages and U937 cells by binding the lipopolysaccharide-interacting proteins CD14 and MD-2**. *J Biol Chem* 2009, **284**:25488–25500, https://doi.org/10.1074/jbc.M109.040832.

26. ** Numata M, Voelker DR: **Anti-inflammatory and anti-viral actions of anionic pulmonary surfactant phospholipids**. *Biochim Biophys Acta Mol Cell Biol Lipids* 2022, **1867**, 159139, https://doi.org/10.1016/j.bbalip.2022.159139.
A review of reference regarding the role of surfactant lipids against pulmonary infections by the most important research group contributing to this field.

27. Wright JR: **Immunomodulatory functions of surfactant**. *Physiol Rev* 1997, **77**:931–962, https://doi.org/10.1152/physrev.1997.77.4.931.

28. Kuroki Y, Takahashi M, Nishitani C: **Pulmonary collectins in innate immunity of the lung**. *Cell Microbiol* 2007, **9**:1871–1879, https://doi.org/10.1111/j.1462-5822.2007.00953.x.

29. ** Cañadas O, Olmeda B, Alonso A, Pérez-Gil J: **Lipid–protein and protein–protein interactions in the pulmonary surfactant system and their role in lung homeostasis**. *Int J Mol Sci* 2020, **21**:3708, https://doi.org/10.3390/ijms21103708.
All pulmonary surfactant components and the network of interactions among them are deeply analyzed in this review by the same group responsible of the present study. It integrates a vision of pulmonary surfactant as a whole.

30. Herías MV, Hogenkamp A, van Asten AJAM, Tersteeg MHG, van Eijk M, Haagsman HP: **Expression sites of the collectin SP-D suggest its importance in first line host defence: power of combining in situ hybridisation, RT-PCR and immunohistochemistry**. *Mol Immunol* 2007, **44**:3324–3332, https://doi.org/10.1016/j.molimm.2007.02.025.

31. Mason RJ: **Surfactant synthesis, secretion, and function in alveoli and small airways**. *Respiration* 1987, **51**, https://doi.org/10.1159/000195267.

32. Singh G, Katyal SL: **Clara cell proteins**. *Ann N Y Acad Sci* 2006, **923**:43–58, https://doi.org/10.1111/j.1749-6632.2000.tb05518.x.

33. Egberts J, Sloot H, Mazure A: **Minimal surface tension, squeeze-out and transition temperatures of binary mixtures of dipalmitoylphosphatidylcholine and unsaturated phospholipids**. *Biochim Biophys Acta Mol Cell Biol Lipids* 1989, **1002**:109–113, https://doi.org/10.1016/0005-2760(89)90072-6.

34. Pastrana-Rios B, Flach CR, Brauner JW, Mautone AJ, Mendelsohn R: **A direct test of the "squeeze-out" hypothesis of lung surfactant function. External reflection FT-IR at the air/wave interface**. *Biochemistry* 1994, **33**:5121–5127, https://doi.org/10.1021/bi00183a016.

35. Xu L, Yang Y, Zuo YY: **Atomic force microscopy imaging of adsorbed pulmonary surfactant films**. *Biophys J* 2020, **119**:756–766, https://doi.org/10.1016/j.bpj.2020.06.033.

36. Serrano AG, Pérez-Gil J: **Protein–lipid interactions and surface activity in the pulmonary surfactant system**. *Chem Phys Lipids* 2006, **141**:105–118, https://doi.org/10.1016/j.chemphyslip.2006.02.017.

37. Autilio C, Pérez-Gil J: **Understanding the principle biophysics concepts of pulmonary surfactant in health and disease**. *Arch Dis Child Fetal Neonatal Ed* 2018, **104**:2018–315413, https://doi.org/10.1136/archdischild-2018-315413.

38. Wright JR: **Clearance and recycling of pulmonary surfactant**. *Am J Physiol Lung Cell Mol Physiol* 1990, **259**, https://doi.org/10.1152/ajplung.1990.259.2.L1. L1–L12.

39. Grabner R, Meerbach W: **Phagocytosis of surfactant by alveolar macrophages in vitro**. *Am J Physiol Lung Cell Mol Physiol* 1991, **261**:L472, https://doi.org/10.1152/ajplung.1991.261.6.L472. –L477.

40. Veldhuizen R, Possmayer F: **Phospholipid metabolism in lung surfactant**. *Subcell Biochem* 2004:359–388, https://doi.org/10.1007/978-1-4757-5806-1_11.

41. Roldan N, Nyholm TKM, Slotte JP, Pérez-Gil J, García-Álvarez B: **Effect of lung surfactant protein SP-C and SP-C-promoted membrane fragmentation on cholesterol dynamics**. *Biophys J* 2016, **111**:1703–1713, https://doi.org/10.1016/j.bpj.2016.09.016.

42. White MK, Strayer DS: **Surfactant protein A regulates pulmonary surfactant secretion via activation of phosphatidylinositol 3-kinase in type II alveolar cells**. *Exp Cell Res* 2000, **255**:67–76, https://doi.org/10.1006/excr.1999.4764.

43. García-Álvarez B, Alonso A, Pérez-Gil J: *Structure and function of pulmonary surfactant proteins*. Chichester: eLS. John Wiley & Sons, Ltd; 2019:1–15, https://doi.org/10.1002/9780470015902.a0027639.

44. Kairys V, Gilson MK, Luy B: **Structural model for an AxxxG-mediated dimer of surfactant-associated protein C**. *Eur J Biochem* 2004, **271**:2086–2092, https://doi.org/10.1111/j.1432-1033.2004.04107.x.

45. Parra E, Pérez-Gil J: **Composition, structure and mechanical properties define performance of pulmonary surfactant membranes and films**. *Chem Phys Lipids* 2015, **185**:153–175, https://doi.org/10.1016/j.chemphyslip.2014.09.002.

46. Epaud R, Ikegami M, Whitsett JA, Jobe AH, Weaver TE, Akinbi HT: **Surfactant protein B inhibits endotoxin-induced lung inflammation**. *Am J Respir Cell Mol Biol* 2003, **28**:373–378, https://doi.org/10.1165/rcmb.2002-0071OC.

47. Fraile-Ágreda V, Cañadas O, Weaver TE, Casals C: **Synergistic action of antimicrobial lung proteins against Klebsiella pneumoniae**. *Int J Mol Sci* 2021, **22**, 11146, https://doi.org/10.3390/ijms222011146.

48. Coya JM, Akinbi HT, Sáenz A, Yang L, Weaver TE, Casals C: **Natural anti-infective pulmonary proteins: in vivo cooperative action of surfactant protein SP-A and the lung antimicrobial peptide SP-BN**. *J Immun* 2015, **195**:1628–1636, https://doi.org/10.4049/jimmunol.1500778.

49. Gómez-Gil L, Pérez-Gil J, Goormaghtigh E: **Cholesterol modulates the exposure and orientation of pulmonary surfactant protein SP-C in model surfactant membranes**. *Biochim Biophys Acta Biomembr* 2009, **1788**:1907, https://doi.org/10.1016/j.bbamem.2009.05.011. –1915.

