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Non-Pulmonary Immune Functions of Surfactant Proteins A and D

Sylvia Ujma[a–c] William G.C. Horsnell[b, d] Arieh A. Katz[a–c] Howard W. Clark[e] Georgia Schäfer[a–c]

[a]UCT Receptor Biology Research Unit, Division of Medical Biochemistry and Structural Biology, Department of Integrative Biomedical Sciences, [b]Institute of Infectious Disease and Molecular Medicine, [c]SAMRC Gynecology Cancer Research Centre, and [d]Division of Immunology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa; [e]Child Health, Division of Clinical and Experimental Sciences, Department of Child Health, University of Southampton, Southampton, UK

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Abstract
Surfactant proteins A (SP-A) and D (SP-D) are established as essential components of our innate immune system for protecting the lung from pathogens and allergens. They essentially exert their protective functions by regulating pulmonary homeostasis. Both proteins are however widely expressed throughout the body, including the female reproductive tract, urinary tract, gastrointestinal tract, the eye, ear, nasal compartment, central nervous system, the coronary artery and the skin. The functions of SP-A and SP-D at these sites are a relatively underinvestigated area, but it is emerging that both SP-A and SP-D contribute significantly to the regulation of inflammation and protection from infection at these sites. This review presents our current understanding of the roles of SP-A and SP-D in non-pulmonary sites.

Introduction

Surfactant proteins A and D (SP-A and SP-D, respectively) are large, collagenous, hydrophilic proteins which belong to a family of proteins known as the collectins. They were first identified in pulmonary surfactant, a phospholipid and protein complex that lines the lungs and is essential for pulmonary function by reducing surface tension [1]. Moreover, the interaction of SP-A and SP-D with airborne pathogens revealed their central role in host defence in the lung [2].

The primary structure of both SPs consists of an N-terminal non-collagenous domain capable of forming intersubunit disulphide bonds, a collagenous region of Gly-X-Y repeats, a helical neck domain and a globular C-terminal carbohydrate recognition domain (CRD), each one with different ligand-binding specificities. Monomers spontaneously self-assemble into trimers which then form the higher-order bunch-like SP-A octadecamers, and the cruciform SP-D dodecamers, respectively [3]. Although structurally very similar, SP-A and SP-D display distinct activities and modes of action which is reflected by their different ligand-binding affinities: SP-A prefer-

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E-Mail karger@karger.com
www.karger.com/jin

Dr. Georgia Schäfer
Institute of Infectious Disease and Molecular Medicine (IDM), Division of Medical Biochemistry, University of Cape Town, Wernher and Beit Building North, Room N2.02 Anzio Road, Observatory 7925, Cape Town (South Africa)
E-Mail georgia.schafer@uct.ac.za

entially binds to monosaccharides and lipid ligands, while SP-D additionally binds disaccharides, complex carbohydrates and anionic phospholipids present on cell surfaces [4]. This might be explained by subtle structural differences between the CRDs of trimeric SP-A and SP-D resulting in the flatter and more hydrophobic surface of SP-A preferentially binding to less polar substrates, while the more hydrophilic surface of SP-D displays a higher affinity for highly polar targets [5, 6]. Moreover, in SP-A, the Gly-X-Y repeats in the collagenous region are interrupted resulting in a kink (and hence the bunch-like structure) and a relatively smaller distance to the distal CRD compared to SP-D which displays more freedom to bind and aggregate target pathogens [3].

SP-A and SP-D play important roles in innate immune responses to a wide range of respiratory pathogens including influenza A virus, respiratory syncytial virus, *Mycobacterium tuberculosis*, *Aspergillus fumigatus, Pseudomonas aeruginosa* and *Haemophilus influenzae* [7–14]. Additionally, it has recently been shown that pulmonary SP-D contributes significantly to host control of infections by the parasitic helminth *Nippostrongylus brasiliensis* [15]. Recognition and binding of this diverse variety of incoming pathogens by SP-A and SP-D trigger various immune responses, including opsonisation leading to enhanced phagocytosis and killing by recruited macrophages and neutrophils via oxidative mechanisms, aggregation of pathogens thereby hindering their entry into host cells, and direct microbicidal activities by increasing cellular membrane permeability (reviewed in [2]). SPs also assist in the clearance of apoptotic cells and in modulating inflammation [2, 16]. The interaction of SPs with immune cells to initiate clearance mechanisms is mediated by SP's collagen region with a number of proposed receptor molecules on these cells [3]. (For a detailed review of SP functions and mechanism in clearance of pathogens in the lung, please refer to Kishore et al. [3]).

