WAPing Out Pathogens and Disease in the Mucosa: Roles for SLPI and Trappin-2

Thomas S. Wilkinson, Ali Roghanian, and Jean-Michel Sallenave

Abstract The interface between the external environment and the body's internal structures is defined by the mucosal tissue and the viscous lining fluid that is responsible for maintaining its integrity and protecting internal structures from damage or infection. Human mucosal fluids include seminal fluid, cervical mucus, bronchial and nasal secretions and tears whose composition is particularly complicated. Here we will focus on just two related molecules that are present in the mucosal lining fluid, namely, secretory leucocyte protease inhibitor (SLPI) and trappin-2/elafin, that are responsible for many of the homeostatic and host defence functions of these uniquely situated viscous sols. This review will focus on our increasing understanding of these two molecules from a simple role as local antibiotics that respond to pathogen invasion to major orchestrators of cellular interplays, host defence mechanisms and immune homeostasis.

Thomas S. Wilkinson and Ali Roghanian contributed equally.

T.S. Wilkinson
Institute of Life Science, Microbiology and Infection, Floor 5, School of Medicine, Swansea University, Singleton Park, Swansea SA2 8PP, UK

A. Roghanian (✉)
Antibody and Vaccine Group, Cancer Sciences Unit, University of Southampton, Faculty of Medicine, Southampton, UK SO16 6YD
e-mail: A.Roghanian@soton.ac.uk

J.-M. Sallenave
Institut Pasteur, Unité Défense Innée et Inflammation, 25, Rue du Dr Roux, 75015 Paris, France

INSERM, U874, 75015 Paris, France

UFR Sciences du Vivant, Université Paris Diderot Paris 7, 75013 Paris, France

P.S. Hiemstra and S.A.J. Zaat (eds.), *Antimicrobial Peptides and Innate Immunity*, Progress in Inflammation Research, DOI 10.1007/978-3-0348-0541-4_6,

1 SLPI

1.1 The Gene and Molecule

Early understanding of SLPI structure and function was complicated by at least four interrelated factors (Seemuller et al. 1986). Firstly, multiple forms seemed to exist in vivo. Secondly, the mucosal environment where SLPI is present is often full of mucous, leucocytes and degradative enzymes. Thirdly, isolation of molecules from tissues often involved the use of trypsin or non-specific protease digestion steps. Finally, inhibitors of similar activity were isolated from anatomically distinct body compartments. Thus, bronchial mucus inhibitor (BMI), human seminal inhibitor I (HUSI-I), cervical mucus inhibitors (CUSI), antileucoprotease (ALP), secretory leucocyte protease inhibitor (SLPI) and mucus proteinase inhibitor (MPI) proved to be identical or derived from a mature inhibitory protein encoded by a single gene of the human genome (Fritz 1988). Human and mouse SLPI are relatively conserved at both the genomic and protein level. The human gene is composed of ~2.6 kb and is organised into four exons, which transcribes a 399-base-pair message to a 132-amino acid protein (Stetler et al. 1986). Similarly, the mouse gene is composed of four exons, which transcribes a 396-base-pair coding sequence to a 131-amino acid protein (Kikuchi et al. 1998). Human SLPI is located on chromosome 20, and the mouse orthologue is located to the syntenic chromosome 2 H (Kikuchi et al. 1998). In both species the functional domains of the SLPI molecule are distributed across the exons; exon 1 codes for the secretion signal, exon 2 the trypsin inhibition domain, exon 3 the elastase inhibitory domain and exon 4 the 3′ untranslated region. Grutter and co-workers eloquently wrote that 'SLPI has a boomerang-like shape with both wings comprising two well separated domains of similar architecture' in their paper outlining the 2.5 Å crystal structure of SLPI binding to bovine α-chymotrypsin (Grutter et al. 1988). Each domain is, relatively conserved, cysteine rich and has high homology to the whey acidic protein (WAP) genes found in rodent milk (Campbell et al. 1984). However, despite the presence of two separate WAP domains, it is the C-terminal that is responsible for the antielastase, antichymotrypsin and antitrypsin activities and that leucine 72 is a key residue involved in the interaction (Kramps et al. 1990; Eisenberg et al. 1990). In keeping with this, both full length and a truncated C-terminal (1/2 SLPI) SLPI could also inhibit cathepsin G activity (Renesto et al. 1993). However, SLPI and its active site variants do not bind or inactivate proteinase 3(PR-3), but instead get cleaved in the N-terminal domain at alanine-16 (Rao et al. 1993). This is further complicated by the species specific potency of SLPI against these proteases (Wright et al. 1999a).

1.2 Expression and Binding Interactions

Numerous studies have evaluated the tissue distribution of SLPI in humans using specimens from surgically treated patients or autopsy where normal tissue is selected

from gross appearance and further examination by light microscopy. These studies utilise specific antisera to localise signal in tissue by immunocytochemistry or to detect in biological fluids using ELISA. SLPI is expressed in numerous areas of the respiratory tract including the submucosal glands of the nose and bronchus, non-ciliated cells of the bronchus, terminal and respiratory bronchioles and alveolar duct (Franken et al. 1989; Fryksmark et al. 1982). Willems et al. used two separate antibodies to localised SLPI along the elastic fibres of the alveolar septa and walls of the bronchi, bronchioles, blood vessels and extracellular matrix (Willems et al. 1986; Kramps et al. 1989). Using a gold labelling technique to demonstrate increased resolution in serous cells of the bronchial submucosal glands, SLPI was located in granules, including structures such as the endoplasmic reticulum and nuclear envelope. This study could only detect SLPI in the Clara cells of bronchial epithelium (De Water et al. 1986). SLPI has also been detected in lung secretions including bronchoalveolar lavage (Kouchi et al. 1993; Ohlsson et al. 1992), broncholavage (Kouchi et al. 1993) and sputum sol phases (Piccioni et al. 1992).

SLPI is also expressed in reproductive mucosa where it has been localised to the epithelium of the upper cervix (Schill et al. 1978) and in seminal fluid (Moriyama et al. 1998). More specifically others have demonstrated SLPI expression in the cervical crypts, together with high concentrations in cervical mucus which varied throughout the menstrual cycle with increased concentrations during the ovulatory compared to follicular phases (Casslen et al. 1981; Moriyama et al. 1999). Interestingly during pregnancy SLPI is increased in cervical tissue and is particularly high in the cervical plug which also has a high molar ratio of SLPI to elastase. Denison and co-workers demonstrated dramatic increases in the levels of SLPI (~200-fold) in amniotic fluid over the course of pregnancy and suggested that the major source is the decidua parietalis cells (Denison et al. 1999). These studies together with demonstration of SLPI in foetal membranes suggest a protective role (involving structural integrity and inhibition of proinflammatory responses) for SLPI during the menstrual cycle and pregnancy (Helmig et al. 1995).

Expression of SLPI has been demonstrated in many other mucosal tissues and lining fluids including salivary glands (Ohlsson et al. 1984; Shugars et al. 2001; Cox et al. 2006), middle ear (Carlsson & Ohlsson 1983; Lee et al. 2006), maxillary sinus (Fryksmark et al. 1985), intestine, (Bergenfeldt et al. 1996), colon, (Nystrom et al. 2001), human skin (Wiedow et al. 1993), nasal secretions (Westin et al. 1999a), peritoneal fluid (Shimoya et al. 2000), stomach (Wex et al. 2004), gingival crevicular fluid (Cox et al. 2006; Nakamura-Minami et al. 2003) and cornea (Nielsen et al. 2005).

The binding interactions of SLPI are not limited to forming 1:1 molar complexes with proteases such as elastase, chymotrypsin and trypsin. Indeed, binding activities for SLPI are not just limited to the extracellular milieu but have also been reported at the plasma membrane and within the intracellular space. Extracellular binding interactions include those to the pathogen-associated molecular patterns bacterial lipopolysaccharide (LPS) (Ding et al. 1999), mannan-capped lipoarabinomannans and phosphatidylinositol mannoside (Gomez et al. 2009) together with numerous glycosaminoglycans (Fath et al. 1998; Ying et al. 1997) and classes of immunoglobulin (Hirano et al. 1999). Intracellular binding interactions include binding to DNA

(Miller et al. 1989; Taggart et al. 2002) and to IRAK, IκBα and IκBβ (Lentsch et al. 1999a). Interactions at the plasma membrane include annexin-II (Ma et al. 2004), scramblase-1 (Tseng & Tseng 2000; Py et al. 2009) and scramblase-4 (Py et al. 2009).

1.3 Antimicrobial Activity

SLPI has moderate antimicrobial actions against a variety of human bacterial pathogens including *Escherichia coli, Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa* and *Mycobacterium bovis* (Wiedow et al. 1998; Hiemstra et al. 1996; Nishimura et al. 2008). SLPI is less potent (on a molar basis) than lysozyme or defensin with 50 % inhibitory concentrations against *E. coli* of 4.7 μM, 1.8 μM and 1.4 μM, respectively, with the antimicrobial domain residing in the N-terminal (Hiemstra et al. 1996). SLPI has also been shown to have antimicrobial activity against metabolically active fungi, in particular *Aspergillus fumigatus* and *Candida albicans*. Interestingly metabolically quiescent *A. fumigatus* conidia were resistant to SLPI in this study. The antifungal activity is reported to be equal to lysozyme and defensins and also appears to reside in the N-terminal portion of the molecule (Wiedow et al. 1998; Tomee et al. 1997).

In contrast to the relatively consistent parallel studies investigating bacterial and fungal activity, the antiviral activity of SLPI has proved much more complicated. McNeely and colleagues identified a protein in saliva that could protect monocytes against HIV infection which following analysis was confirmed to be SLPI (McNeely et al. 1995; Shugars et al. 1997). Since then reports have both supported (McNeely et al. 1997) and refuted (Turpin et al. 1996) this work. Following this, studies focused on two main aspects of this compelling argument: (1) clinical studies attempting to relate SLPI levels to transmission of HIV and viral load and (2) mechanistic studies attempting to explain the precise conditions necessary for activity. Thus, SLPI was increased in saliva and plasma of HIV-infected individuals compared to uninfected controls (Baqui et al. 1999). In a study of pregnant HIV-positive South African women, those who had higher levels of SLPI in vaginal fluid had lower perinatal transmission rates to their babies (Pillay et al. 2001). In a similar but larger study of 602 saliva samples from 188 infants over the first 3 months following birth, increased SLPI was associated with a reduced risk of HIV transmission from breast milk (Farquhar et al. 2002).