50. Gómez-Gil L, Schürch D, Goormaghtigh E, Pérez-Gil J: **Pulmonary surfactant protein SP-C counteracts the deleterious effects of cholesterol on the activity of surfactant films under physiologically relevant compression-expansion dynamics**. *Biophys J* 2009, **97**:2736–2745, https://doi.org/10.1016/j.bpj.2009.08.045.

51. Chaby R, Garcia-Verdugo I, Espinassous Q, Augusto LA: **Interactions between LPS and lung surfactant proteins**. *J Endotoxin Res* 2005, **11**:181–185, https://doi.org/10.1179/096805105X37358.

52. Garcia-Verdugo I, Garcia de Paco E, Espinassous Q, Gonzalez-Horta A, Synguelakis M, Kanellopoulos J, *et al.*: **Synthetic peptides representing the N-terminal segment of surfactant protein C modulate LPS-stimulated TNF-α production by macrophages**. *Innate Immun* 2009, **15**:53–62, https://doi.org/10.1177/1753425908100500.

53. Crouch EC: **Collectins and pulmonary host defense**. *Am J Respir Cell Mol Biol* 1998, **19**:177–201, https://doi.org/10.1165/ajrcmb.19.2.140.

54. Watson A, Phipps MJS, Clark HW, Skylaris C-K, Madsen J: **Surfactant proteins A and D: trimerized innate immunity proteins with an affinity for viral fusion proteins**. *J Innate Immun* 2019, **11**:13–28, https://doi.org/10.1159/000492974.

55. Hoppe H-J, Reid KBM: **Collectins - soluble proteins containing collagenous regions and lectin domains - and their roles in innate immunity**. *Protein Sci* 1994, **3**:1143–1158, https://doi.org/10.1002/pro.5560030801.

56. Haagsman HP, White RT, Schilling J, Lau K, Benson BJ, Golden J, *et al.*: **Studies of the structure of lung surfactant protein SP-A**. *Am J Physiol Lung Cell Mol Physiol* 1989, **257**:L421–L429, https://doi.org/10.1152/ajplung.1989.257.6.L421.

57. Vang Petersen S: **The mannan-binding lectin pathway of complement activation: biology and disease association**. *Mol Immunol* 2001, **38**:133–149, https://doi.org/10.1016/S0161-5890(01)00038-4.

58. Sano H, Kuroki Y: **The lung collectins, SP-A and SP-D, modulate pulmonary innate immunity**. *Mol Immunol* 2005, **42**:279–287, https://doi.org/10.1016/j.molimm.2004.07.014.

59. Haagsman H: **Synthesis and assembly of lung surfactant**. *Annu Rev Physiol* 1991, **53**:441–464, https://doi.org/10.1146/annurev.physiol.53.1.441.

60. Kingma PS, Whitsett JA: **In defense of the lung: surfactant protein A and surfactant protein D**. *Curr Opin Pharmacol* 2006, **6**:277–283, https://doi.org/10.1016/j.coph.2006.02.003.

61. Arroyo R, Martín-González A, Echaide M, Jain A, Brondyk WH, Rosenbaum J, *et al.*: **Supramolecular assembly of human pulmonary surfactant protein SP-D**. *J Mol Biol* 2018, **430**:1495–1509, https://doi.org/10.1016/j.jmb.2018.03.027.

62. Voss T, Eistetter H, Schäfer KP, Engel J: **Macromolecular organization of natural and recombinant lung surfactant protein SP 28–36, Structural homology with the complement factor C1q**. *J Mol Biol* 1988, **201**:219–227, https://doi.org/10.1016/0022-2836(88)90448-2.

63. Guillot L, Balloy V, McCormack FX, Golenbock DT, Chignard M, Si-Tahar M: **Cutting edge: the immunostimulatory activity of the lung surfactant protein-A involves toll-like receptor 4**. *J Immun* 2002, **168**:5989–5992, https://doi.org/10.4049/jimmunol.168.12.5989.

64. Labarrere CA, Kassab GS: **Pattern recognition proteins: first line of defense against coronaviruses**. *Front Immunol* 2021, **12**, 652252, https://doi.org/10.3389/fimmu.2021.652252.

65. Reid KBM: **Functional roles of the lung surfactant proteins SP-A and SP-D in innate immunity**. *Immunobiology* 1998, **199**:200–207, https://doi.org/10.1016/S0171-2985(98)80027-2.

66. Vieira F, Kung JW, Bhatti F: **Structure, genetics and function of the pulmonary associated surfactant proteins A and D: the extra-pulmonary role of these C type lectins**. *Ann Anat* 2017, **211**:184–201, https://doi.org/10.1016/j.aanat.2017.03.002.

67. Subbarao K, Mahanty S: **Respiratory virus infections: understanding COVID-19**. *Immunity* 2020, **52**:905–909, https://doi.org/10.1016/j.immuni.2020.05.004.

68. Hewitt R, Farne H, Ritchie A, Luke E, Johnston SL, Mallia P: **The role of viral infections in exacerbations of chronic obstructive pulmonary disease and asthma**. *Ther Adv Respir Dis* 2016, **10**:158–174, https://doi.org/10.1177/1753465815618113.

69. Avendaño Carvajal L, Perret Pérez C: **Epidemiology of Respiratory Infections**. In *Pediatric Respiratory Diseases*. Edited by Bertrand P, Sánchez I, Cham: Springer; 2020. https://doi.org/10.1007/978-3-030-26961-6_28.

70. Nunes-Silva C, Vilares AT, Schweitzer V, Castanhinha S, Martins A, Lopes MJ, *et al.*: **Non-COVID-19 respiratory viral infection**. *Breathe* 2022, **18**, 210151, https://doi.org/10.1183/20734735.0151-2021.

71. Al-Qahtani AA, Murugaiah V, Bashir HA, Pathan AA, Abozaid SM, Makarov E, *et al.*: **Full-length human surfactant protein A inhibits influenza A virus infection of A549 lung epithelial cells: a recombinant form containing neck and lectin domains promotes infectivity**. *Immunobiology* 2019, **224**:408–418, https://doi.org/10.1016/j.imbio.2019.02.006.

72. Hartshorn KL: **Role of surfactant protein A and D (SP-A and SP-D) in human antiviral host defense**. *Front Biosci* 2010, **S2**:83, https://doi.org/10.2741/s83.

73. Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, *et al.*: **Influenza**. *Nat Rev Dis Primers.* 2018, **4**:1–21, https://doi.org/10.1038/s41572-018-0002-y.

74. Zhang Y, Xu Z, Cao Y: **Host–virus interaction: how host cells defend against influenza A virus infection**. *Viruses* 2020, **12**:376, https://doi.org/10.3390/v12040376.

75. Kishore U, Greenhough TJ, Waters P, Shrive AK, Ghai R, Kamran MF, *et al.*: **Surfactant proteins SP-A and SP-D: structure, function and receptors**. *Mol Immunol* 2006, **43**:1293–1315, https://doi.org/10.1016/j.molimm.2005.08.004.

76. Hillaire MLB, Haagsman HP, Osterhaus ADME, Rimmelzwaan GF, van Eijk M: **Pulmonary surfactant protein D in first-line innate defence against influenza A virus infections**. *J Innate Immun* 2013, **5**:197–208, https://doi.org/10.1159/000346374.