SP-A and SP-D are also important for maintaining immune homeostasis in the lung by either enhancing or suppressing the production of inflammatory molecules by, for example, alveolar macrophages [17]. SP-A and SP-D mediate this protection in part at least via CRD binding to signal inhibitory regulatory protein α (or CD172a), and this interaction can maintain macrophages in a homeostatic state [17]. The significance of SP-D in maintaining lung homeostasis is particularly well demonstrated by pulmonary pathological phenotypes in SP-D-deficient mice; these display substantial abnormalities in surfactant homeostasis and alveolar cell morphology and spontaneously develop chronic inflammation and emphysema-like pathology [18]. This observation also supports the perceived stronger potency of SP-D compared to SP-A: the absence of SP-A does not result in significant physiological effects in mice [19]. The significance of these preclinical findings is also supported by clinical studies which have shown that individuals with reduced or altered SP-D present with severer chronic obstructive pulmonary diseases or asthma [20].

SP-A and SP-D functions are therefore important in both maintaining lung homeostasis and protecting this site from infection. However, SP-A and SP-D are also widely expressed at other mucosal surfaces throughout the body, but their contribution to host immunity and immune homeostasis at these sites is not as well understood. In this review, we will give a short overview of our current understanding of the non-pulmonary locations of SP-A and SP-D and their proposed functions in these regions, and identify key areas where understanding their function may be of biomedical importance.

The Female Reproductive Tract

SP-A and SP-D have been detected throughout the female reproductive tract by immunohistochemistry and RT-PCR. In humans, SP-A has been found in the myometrium, vaginal epithelial tissue as well as vaginal lavage fluid, and SP-D has been detected in the epithelial layers of the vagina, cervix, endometrium, fallopian tubes and ovaries [21–23]. By sampling at different times of the menstrual cycle, it was shown that levels of SP-A vary in vaginal lavage fluid: peak levels are observed during the follicular phase, which may be beneficial as in the postmenses period the vagina is more susceptible to infection due to the vaginal mucosa being thinned with unstable vaginal flora [24]. SP-D levels in the endometrium also vary according to the menstrual cycle, as here SP-D levels peak in the secretory phase [23]. In the murine reproductive tract, SP expression is most prominent in the oviduct, with lower expression observed in the uterus, cervix and vagina. Expression of SP-D in the murine uterus is highest during oestrus, and it is speculated that SP-D expression may here be regulated by oestrogen, which peaks late in pro-oestrus. The increase in SP-D during oestrus may also be linked to the increased neutrophilia associated with di-oestrus, with SP-D potentially functioning as a chemoattractant [25].

SP-A and SP-D expression in the female reproductive tract has naturally been suggested to contribute to innate immune protection against sexually transmitted infections

[22, 23], and evidence exists to support this. In vitro, SP-D can inhibit infection of the human cervical epithelial cell line HeLa by *Chlamydia trachomatis* [26]. In vivo, murine infections with *Chlamydia muridarum* have been shown to enhance SP-D expression in the cervix, supporting a role for SP-D in defence against *Chlamydia* infections [25]. SP-A and SP-D also interact with human immunodeficiency virus (HIV). The glycosylated HIV envelope protein gp120 can be bound by both SP-A and SP-D to potentially inhibit HIV infectivity of CD4 T cells [27, 28]. SP-A and SP-D also enhance HIV uptake by dendritic cells, which may be an unfavourable interaction in terms of host immunity against HIV, as dendritic cells transfer the virus to CD4 T cells [27, 28]. It therefore seems that SP-A and SP-D inhibit direct infection as well as enhance indirect infection of CD4 cells by HIV, showing that they may have contrasting effects with regard to immunity against HIV.