1.4 Unique Role in Inflammation: Priming Innate Immunity and Tissue Remodelling

Cell culture studies have identified a plethora of cytokines, drugs and hormones that modulate the levels of SLPI when introduced to the bathing medium. In human

airway cells, Abbinante-Nissen et al. found that neutrophil elastase (NE) was a potent inducer of SLPI transcript. Furthermore, other neutrophil products, such as cathepsin G, myeloperoxidase and lysozyme, had little or no effect on SLPI transcript levels. In contrast, two non-neutrophil proteases, trypsin and pancreatic elastase, also increased SLPI transcript levels at higher doses than that required of NE. These authors also showed that tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-8 induced little or no SLPI transcript levels (Abbinante-Nissen et al. 1993). Using Clara cells and alveolar type II cells and measuring SLPI protein as an end point, we showed both a constitutive and IL-1β- or TNF-α-induced production of SLPI (Sallenave et al. 1994). Interestingly, glucocorticoids can induce SLPI transcript in human airway epithelial cells with a descending potency of fluticasone > triamcinolone > or = dexamethasone > methylprednisolone > hydrocortisone (Abbinante-Nissen et al. 1995). This study also demonstrated that elastase and fluticasone together induce synergistic increases in SLPI. Indeed, the ability of glucocorticoids to induce SLPI may be partly responsible for their anti-inflammatory action. Furthermore, progesterone has been shown to upregulate SLPI mRNA and protein through a mechanism involving its transactivation of the SLPI gene through the progesterone receptor (PR), via induction of basic transcription element-binding protein-1 (BTEB1) gene and co-recruitment of BTEB1 and the PR coactivator cAMP-response element-binding protein (CBP) to the SLPI promoter (Velarde et al. 2006; King et al. 2003).

The late 1990s saw a dramatic change in the way we viewed SLPI. Before then SLPI was considered an antimicrobial molecule with potent antiprotease activity; however, the seminal work of Jin and colleagues in macrophages demonstrating the ability of LPS to induce SLPI and furthermore that SLPI could suppress LPS-induced activation of NF-κB and synthesis of TNF-α/nitric oxide suggested that SLPI had immunomodulatory activity as well (Jin et al. 1997). In a later paper, the same group also demonstrated that LPS-induced SLPI was an early (~30 min) and prolonged response (remaining at 72 h). The LPS inducible proteins IL-10 and IL-6 could also upregulate SLPI but IL-1β and TNF-α could not. Finally, the Gram-positive cell wall constituent LTA could also stimulate SLPI production (Jin et al. 1998). There are multiple mechanisms responsible for these effects including the ability of SLPI to inhibit NF-κB activation by stabilisation of IRAK, IκBα and IκBβ proteins, despite increasing the amount of phosphorylated and polyubiquitinated IκBα (Taggart et al. 2002; Lentsch et al. 1999a). This is supported by the anti-inflammatory activity of a non-secretable form of SLPI when transfected into macrophages (Zhu et al. 1999). Others have suggested that SLPI can prevent the p65 subunit of NF-κB binding to its consensus sequence in the promoters regions of target genes. It is unclear which domain of the SLPI molecule mediates the anti-inflammatory action as one study suggests that oxidation of SLPI inhibits this action (Taggart et al. 2002), whereas site-directed mutants of the oxidisable methionine residue (Met 73) could still inhibit LPS-induced TNF and nitric oxide responses (Yang et al. 2005). In vivo Mulligan and co-workers have suggested that the leucine 72 residue which is essential in determining antiprotease

function is vital, and their studies implicate the antitrypsin activity in SLPI (through a Lysine 72 mutant) to have a greater suppressive effect on the inflammatory response than wild-type SLPI (Mulligan et al. 2000).

Generation of mice deficient in SLPI at the beginning of the millennium has enhanced our knowledge of the in vivo effects of this molecule. The first of these studies suggested a role for SLPI in linking host defence with wound repair (Ashcroft et al. 2000). SLPI-deficient mice have deficient cutaneous wound healing with increased inflammation and elastase activity with enhanced local production of TGF-beta. In a similar model, Zhu has suggested an alternative pathway dependent on proepithelin and its cleaved product epithelin which have opposite effects during inflammation (Zhu et al. 2002). Proepithelin blocks TNF-α-induced neutrophil activation and oxidant and protease release, whereas epithelin inhibits the growth of epithelial cells and induces IL-8 production by neutrophils. In this way proepithelin complexed with SLPI cannot be cleaved with elastase to epithelin, and SLPI null mice can be rescued with proepithelin. Angelov and co-workers identified differences in the mechanisms of wound healing in a combined dermal scarring and oral non-scarring model (Angelov et al. 2004). Here an absence of SLPI results in markedly impaired oral wound healing associated with increased inflammation, raised elastase activity and decreased matrix deposition through increased MMP activity suggesting deregulated proteolysis. Intriguingly, TGF-beta expression is increased in cutaneous model (Zhu et al. 2002), but decreased in the oral model (Angelov et al. 2004) pointing to the ability of SLPI to improve wound healing by very different local mechanisms. The link is particularly pertinent in a cardiac transplant model of ischemia/reperfusion injury where SLPI-deficient hearts had profound abnormalities in early contraction and high protease expression and TGF-beta expression (Schneeberger et al. 2008). Interestingly, systemic SLPI could not rescue this phenotype, whereas including SLPI in the preservation solution prior to transplantation reversed the phenotype suggesting a dual inhibitory effect on protease and TGF-beta expression might be the underlying mechanism (Schneeberger et al. 2008).

The identification of these homeostatic functions for SLPI encouraged others to investigate its role during inflammation and infection. In a model of endotoxin shock, SLPI-deficient mice had significantly higher mortality possibly due to increased levels of macrophage IL-6, HMG-1 and NF-κB compared to wild-type cells (Nakamura et al. 2003). Similarly B cells isolated from null mice showed increased proliferation and IgM production suggesting that SLPI acts to attenuate excessive inflammatory responses. However, in a model of infection, SLPI null mice were highly susceptible to *Mycobacterium bovis*, when given by the respiratory route, suggesting a role in driving the local inflammatory response to clear pathogens (Nishimura et al. 2008).

In addition to gene deletion, functional studies have also supplemented recombinant SLPI either through overexpression (e.g. adenoviral) or administration of the purified protein. Thus, adenoviral gene delivery of SLPI can protect against ischemic

brain injury (Wang et al. 2003) and has also been shown to attenuate NF-κB-dependent inflammatory responses to atherogenic stimuli (Henriksen et al. 2004). By transfecting multiple clones of the highly metastatic subline (H-59) to overexpress SLPI, Wang and colleagues showed that these cells' ability to elicit a host proinflammatory response in the liver was markedly decreased, as evidenced by reduced TNF-α production and vascular E-selectin expression, relative to controls (Wang et al. 2006). Consistent with these findings, recombinant SLPI administered systemically could suppress inflammation associated with joint damage (Song et al. 1999) and attenuate hepatic ischemia/reperfusion injury (Lentsch et al. 1999b) in rats and mice, respectively. Furthermore, local delivery of SLPI to ovine lung by aerosol has been shown to prevent allergen-induced pulmonary responses in a model of asthma (Wright et al. 1999b), and topical administration to the eye in guinea pigs suppressed the recruitment of eosinophils and decreased the severity of conjunctivitis (Murata et al. 2003).

It has been unclear for sometime whether SLPI has proinflammatory/immune stimulatory effects that are distinct from its direct antimicrobial activity. In models of resolving inflammation where neutrophil apoptosis has been shown to stimulate macrophage clearance (Savill et al. 1989), SLPI seems to play a proinflammatory role. Firstly, murine macrophages produce SLPI during clearance of apoptotic cells (Odaka et al. 2003), and SLPI (together with lactoferrin) is secreted by activated neutrophils (Jacobsen et al. 2008). Recently a functional study by Subramaniyam and colleagues has suggested that SLPI inhibits apoptosis therefore prolonging the life of neutrophils during inflammation (Subramaniyam et al. 2011). In support of an immune stimulatory role for SLPI, Gomez and co-workers have shown that SLPI may act as a pattern recognition receptor for mycobacteria which acts to stimulate phagocytosis (Gomez et al. 2009). Thus, it seems that the proinflammatory actions of SLPI are dependent on the type of pathogen and on the progress of the inflammatory response.

1.5 Key Roles in Mucosal Tissue: Ovarian and Gastric Cancer

An emerging literature identifying a role for SLPI in cancer has developed over the last few years. Initial evidence for this came from mRNA differential display systems identifying changes in a SLPI gene variant in the highly metastatic murine carcinoma cell line IMC-HA1 (Morita et al. 1999). The gene was isolated as SLPI-α and SLPI-β and was found to be differentially expressed with SLPI-α expressed ubiquitously in tumours but SLPI-β having lower expression in normal tissues and distinct expression in certain tumours (e.g. P388 leukemias). In a separate study, the repression of SLPI was shown to be under the control of the cell growth regulator interferon regulatory factor (IRF)-1 suggesting that it might be a downstream target modulating cell growth properties (Nguyen et al. 1999). Changes in SLPI levels have been associated with cancer. For instance, SLPI is expressed in a number of tumour environments including ovarian endometriomas (Suzumori et al. 2001;

Shigemasa et al. 2001), head and neck squamous cell carcinomas (Westin et al. 1999b), cervical adenocarcinoma (Tian et al. 2004) and gastric cancer (Cheng et al. 2008) but decreased in prostate cancer (Xuan et al. 2008).

A role for SLPI in cancer has been suggested in a variety of studies. Devoogdt et al. have suggested a pro-malignant role as transfection of low malignant lung carcinomas with SLPI produced a highly malignant phenotype both in vitro and in vivo (Devoogdt et al. 2003), and moreover, the protease inhibitory function was essential for that activity. In a later study, the same author has found that overexpression of a protease-dead SLPI resulted in more aggressive ovarian cancers (Devoogdt et al. 2009). This tumour-promoting effect of SLPI is thought to mediate the pro-tumourigenic effects of TNF-α as SLPI expression and tumour size was decreased in TNF-α-deficient mice (Devoogdt et al. 2006). In being a TNF responsive gene, SLPI may even impact on the 'cancer immunoediting hypothesis' which suggests that the local tumour microenvironment might induce cancer cell variants with increased resistance to the immune system (Dunn et al. 2002).

2 Trappin-2/Elafin

2.1 The Gene and Molecule

Trappin-2 protein was purified and characterised from human lung secretions and skin tissues in the 1980s and 1990s under a variety of names, such as elafin, BSI-E, elastase-specific inhibitor (ESI), precursor of elafin–ESI (PELESI) and skin-derived antileucoprotease (SKALP) (Hochstrasser et al. 1981; Kramps & Klasen 1985; Wiedow et al. 1990; Sallenave & Ryle 1991; Sallenave et al. 1992). The trappin-2 gene encodes a secreted 9.9-kDa non-glycosylated 95-residue cationic protein (Saheki et al. 1992; Sallenave & Silva 1993), comprising an N-terminal domain (38 residues) or cementoin domain (Nara et al. 1994) and a C-terminal inhibitory whey acidic protein (WAP)-type domain (57 residues) (Bairoch & Apweiler 1997). The N-terminal domain contains several repeated motifs with the consensus sequence Gly–Gln–Asp–Pro–Val–Lys that can anchor the whole molecule to extracellular matrix proteins by transglutaminase-catalysed cross-links. By doing so, it is believed trappin-2 shields the elastic tissues from locally secreted enzymes (e.g., NE), which overwhelm the tissues at times of inflammation/infection (Nara et al. 1994). The C-terminal domain is structurally similar to the SLPI domains (about 40 % sequence identity with each SLPI domain). Trappin-2 is encoded by the PI3 gene in the same chromosome region 20q12–13 as SLPI gene and is composed of three exons spanning about 2 kb. The first exon codes for the 5′ untranslated region, the signal peptide and the first few amino acids of the mature protein; the second exon encodes most of the mature protein, and the third exon encodes the 3′ untranslated region (Molhuizen et al. 1993). Trappin-2 is translated with a signal peptide that is cleaved during secretion and proteolytically processed

to form a ~6-kDa peptide referred to as elafin. Although the antiprotease activity of trappin-2 was initially identified in both the intact 9.9-kDa peptide and its cleaved 6-kDa C-terminus product (elafin), trappin-2 has a reduced protective effect in an in vivo model of elastase-induced lung injury when it is cleaved of its cementoin domain (Tremblay et al. 2002).