77. Herold S, Becker C, Ridge KM, Budinger GRS: **Influenza virus-induced lung injury: pathogenesis and implications for treatment**. *Eur Respir J* 2015, **45**:1463–1478, https://doi.org/10.1183/09031936.00186214.

78. Haagsman HP, Hogenkamp A, van Eijk M, Veldhuizen EJA: **Surfactant collectins and innate immunity**. *Neonatology* 2008, **93**:288–294, https://doi.org/10.1159/000121454.

79. Johansson C. *Respiratory syncytial virus infection: an innate perspective, F1000Res*, **5**; 2016:1–10, https://doi.org/10.12688/f1000research.9637.1.

80. Carvajal JJ, Avellaneda AM, Salazar-Ardiles C, Maya JE, Kalergis AM, Lay MK: **Host components contributing to respiratory syncytial virus pathogenesis**. *Front Immunol* 2019, **10**: 1–19, https://doi.org/10.3389/fimmu.2019.02152.

81. Smallcombe CC, Linfield DT, Harford TJ, Bokun V, Ivanov AI, Piedimonte G, *et al.*: **Disruption of the airway epithelial barrier in a murine model of respiratory syncytial virus infection**. *Am J Physiol Lung Cell Mol Physiol* 2019, **316**:L358–L368, https://doi.org/10.1152/ajplung.00345.2018.

82. Fung TS, Liu DX: **Coronavirus infection, ER stress, apoptosis and innate immunity**. *Front Microbiol* 2014, **5**:296, https://doi.org/10.3389/fmicb.2014.00296.

83. Qian Z, Travanty EA, Oko L, Edeen K, Berglund A, Wang J, *et al.*: **Innate immune response of human alveolar type II cells infected with severe acute respiratory syndrome–coronavirus**. *Am J Respir Cell Mol Biol* 2013, **48**:742–748, https://doi.org/10.1165/rcmb.2012-0339OC.

84. Yuan M, Wu NC, Zhu X, Lee C-CD, So RTY, Lv H, *et al.*: **A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV**. *Science* 2020, **368**:630–633, https://doi.org/10.1126/science.abb7269.

85. Lang J, Yang N, Deng J, Liu K, Yang P, Zhang G, *et al.*: **Inhibition of SARS pseudovirus cell entry by lactoferrin binding to heparan sulfate proteoglycans**. *PLoS One* 2011, **6**, e23710, https://doi.org/10.1371/journal.pone.0023710.

86. Bochkov YA, Gern JE: **Rhinoviruses and their receptors: implications for allergic disease**. *Curr Allergy Asthma Rep* 2016, **16**:30, https://doi.org/10.1007/s11882-016-0608-7.

87. Barral S, Mamin A, Dantin C, Masouridi-Levrat S, Chalandon Y, Kaiser L, *et al.*: **Rhinovirus infections among hematopoietic stem cell transplant recipients: a pre-transplant dilemma?** *Viruses* 2022, **14**:267, https://doi.org/10.3390/v14020267.

88. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, *et al.*: **Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5**. *Science* 1979, **275**:1320–1323, https://doi.org/10.1126/science.275.5304.1320.

89. Branche A, Falsey A: **Parainfluenza virus infection**. *Semin Respir Crit Care Med* 2016, **37**:538–554, https://doi.org/10.1055/s-0036-1584798.

90. Fanales-Belasio E, Raimondo M, Suligoi B, Buttò S: **HIV virology and pathogenetic mechanisms of infection: a brief overview**. *Ann Ist Super Sanita* 2010, **46**:5–14, https://doi.org/10.4415/ANN_10_01_02.

91. Alexandrova Y, Costiniuk CT, Jenabian MA: **Pulmonary immune dysregulation and viral persistence during HIV infection**. *Front Immunol* 2022, **12**, 808722, https://doi.org/10.3389/fimmu.2021.808722.

92. Brito LF, Brune W, Stahl FR: **Cytomegalovirus (CMV) pneumonitis: cell tropism, inflammation, and immunity**. *Int J Mol Sci* 2019, **20**:3865, https://doi.org/10.3390/ijms20163865.

93. Gonçalves C, Cipriano A, Videira Santos F, Abreu M, Méndez J, Sarmento e Castro R: **Cytomegalovirus acute infection with pulmonary involvement in an immunocompetent patient**. *IDCases* 2018, **14**, e00445, https://doi.org/10.1016/j.idcr.2018.e00445.

94. Leung NHL: **Transmissibility and transmission of respiratory viruses**. *Nat Rev Microbiol* 2021, **19**:528–545, https://doi.org/10.1038/s41579-021-00535-6.

95. Majdoul S, Compton AA: **Lessons in self-defence: inhibition of virus entry by intrinsic immunity**. *Nat Rev Immunol* 2022, **22**: 339–352, https://doi.org/10.1038/s41577-021-00626-8.

96. ** Ghati A, Dam P, Tasdemir D, Kati A, Sellami H, Sezgin GC, *et al.*: **Exogenous pulmonary surfactant: a review focused on adjunctive therapy for severe acute respiratory syndrome coronavirus 2 including SP-A and SP-D as added clinical marker**. *Curr Opin Colloid Interface Sci* 2021, **51**, 101413, https://doi.org/10.1016/j.cocis.2020.101413.
Based on the clinical uses of surfactant replacement therapy and the recent studies involving pulmonary surfactant collectins and SARS-CoV2, this review points out the potential applications of pulmonary surfactant to develop therapeutic strategies against the coronavirus-induced respiratory pathology and the necessity of its optimization.

97. Hui DS, Chan PKS: *Viral pneumonia, encyclopedia of respiratory medicine*. Elsevier; 2006:456–466, https://doi.org/10.1016/B0-12-370879-6/00317-3.

98. Gao N, Rezaee F: **Airway epithelial cell junctions as targets for pathogens and antimicrobial therapy**. *Pharmaceutics* 2022, **14**:2619, https://doi.org/10.3390/pharmaceutics14122619.

99. Leiva-Juárez MM, Kolls JK, Evans SE: **Lung epithelial cells: therapeutically inducible effectors of antimicrobial defense**. *Mucosal Immunol* 2018, **11**:21–34, https://doi.org/10.1038/mi.2017.71.

100. Barbier D, Garcia-Verdugo I, Pothlichet J, Khazen R, Descamps D, Rousseau K, *et al.*: **Influenza A induces the major secreted airway mucin MUC5AC in a protease–EGFR–extracellular regulated kinase–sp1–dependent pathway**. *Am J Respir Cell Mol Biol* 2012, **47**:149–157, https://doi.org/10.1165/rcmb.2011-0405OC.

101. Tamura S, Kurata T: **Defense mechanisms against influenza virus infection in the respiratory tract mucosa**. *Jpn J Infect Dis* 2004, **57**:236–247. http://www.ncbi.nlm.nih.gov/pubmed/15623947.

102. Hallstrand TS, Hackett TL, Altemeier WA, Matute-Bello G, Hansbro PM, Knight DA: **Airway epithelial regulation of pulmonary immune homeostasis and inflammation**. *Clin Immunol* 2014, **151**:1–15, https://doi.org/10.1016/j.clim.2013.12.003.

