WAP domain proteins as modulators of mucosal immunity

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Abstract

WAP (whey acidic protein) is an important whey protein present in milk of mammals. This protein has characteristic domains, rich in cysteine residues, called 4-DSC (four-disulfide core domain). Other proteins, mainly present at mucosal surfaces, have been shown to also possess these characteristic WAP-4-DSC domains. The present review will focus on two WAP-4-DSC containing proteins, namely SLPI (secretory leucocyte protease inhibitor) and trappin-2/elafin. Although first described as antiproteases able to inhibit in particular host neutrophil proteases [NE (neutrophil elastase), cathepsin-G and proteinase-3] and as such, able to limit maladaptive tissue damage during inflammation, it has become apparent that these molecules have a variety of other functions (direct antimicrobial activity, bacterial opsonization, induction of adaptive immune responses, promotion of tissue repair, etc.). After providing information about the ‘classical’ antiprotease role of these molecules, we will discuss the evidence pertaining to their pleiotropic functions in inflammation and immunity.

Introduction

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. In the present review, we focus on two WAPs (whey acid proteins), SLPI (secretory leucocyte protease inhibitor) and trappin-2/elafin, that are responsible for many of the homeostatic and host defence functions of these uniquely situated viscous sols. Additionally, the present review discusses our increasing understanding of these two molecules from a simple role as local antibiotics that respond to pathogen invasion to major orchestrators of cellular interplay, host defence mechanisms and immune homeostasis.

SLPI

Gene and protein

This molecule has been isolated from different body compartments under different names: BMI (bronchial mucus inhibitor), HUSI-I (human seminal inhibitor I), CUSI (cervical mucus inhibitors), ALP (antileukoprotease), SLPI and MPI (mucus proteinase inhibitor) [1]. Human and mouse SLPI are relatively conserved at both the genomic and protein levels. The human gene is composed of ∼2.6 kb and is organized into four exons, which transcribes a 399 bp mRNA to a 132 amino acid protein [2]. Similarly, the mouse gene is composed of four exons, which transcribes a 396 bp coding sequence to a 131 amino acid protein [3] and the crystal structure of human SLPI is known [4]. Each domain is relatively conserved, is cysteine rich and has high homology with the WAP genes found in rodent milk [5]. The molecule is indeed composed of two different WAP domains, and the antiprotease activity is situated in the C-terminal one [6–8].

Targets, receptors and binding interactions

The binding interactions of SLPI are not limited to forming 1:1 molar complexes with proteases such as NE (neutrophil elastase), chymotrypsin and trypsin. Extracellular binding interactions include those to the pathogen-associated molecular patterns LPS (lipopolysaccharide) [9], mannan-capped lipoarabinomannans and phosphatidylinositol mannoside [10] together with numerous GAGs (glycosaminoglycans) [11,12] and classes of immunoglobulin [13]. Intracellular binding interactions include binding to DNA [14] and
Expression and distribution in cells and tissues
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 [19–23] as well as in the alveolar septa [25,26]. SLPI expression is not, however, confined to the lungs and has also been demonstrated in the reproductive mucosa, seminal and amniotic fluids [27–31], as well as in salivary glands [32–34], middle ear [35,36], maxillary sinus [37], intestine [38], colon [39], human skin [40], nasal secretions [41], peritoneal fluid [42], stomach [43], gingival crevicular fluid [34,44] and cornea [45].

Induction and regulation in response to physiological and pathological stimuli
Cell culture studies have identified a variety 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. [46] and Sallenave et al. [47] found that 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, TNFα (tumour necrosis factor α) and IL-8, induced little or no SLPI transcript levels [46]. Using Clara cells and alveolar type II cells and measuring SLPI protein as an endpoint, we showed both a constitutive and IL-1β- or TNFα-induced production of SLPI [47]. Interestingly, glucocorticoids can induce SLPI transcript in human airway epithelial cells with a descending potency of fluticasone>triamcinolone>or = dexamethasone>methylprednisolone>hydrocortisone [48]. This study also demonstrated that NE 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 up-regulate SLPI mRNA and protein through a mechanism involving its transactivation of the SLPI gene through the PR (progesterone receptor), via induction of the BTEB1 (basic transcription element binding protein-1) gene and co-recruitment of BTEB1 and the PR co-activator CBP (cAMP-response element binding protein) to the SLPI promoter [49,50].

Antimicrobial actions
The antimicrobial activity of SLPI (and trappin-2/elafin, see below) has been extensively reviewed elsewhere [51–53] and we therefore refer the reader to these reviews for further information.

Disease

Phenotype of SLPI-deficient mice
SLPI-deficient mice have impaired cutaneous wound healing with increased inflammation and NE activity with enhanced local production of TGFβ (transforming growth factor β) [54]. In a similar model, Zhu et al. [55] have suggested an alternative pathway dependent on proepithelin and its cleaved product epithelin that have opposite effects during inflammation. Intriguingly, TGFβ expression is increased in a cutaneous model [55], but decreased in the oral model [56], pointing to the ability of SLPI to improve wound healing, but by very different local mechanisms. The link is particularly pertinent in a cardiac transplant model of ischaemia/reperfusion injury, where SLPI-deficient hearts had profound abnormalities in early contraction and exhibited high protease expression and TGFβ expression [57]. Interestingly, systemic SLPI could not rescue this phenotype, whereas including SLPI in the preservation solution prior to transplantation reversed the phenotype suggesting that a dual inhibitory effect on protease and TGFβ expression might be the underlying mechanism [57].