2.2 Expression and Binding Interactions

Trappin-2 or its orthologues are also found in other mammals and is expressed both in foetal and adult tissues (Pfundt et al. 1996). Interestingly, the expression trappin-2/elafin has not been demonstrated in rat or mouse tissues (Williams et al. 2006). Trappin-2 inhibits NE, porcine pancreatic elastase and PR-3 with a low degree of reversibility but does not inhibit cathepsin G, trypsin or chymotrypsin and, hence, has a more restricted spectrum of inhibition than SLPI (Williams et al. 2006). The regulation of trappin-2 expression by healthy and inflamed tissues has attracted much attention. Unlike SLPI, low levels of trappin-2 is secreted by bronchial and alveolar epithelial cells as well as keratinocytes in steady state, but its production is significantly increased under the influence of LPS as well as inflammatory cytokines IL-1 and TNF-α (Sallenave et al. 1994). A few signalling pathways, namely, c-jun, p38 mitogen-activated protein (MAP) kinase and NF-κB pathways, are implicated in the trappin-2 response to inflammatory molecules (Pfundt et al. 2000, 2001; Bingle et al. 2001). Likewise, trappin-2/elafin mRNA expression is increased by free NE in bronchial epithelial cells, which is found at high concentrations (μM levels) at inflammatory sites (Reid et al. 1999; van Wetering et al. 2000). In recent years, trappin-2 has increasingly been shown to display functions beyond its protease inhibition [reviewed in (Williams et al. 2006; Roghanian & Sallenave 2008; Sallenave 2010)] such as antimicrobial and mmunomodulatory activities, as will be discussed below.

2.3 Antimicrobial Activity

Our group first ascribed antimicrobial activity to trappin-2 in the late 1990s. Importantly, we demonstrated that trappin-2 was active against two major respiratory pathogens, the Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Staphylococcus aureus* both in vitro and in vivo (Simpson et al. 1999; McMichael et al. 2005a). To this end, overexpression of trappin-2 by adenovirus-mediated gene transfer dramatically increased the local antibacterial defences against *P. aeruginosa* and *S. aureus* infections (Simpson et al. 1999; McMichael et al. 2005a). On the other hand, supernatants of *P. aeruginosa* could induce trappin-2 production in human keratinocytes, and trappin-2 inhibits growth of *P. aeruginosa* in vitro, but not *E. coli* (Meyer-Hoffert et al. 2003; Bellemare et al. 2008). *P. aeruginosa* is an opportunistic pathogen and commonly resistant to conventional antibiotics that is life-threatening

for immunocompromised individuals and for patients suffering from chronic respiratory diseases such as cystic fibrosis (CF). *P. aeruginosa* is also the predominant bacteria associated with nosocomial infections, and acute *P. aeruginosa* infection may result in sepsis and death (Hancock 1998; Erwin & VanDevanter 2002). Similarly, *Staphylococcus aureus* infections are closely associated with pneumonia and sepsis, particularly in nosocomial infections, and its increasing association with antimicrobial resistance has become a major concern for clinicians (Butterly et al. 2010). In addition to the above-mentioned pathogens, trappin-2 has significant bactericidal activity against *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Branhamella catarrhalis* which are also common features of inflammatory lung disorders such as CF and chronic obstructive pulmonary disease (COPD) (Baranger et al. 2008). Furthermore, trappin-2 and its cleaved product elafin possess potent fungicidal activities against pathogenic *Aspergillus fumigatus* and *Candida albicans*, which have preferential tropism for human lungs and other mucosae (Baranger et al. 2008).

Trappin-2/elafin has also been shown to possess anti-human immunodeficiency virus (HIV) activity, although the mechanism(s) of this inhibition is currently unknown. Both trappin-2 and SLPI have been detected in cervicovaginal lavage samples of HIV-negative and HIV-positive women (Moreau et al. 2008; Ghosh et al. 2010a). Reportedly, the 6-kDa elafin was amongst factors that correlated with protective immunity to HIV infection in the genital tract secretions of a group of African women who remain virus-free despite multiple high-risk exposures to HIV infection (Iqbal et al. 2009). Recombinant trappin-2/elafin is able to inhibit both T cell-tropic X4/IIIB and macrophage-tropic R5/BaL HIV-1 in a dose-dependent manner (Ghosh et al. 2010b). This inhibitory activity was observed when virus was incubated with trappin-2/elafin but not when trappin-2/elafin was added to cells either before infection or after infection. This indicates that the inhibitory activity of trappin-2/elafin occurs through a direct interaction with the virus rather than at the level of the target cell surface, for example, through the blocking of receptors.

Collectively, these findings propose that trappin-2/elafin may play an important protective role in vivo against the transmission of HIV from men to women. In the latest attempt to develop preventive anti-HIV therapeutics, engineered commensal bacteria secreting elafin have been utilised that appear to confer protection against HIV infection in vitro (Fahey et al. 2011). These innovative yet unproven approaches are designed to regulate immunity in the female reproductive tract in ways that will potentially reduce HIV infection in women.

2.4 *Unique Roles in Inflammation: Linking Innate and Adaptive Immunity*

The initial interaction between antimicrobial peptides and pathogens is due to electrostatic forces, since the host defence peptide is positively charged and

molecules such as LPS and lipoteichoic acid are negatively charged. Indeed, trappin-2 and its C- and N-terminus peptides are capable of binding both smooth and rough forms of LPS at the lipid A portion of the molecule, with N-terminus binding both forms of LPS more avidly, thus modulating immune responses (McMichael et al. 2005b). Moreover, binding of trappin-2 and cementoin (trappin-2N-terminal domain) to *P. aeruginosa* elicits morphological changes such as wrinkling and blister formation on the cell surface and the presence of pore-like structures (Baranger et al. 2008; Bellemare et al. 2010; Wilkinson et al. 2009). It is commonly assumed that the presence of pore-like structures is indicative of cell lysis. However, several lines of evidence suggest that the membrane disruption properties of cementoin, trappin-2 and elafin are considerably weaker compared to other antimicrobial peptides, such as the amphibian lytic magainin 2 (Bellemare et al. 2010). Moreover, recent evidence indicates that trappin-2 and elafin, but not cementoin, are capable of reducing biofilm development and the secretion of pyoverdine, which correlates with the ability of these peptides to bind DNA in vitro and to accumulate within the bacterial cytosol (Bellemare et al. 2010; Li et al. 2010a). Thus, in addition to bacterial opsonisation and induction of cell lysis, trappin-2 and elafin attenuate the expression of some *P. aeruginosa* virulence factors, possibly through acting on intracellular pathways (Bellemare et al. 2010). Interestingly, it has been suggested that trappin-2 WAP domain also specifically inhibits a *P. aeruginosa*-secreted peptidase with the characteristics of arginyl peptidase (protease IV) and prevents bacterial growth in vitro (Bellemare et al. 2008).

In an effort to further address the mechanisms by which trappin-2 exerts its antimicrobial/immunomodulatory effects in the host, in vitro and in vivo models of the very earliest interactions between *P. aeruginosa* and macrophages were developed by us to mimic the presumed environment encountered in the initial stages of lung infection (Wilkinson et al. 2009). Consequently, subantimicrobial concentrations (nanomolar range) of trappin-2 enhanced clearance of *P. aeruginosa* (strain PA01) by macrophages, which was dependent on prior opsonisation of the bacteria by trappin-2 in vitro. Similarly, wild-type mice receiving an intratracheal dose of trappin-2-opsonised *P. aeruginosa* had significantly decreased bacterial burden compared with mice receiving nonopsonised *P. aeruginosa*. In striking contrast, CD14-deficient mice were resistant to the *P. aeruginosa*-opsonising effects of trappin-2 and were unable to clear the bacteria as effectively (Wilkinson et al. 2009). Hence, CD14, a promiscuous pattern recognition receptor, is the only described receptor involved in mediating the effect of trappin-2 to date. CD14 has a broad ligand specificity allowing it to bind Gram-positive, Gram-negative and viral pathogens (Anas et al. 2010). CD14 can also participate in non-inflammatory or anti-inflammatory responses by acting as a macrophage receptor for engulfment of apoptotic cells (Anas et al. 2010). Furthermore, trappin-2-opsonised *P. aeruginosa* simultaneously promoted a CD14-dependant fivefold increase in CXCL1 compared with nonopsonised bacteria, which led to a rapid recruitment of neutrophils soon after, as previously observed in other experimental models (Simpson et al. 1999; Sallenave et al. 2003; Roghanian et al. 2006). Both CXCL1 and CXCL2 act through the chemokine receptor CXCR2, which has been shown to be essential for host

protection against *P. aeruginosa* pneumonia (Tsai et al. 2000). Thus, in the early stages of infection, trappin-2 simultaneously delivers pathogens to resident alveolar macrophages, while contributing to activation of the neutrophil/CXCR2 axis should the bacterial inoculum appear sufficient to drive significant infection. These recent findings further strengthen the notion that trappin-2 is able to augment clearance of pathogens at early onset of infection, even before recruitment of neutrophils and other effector cells to the inflammatory site.

It is noteworthy to point out that *P. aeruginosa* PAO1-conditioned medium and two purified *Pseudomonas* metalloproteases, pseudolysin (elastase) and aeruginolysin (alkaline protease), are able to cleave recombinant elafin leading to loss of its antiprotease activity and binding to fibronectin following transglutaminase activity, respectively (Ghosh et al. 2010b). Moreover, elafin is cleaved by its cognate enzyme NE, present at excessive concentration at inflammatory milieu, and that *P. aeruginosa* infection promotes this effect (Guyot et al. 2008). Consequently, such cleavages may have repercussions on the innate immune function of elafin.