103. Wu NH, Yang W, Beineke A, Dijkman R, Matrosovich M, Baumgärtner W, *et al.*: **The differentiated airway epithelium infected by influenza viruses maintains the barrier function despite a dramatic loss of ciliated cells**. *Sci Rep* 2016, **6**, 39668, https://doi.org/10.1038/srep39668.

104. Vareille M, Kieninger E, Edwards MR, Regamey N: **Pattern recognition receptors in health and diseases**. *Signal Transduct Targeted Ther* 2021, **6**:291, https://doi.org/10.1038/s41392-021-00687-0.

105. Kumar H, Kawai T, Akira S: **Toll-like receptors and innate immunity**. *Biochem Biophys Res Commun* 2009, **388**:621–625, https://doi.org/10.1016/j.bbrc.2009.08.062.

106. Groskreutz DJ, Monick MM, Powers LS, Yarovinsky TO, Look DC, Hunninghake GW: **Respiratory syncytial virus induces TLR3 protein and protein kinase R, leading to increased double-stranded RNA responsiveness in airway epithelial cells**. *J Immun* 2006, **176**:1733–1740, https://doi.org/10.4049/jimmunol.176.3.1733.

107. Hewson CA, Jardine A, Edwards MR, Laza-Stanca V, Johnston SL: **Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells**. *J Virol* 2005, **79**:12273–12279, https://doi.org/10.1128/JVI.79.19.12273-12279.2005.

108. Guillot L, le Goffic R, Bloch S, Escriou N, Akira S, Chignard M, *et al.*: **Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus**. *J Biol Chem* 2005, **280**:5571–5580, https://doi.org/10.1074/jbc.M410592200.

109. Haynes LM, Moore DD, Kurt-Jones EA, Finberg RW, Anderson LJ, Tripp RA: **Involvement of toll-like receptor 4 in innate immunity to respiratory syncytial virus**. *J Virol* 2001, **75**:10730–10737, https://doi.org/10.1128/jvi.75.22.10730-10737.2001.

110. Levy BD, De Sanctis GT, Devchand PR, Kim E, Ackerman K, Schmidt BA, *et al.*: **Multi-pronged inhibition of airway hyper-**

responsiveness and inflammation by lipoxin A4. *Nat Med* 2002, **8**:1018–1023, https://doi.org/10.1038/nm748.

111. Clementi N, Ghosh S, De Santis M, Castelli M, Criscuolo E, * Zanoni I, *et al.*: **Viral respiratory pathogens and lung injury**. *Clin Microbiol Rev* 2021, **34**, e00103-e00120, https://doi.org/10.1128/CMR.00103-20.
A very complete review on respiratory viruses and the response of the respiratory system beyond pulmonary surfactant. It focuses on the different cellular components of the whole respiratory tract and their involvement in defense and immune mechanisms.

112. Glasser JR, Mallampalli RK: **Surfactant and its role in the pathobiology of pulmonary infection**. *Microb Infect* 2012, **14**: 17–25, https://doi.org/10.1016/j.micinf.2011.08.019.

113. Barrow AD, Palarasah Y, Bugatti M, Holehouse AS, Byers DE, Holtzman MJ, *et al.*: **OSCAR is a receptor for surfactant protein D that activates TNF-α release from human CCR2+ inflammatory monocytes**. *J Immun* 2015, **194**:3317–3326, https://doi.org/10.4049/jimmunol.1402289.

114. 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, https://doi.org/10.1074/jbc.M001107200.

115. Minutti CM, García-Fojeda B, Sáenz A, de las Casas-Engel M, Guillamat-Prats R, de Lorenzo A, *et al.*: **Surfactant protein A prevents IFN-γ/IFN-γ receptor interaction and attenuates classical activation of human alveolar macrophages**. *J Immun* 2016, **197**:590–598, https://doi.org/10.4049/jimmunol.1501032.

116. Arroyo R, Khan MA, Echaide M, Pérez-Gil J, Palaniyar N: **SP-D attenuates LPS-induced formation of human neutrophil extracellular traps (NETs), protecting pulmonary surfactant inactivation by NETs**. *Commun Biol* 2019, **2**:470, https://doi.org/10.1038/s42003-019-0662-5.

117. Gil M, McCormack FX, LeVine AM: **Surfactant protein A modulates cell surface expression of CR3 on alveolar macrophages and enhances CR3-mediated phagocytosis**. *J Biol Chem* 2009, **284**:7495–7504, https://doi.org/10.1074/jbc.M808643200.

118. Coya JM, Fraile-Ágreda V, de Tapia L, García-Fojeda B, Sáenz A, Bengoechea JA, *et al.*: **Cooperative action of SP-A and its trimeric recombinant fragment with polymyxins against Gram-negative respiratory bacteria**. *Front Immunol* 2022, **13**, 927017, https://doi.org/10.3389/fimmu.2022.927017.

119. Mulugeta S, Beers MF: **Surfactant protein C: its unique properties and emerging immunomodulatory role in the lung**. *Microb Infect* 2006, **8**:2317–2323, https://doi.org/10.1016/j.micinf.2006.04.009.

120. Yang L, Johansson J, Ridsdale R, Willander H, Fitzen M, Akinbi HT, *et al.*: **Surfactant protein B propeptide contains a saposin-like protein domain with antimicrobial activity at low pH**. *J Immun* 2010, **184**:975–983, https://doi.org/10.4049/jimmunol.0900650.

121. Watson A, Madsen J, Clark HW: **SP-A and SP-D: Dual functioning immune molecules with antiviral and immunomodulatory properties**. *Front Immunol* 2021, **11**, 622598, https://doi.org/10.3389/fimmu.2020.622598. **
This review offers an extensive view of the role of collectins against respiratory virus, including SARS-CoV2, specially from a molecular perspective. It mentions most of the studies performed with these proteins and their variants in different in vitro and in vivo contexts.

122. Gardai SJ, Xiao Y-Q, Dickinson M, Nick JA, Voelker DR, Greene KE, *et al.*: **By binding SIRPα or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation**. *Cell* 2003, **115**:13–23, https://doi.org/10.1016/S0092-8674(03)00758-X.

123. McCormack FX, Whitsett JA: **The pulmonary collectins, SP-A and SP-D, orchestrate innate immunity in the lung**. *J Clin Investig* 2002, **109**:707–712, https://doi.org/10.1172/JCI200215293.

124. Hartshorn KL, White MR, Voelker DR, Coburn J, Zaner K, Crouch EC: **Mechanism of binding of surfactant protein D to influenza A viruses: importance of binding to haemagglutinin to antiviral activity**. *Biochem J* 2000, **351 Pt 2**:449–458.

125. Hartshorn KL, Crouch EC, White MR, Eggleton P, Tauber AI, Chang D, *et al.*: **Evidence for a protective role of pulmonary surfactant protein D (SP-D) against influenza A viruses**. *J Clin Investig* 1994, **94**:311–319, https://doi.org/10.1172/JCI117323.