As mentioned above, SP-D levels are increased in the endometrium in the secretory stage of menstruation, which may help prevent intrauterine infections [23]. This is especially important in ensuring successful pregnancies, as these infections can lead to complications of pregnancy such as premature birth, still birth and neonatal sepsis [29]. In pregnancy, SP-A and SP-D are present in the amniotic fluid, placenta, amnion and chorion [23, 30]. The potential protective role of these proteins in pregnancy has been described by several studies. Expression of SP-A and SP-D is elevated in the first trimester decidua (maternal part of the placenta) by trophoblasts and decidual stromal cells [31]. The potential function of this increase in SP-A and SP-D in protection from bacterial infection is supported by the SP-A- and SP-D-mediated reduction of lipopolysaccharide (LPS)-induced inflammation associated with TNF-α release by decidual macrophages [29]. At term, SP-A has been shown to decrease in the amniotic fluid, possibly due to it being sequestered by receptors in the amnion [32]. Additionally, amnion explants treated with SP-A exhibit decreased IL-1β, CXCL2, and CXCL5 mRNA expression. It has therefore been suggested that SP-A in the amniotic fluid protects the amnion and amniotic cavity from inflammation, thus protecting the foetus [32]. SP-A1 mRNA is significantly upregulated in the foetal membranes of patients who experience preterm birth with chorioamnionitis (as compared to preterm birth without chorioamnionitis), suggesting that SP-A may be involved in regulating inflammation in this compartment [33]. Furthermore, a genetic polymorphism in the SP-D gene (Met31Thr, which has previously been linked to influence serum concentrations and oligomerisation of SP-D) has been associated with preterm birth [34]. Taken together, these findings suggest that SP-A and SP-D have a potential role in protecting the foetus during pregnancy and preventing preterm birth.

Some studies have implicated SP-A production in the onset of labour: in mice, increased production of SP-A by the foetal lung towards the end of term was shown to drive the activation of amniotic fluid macrophages as well as their migration to the uterus, resulting in an increased production of IL-1β and NF-κB which contributes to the initiation of labour [35]. Additionally, injection of SP-A into the amniotic fluid has been shown to both cause and prevent preterm birth [35, 36]. As mentioned above, SP-A has been shown to have an anti-inflammatory effect on amnion explants, further contradicting the role of SP-A in the onset of labour. These contrasting results may be explained by species-specific differences of SP functions in the various reproductive compartments [3].

The Urinary Tract

Similarly to the reproductive tract, the urinary tract can be infected by external pathogens causing urinary tract infections. SP-A and SP-D have been detected by RT-PCR and immunohistochemistry in the kidney and epithelium of the ureter and bladder [37, 38], and they are likely to have a protective role in these regions. In vitro, SP-A and SP-D inhibit the growth of uropathogenic *Escherichia coli* [37], and SP-D reduces bacterial adherence to human bladder cells [39]. In vivo experiments on SP-A and SP-D double-knockout mice showed that these mice have an increased susceptibility to urinary tract infections by uropathogenic *E. coli*, as measured by higher bacterial loads in the kidney and urine [37]. The kidneys of wild-type mice were histologically normal after infection, whereas in the knockout mice, neutrophil and monocyte infiltrates were noted in the medulla, indicating a higher inflammatory response in these mice [37]. Additionally, mice infected with uropathogenic *E. coli* exhibited higher levels of SP-D mRNA in the bladder as compared to uninfected mice [39]. SP-D may also play a protective role against tubulointerstitial fibrosis (the common pathway to end-stage renal disease), as overexpressing SP-D in human kidney proximal tubular cells inhibits the expression of monocyte chemoattractant protein-1, a protein that when upregulated leads to disease progression [40]. In a study involving Chinese women, it was found that individuals carrying an Ala19Val polymorphism in SP-A1 or a Lys223Gln polymorphism in SP-A2 show increased susceptibility to recurring urinary tract infections [41],

further emphasising that SPs seem to have important roles in preventing infection of the urinary tract.