When secreted locally at mucosal sites, trappin-2 promotes recruitment or priming of innate immunity. Expression of the human trappin-2 gene in the murine lungs results in an increased influx of inflammatory cells in response to infection/inflammation (Wilkinson et al. 2009; Sallenave et al. 2003; Roghanian et al. 2006; Simpson et al. 2001), and the interaction of trappin-2 with LPS results in an augmentation of the LPS-induced TNF-α response in a murine macrophage cell line (McMichael et al. 2005b). Interestingly, transgenic mice expressing human trappin-2 show lower serum-to-bronchoalveolar lavage ratios of proinflammatory cytokines, including TNF-α, macrophage inflammatory protein 2 and monocyte chemoattractant protein 1, than wild-type mice in response to local intratracheal LPS stimulation with a concomitant increase in inflammatory cell influx (Sallenave et al. 2003). Conversely, trappin-2 transgenic mice show lower TNF-α serum levels in response to systemic LPS, indicating that trappin-2 may have a dual function, that is, promoting upregulation of local lung innate immunity while simultaneously downregulating potentially unwanted systemic inflammatory responses in the circulation (e.g. preventing septic shock) (Sallenave et al. 2003).

As discussed above, trappin-2 was first identified as being able to protect tissues from the damaging effects of proteases released during inflammation and was later shown to be functionally active in the regulation of both inflammation and innate immunity (Williams et al. 2006). However, emerging data expand upon the previously described functions for trappin-2/elafin, by showing that the influence of trappin-2 actually extends to include modulation of adaptive immune responses. To this end, using the dual system of trappin-2 expression (either provided as an adenoviral construct or in an elafin-transgenic model), our laboratory provided novel evidence that trappin-2 induces a type 1-biased inflammatory and immunological response (cellular and humoral) in the lungs and spleens of mice overexpressing elafin (Roghanian et al. 2006). The demonstrated Th1 skewing effect of trappin-2 is likely to be mediated through the increase in numbers and/or activation status of lung antigen-presenting cells, as elafin overexpressers exhibited higher numbers of total lung $CD11c^{+high}$ cells and $CD11c^{+high}$ $MHCII^{+high}$ cells (dendritic cells; DCs), expressing

higher levels of the B7 family costimulatory molecules CD80 and CD86 (indicative of activated DCs). In accordance with the increase in the number of activated DCs, increased levels of proinflammatory cytokines IL-12, TNF-α and IFN-γ were observed in BALF of trappin-2 overexpressers (Roghanian et al. 2006). Clinical evidence to support a role for trappin-2 in the augmentation of a Th1 phenotype is also available, for example, increased levels of trappin-2 are found in pathological conditions associated with a type I immune response, such as in the bronchoalveolar lavage of farmer's lung sufferers (Tremblay et al. 1996) and psoriatic skin (Schalkwijk et al. 1993).

More recently, human γδ T cells have been shown to produce trappin-2/elafin (both mRNA and protein) upon stimulation with supernatant of *P. aeruginosa* grown in culture. Between 2 and 5 % of $CD3^+$ T cells in the peripheral blood express the γδ T cell receptor (TCR) instead of the conventional αβ TCR. In contrast to the peripheral blood, γδ T cells represent a major T cell population in other anatomical localisations such as the small intestine where 20–30 % of local T cells are γδ T cells (Kabelitz et al. 2000; Hayday 2000). γδ T cells have the capacity to act as antigen-presenting cells (Brandes et al. 2005) and to secrete antimicrobial effector molecules such as granulysin (Dieli et al. 2001) and the cationic antimicrobial peptide LL37/cathelicidin, which is typically produced by epithelial cells (Agerberth et al. 2000; Selsted and Ouellette 2005). Due to certain features, which γδ T cells share with cells of both the adaptive (e.g. TCR expression) and the innate immune system (e.g. Toll-like receptor expression, antigen-presenting capacity), γδ T cells are thought to bridge innate and adaptive immunity (Hayday 2000). The secretion of elafin by γδ T cells might contribute to the recruitment of neutrophils or the opsonisation of the pathogens in sites of inflammation where access is restricted.

2.5 Key Roles in Mucosal Tissue

2.5.1 Reproductive Tract

In addition to the lung mucosa and skin, trappin-2 expression and regulation has received much interest in the female genital tracts, as it represents a major mucosal site. The mucosal immune system in the female reproductive tract has evolved to meet the unique requirements arising from the need to deal with sexually transmitted bacterial and viral pathogens, allogeneic spermatozoa and the immunologically distinct foetus. In this regard, a wide range of antimicrobial peptides including trappin-2 are expressed throughout the female genital tract [(Nishimura et al. 2008; Tomee et al. 1997), reviewed in (Horne et al. 2008)]. Trappin-2 and SLPI are expressed in the vagina (Draper et al. 2000) and cervix, with high concentrations of SLPI demonstrated in the cervical mucus (12, 57). SLPI is expressed in endometrium from the mid-late secretory phase of the menstrual cycle when it is localised predominantly to the glandular epithelium (King et al. 2000). In contrast,

trappin-2 is expressed primarily in endometrial neutrophils during menstruation (Turpin et al. 1996). Trappin-2 and SLPI are also detectable in the vaginal secretions throughout pregnancy (Shugars et al. 1997). Trappin-2 levels are diminished in bacterial vaginosis, suggesting that it may be an important component of innate immunity in the lower genital tract (Stock et al. 2009). In the Fallopian tube, trappin-2 and SLPI mRNA are upregulated in ectopic pregnancy. In contrast to endometrium, trappin-2 and SLPI are not regulated in a cycle-dependent manner at the mRNA level in the Fallopian tube. The pathology underlying ectopic pregnancy is unclear although previous infection with *Chlamydia trachomatis* is a risk factor. In line with this, the mRNA message for trappin-2 is increased during in vitro chlamydial infection of an oviductal cell line (King et al. 2009).

Natural antimicrobial production is also an important part of the innate immune response of the amnion. Indeed, the primary amnion epithelial cells produce potent natural antimicrobials, including trappin-2 and SLPI, which may help protect the pregnancy from infection (Stock et al. 2007). Taken together, these observations suggest that trappin-2 and SLPI play important roles in the maintenance of the female reproductive tract physiology via regulation of protease activity, wound healing and tissue remodelling. Trappin-2 and SLPI may also be implicated in the event of pathological conditions, such as infection and ectopic implantation (King et al. 2009), and abnormal expression of these peptides may predispose to infection or ectopic pregnancy.

2.5.2 Gastrointestinal Tract

Recent limited studies also point out to the important roles played by antimicrobial peptides, including trappin2/elafin and SLPI, in the gastrointestinal tract and associated pathologies. In a rhesus macaque host–pathogen model, microarray analysis revealed that in *Helicobacter pylori*-infected animals, several innate antimicrobial effector proteins, including elafin and siderocalin, and several novel paralogues of human β-defensin-2 were upregulated, which depended on the presence of the *cag* pathogenicity island (Baqui et al. 1999). In another study, investigating the presence of antimicrobial peptides in biopsies from the healthy oesophagus, stomach and the duodenum, trappin-2 was found to be predominantly expressed in the oesophagus (Hosaka et al. 2008).

Antimicrobial peptide imbalance appears to contribute to aetiology and pathogenesis of inflammatory bowel disease (IBD) (Pillay et al. 2001; Farquhar et al. 2002; Abbinante-Nissen et al. 1993). Interestingly, in biopsies taken from patients with Crohn's disease, the expression of trappin-2 and SLPI was shown to be attenuated upon inflammation, thereby suggesting a disruption of the protease/antiprotease balance in chronic inflammatory status of the gut (Schmid et al. 2007). By taking advantage of the adenoviral construct and two trappin-2 transgenic murine models, we established that restoring this proteolytic imbalance by the expression of the trappin-2 is associated with a strong protective effect against the development of colitis in experimental models (Motta et al. 2011). This

protection appears to be both due to reduced NE/PR-3 and trypsin-like activities and also due to the inhibition of NF-κB proinflammatory pathway by trappin-2, as observed in other models (Velarde et al. 2006; King et al. 2003). Collectively, these results not only provide definitive insights into the importance of the proteolytic balance in gut inflammation but also point to trappin-2 as a possible protective molecule in chronic inflammatory disorders of the gut (Motta et al. 2011).

3 WAP as Therapeutics, Drug Targets or Biomarkers

In vitro and in vivo experimental modelling has identified the activities of SLPI and trappin-2/elafin, but transforming these results into medicines is only just becoming a reality.

Numerous studies in the 1990s reported the effects of giving recombinant SLPI to humans (McElvaney et al. 1993; Bergenfeldt et al. 1990; Stolk et al. 1995; McElvaney et al. 1992) with a view to treating lung disease. These studies confirmed elimination half-lives of 10 min (Bergenfeldt et al. 1990) and 0.2–2.8 h (Stolk et al. 1995) for intravenous administration and inhalation, respectively. Inhaled therapy appears to be the way forward due to increased lung targeting and decreased systemic effects although repeated dosing was necessary to maintain therapeutic levels in CF patients (McElvaney et al. 1993). Analysis of epithelial lining fluid from patients with emphysema has suggested this may be due to SLPI cleavage by cathepsins (Taggart et al. 2001). To improve delivery to the diseased lung, Gibbons and co-workers have developed a dry powder formulation of liposome-encapsulated recombinant SLPI that proved better at retaining a protective function against cathepsin L-induced rSLPI inactivation compared to an aqueous DOPS–rSLPI liposome dispersion and was also more stable under storage (Gibbons et al. 2010).

Improvements have also been made with regard to the production of recombinant protein. Expression of SLPI in bacteria required extensive denaturation and renaturation to refold the disulphide-rich protein into its biologically active form (Lucey et al. 1990). Recently two alternative methods of production have been developed using baculovirus expression (Gray et al. 2002) and the yeast *Pichia pastoris* (Li et al. 2009, 2010b) with purification under non-denaturing conditions. These advances have suggested that SLPI can be produced in an efficient and cost-effective manner for therapeutic purposes. These methods have resulted in a greater yield of protein with improved biological activity. Interestingly Zani and co-workers have produced fusion proteins to create antiproteases with activities overlapping with SLPI and elafin so that elastase, cathepsin G and PR-3 could be inhibited by one molecule (Zani et al. 2009). Such manipulation of these molecules will hopefully result in designer therapeutics directed at lung diseases (e.g. COPD) where protease/antiprotease balance is destructive to the host.

Further advances moving SLPI therapeutics one step closer to reality have been reported recently: firstly, the development of four hybridomas that produce anti-

human SLPI monoclonal antibodies (Chen et al. 2006); secondly, the specificity of serum SLPI levels to differentiate between benign ovarian cysts and malignancies (Tsukishiro et al. 2005); thirdly, the development of cleaved SLPI (cSLPI) as a biomarker of chymase activity in allergic disease (Belkowski et al. 2008); and, finally, the exciting potential of the SLPI promoter as a tissue-specific promoter in the development of ovarian cancer gene therapy (Barker et al. 2003).