126. Hartshorn KL, Reid KB, White MR, Jensenius JC, Morris SM, Tauber AI, *et al.*: **Neutrophil deactivation by influenza A viruses: mechanisms of protection after viral opsonization with collectins and hemagglutination-inhibiting antibodies**. *Blood* 1996, **87**:3450–3461. http://www.ncbi.nlm.nih.gov/pubmed/8605364.

127. Hsieh I-N, de Luna X, White MR, Hartshorn KL: **The role and molecular mechanism of action of surfactant protein D in innate host defense against influenza A virus**. *Front Immunol* 2018, **9**:1368, https://doi.org/10.3389/fimmu.2018.01368.

128. Tecle T, White MR, Crouch EC, Hartshorn KL: **Inhibition of influenza viral neuraminidase activity by collectins**. *Arch Virol* 2007, **152**:1731–1742, https://doi.org/10.1007/s00705-007-0983-4.

129. LeVine AM, Whitsett JA, Hartshorn KL, Crouch EC, Korfhagen TR: **Surfactant protein D enhances clearance of influenza A virus from the lung in vivo**. *J Immun* 2001, **167**: 5868–5873, https://doi.org/10.4049/jimmunol.167.10.5868.

130. Hawgood S, Brown C, Edmondson J, Stumbaugh A, Allen L, Goerke J, *et al.*: **Pulmonary collectins modulate strain-specific influenza A virus infection and host responses**. *J Virol* 2004, **78**:8565–8572, https://doi.org/10.1128/JVI.78.16.8565-8572.2004.

131. Hartshorn KL, White MR, Shepherd V, Reid K, Jensenius JC, Crouch EC: **Mechanisms of anti-influenza activity of surfactant proteins A and D: comparison with serum collectins**. *Am J Physiol Lung Cell Mol Physiol* 1997, **273**:L1156–L1166, https://doi.org/10.1152/ajplung.1997.273.6.L1156.

132. Benne CA, Benaissa-Trouw B, van Strijp JAG, Kraaijeveld CA, van Iwaarden JFF: **Surfactant protein A, but not surfactant protein D, is an opsonin for influenza A virus phagocytosis by rat alveolar macrophages**. *Eur J Immunol* 1997, **27**: 886–890, https://doi.org/10.1002/eji.1830270413.

133. LeVine AM, Elliott J, Whitsett JA, Srikiatkhachorn A, Crouch E, DeSilva N, *et al.*: **Surfactant protein-D enhances phagocytosis and pulmonary clearance of respiratory syncytial virus**. *Am J Respir Cell Mol Biol* 2004, **31**:193–199, https://doi.org/10.1165/rcmb.2003-0107OC.

134. Hickling TP, Bright H, Wing K, Gower D, Martin SL, Sim RB, *et al.*: **A recombinant trimeric surfactant protein D carbohydrate recognition domain inhibits respiratory syncytial virus infectionin vitro and in vivo**. *Eur J Immunol* 1999, **29**: 3478–3484, https://doi.org/10.1002/(SICI)1521-4141(199911)29:11<3478::AID-IMMU3478>3.0.CO;2-W.

135. Kerr MH, Paton JY: **Surfactant protein levels in severe respiratory syncytial virus infection**. *Am J Respir Crit Care Med* 1999, **159**:1115–1118, https://doi.org/10.1164/ajrccm.159.4.9709065.

136. Ghildyal R, Hartley C, Varrasso A, Meanger J, Voelker DR, Anders EM, *et al.*: **Surfactant protein A binds to the fusion glycoprotein of respiratory syncytial virus and neutralizes virion infectivity**. *J Infect Dis* 1999, **180**:2009–2013, https://doi.org/10.1086/315134.

137. LeVine AM, Gwozdz J, Stark J, Bruno M, Whitsett J, Korfhagen T: **Surfactant protein-A enhances respiratory syncytial virus clearance in vivo**. *J Clin Investig* 1999, **103**:1015–1021, https://doi.org/10.1172/JCI5849.

138. Barr FE, Pedigo H, Johnson TR, Shepherd VL: **Surfactant protein-A enhances uptake of respiratory syncytial virus by monocytes and U937 macrophages**. *Am J Respir Cell Mol Biol* 2000, **23**:586–592, https://doi.org/10.1165/ajrcmb.23.5.3771.

139. el Saleeby CM, Li R, Somes GW, Dahmer MK, Quasney MW, DeVincenzo JP: **Surfactant protein A2 polymorphisms and disease severity in a respiratory syncytial virus-infected population**. *J Pediatr* 2010, **156**:409–414, https://doi.org/10.1016/j.jpeds.2009.09.043.

140. Harrod KS, Trapnell BC, Otake K, Korfhagen TR, Whitsett JA: **SP-A enhances viral clearance and inhibits inflammation**

after pulmonary adenoviral infection. *Am J Physiol Lung Cell Mol Physiol* 1999, **277**:L580, https://doi.org/10.1152/ajplung.1999.277.3.L580. –L588.

141. Grubor B, Gallup JM, Meyerholz DK, Crouch EC, Evans RB, Brogden KA, *et al.*: **Enhanced surfactant protein and defensin mRNA levels and reduced viral replication during parainfluenza virus type 3 pneumonia in neonatal lambs**. *Clin Vaccine Immunol* 2004, **11**:599–607, https://doi.org/10.1128/CDLI.11.3.599-607.2004.

142. Leth-Larsen R, Zhong F, Chow VTK, Holmskov U, Lu J: **The SARS coronavirus spike glycoprotein is selectively recognized by lung surfactant protein D and activates macrophages**. *Immunobiology* 2007, **212**:201–211, https://doi.org/10.1016/j.imbio.2006.12.001.

143. Funk CJ, Wang J, Ito Y, Travanty EA, Voelker DR, Holmes Kv, *et al.*: **Infection of human alveolar macrophages by human coronavirus strain 229E**. *J Gen Virol* 2012, **93**:494–503, https://doi.org/10.1099/vir.0.038414-0.

144. Wu YP, Liu ZH, Wei R, Pan SD, Mao NY, Chen B, *et al.*: **Elevated plasma surfactant protein D (SP-D) levels and a direct correlation with anti-severe acute respiratory syndrome coronavirus-specific IgG antibody in SARS patients**. *Scand J Immunol* 2009, **69**:508–515, https://doi.org/10.1111/j.1365-3083.2009.02245.x.

145. Kerget B, Kerget F, Koçak AO, Kõzõltunç A, Araz Ö, Uçar EY, *et al.*: **Are serum interleukin 6 and surfactant protein D levels associated with the clinical course of COVID-19?** *Lung* 2020, **198**:777–784, https://doi.org/10.1007/s00408-020-00393-8.

146. Tong M, Xiong Y, Zhu C, Xu H, Zheng Q, Jiang Y, *et al.*: **Serum surfactant protein D in COVID-19 is elevated and correlated with disease severity**. *BMC Infect Dis* 2021, **21**:737, https://doi.org/10.1186/s12879-021-06447-3.

147. * Madan T, Biswas B, Varghese PM, Subedi R, Pandit H, Idicula-Thomas S, *et al.*: **A recombinant fragment of human surfactant protein D binds spike protein and inhibits infectivity and replication of SARS-CoV-2 in clinical samples**. *Am J Respir Cell Mol Biol* 2021, **65**:41–53, https://doi.org/10.1165/rcmb.2021-0005OC.