The Gastrointestinal Tract

In the gastrointestinal tract (GIT), SP-A is expressed in the columnar epithelial cells lining the villi and crypts of the small intestine, as well as in the surface epithelial cells of the large intestine [42], while SP-D is expressed in the epithelial lining of the small intestine as well as at low levels in the large intestine and stomach [38]. However, our understanding of the role these proteins have in these compartments is surprisingly limited. Functionally, both SP-A and SP-D in the GIT appear to act both in the control of pathogens and in the regulation of immunity. In vivo experiments on the porcine GIT showed that SP-D aggregates a variety of Gram-negative bacteria, thereby enhancing the uptake of these pathogens by intestinal epithelial cells, allowing for a more rapid immune response [43]. SP-D may also contribute to the control of parasitic helminth infections based on observations that non-lung SP-D also contributes to the resolution of *N. brasiliensis* infection; however, we do not currently understand how this effect may be mediated [15].

Both SP-A and SP-D have been implicated in controlling inflammatory bowel disease and necrotising enterocolitis. In the case of inflammatory bowel disease, there is an overexpression of SP-A [44], and a single-nucleotide polymorphism of SP-D (G/A Ala160Thr) has been associated with susceptibility to Crohn's disease, one of the subtypes of inflammatory bowel disease [45]. SP-A and SP-D have also been shown to contribute to protection against necrotising enterocolitis, a disease of preterm infants which is associated with an overexpression of Toll-like receptor 4 in the intestine. For example, in vitro pretreatment of intestinal cell cultures with SP-D reduced the LPS-induced release of proinflammatory cytokine IL-8 [46], while in a rat model of necrotising enterocolitis, oral administration of SP-A reduced mortality and intestinal pathology, as well as levels of proinflammatory cytokines (IL-1β, TNF-α and INF-γ) and Toll-like receptor 4 [47]. These studies emphasise the potential importance of SP-A and SP-D in controlling inflammation to prevent pathological states.

SP-A and SP-D are also expressed in the oral cavity of the upper GIT. They have been detected in the gingiva, saliva, as well as the parotid and submandibular glands, especially in serous acinus cells and epithelial cells lining the ducts [48]. ELISA experiments have shown that SP-A and SP-D expression is upregulated in saliva from patients suffering from periodontal disease as compared to saliva from healthy patients [49]. Additionally, in chronic sialadenitis (inflammation of the salivary glands), SP-A expression is upregulated [50]. This suggests that during pathological conditions of the mouth, SP-A and SP-D are upregulated, possibly to assist in combating infection.

Other Sites of SP-A and SP-D Expression

The Eye

SP-A and SP-D have been located in various parts of the eye: the conjunctiva, superficial layers of the cornea, the lacrimal and nasolacrimal glands, as well as in tear fluid [51]. SP-D knockout mice are less efficient at clearing *Staphylococcus aureus* and *P. aeruginosa* infection from the ocular surface as compared to wild-type mice, as determined by quantifying the bacterial colony-forming units in tear fluid [52, 53]. *S. aureus*-infected SP-D knockout mice also have severer ocular surface injury as compared to infected wild-type mice, and SP-D levels in wild-type mice increase after infection [53]. It has also been noted that cultured human corneal epithelial cells express higher levels of SP-D when exposed to heat-killed *P. aeruginosa* or *A. fumigatus* spores [54, 55]. Additionally, higher levels of SP-A and SP-D have been detected in corneal endothelial and epithelial cells as well as in the corneal stroma of eyes with corneal ulceration and herpetic keratitis [51]. These findings suggest that SP-A and SP-D have important roles in the innate defence of the eye, and that their production in the eye can be induced or upregulated due to infection.

The Middle Ear

By using electron and immunoelectron microscopy as well as in situ hybridisation, it was found that SP-A and SP-D are expressed in the eustachian tube epithelium as well as lavage fluid [56]. The eustachian tube links the middle ear and nasopharynx, and can potentially be infected by pathogens from the lungs, which may lead to otitis media, a middle ear inflammatory disease which most commonly occurs in children [56]. In vivo, otitis media was induced in mice by injection of LPS derived from *Klebsiella pneumoniae* directly to the middle ear, and it was noted that these mice had significantly increased SP-A expression in the middle ear, indicating that SP-A may be upregulated due to infection [57].