4 Concluding Remarks

Recent publications on the WAP SLPI and trappin-2/elafin have dramatically changed our current view of these molecules. They are no longer 'just' antiproteases expressed in lining fluid but major modulators of immunity. Moreover, their actions seem temporally regulated to be expressed during stages of the immune response. They have roles in innate immune priming, which links to the adaptive immune system and also to immune homeostasis and tissue remodelling, suggesting that their plethora of activities are essential throughout the inflammatory response. Understanding how they can produce such varying activities over the course of the inflammatory response is not so well understood and will form the basis for the next generation of literature on these quite extraordinary pleiotropic molecules.

References

Abbinante-Nissen JM, Simpson LG, Leikauf GD (1993) Neutrophil elastase increases secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. Am J Physiol 265: L286–L292

Abbinante-Nissen JM, Simpson LG, Leikauf GD (1995) Corticosteroids increase secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. Am J Physiol 268: L601–L606

Agerberth B, Charo J, Werr J, Olsson B, Idali F, Lindbom L, Kiessling R, Jornvall H, Wigzell H, Gudmundsson GH (2000) The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. Blood 96:3086–3093

Anas A, van der Poll T, de Vos AF (2010) Role of CD14 in lung inflammation and infection. Crit Care 14:209

Angelov N, Moutsopoulos N, Jeong MJ, Nares S, Ashcroft G, Wahl SM (2004) Aberrant mucosal wound repair in the absence of secretory leukocyte protease inhibitor. Thromb Haemost 92:288–297

Ashcroft GS, Lei K, Jin W, Longenecker G, Kulkarni AB, Greenwell-Wild T, Hale-Donze H, McGrady G, Song XY, Wahl SM (2000) Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. Nat Med 6:1147–1153

Bairoch A, Apweiler R (1997) The SWISS-PROT protein sequence database: its relevance to human molecular medical research. J Mol Med 75:312–316

Baqui AA, Meiller TF, Falkler WA Jr (1999) Enhanced secretory leukocyte protease inhibitor in human immunodeficiency virus type 1-infected patients. Clin Diagn Lab Immunol 6:808–811

Baranger K, Zani ML, Chandenier J, Dallet-Choisy S, Moreau T (2008) The antibacterial and antifungal properties of trappin-2 (pre-elafin) do not depend on its protease inhibitory function. FEBS J 275:2008–2020

Barker SD, Coolidge CJ, Kanerva A, Hakkarainen T, Yamamoto M, Liu B, Rivera AA, Bhoola SM, Barnes MN, Alvarez RD et al (2003) The secretory leukoprotease inhibitor (SLPI) promoter for ovarian cancer gene therapy. J Gene Med 5:300–310

Belkowski SM, Masucci J, Mahan A, Kervinen J, Olson M, de Garavilla L, D'Andrea MR (2008) Cleaved SLPI, a novel biomarker of chymase activity. Biol Chem 389:1219–1224

Bellemare A, Vernoux N, Morisset D, Bourbonnais Y (2008) Human pre-elafin inhibits a Pseudomonas aeruginosa-secreted peptidase and prevents its proliferation in complex media. Antimicrob Agents Chemother 52:483–490

Bellemare A, Vernoux N, Morin S, Gagne SM, Bourbonnais Y (2010) Structural and antimicrobial properties of human pre-elafin/trappin-2 and derived peptides against Pseudomonas aeruginosa. BMC Microbiol 10:253

Bergenfeldt M, Bjork P, Ohlsson K (1990) The elimination of secretory leukocyte protease inhibitor (SLPI) after intravenous injection in dog and man. Scand J Clin Lab Invest 50:729–737

Bergenfeldt M, Nystrom M, Bohe M, Lindstrom C, Polling A, Ohlsson K (1996) Localization of immunoreactive secretory leukocyte protease inhibitor (SLPI) in intestinal mucosa. J Gastroenterol 31:18–23

Bingle L, Tetley TD, Bingle CD (2001) Cytokine-mediated induction of the human elafin gene in pulmonary epithelial cells is regulated by nuclear factor-kappaB. Am J Respir Cell Mol Biol 25:84–91

Brandes M, Willimann K, Moser B (2005) Professional antigen-presentation function by human gammadelta T Cells. Science 309:264–268

Butterly A, Schmidt U, Wiener-Kronish J (2010) Methicillin-resistant Staphylococcus aureus colonization, its relationship to nosocomial infection, and efficacy of control methods. Anesthesiology 113:1453–1459

Campbell SM, Rosen JM, Hennighausen LG, Strech-Jurk U, Sippel AE (1984) Comparison of the whey acidic protein genes of the rat and mouse. Nucleic Acids Res 12:8685–8697

Carlsson B, Ohlsson K (1983) Localization of antileukoprotease in middle ear mucosa. Acta Otolaryngol 95:111–116

Casslen B, Rosengren M, Ohlsson K (1981) Localization and quantitation of a low molecular weight proteinase inhibitor, antileukoprotease, in the human uterus. Hoppe Seylers Z Physiol Chem 362:953–961

Chen YZ, He SH, Zhou YC, Huang T, Liu YN, Chen LF (2006) Preparation and characterization of monoclonal antibodies against human secretory leukocyte protease inhibitor. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 22:54–57

Cheng WL, Wang CS, Huang YH, Liang Y, Lin PY, Hsueh C, Wu YC, Chen WJ, Yu CJ, Lin SR et al (2008) Overexpression of a secretory leukocyte protease inhibitor in human gastric cancer. Int J Cancer 123:1787–1796

Cox SW, Rodriguez-Gonzalez EM, Booth V, Eley BM (2006) Secretory leukocyte protease inhibitor and its potential interactions with elastase and cathepsin B in gingival crevicular fluid and saliva from patients with chronic periodontitis. J Periodontal Res 41:477–485

De Water R, Willems LN, Van Muijen GN, Franken C, Fransen JA, Dijkman JH, Kramps JA (1986) Ultrastructural localization of bronchial antileukoprotease in central and peripheral human airways by a gold-labeling technique using monoclonal antibodies. Am Rev Respir Dis 133:882–890

Denison FC, Kelly RW, Calder AA, Riley SC (1999) Secretory leukocyte protease inhibitor concentration increases in amniotic fluid with the onset of labour in women: characterization of sites of release within the uterus. J Endocrinol 161:299–306

Devoogdt N, Hassanzadeh Ghassabeh G, Zhang J, Brys L, De Baetselier P, Revets H (2003) Secretory leukocyte protease inhibitor promotes the tumorigenic and metastatic potential of cancer cells. Proc Natl Acad Sci USA 100:5778–5782

Devoogdt N, Revets H, Kindt A, Liu YQ, De Baetselier P, Ghassabeh GH (2006) The tumor-promoting effect of TNF-alpha involves the induction of secretory leukocyte protease inhibitor. J Immunol 177:8046–8052

Devoogdt N, Rasool N, Hoskins E, Simpkins F, Tchabo N, Kohn EC (2009) Overexpression of protease inhibitor-dead secretory leukocyte protease inhibitor causes more aggressive ovarian cancer in vitro and in vivo. Cancer Sci 100:434–440

Dieli F, Troye-Blomberg M, Ivanyi J, Fournie JJ, Krensky AM, Bonneville M, Peyrat MA, Caccamo N, Sireci G, Salerno A (2001) Granulysin-dependent killing of intracellular and extracellular Mycobacterium tuberculosis by Vgamma9/Vdelta2 T lymphocytes. J Infect Dis 184:1082–1085

Ding A, Thieblemont N, Zhu J, Jin F, Zhang J, Wright S (1999) Secretory leukocyte protease inhibitor interferes with uptake of lipopolysaccharide by macrophages. Infect Immun 67:4485–4489

Draper DL, Landers DV, Krohn MA, Hillier SL, Wiesenfeld HC, Heine RP (2000) Levels of vaginal secretory leukocyte protease inhibitor are decreased in women with lower reproductive tract infections. Am J Obstet Gynecol 183:1243–1248

Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991–998

Eisenberg SP, Hale KK, Heimdal P, Thompson RC (1990) Location of the protease-inhibitory region of secretory leukocyte protease inhibitor. J Biol Chem 265:7976–7981

Erwin AL, VanDevanter DR (2002) The Pseudomonas aeruginosa genome: how do we use it to develop strategies for the treatment of patients with cystic fibrosis and Pseudomonas infections? Curr Opin Pulm Med 8:547–551

Fahey JV, Bodwell JE, Hickey DK, Ghosh M, Muia MN, Wira CR (2011) New approaches to making the microenvironment of the female reproductive tract hostile to HIV. Am J Reprod Immunol 65:334–343

Farquhar C, VanCott TC, Mbori-Ngacha DA, Horani L, Bosire RK, Kreiss JK, Richardson BA, John-Stewart GC (2002) Salivary secretory leukocyte protease inhibitor is associated with reduced transmission of human immunodeficiency virus type 1 through breast milk. J Infect Dis 186:1173–1176

Fath MA, Wu X, Hileman RE, Linhardt RJ, Kashem MA, Nelson RM, Wright CD, Abraham WM (1998) Interaction of secretory leukocyte protease inhibitor with heparin inhibits proteases involved in asthma. J Biol Chem 273:13563–13569

Franken C, Meijer CJ, Dijkman JH (1989) Tissue distribution of antileukoprotease and lysozyme in humans. J Histochem Cytochem 37:493–498

Fritz H (1988) Human mucus proteinase inhibitor (human MPI). Human seminal inhibitor I (HUSI-I), antileukoprotease (ALP), secretory leukocyte protease inhibitor (SLPI). Biol Chem Hoppe Seyler 369 Suppl:79–82

Fryksmark U, Ohlsson K, Polling A, Tegner H (1982) Distribution of antileukoprotease in upper respiratory mucosa. Ann Otol Rhinol Laryngol 91:268–271

Fryksmark U, Jannert M, Ohlsson K, Tegner H (1985) Antileukoprotease in patients with maxillary sinusitis. Rhinology 23:247–251

Ghosh M, Fahey JV, Shen Z, Lahey T, Cu-Uvin S, Wu Z, Mayer K, Wright PF, Kappes JC, Ochsenbauer C et al (2010a) Anti-HIV activity in cervical-vaginal secretions from HIV-positive and -negative women correlate with innate antimicrobial levels and IgG antibodies. PLoS One 5:e11366

Ghosh M, Shen Z, Fahey JV, Cu-Uvin S, Mayer K, Wira CR (2010b) Trappin-2/Elafin: a novel innate anti-human immunodeficiency virus-1 molecule of the human female reproductive tract. Immunology 129:207–219

Gibbons A, McElvaney NG, Cryan SA (2010) A dry powder formulation of liposome-encapsulated recombinant secretory leukocyte protease inhibitor (rSLPI) for inhalation: preparation and characterisation. AAPS PharmSciTech 11:1411–1421

Gomez SA, Arguelles CL, Guerrieri D, Tateosian NL, Amiano NO, Slimovich R, Maffia PC, Abbate E, Musella RM, Garcia VE et al (2009) Secretory leukocyte protease inhibitor: a secreted pattern recognition receptor for mycobacteria. Am J Respir Crit Care Med 179:247–253