148. ** Hsieh M-H, Beirag N, Murugaiah V, Chou Y-C, Kuo W-S, Kao H-F, *et al.*: **Human surfactant protein D binds spike protein and acts as an entry inhibitor of SARS-CoV-2 pseudotyped viral particles**. *Front Immunol* 2021, **12**, 641360, https://doi.org/10.3389/fimmu.2021.641360.

These two recent studies bring to light the potential of pulmonary surfactant and its components against SAR-CoV-2 infections. They both focus on the direct interaction of Surfactant Protein D with this virus.

149. White MR, Crouch E, van Eijk M, Hartshorn M, Pemberton L, Tornoe I, *et al.*: **Cooperative anti-influenza activities of respiratory innate immune proteins and neuraminidase inhibitor**. *Am J Physiol Lung Cell Mol Physiol* 2005, **288**, https://doi.org/10.1152/ajplung.00365.2004. L831–L840.

150. Bruce SR, Atkins CL, Colasurdo GN, Alcorn JL: **Respiratory syncytial virus infection alters surfactant protein A expression in human pulmonary epithelial cells by reducing translation efficiency**. *Am J Physiol Lung Cell* 2009, **297**:L559–L567, https://doi.org/10.1152/ajplung.90507.2008.

151. Arroyo R, Grant SN, Colombo M, Salvioni L, Corsi F, Truffi M, *et al.*: **Full-Length recombinant hSP-D binds and inhibits SARS-CoV-2**. *Biomolecules* 2021, **11**:1114, https://doi.org/10.3390/biom11081114.

152. Voelker DR, Numata M: **Phospholipid regulation of innate immunity and respiratory viral infection**. *J Biol Chem* 2019, **294**:4282–4289, https://doi.org/10.1074/jbc.AW118.003229.

153. ** Numata M, Mitchell JR, Tipper JL, Brand JD, Trombley JE, Nagashima Y, *et al.*: **Pulmonary surfactant lipids inhibit infections with the pandemic H1N1 influenza virus in several animal models**. *J Biol Chem* 2020, **295**:1704–1715, https://doi.org/10.1074/jbc.RA119.012053.

This paper from Voelker's group is focused on the study of pulmonary surfactant lipids in viral infections. Here they show their potential against Influenza A virus in a very complete study including cellular and animal models.

154. Numata M, Kandasamy P, Nagashima Y, Posey J, Hartshorn K, Woodland D, *et al.*: **Phosphatidylglycerol suppresses influenza A virus infection**. *Am J Respir Cell Mol Biol* 2012, **46**:479–487, https://doi.org/10.1165/rcmb.2011-0194OC.

155. Numata M, Kandasamy P, Nagashima Y, Fickes R, Murphy RC, Voelker DR: **Phosphatidylinositol inhibits respiratory syncytial virus infection**. *J Lipid Res* 2015, **56**:578–587, https://doi.org/10.1194/jlr.M055723.

156. Numata M, Chu HW, Dakhama A, Voelker DR: **Pulmonary surfactant phosphatidylglycerol inhibits respiratory syncytial virus–induced inflammation and infection**. *Proc Natl Acad Sci USA* 2010, **107**:320–325, https://doi.org/10.1073/pnas.0909361107.

157. Glasser SW, Witt TL, Senft AP, Baatz JE, Folger D, Maxfield MD, *et al.*: **Surfactant protein C-deficient mice are susceptible to respiratory syncytial virus infection**. *Am J Physiol Lung Cell Mol Physiol* 2009, **297**:64–72, https://doi.org/10.1152/ajplung.90640.2008.-Patients.

158. Puthothu B, Forster J, Heinze J, Heinzmann A, Krueger M: **Surfactant protein B polymorphisms are associated with severe respiratory syncytial virus infection, but not with asthma**. *BMC Pulm Med* 2007, **7**:6, https://doi.org/10.1186/1471-2466-7-6.

159. Singh D, McCann KL, Imani F: **MAPK and heat shock protein 27 activation are associated with respiratory syncytial virus induction of human bronchial epithelial monolayer disruption**. *Am J Physiol Lung Cell Mol Physiol* 2007, **293**:L436–L445, https://doi.org/10.1152/ajplung.00097.2007.

160. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM: **Asthma. From bronchoconstriction to airways inflammation and remodeling**. *Am J Respir Crit Care Med* 2000, **161**:1720–1745, https://doi.org/10.1164/ajrccm.161.5.9903102.

161. Folkerts G, van der Linde HJ, Nijkamp FP: **Virus-induced airway hyperresponsiveness in Guinea pigs is related to a deficiency in nitric oxide**. *J Clin Investig* 1995, **95**:26–30, https://doi.org/10.1172/JCI117649.

162. Mori I, Komatsu T, Takeuchi K, Nakakuki K, Sudo M, Kimura Y: **In vivo induction of apoptosis by influenza virus**. *J Gen Virol* 1995, **76**:2869–2873, https://doi.org/10.1099/0022-1317-76-11-2869.

163. Huang WJ, Tang XX: **Virus infection induced pulmonary fibrosis**. *J Transl Med* 2021, **19**:496, https://doi.org/10.1186/s12967-021-03159-9.

164. Boyd DF, Allen EK, Randolph AG, Guo XJ, Weng Y, Sanders CJ, *et al.*: **Exuberant fibroblast activity compromises lung function via ADAMTS4**. *Nature* 2020, **587**:466–471, https://doi.org/10.1038/s41586-020-2877-5.

165. Li W, Shen H-H: **Effect of respiratory syncytial virus on the activity of matrix metalloproteinase in mice**. *Chin Med J* 2007, **120**:5–11. http://www.ncbi.nlm.nih.gov/pubmed/17254480.

166. Hashimoto K, Graham BS, Ho SB, Adler KB, Collins RD, Olson SJ, *et al.*: **Respiratory syncytial virus in allergic lung inflammation increases Muc5ac and Gob-5**. *Am J Respir Crit Care Med* 2004, **170**:306–312, https://doi.org/10.1164/rccm.200301-030OC.

167. He S-H, Zheng J, Duan M-K: **Induction of mucin secretion from human bronchial tissue and epithelial cells by rhinovirus and lipopolysaccharide**. *Acta Pharmacol Sin* 2004, **25**:1176–1181. http://www.ncbi.nlm.nih.gov/pubmed/15339394.

168. Jiang H, White EJ, Ríos-Vicil CI, Xu J, Gomez-Manzano C, Fueyo J: **Human adenovirus type 5 induces cell lysis through autophagy and autophagy-triggered caspase activity**. *J Virol* 2011, **85**:4720–4729, https://doi.org/10.1128/JVI.02032-10.

169. Julkunen I: **Molecular pathogenesis of influenza A virus infection and virus-induced regulation of cytokine gene expression**. *Cytokine Growth Factor Rev* 2001, **12**:171–180, https://doi.org/10.1016/S1359-6101(00)00026-5.