The Nasal Compartment

The innate immune components of the nasal epithelium are vital to prevent upper respiratory tract infec-

tions. Immunohistochemistry has shown that SP-A and SP-D are expressed in the nasal epithelium, more specifically in the cytoplasm of ciliated epithelial cells and in the serous acini of the submucosal gland [58]. Additionally, both SP-A and SP-D are present in the nasal lavage fluid [59]. Their possible role in immunity in the nasal mucosa has been supported by studies showing that they are differentially expressed in individuals with certain nasal pathologies. In cystic fibrosis, SP-A1, SP-A2 and SP-D mRNA expression is significantly upregulated in the sinus mucosa [60]. Both SP-A and SP-D are more strongly expressed in the submucosal glands of patients with chronic rhinosinusitis (CRS) [59]. These increased levels of SP-A and SP-D are also seen in the nasal lavage fluid of patients with CRS without nasal polyps, but not in CRS with nasal polyps, and this is likely due to the secretion of the proteins being impaired due to severe damage of the mucosa in this case [59]. Experiments on CRS nasal tissue explants have shown that SP-D mRNA is upregulated in response to *A. fumigatus* and *Alternaria tenuis* [61]. Additionally, in patients with CRS without polyps, there is an association between colonisation of the nasal passages with pathogenic bacteria and higher levels of SP-A and SP-D in the nasal lavage fluid, indicating that SP-A and SP-D are upregulated in these cases due to infection [59].

The Central Nervous System

Recently, by using RT-PCR and Western blotting, it was determined that both SP-A and SP-D are located in the brain stem, choroid plexus, cerebellum, subventricular cortex, pia mater and cerebral spinal fluid (CSF) of the central nervous system (CNS) [62]. ELISAs were used to determine whether there is a variation in SP-A and SP-D levels in CSF depending on the state of health, and it was seen that they did indeed vary among healthy, autoimmune CNS disease and CNS infection samples (infection samples were derived from patients diagnosed with meningitis, encephalitis or meningoencephalitis). As compared to samples from healthy individuals, there was a significant decrease in SP-A and SP-D levels in the CSF of individuals with CNS autoimmune disease or CNS infection. It has been suggested that SP-A and SP-D may function by binding and removing microbes and exhausted neutrophils in the CSF, which results in decreased SPs being detected [62].

Coronary Artery

SP-D has been detected in endothelial and smooth muscle cells of the coronary arteries by immunostaining of tissue sections [63]. The possible role of SP-D in this tissue was explored using human coronary artery smooth muscle cells, whereby inflammation was stimulated by addition of LPS. During inflammation, SP-D expression was increased, and overexpression or administration of SP-D reduced the expression of IL-8, indicating that SP-D plays an important role in modulating inflammation in this tissue. Low levels of inflammation and IL-8 release have been associated with the development of atherosclerosis, and therefore, it seems that SP-D may have a role in modulating this disease. Additionally, it was observed that after infection with *Chlamydia pneumoniae*, there are reduced viable *C. pneumoniae* inclusions in the presence of SP-D [63], suggesting that SP-D may be important in controlling infection in the coronary artery. The expression and function of SP-A in the coronary artery has not yet been explored in depth, but it was seen that SP-A mRNA was detectable in human coronary artery smooth muscle cells and that the administration of SP-A also inhibited the LPS-induced release of IL-8, thus SP-A is likely to play a similar role to SP-D here [63].

The Skin

SP-A and SP-D expression has been detected in the skin using both RT-PCR as well as immunohistochemistry to further localise their expression in different layers of the skin. Both proteins were found in the epidermis, with SP-A detected in the stratum corneum and stratum granulosum and SP-D detected in all epidermal layers as well as the dermis [64]. Interestingly, it is within these outer skin layers that contact with pathogens often occurs, and SP-A and SP-D may be contributing to the innate defences already located here. It has been noted that SP-A and SP-D may also play a role in certain inflammatory skin diseases. Skin biopsy samples from individuals with psoriasis, atopic dermatitis, lichen planus or Behçet's disease were observed to have higher levels of SP-A and SP-D present compared to normal skin samples [65]. The strong expression of SP-A and SP-D in these inflammatory pathologies further highlights the role of SP-A and SP-D in inflammation.