Gray LR, Alexander AL, Shugars DC (2002) Construction, non-denaturing affinity purification, and characterization of baculovirally expressed human secretory leukocyte protease inhibitor. Protein Expr Purif 26:179–186

Grutter MG, Fendrich G, Huber R, Bode W (1988) The 2.5 A X-ray crystal structure of the acid-stable proteinase inhibitor from human mucous secretions analysed in its complex with bovine alpha-chymotrypsin. EMBO J 7:345–351

Guyot N, Butler MW, McNally P, Weldon S, Greene CM, Levine RL, O'Neill SJ, Taggart CC, McElvaney NG (2008) Elafin, an elastase-specific inhibitor, is cleaved by its cognate enzyme neutrophil elastase in sputum from individuals with cystic fibrosis. J Biol Chem 283:32377–32385

Hancock RE (1998) Resistance mechanisms in Pseudomonas aeruginosa and other nonfermentative gram-negative bacteria. Clin Infect Dis 27(Suppl 1):S93–S99

Hayday AC (2000) [gamma][delta] cells: a right time and a right place for a conserved third way of protection. Annu Rev Immunol 18:975–1026

Helmig R, Uldbjerg N, Ohlsson K (1995) Secretory leukocyte protease inhibitor in the cervical mucus and in the fetal membranes. Eur J Obstet Gynecol Reprod Biol 59:95–101

Henriksen PA, Hitt M, Xing Z, Wang J, Haslett C, Riemersma RA, Webb DJ, Kotelevtsev YV, Sallenave JM (2004) Adenoviral gene delivery of elafin and secretory leukocyte protease inhibitor attenuates NF-kappa B-dependent inflammatory responses of human endothelial cells and macrophages to atherogenic stimuli. J Immunol 172:4535–4544

Hiemstra PS, Maassen RJ, Stolk J, Heinzel-Wieland R, Steffens GJ, Dijkman JH (1996) Antibacterial activity of antileukoprotease. Infect Immun 64:4520–4524

Hirano M, Kamada M, Maegawa M, Gima H, Aono T (1999) Binding of human secretory leukocyte protease inhibitor in uterine cervical mucus to immunoglobulins: pathophysiology in immunologic infertility and local immune defense. Fertil Steril 71:1108–1114

Hochstrasser K, Albrecht GJ, Schonberger OL, Rasche B, Lempart K (1981) An elastase-specific inhibitor from human bronchial mucus. Isolation and characterization. Hoppe Seylers Z Physiol Chem 362:1369–1375

Horne AW, Stock SJ, King AE (2008) Innate immunity and disorders of the female reproductive tract. Reproduction 135:739–749

Hosaka Y, Koslowski M, Nuding S, Wang G, Schlee M, Schafer C, Saigenji K, Stange EF, Wehkamp J (2008) Antimicrobial host defense in the upper gastrointestinal tract. Eur J Gastroenterol Hepatol 20:1151–1158

Iqbal SM, Ball TB, Levinson P, Maranan L, Jaoko W, Wachihi C, Pak BJ, Podust VN, Broliden K, Hirbod T et al (2009) Elevated elafin/trappin-2 in the female genital tract is associated with protection against HIV acquisition. AIDS 23:1669–1677

Jacobsen LC, Sorensen OE, Cowland JB, Borregaard N, Theilgaard-Monch K (2008) The secretory leukocyte protease inhibitor (SLPI) and the secondary granule protein lactoferrin are synthesized in myelocytes, colocalize in subcellular fractions of neutrophils, and are coreleased by activated neutrophils. J Leukoc Biol 83:1155–1164

Jin FY, Nathan C, Radzioch D, Ding A (1997) Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. Cell 88:417–426

Jin F, Nathan CF, Radzioch D, Ding A (1998) Lipopolysaccharide-related stimuli induce expression of the secretory leukocyte protease inhibitor, a macrophage-derived lipopolysaccharide inhibitor. Infect Immun 66:2447–2452

Kabelitz D, Glatzel A, Wesch D (2000) Antigen recognition by human gammadelta T lymphocytes. Int Arch Allergy Immunol 122:1–7

Kikuchi T, Abe T, Hoshi S, Matsubara N, Tominaga Y, Satoh K, Nukiwa T (1998) Structure of the murine secretory leukoprotease inhibitor (Slpi) gene and chromosomal localization of the human and murine SLPI genes. Am J Respir Cell Mol Biol 19:875–880

King AE, Critchley HO, Kelly RW (2000) Presence of secretory leukocyte protease inhibitor in human endometrium and first trimester decidua suggests an antibacterial protective role. Mol Hum Reprod 6:191–196

King AE, Morgan K, Sallenave JM, Kelly RW (2003) Differential regulation of secretory leukocyte protease inhibitor and elafin by progesterone. Biochem Biophys Res Commun 310:594–599

King AE, Wheelhouse N, Cameron S, McDonald SE, Lee KF, Entrican G, Critchley HO, Horne AW (2009) Expression of secretory leukocyte protease inhibitor and elafin in human fallopian tube and in an in-vitro model of Chlamydia trachomatis infection. Hum Reprod 24:679–686

Kouchi I, Yasuoka S, Ueda Y, Ogura T (1993) Analysis of secretory leukocyte protease inhibitor (SLPI) in bronchial secretions from patients with hypersecretory respiratory diseases. Tokushima J Exp Med 40:95–107

Kramps JA, Klasen EC (1985) Characterization of a low molecular weight anti-elastase isolated from human bronchial secretion. Exp Lung Res 9:151–165

Kramps JA, Te Boekhorst AH, Fransen JA, Ginsel LA, Dijkman JH (1989) Antileukoprotease is associated with elastin fibers in the extracellular matrix of the human lung. An immunoelectron microscopic study. Am Rev Respir Dis 140:471–476

Kramps JA, van Twisk C, Appelhans H, Meckelein B, Nikiforov T, Dijkman JH (1990) Proteinase inhibitory activities of antileukoprotease are represented by its second COOH-terminal domain. Biochim Biophys Acta 1038:178–185

Lee JK, Chae SW, Cho JG, Lee HM, Hwang SJ, Jung HH (2006) Expression of secretory leukocyte protease inhibitor in middle ear cholesteatoma. Eur Arch Otorhinolaryngol 263:1077–1081

Lentsch AB, Jordan JA, Czermak BJ, Diehl KM, Younkin EM, Sarma V, Ward PA (1999a) Inhibition of NF-kappaB activation and augmentation of IkappaBbeta by secretory leukocyte protease inhibitor during lung inflammation. Am J Pathol 154:239–247

Lentsch AB, Yoshidome H, Warner RL, Ward PA, Edwards MJ (1999b) Secretory leukocyte protease inhibitor in mice regulates local and remote organ inflammatory injury induced by hepatic ischemia/reperfusion. Gastroenterology 117:953–961

Li Z, Moy A, Sohal K, Dam C, Kuo P, Whittaker J, Whittaker M, Duzgunes N, Konopka K, Franz AH et al (2009) Expression and characterization of recombinant human secretory leukocyte protease inhibitor (SLPI) protein from Pichia pastoris. Protein Expr Purif 67:175–181

Li Q, Zhou X, Nie X, Yang J (2010a) The role of recombinant human elafin in the resistance of A549 cells against Pseudomonas aeruginosa biofilm. Respiration 79:68–75

Li Z, Moy A, Gomez SR, Franz AH, Lin-Cereghino J, Lin-Cereghino GP (2010b) An improved method for enhanced production and biological activity of human secretory leukocyte protease inhibitor (SLPI) in Pichia pastoris. Biochem Biophys Res Commun 402:519–524

Lucey EC, Stone PJ, Ciccolella DE, Breuer R, Christensen TG, Thompson RC, Snider GL (1990) Recombinant human secretory leukocyte-protease inhibitor: in vitro properties, and amelioration of human neutrophil elastase-induced emphysema and secretory cell metaplasia in the hamster. J Lab Clin Med 115:224–232

Ma G, Greenwell-Wild T, Lei K, Jin W, Swisher J, Hardegen N, Wild CT, Wahl SM (2004) Secretory leukocyte protease inhibitor binds to annexin II, a cofactor for macrophage HIV-1 infection. J Exp Med 200:1337–1346

McElvaney NG, Nakamura H, Birrer P, Hebert CA, Wong WL, Alphonso M, Baker JB, Catalano MA, Crystal RG (1992) Modulation of airway inflammation in cystic fibrosis. In vivo suppression of interleukin-8 levels on the respiratory epithelial surface by aerosolization of recombinant secretory leukoprotease inhibitor. J Clin Invest 90:1296–1301

McElvaney NG, Doujaiji B, Moan MJ, Burnham MR, Wu MC, Crystal RG (1993) Pharmacokinetics of recombinant secretory leukoprotease inhibitor aerosolized to normals and individuals with cystic fibrosis. Am Rev Respir Dis 148:1056–1060

McMichael JW, Maxwell AI, Hayashi K, Taylor K, Wallace WA, Govan JR, Dorin JR, Sallenave JM (2005a) Antimicrobial activity of murine lung cells against Staphylococcus aureus is increased in vitro and in vivo after elafin gene transfer. Infect Immun 73:3609–3617

McMichael JW, Roghanian A, Jiang L, Ramage R, Sallenave JM (2005b) The antimicrobial antiproteinase elafin binds to lipopolysaccharide and modulates macrophage responses. Am J Respir Cell Mol Biol 32:443–452

McNeely TB, Dealy M, Dripps DJ, Orenstein JM, Eisenberg SP, Wahl SM (1995) Secretory leukocyte protease inhibitor: a human saliva protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. J Clin Invest 96:456–464

McNeely TB, Shugars DC, Rosendahl M, Tucker C, Eisenberg SP, Wahl SM (1997) Inhibition of human immunodeficiency virus type 1 infectivity by secretory leukocyte protease inhibitor occurs prior to viral reverse transcription. Blood 90:1141–1149

Meyer-Hoffert U, Wichmann N, Schwichtenberg L, White PC, Wiedow O (2003) Supernatants of Pseudomonas aeruginosa induce the Pseudomonas-specific antibiotic elafin in human keratinocytes. Exp Dermatol 12:418–425

Miller KW, Evans RJ, Eisenberg SP, Thompson RC (1989) Secretory leukocyte protease inhibitor binding to mRNA and DNA as a possible cause of toxicity to Escherichia coli. J Bacteriol 171:2166–2172

Molhuizen HO, Alkemade HA, Zeeuwen PL, de Jongh GJ, Wieringa B, Schalkwijk J (1993) SKALP/elafin: an elastase inhibitor from cultured human keratinocytes. Purification, cDNA sequence, and evidence for transglutaminase cross-linking. J Biol Chem 268:12028–12032

Moreau T, Baranger K, Dade S, Dallet-Choisy S, Guyot N, Zani ML (2008) Multifaceted roles of human elafin and secretory leukocyte proteinase inhibitor (SLPI), two serine protease inhibitors of the chelonianin family. Biochimie 90:284–295