170. Martínez-Girón R, Pantanowitz L: **Lower respiratory tract viral infections: diagnostic role of exfoliative cytology**. *Diagn Cytopathol* 2017, **45**:614–620, https://doi.org/10.1002/dc.23697.

171. Balakireva L, Schoehn G, Thouvenin E, Chroboczek J: **Binding of adenovirus capsid to dipalmitoyl phosphatidylcholine provides a novel pathway for virus entry**. *J Virol* 2003, **77**: 4858–4866, https://doi.org/10.1128/JVI.77.8.4858-4866.2003.

172. Miakotina OL, McCoy DM, Shi L, Look DC, Mallampalli RK: **Human adenovirus modulates surfactant phospholipid trafficking**. *Traffic* 2007, **8**:1765–1777, https://doi.org/10.1111/j.1600-0854.2007.00641.x.

173. Mason RJ: **Biology of alveolar type II cells**. *Respirology* 2006, **11**:S12–S15, https://doi.org/10.1111/j.1440-1843.2006.00800.x.

174. Yamagata T, Yamagata Y, Nishimoto T, Hirano T, Nakanishi M, Minakata Y, *et al.*: **The regulation of amiloride-sensitive epithelial sodium channels by tumor necrosis factor-alpha in injured lungs and alveolar type II cells**. *Respir Physiol Neurobiol* 2009, **166**:16–23, https://doi.org/10.1016/j.resp.2008.12.008.

175. Echaide M, Autilio C, Arroyo R, Perez-Gil J: **Restoring pulmonary surfactant membranes and films at the respiratory surface**. *Biochim Biophys Acta Biomembr* 2017, **1859**:1725–1739, https://doi.org/10.1016/j.bbamem.2017.03.015.

176. Zasadzinski JA, Alig TF, Alonso C, de la Serna JB, Perez-Gil J, Taeusch HW: **Inhibition of pulmonary surfactant adsorption by serum and the mechanisms of reversal by hydrophilic polymers: theory**. *Biophys J* 2005, **89**:1621–1629, https://doi.org/10.1529/biophysj.105.062646.

177. Taeusch HW, de la Serna JB, Perez-Gil J, Alonso C, Zasadzinski JA: **Inactivation of pulmonary surfactant due to serum-inhibited adsorption and reversal by hydrophilic polymers: experimental**. *Biophys J* 2005, **89**:1769–1779, https://doi.org/10.1529/biophysj.105.062620.

178. Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, *et al.*: **Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage**. *J Transl Med* 2020, **18**: 206, https://doi.org/10.1186/s12967-020-02374-0.

179. Dennis EA, Cao J, Hsu Y-H, Magrioti V, Kokotos G: **Phospholipase A 2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention**. *Chem Rev* 2011, **111**:6130–6185, https://doi.org/10.1021/cr200085w.

180. Liu T, Zaman W, Kaphalia BS, Ansari GAS, Garofalo RP, Casola A: **RSV-induced prostaglandin E2 production occurs via cPLA2 activation: role in viral replication**. *Virology* 2005, **343**:12–24, https://doi.org/10.1016/j.virol.2005.08.012.

181. Letsiou E, Htwe YM, Dudek SM: **Secretory phospholipase A2 enzymes in acute lung injury**. *Cell Biochem Biophys* 2021, **79**: 609–617, https://doi.org/10.1007/s12013-021-01003-x.

182. Kuypers FA, Rostad CA, Anderson EJ, Chahroudi A, Jaggi P, Wrammert J, *et al.*: **Secretory phospholipase A2 in SARS-CoV-2 infection and multisystem inflammatory syndrome in children (MIS-C)**. *Exp Biol Med* 2021, **246**:2543–2552, https://doi.org/10.1177/15353702211028560.

183. Murakami M, Sato H, Miki Y, Yamamoto K, Taketomi Y: **A new era of secreted phospholipase A2**. *J Lipid Res* 2015, **56**: 1248–1261, https://doi.org/10.1194/jlr.R058123.

184. Ohtsuki M, Taketomi Y, Arata S, Masuda S, Ishikawa Y, Ishii T, *et al.*: **Transgenic expression of group V, but not group X, secreted phospholipase A2 in mice leads to neonatal lethality because of lung dysfunction**. *J Biol Chem* 2006, **281**: 36420–36433, https://doi.org/10.1074/jbc.M607975200.

185. Saenz A, López-Sánchez A, Mojica-Lázaro J, Martínez-Caro L, Nin N, Bagatolli LA, *et al.*: **Fluidizing effects of C-reactive protein on lung surfactant membranes: protective role of surfactant protein A**. *Faseb J* 2010, **24**:3662–3673, https://doi.org/10.1096/fj.09-142646.

186. Malcharek S, Hinz A, Hilterhaus L, Galla H-J: **Multilayer structures in lipid monolayer films containing surfactant protein C: effects of cholesterol and POPE**. *Biophys J* 2005, **88**: 2638–2649, https://doi.org/10.1529/biophysj.104.050823.

187. Hite RD, Seeds MC, Jacinto RB, Grier BL, Waite BM, Bass DA: **Lysophospholipid and fatty acid inhibition of pulmonary surfactant: non-enzymatic models of phospholipase A2 surfactant hydrolysis**. *Biochim Biophys Acta Biomembr* 2005, **1720**:14–21, https://doi.org/10.1016/j.bbamem.2005.10.014.

188. Gunasekara L, Schürch S, Schoel WM, Nag K, Leonenko Z, Haufs M, *et al.*: **Pulmonary surfactant function is abolished by an elevated proportion of cholesterol**. *Biochim Biophys Acta Mol Cell Biol Lipids* 2005, **1737**:27–35, https://doi.org/10.1016/j.bbalip.2005.09.002.

189. Lopez-Rodriguez E, Pérez-Gil J: **Structure-function relationships in pulmonary surfactant membranes: from biophysics to therapy**. *Biochim Biophys Acta Biomembr* 2014, **1838**: 1568–1585, https://doi.org/10.1016/j.bbamem.2014.01.028.

190. De Luca D, Lopez-Rodriguez E, Minucci A, Vendittelli F, Gentile L, Stival E, *et al.*: **Clinical and biological role of secretory phospholipase A2 in acute respiratory distress syndrome infants**. *Crit Care* 2013, **17**:R163, https://doi.org/10.1186/cc12842.

191. De Luca D, Shankar-Aguilera S, Autilio C, Raschetti R, Vedovelli L, Fitting C, *et al.*: **Surfactant-secreted phospholipase A2 interaction and and respiratory outcome in preterm neonates**. *Am J Physiol Lung Cell Mol Physiol* 2020, **319**, https://doi.org/10.1152/ajplung.00462.2019. L95–L104.

192. Zuo YY, Veldhuizen RAW, Neumann AW, Petersen NO, Possmayer F: **Current perspectives in pulmonary surfactant — inhibition, enhancement and evaluation**. *Biochim Biophys Acta Biomembr* 2008, **1778**:1947, https://doi.org/10.1016/j.bbamem.2008.03.021. –1977.

193. Mizgerd JP: **Respiratory infection and the impact of pulmonary immunity on lung health and disease**. *Am J Respir Crit Care Med* 2012, **186**:824–829, https://doi.org/10.1164/rccm.201206-1063PP.