Conclusion

It is now known that expression of the immune SPs is not exclusive to the lungs. Their expression in other regions including the reproductive tract, urinary tract, and GIT and their interaction with various pathogens (ta-

Table 1. Relationships between surfactant proteins A and D and different pathogens in non-pulmonary sites

Pathogen	Site of infection	SP-A	SP-D	Proposed effect and/or function	Reference
Alternaria tenuis	Nasal tissue	?	+	Infection with *A. tenuis* is associated with increased SP-D expression during chronic rhinosinusitis	Ooi et al. [61], 2007
Aspergillus fumigatus	Nasal tissue	?	+	Infection with *A. fumigatus* is associated with increased SP-D expression during chronic rhinosinusitis	Ooi et al. [61], 2007
Chlamydia muridarum	Murine reproductive tract	?	+	Infection with *C. muridarum* increases SP-D expression in the cervix	Oberley et al. [25], 2007
Chlamydia pneumoniae	Coronary artery	?	+	SP-D reduces the amount of viable *C. pneumoniae* inclusions	Snyder et al. [63], 2008
Chlamydia trachomatis	Female reproductive tract	+	+	SP-D prevents HeLa cell infection by *C. trachomatis*. SP-A and SP-D enhance uptake of *C. trachomatis* by THP-1 macrophages	Oberley et al. [26], 2004
Escherichia coli (uropathogenic)	Urinary tract	+	+	SP-A and SP-D inhibit *E. coli* growth, and SP-D reduces bacterial adherence to bladder cells. SP-D is upregulated following infection	Hu et al. [37], 2016
Escherichia coli (various strains)	GIT	?	+	SP-D aggregates *E. coli*, enhancing its uptake by intestinal epithelial cells	Hogenkamp et al. [43], 2007
Klebsiella pneumoniae	Ear	+	?	Infection with *K. pneumoniae*-derived LPS increases SP-A expression in the middle ear	Li et al. [57], 2015
Pseudomonas aeruginosa	Eye	?	+	SP-D knockout mice do not clear infection as efficiently	Mun et al. [52], 2009
Salmonella enteritidis, Salmonella typhimurium, group B *Salmonella*	GIT	?	+	SP-D aggregates *Salmonella*, enhancing its uptake by intestinal epithelial cells	Hogenkamp et al. [43], 2007
Staphylococcus aureus	Eye	?	+	SP-D knockout mice are less efficient at clearing ocular *S. aureus*	Zhang et al. [53], 2015
Ureaplasma parvum and *Ureaplasma urealyticum*	Vagina	+	?	SP-A increases phagocytosis and ureaplasmacidal activity of RAW264.7 macrophages	Okogbule-Wonodi et al. [66], 2011
Herpes simplex virus 1	Mouth	+	?	SP-A is shown to interact with HSV-1, but the consequences of this are unknown	van Iwaarden et al. [67], 1992
Human immunodeficiency virus	Reproductive tract	+	+	SP-A and SP-D inhibit HIV infectivity of CD4 cells but enhance uptake by dendritic cells	Gaiha et al. [27], 2008; Madsen et al. [28], 2013
Aspergillus fumigatus	Eye	?	+	Infection with *A. fumigatus* spores increases SP-D expression in human corneal epithelial cells	Che et al. [54], 2012

+ indicates a relationship between pathogen and protein; ? indicates a relationship is unknown. Only non-pulmonary interactions are stated here.

ble 1) suggest that they play a more general role in innate immunity, with SP-D generally displaying more widespread activities. It may therefore be possible to develop SPs as broad-spectrum alternative means for the prevention and/or novel therapeutic approaches for the treatment of common infections, which may be especially beneficial for individuals who are immunocompromised.

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Disclosure Statement

The authors state no conflict of interest.

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