Morita M, Arakawa H, Nishimura S (1999) Identification and cloning of a novel isoform of mouse secretory leukocyte protease inhibitor, mSLPI-beta, overexpressed in murine leukemias and a highly liver metastatic tumor, IMC-HA1 cells. Adv Enzyme Regul 39:341–355

Moriyama A, Shimoya K, Kawamoto A, Hashimoto K, Ogata I, Kunishige I, Ohashi K, Azuma C, Saji F, Murata Y (1998) Secretory leukocyte protease inhibitor (SLP) concentrations in seminal plasma: SLPI restores sperm motility reduced by elastase. Mol Hum Reprod 4:946–950

Moriyama A, Shimoya K, Ogata I, Kimura T, Nakamura T, Wada H, Ohashi K, Azuma C, Saji F, Murata Y (1999) Secretory leukocyte protease inhibitor (SLPI) concentrations in cervical mucus of women with normal menstrual cycle. Mol Hum Reprod 5:656–661

Motta JP, Magne L, Descamps D, Rolland C, Squarzoni-Dale C, Rousset P, Martin L, Cenac N, Balloy V, Huerre M et al. (2011) Overexpression of elastin affects the protease to anti-protease balance and protects mice from colitis. Gastroenterology 140:1272-82

Mulligan MS, Lentsch AB, Huber-Lang M, Guo RF, Sarma V, Wright CD, Ulich TR, Ward PA (2000) Anti-inflammatory effects of mutant forms of secretory leukocyte protease inhibitor. Am J Pathol 156:1033–1039

Murata E, Sharmin S, Shiota H, Shiota M, Yano M, Kido H (2003) The effect of topically applied secretory leukocyte protease inhibitor on the eosinophil response in the late phase of allergic conjunctivitis. Curr Eye Res 26:271–276

Nakamura A, Mori Y, Hagiwara K, Suzuki T, Sakakibara T, Kikuchi T, Igarashi T, Ebina M, Abe T, Miyazaki J et al (2003) Increased susceptibility to LPS-induced endotoxin shock in secretory leukoprotease inhibitor (SLPI)-deficient mice. J Exp Med 197:669–674

Nakamura-Minami M, Furuichi Y, Ishikawa K, Mitsuzono-Tofuku Y, Izumi Y (2003) Changes of alpha1-protease inhibitor and secretory leukocyte protease inhibitor levels in gingival crevicular fluid before and after non-surgical periodontal treatment. Oral Dis 9:249–254

Nara K, Ito S, Ito T, Suzuki Y, Ghoneim MA, Tachibana S, Hirose S (1994) Elastase inhibitor elafin is a new type of proteinase inhibitor which has a transglutaminase-mediated anchoring sequence termed "cementoin". J Biochem 115:441–448

Nguyen H, Teskey L, Lin R, Hiscott J (1999) Identification of the secretory leukocyte protease inhibitor (SLPI) as a target of IRF-1 regulation. Oncogene 18:5455–5463

Nielsen K, Heegaard S, Vorum H, Birkenkamp-Demtroder K, Ehlers N, Orntoft TF (2005) Altered expression of CLC, DSG3, EMP3, S100A2, and SLPI in corneal epithelium from keratoconus patients. Cornea 24:661–668

Nishimura J, Saiga H, Sato S, Okuyama M, Kayama H, Kuwata H, Matsumoto S, Nishida T, Sawa Y, Akira S et al (2008) Potent antimycobacterial activity of mouse secretory leukocyte protease inhibitor. J Immunol 180:4032–4039

Nystrom M, Westin UP, Linder C, Ohlsson K (2001) Secretory leukocyte protease inhibitor in punch biopsies from human colonic mucosa. Mediators Inflamm 10:269–272

Odaka C, Mizuochi T, Yang J, Ding A (2003) Murine macrophages produce secretory leukocyte protease inhibitor during clearance of apoptotic cells: implications for resolution of the inflammatory response. J Immunol 171:1507–1514

Ohlsson M, Fryksmark U, Polling A, Tegner H, Ohlsson K (1984) Localization of antileukoprotease in the parotid and the submandibular salivary glands. Acta Otolaryngol 98:147–151

Ohlsson K, Sveger T, Svenningsen N (1992) Protease inhibitors in bronchoalveolar lavage fluid from neonates with special reference to secretory leukocyte protease inhibitor. Acta Paediatr 81:757–759

Pfundt R, van Ruissen F, van Vlijmen-Willems IM, Alkemade HA, Zeeuwen PL, Jap PH, Dijkman H, Fransen J, Croes H, van Erp PE et al (1996) Constitutive and inducible expression of SKALP/elafin provides anti-elastase defense in human epithelia. J Clin Invest 98:1389–1399

Pfundt R, Wingens M, Bergers M, Zweers M, Frenken M, Schalkwijk J (2000) TNF-alpha and serum induce SKALP/elafin gene expression in human keratinocytes by a p38 MAP kinase-dependent pathway. Arch Dermatol Res 292:180–187

Pfundt R, van Vlijmen-Willems I, Bergers M, Wingens M, Cloin W, Schalkwijk J (2001) In situ demonstration of phosphorylated c-jun and p38 MAP kinase in epidermal keratinocytes following ultraviolet B irradiation of human skin. J Pathol 193:248–255

Piccioni PD, Kramps JA, Rudolphus A, Bulgheroni A, Luisetti M (1992) Proteinase/proteinase inhibitor imbalance in sputum sol phases from patients with chronic obstructive pulmonary disease. Suggestions for a key role played by antileukoprotease. Chest 102:1470–1476

Pillay K, Coutsoudis A, Agadzi-Naqvi AK, Kuhn L, Coovadia HM, Janoff EN (2001) Secretory leukocyte protease inhibitor in vaginal fluids and perinatal human immunodeficiency virus type 1 transmission. J Infect Dis 183:653–656

Py B, Basmaciogullari S, Bouchet J, Zarka M, Moura IC, Benhamou M, Monteiro RC, Hocini H, Madrid R, Benichou S (2009) The phospholipid scramblases 1 and 4 are cellular receptors for the secretory leukocyte protease inhibitor and interact with CD4 at the plasma membrane. PLoS One 4:e5006

Rao NV, Marshall BC, Gray BH, Hoidal JR (1993) Interaction of secretory leukocyte protease inhibitor with proteinase-3. Am J Respir Cell Mol Biol 8:612–616

Reid PT, Marsden ME, Cunningham GA, Haslett C, Sallenave JM (1999) Human neutrophil elastase regulates the expression and secretion of elafin (elastase-specific inhibitor) in type II alveolar epithelial cells. FEBS Lett 457:33–37

Renesto P, Balloy V, Kamimura T, Masuda K, Imaizumi A, Chignard M (1993) Inhibition by recombinant SLPI and half-SLPI (Asn55-Ala107) of elastase and cathepsin G activities: consequence for neutrophil-platelet cooperation. Br J Pharmacol 108:1100–1106

Roghanian A, Sallenave JM (2008) Neutrophil elastase (NE) and NE inhibitors: canonical and noncanonical functions in lung chronic inflammatory diseases (cystic fibrosis and chronic obstructive pulmonary disease). J Aerosol Med Pulm Drug Deliv 21:125–144

Roghanian A, Williams SE, Sheldrake TA, Brown TI, Oberheim K, Xing Z, Howie SE, Sallenave JM (2006) The antimicrobial/elastase inhibitor elafin regulates lung dendritic cells and adaptive immunity. Am J Respir Cell Mol Biol 34:634–642

Saheki T, Ito F, Hagiwara H, Saito Y, Kuroki J, Tachibana S, Hirose S (1992) Primary structure of the human elafin precursor preproelafin deduced from the nucleotide sequence of its gene and the presence of unique repetitive sequences in the prosegment. Biochem Biophys Res Commun 185:240–245

Sallenave JM (2010) Secretory leukocyte protease inhibitor and elafin/trappin-2: versatile mucosal antimicrobials and regulators of immunity. Am J Respir Cell Mol Biol 42:635–643

Sallenave JM, Ryle AP (1991) Purification and characterization of elastase-specific inhibitor. Sequence homology with mucus proteinase inhibitor. Biol Chem Hoppe Seyler 372:13–21

Sallenave JM, Silva A (1993) Characterization and gene sequence of the precursor of elafin, an elastase-specific inhibitor in bronchial secretions. Am J Respir Cell Mol Biol 8:439–445

Sallenave JM, Marsden MD, Ryle AP (1992) Isolation of elafin and elastase-specific inhibitor (ESI) from bronchial secretions. Evidence of sequence homology and immunological cross-reactivity. Biol Chem Hoppe Seyler 373:27–33

Sallenave JM, Shulmann J, Crossley J, Jordana M, Gauldie J (1994) Regulation of secretory leukocyte proteinase inhibitor (SLPI) and elastase-specific inhibitor (ESI/elafin) in human airway epithelial cells by cytokines and neutrophilic enzymes. Am J Respir Cell Mol Biol 11:733–741

Sallenave JM, Cunningham GA, James RM, McLachlan G, Haslett C (2003) Regulation of pulmonary and systemic bacterial lipopolysaccharide responses in transgenic mice expressing human elafin. Infect Immun 71:3766–3774

Savill JS, Wyllie AH, Henson JE, Walport MJ, Henson PM, Haslett C (1989) Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. J Clin Invest 83:865–875

Schalkwijk J, van Vlijmen IM, Alkemade JA, de Jongh GJ (1993) Immunohistochemical localization of SKALP/elafin in psoriatic epidermis. J Invest Dermatol 100:390–393

Schill WB, Wallner O, Schiessler H, Fritz H (1978) Immunofluorescent localization of the acid-stable proteinase inhibitor (antileukoprotease) of human cervical mucus. Experientia 34:509–510

Schmid M, Fellermann K, Fritz P, Wiedow O, Stange EF, Wehkamp J (2007) Attenuated induction of epithelial and leukocyte serine antiproteases elafin and secretory leukocyte protease inhibitor in Crohn's disease. J Leukoc Biol 81:907–915

Schneeberger S, Hautz T, Wahl SM, Brandacher G, Sucher R, Steinmassl O, Steinmassl P, Wright CD, Obrist P, Werner ER et al (2008) The effect of secretory leukocyte protease inhibitor (SLPI) on ischemia/reperfusion injury in cardiac transplantation. Am J Transplant 8:773–782

Seemuller U, Arnhold M, Fritz H, Wiedenmann K, Machleidt W, Heinzel R, Appelhans H, Gassen HG, Lottspeich F (1986) The acid-stable proteinase inhibitor of human mucous secretions (HUSI-I, antileukoprotease). Complete amino acid sequence as revealed by protein and cDNA sequencing and structural homology to whey proteins and Red Sea turtle proteinase inhibitor. FEBS Lett 199:43–48

Selsted ME, Ouellette AJ (2005) Mammalian defensins in the antimicrobial immune response. Nat Immunol 6:551–557