194. Li W, Moltedo B, Moran TM: **Type I interferon induction during influenza virus infection increases susceptibility to secondary Streptococcus pneumoniae infection by negative regulation of γδ T cells**. *J Virol* 2012, **86**:12304–12312, https://doi.org/10.1128/JVI.01269-12.

195. Redford PS, Mayer-Barber KD, McNab FW, Stavropoulos E, Wack A, Sher A, *et al.*: **Influenza A virus impairs control of Mycobacterium tuberculosis coinfection through a type I interferon receptor–dependent pathway**. *J Infect Dis* 2014, **209**:270–274, https://doi.org/10.1093/infdis/jit424.

196. Chen X, Liao B, Cheng L, Peng X, Xu X, Li Y, *et al.*: **The microbial coinfection in COVID-19**. *Appl Microbiol Biotechnol* 2020, **104**:7777–7785, https://doi.org/10.1007/s00253-020-10814-6.

197. Klugman KP, Chien Y-W, Madhi SA: **Pneumococcal pneumonia and influenza: a deadly combination**. *Vaccine* 2009, **27**, https://doi.org/10.1016/j.vaccine.2009.06.007. C9–C14.

198. Clark H: **The potential of recombinant surfactant protein D therapy to reduce inflammation in neonatal chronic lung disease, cystic fibrosis, and emphysema**. *Arch Dis Child* 2003, **88**:981–984, https://doi.org/10.1136/adc.88.11.981.

199. Clark H, Palaniyar N, Strong P, Edmondson J, Hawgood S, Reid KBM: **Surfactant protein D reduces alveolar macrophage apoptosis in vivo**. *J Immun* 2002, **169**:2892–2899, https://doi.org/10.4049/jimmunol.169.6.2892.

200. Ahmed S, Akter MdS, Roy K, Islam MdS: **Role of surfactant for the treatment of alveolar cells against coronavirus (Covid-19)**. *Annu Res Rev Biol* 2020:34–39, https://doi.org/10.9734/arrb/2020/v35i630233.

201. Zhang L, Cao H-Y, Zhao S, Yuan L-J, Han D, Jiang H, *et al.*: **Effect of exogenous pulmonary surfactants on mortality rate in neonatal respiratory distress syndrome: a network meta-analysis of randomized controlled trials**. *Pulm Pharmacol Ther* 2015, **34**:46–54, https://doi.org/10.1016/j.pupt.2015.08.005.

202. Herting E, Härtel C, Göpel W: **Less invasive surfactant administration**. *Curr Opin Pediatr* 2020, **32**:228–234, https://doi.org/10.1097/MOP.0000000000000878.

203. Mirastschijski U, Dembinski R, Maedler K: **Lung surfactant for pulmonary barrier restoration in patients with COVID-19 pneumonia**. *Front Med* 2020, **7**:254, https://doi.org/10.3389/fmed.2020.00254.

204. Meng S-S, Chang W, Lu Z-H, Xie J-F, Qiu H-B, Yang Y, *et al.*: **Effect of surfactant administration on outcomes of adult patients in acute respiratory distress syndrome: a meta-analysis of randomized controlled trials**. *BMC Pulm Med* 2019, **19**:9, https://doi.org/10.1186/s12890-018-0761-y.

205. Willson DF, Truwit JD, Conaway MR, Traul CS, Egan EE: **The adult calfactant in acute respiratory distress syndrome trial**. *Chest* 2015, **148**:356–364, https://doi.org/10.1378/chest.14-1139.

206. Heching M, Lev S, Shitenberg D, Dicker D, Kramer MR: **Surfactant for the treatment of ARDS in a patient with COVID-19**. *Chest* 2021, **160**, https://doi.org/10.1016/j.chest.2021.01.028. e9–e12.

207. National Institutes of Health Clinical Center: *Curosurf® in adult acute respiratory distress syndrome due to COVID-19 (Caards-1)., NCT04384731*. ClinicalTrials.Gov. National Institutes of Health; 2020. Updated, https://Clinicaltrials.Gov/Ct2/Show/NCT04384731. Accessed 3 February 2021. 2021.

208. National Institutes of Health Clinical Center: *A clinical trial of nebulized surfactant for the treatment of moderate to severe COVID-19 (COVSurf)., NCT04362059*. ClinicalTrials.Gov. National Institutes of Health; 2020. Updated, https://Clinicaltrials.Gov/Ct2/Show/NCT04362059. Accessed 5 May 2022. 2020.

209. National Institutes of Health Clinical Center: *London's exogenous surfactant study for COVID19 (LESSCOVID)., NCT04375735. ClinicalTrials.Gov*. National Institutes of Health; 2020. Updated, https://Clinicaltrials.Gov/Ct2/Show/NCT04375735. Accessed 11 November 2021. 2020.

210. National Institutes of Health: *Poractant alfa - curosurf and SARSCOV- 19 ARDS (Covid-19)., NCT04502433*. ClinicalTrials.Gov. National Institutes of Health; 2020. Updated, https://Clinicaltrials.Gov/Ct2/Show/NCT04502433. Accessed 6 April 2022. 2020.

211. National Institutes of Health Clinical Center: *The safety and preliminary efficacy of lucinactant in adults with COVID-19., NCT04389671*. ClinicalTrials.Gov. National Institutes of Health; 2020. Updated, https://Clinicaltrials.Gov/Ct2/Show/NCT04389671. Accessed 12 July 2022.

212. Kimoto T, Kim H, Sakai S, Takahashi E, Kido H: **Oral vaccination with influenza hemagglutinin combined with human pulmonary surfactant-mimicking synthetic adjuvant SF-10 induces efficient local and systemic immunity compared with nasal and subcutaneous vaccination and provides protective immunity in mice**. *Vaccine* 2019, **37**:612–622, https://doi.org/10.1016/j.vaccine.2018.12.002.

213. Herold S, Sander L-E: **Toward a universal flu vaccine**. *Science* 2020, **367**:852–853, https://doi.org/10.1126/science.aba2754.

214. Wang J, Li P, Yu Y, Fu Y, Jiang H, Lu M, *et al.*: **Pulmonary surfactant-biomimetic nanoparticles potentiate heterosubtypic influenza immunity**. *Science* 2020:367, https://doi.org/10.1126/science.aau0810. eaau0810.

215. * Hidalgo A, Garcia-Mouton C, Autilio C, Carravilla P, Orellana G, Islam MN, *et al.*: **Pulmonary surfactant and drug delivery: vehiculization, release and targeting of surfactant/tacrolimus formulations**. *J Contr Release* 2021, **329**:205–222, https://doi.org/10.1016/j.jconrel.2020.11.042.
The study of drug vehiculization by pulmonary surfactant is described here combining in vitro experiments with a novel device and an in vivo proof of concept. This opens novel perspectives on potential clinical applications using inhaled surfactant/drug combinations.

216. Hidalgo A, Salomone F, Fresno N, Orellana G, Cruz A, Perez-Gil J: **Efficient interfacially driven vehiculization of corticosteroids by pulmonary surfactant**. *Langmuir* 2017, **33**:7929–7939, https://doi.org/10.1021/acs.langmuir.7b01177.