Shigemasa K, Tanimoto H, Underwood LJ, Parmley TH, Arihiro K, Ohama K, O'Brien TJ (2001) Expression of the protease inhibitor antileukoprotease and the serine protease stratum corneum chymotryptic enzyme (SCCE) is coordinated in ovarian tumors. Int J Gynecol Cancer 11:454–461

Shimoya K, Moriyama A, Ogata I, Nobunaga T, Koyama M, Azuma C, Murata Y (2000) Increased concentrations of secretory leukocyte protease inhibitor in peritoneal fluid of women with endometriosis. Mol Hum Reprod 6:829–834

Shugars DC, Sauls DL, Weinberg JB (1997) Secretory leukocyte protease inhibitor blocks infectivity of primary monocytes and mononuclear cells with both monocytotropic and

lymphocytotropic strains of human immunodeficiency virus type I. Oral Dis 3(Suppl 1): S70–S72

Shugars DC, Watkins CA, Cowen HJ (2001) Salivary concentration of secretory leukocyte protease inhibitor, an antimicrobial protein, is decreased with advanced age. Gerontology 47:246–253

Simpson AJ, Maxwell AI, Govan JR, Haslett C, Sallenave JM (1999) Elafin (elastase-specific inhibitor) has anti-microbial activity against gram-positive and gram-negative respiratory pathogens. FEBS Lett 452:309–313

Simpson AJ, Cunningham GA, Porteous DJ, Haslett C, Sallenave JM (2001) Regulation of adenovirus-mediated elafin transgene expression by bacterial lipopolysaccharide. Hum Gene Ther 12:1395–1406

Song X, Zeng L, Jin W, Thompson J, Mizel DE, Lei K, Billinghurst RC, Poole AR, Wahl SM (1999) Secretory leukocyte protease inhibitor suppresses the inflammation and joint damage of bacterial cell wall-induced arthritis. J Exp Med 190:535–542

Stetler G, Brewer MT, Thompson RC (1986) Isolation and sequence of a human gene encoding a potent inhibitor of leukocyte proteases. Nucleic Acids Res 14:7883–7896

Stock SJ, Kelly RW, Riley SC, Calder AA (2007) Natural antimicrobial production by the amnion. Am J Obstet Gynecol 196(255):e251–e256

Stock SJ, Duthie L, Tremaine T, Calder AA, Kelly RW, Riley SC (2009) Elafin (SKALP/Trappin-2/proteinase inhibitor-3) is produced by the cervix in pregnancy and cervicovaginal levels are diminished in bacterial vaginosis. Reprod Sci 16:1125–1134

Stolk J, Camps J, Feitsma HI, Hermans J, Dijkman JH, Pauwels EK (1995) Pulmonary deposition and disappearance of aerosolised secretory leucocyte protease inhibitor. Thorax 50:645–650

Subramaniyam D, Hollander C, Westin U, Erjefalt J, Stevens T, Janciauskiene S (2011) Secretory leukocyte protease inhibitor inhibits neutrophil apoptosis. Respirology 16(2):300–307

Suzumori N, Sato M, Ikuta K, Suzumori K (2001) Secretory leukocyte protease inhibitor in ovarian endometriomas following GnRH agonist therapy. Obstet Gynecol 97:561–566

Taggart CC, Lowe GJ, Greene CM, Mulgrew AT, O'Neill SJ, Levine RL, McElvaney NG (2001) Cathepsin B, L, and S cleave and inactivate secretory leucoprotease inhibitor. J Biol Chem 276:33345–33352

Taggart CC, Greene CM, McElvaney NG, O'Neill S (2002) Secretory leucoprotease inhibitor prevents lipopolysaccharide-induced IkappaBalpha degradation without affecting phosphorylation or ubiquitination. J Biol Chem 277:33648–33653

Tian X, Shigemasa K, Hirata E, Gu L, Uebaba Y, Nagai N, O'Brien TJ, Ohama K (2004) Expression of human kallikrein 7 (hK7/SCCE) and its inhibitor antileukoprotease (ALP/SLPI) in uterine endocervical glands and in cervical adenocarcinomas. Oncol Rep 12:1001–1006

Tomee JF, Hiemstra PS, Heinzel-Wieland R, Kauffman HF (1997) Antileukoprotease: an endogenous protein in the innate mucosal defense against fungi. J Infect Dis 176:740–747

Tremblay GM, Sallenave JM, Israel-Assayag E, Cormier Y, Gauldie J (1996) Elafin/elastase-specific inhibitor in bronchoalveolar lavage of normal subjects and farmer's lung. Am J Respir Crit Care Med 154:1092–1098

Tremblay GM, Vachon E, Larouche C, Bourbonnais Y (2002) Inhibition of human neutrophil elastase-induced acute lung injury in hamsters by recombinant human pre-elafin (trappin-2). Chest 121:582–588

Tsai WC, Strieter RM, Mehrad B, Newstead MW, Zeng X, Standiford TJ (2000) CXC chemokine receptor CXCR2 is essential for protective innate host response in murine Pseudomonas aeruginosa pneumonia. Infect Immun 68:4289–4296

Tseng CC, Tseng CP (2000) Identification of a novel secretory leukocyte protease inhibitor-binding protein involved in membrane phospholipid movement. FEBS Lett 475:232–236

Tsukishiro S, Suzumori N, Nishikawa H, Arakawa A, Suzumori K (2005) Use of serum secretory leukocyte protease inhibitor levels in patients to improve specificity of ovarian cancer diagnosis. Gynecol Oncol 96:516–519

Turpin JA, Schaeffer CA, Bu M, Graham L, Buckheit RW Jr, Clanton D, Rice WG (1996) Human immunodeficiency virus type-1 (HIV-1) replication is unaffected by human secretory leukocyte protease inhibitor. Antiviral Res 29:269–277

van Wetering S, van der Linden AC, van Sterkenburg MA, de Boer WI, Kuijpers AL, Schalkwijk J, Hiemstra PS (2000) Regulation of SLPI and elafin release from bronchial epithelial cells by neutrophil defensins. Am J Physiol Lung Cell Mol Physiol 278:L51–L58

Velarde MC, Iruthayanathan M, Eason RR, Zhang D, Simmen FA, Simmen RC (2006) Progesterone receptor transactivation of the secretory leukocyte protease inhibitor gene in Ishikawa endometrial epithelial cells involves recruitment of Kruppel-like factor 9/basic transcription element binding protein-1. Endocrinology 147:1969–1978

Wang X, Li X, Xu L, Zhan Y, Yaish-Ohad S, Erhardt JA, Barone FC, Feuerstein GZ (2003) Up-regulation of secretory leukocyte protease inhibitor (SLPI) in the brain after ischemic stroke: adenoviral expression of SLPI protects brain from ischemic injury. Mol Pharmacol 64:833–840

Wang N, Thuraisingam T, Fallavollita L, Ding A, Radzioch D, Brodt P (2006) The secretory leukocyte protease inhibitor is a type 1 insulin-like growth factor receptor-regulated protein that protects against liver metastasis by attenuating the host proinflammatory response. Cancer Res 66:3062–3070

Westin U, Lundberg E, Wihl JA, Ohlsson K (1999a) The effect of immediate-hypersensitivity reactions on the level of SLPI, granulocyte elastase, alpha1-antitrypsin, and albumin in nasal secretions, by the method of unilateral antigen challenge. Allergy 54:857–864

Westin U, Polling A, Ljungkrantz I, Ohlsson K (1999b) Identification of SLPI (secretory leukocyte protease inhibitor) in human mast cells using immunohistochemistry and in situ hybridisation. Biol Chem 380:489–493

Wex T, Treiber G, Nilius M, Vieth M, Roessner A, Malfertheiner P (2004) Helicobacter pylori-mediated gastritis induces local downregulation of secretory leukocyte protease inhibitor in the antrum. Infect Immun 72:2383–2385

Wiedow O, Schroder JM, Gregory H, Young JA, Christophers E (1990) Elafin: an elastase-specific inhibitor of human skin. Purification, characterization, and complete amino acid sequence. J Biol Chem 265:14791–14795

Wiedow O, Young JA, Davison MD, Christophers E (1993) Antileukoprotease in psoriatic scales. J Invest Dermatol 101:305–309

Wiedow O, Harder J, Bartels J, Streit V, Christophers E (1998) Antileukoprotease in human skin: an antibiotic peptide constitutively produced by keratinocytes. Biochem Biophys Res Commun 248:904–909

Wilkinson TS, Dhaliwal K, Hamilton TW, Lipka AF, Farrell L, Davidson DJ, Duffin R, Morris AC, Haslett C, Govan JR et al (2009) Trappin-2 promotes early clearance of Pseudomonas aeruginosa through CD14-dependent macrophage activation and neutrophil recruitment. Am J Pathol 174:1338–1346

Willems LN, Otto-Verberne CJ, Kramps JA, ten Have-Opbroek AA, Dijkman JH (1986) Detection of antileukoprotease in connective tissue of the lung. Histochemistry 86:165–168

Williams SE, Brown TI, Roghanian A, Sallenave JM (2006) SLPI and elafin: one glove, many fingers. Clin Sci (Lond) 110:21–35

Wright CD, Kennedy JA, Zitnik RJ, Kashem MA (1999a) Inhibition of murine neutrophil serine proteinases by human and murine secretory leukocyte protease inhibitor. Biochem Biophys Res Commun 254:614–617

Wright CD, Havill AM, Middleton SC, Kashem MA, Lee PA, Dripps DJ, O'Riordan TG, Bevilacqua MP, Abraham WM (1999b) Secretory leukocyte protease inhibitor prevents allergen-induced pulmonary responses in animal models of asthma. J Pharmacol Exp Ther 289:1007–1014

Xuan Q, Yang X, Mo L, Huang F, Pang Y, Qin M, Chen Z, He M, Wang Q, Mo ZN (2008) Expression of the serine protease kallikrein 7 and its inhibitor antileukoprotease is decreased in prostate cancer. Arch Pathol Lab Med 132:1796–1801

Yang J, Zhu J, Sun D, Ding A (2005) Suppression of macrophage responses to bacterial lipopolysaccharide (LPS) by secretory leukocyte protease inhibitor (SLPI) is independent of its anti-protease function. Biochim Biophys Acta 1745:310–317

Ying QL, Kemme M, Saunders D, Simon SR (1997) Glycosaminoglycans regulate elastase inhibition by oxidized secretory leukoprotease inhibitor. Am J Physiol 272:L533–L541

Zani ML, Baranger K, Guyot N, Dallet-Choisy S, Moreau T (2009) Protease inhibitors derived from elafin and SLPI and engineered to have enhanced specificity towards neutrophil serine proteases. Protein Sci 18:579–594

Zhu J, Nathan C, Ding A (1999) Suppression of macrophage responses to bacterial lipopolysaccharide by a non-secretory form of secretory leukocyte protease inhibitor. Biochim Biophys Acta 1451:219–223

Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, Wahl SM, Lacomis L, Erdjument-Bromage H, Tempst P, Wright CD et al (2002) Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. Cell 111:867–878