Modulation of inflammation
The late 1990s saw a dramatic change in the way SLPI was viewed. Before then SLPI was considered an antimicrobial molecule with potent antiprotease activity, but the seminal work of Jin et al. [58] in macrophages showed that SLPI could suppress LPS-induced activation of NF-κB (nuclear factor κB) and the resultant synthesis of TNFα/nitric oxide, suggesting that SLPI was an important immunomodulatory molecule. In a later paper, the same group also demonstrated that SLPI induction by LPS was an early (∼30 min) and prolonged response (remaining at 72 h). Finally, the Gram-positive cell wall constituent lipoteichoic acid could also stimulate SLPI production [59]. There are multiple mechanisms responsible for these effects including the ability of SLPI to inhibit NF-κB activation by stabilization of IRAK, IκBα and IκBβ proteins despite increasing the amount of phosphorylated and polyubiquinated IκBα [14,15]. This is supported by the anti-inflammatory activity of a non-secretable form of SLPI when transfected into macrophages [60]. Others have suggested that SLPI can prevent the p65 subunit of NF-κB binding to its consensus sequence in the promoter 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 [14], whereas another study showed that site-directed mutants of the oxidizable methionine residue (Met72) could still inhibit LPS-induced TNFα and nitric oxide responses [61]. In vivo Mulligan et al. [62] have suggested that the Leu72 residue that is essential in determining antiprotease function is critical for SLPI immunomodulatory activity. Also, adenosiral gene delivery of SLPI can protect against ischaemic brain injury [63] and has also been shown to attenuate NF-κB-dependent inflammatory responses to atherogenic stimuli [64]. Consistent with these findings...
recombinant SLPI administered systemically could suppress inflammation associated with joint damage [65] and attenuate hepatic ischaemia/reperfusion injury [66] in rats and mice respectively. Furthermore, local delivery of SLPI to ovine lung by aerosol was shown to prevent allergen-induced pulmonary responses in a model of asthma [67] and topical administration to the eye in guinea pigs suppressed the recruitment of eosinophils and decreased the severity of conjunctivitis [68]. It has been recently shown also that SLPI may play a role in adaptive immunity through maintenance of mucosal tolerance threshold [69,70].

Although the findings described above suggest that SLPI can be considered as a ‘tolerogenic’ immunomodulatory molecule, other data support an immune-stimulatory role for SLPI: Gomez et al. [10] have shown that SLPI may act as a pattern recognition receptor for mycobacteria that acts to stimulate phagocytosis. Thus it seems that the pro- or anti-inflammatory actions of SLPI are dependent on the type of pathogen and on the progress of the inflammatory response.

Trappin-2/elafin

Gene and protein
As for SLPI (see above), trappin-2/elafin protein was purified from human lung secretions and skin tissues in the 1980s and 1990s under a variety of names, such as elafin, BSI-E, ESI (elastase-specific inhibitor), PELESI (precursor of elafin-ESI), SKALP (skin-derived ALP) [71–75]. The trappin-2 gene encodes a secreted 9.9 kDa non-glycosylated 95-residue cationic protein [76,77] comprising an N-terminal domain (38 residues) or cementoin domain [78] and a C-terminal inhibitory WAP-type domain (57 residues) [79]. 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. The C-terminal WAP domain is structurally similar to the SLPI domains (approximately 40% sequence identity with each SLPI domain). Trappin-2 is encoded by the PI3 gene in the same chromosome region 2q12-13 as the SLPI gene and is composed of three exons spanning approximately 2 kb. Trappin-2 is translated with a signal peptide that is cleaved during secretion and proteolytically processed in inflammatory secretions 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 [80].

Targets, receptors and binding interactions
Trappin-2 inhibits NE, porcine pancreatic elastase and proteinase-3, but does not inhibit cathepsin G, trypsin or chymotrypsin and, hence, has a more restricted spectrum of inhibition than SLPI [51]. In recent years, trappin-2 has increasingly been shown to display functions beyond its protease inhibition (reviewed in [51–53]) such as antimicrobial and immunomodulatory activities, as will be discussed below.

Expression and distribution in cells and tissues
As mentioned above for SLPI, trappin-2 is mainly derived from the mucosae, and in particular from epithelial cells and cells lining body cavities, as well as inflammatory cells [51–53]. The same mucosal surfaces producing SLPI (lung, skin, genital tract, etc.) have also been shown to be a source of trappin-2. We have also shown recently, in murine models of trappin-2 overexpression (using adenovirus constructs and elafin-transgenic mice) that this molecule is highly protective against gut-colitis inflammation [81]. In addition, these results echo clinical findings demonstrating a reduced expression of this molecule in human samples from IBD (inflammatory bowel disease) patients [81–83].

Induction and regulation in response to physiological and pathological stimuli
The regulation of trappin-2 expression by healthy and inflamed tissues has attracted much attention. In vitro, bronchial, alveolar epithelial cells and keratinocytes produce little trappin-2 protein, but production is greatly increased in the presence of IL-1β and TNFα [47,84]. The c-Jun, p38 MAPK (mitogen-activated protein kinase) and NF-κB pathways are thought to be implicated in these responses [85–87]. Interestingly, the cytokine-mediated increase in trappin-2 production by epithelial cells is greater than SLPI production [47], suggesting that, whereas SLPI provides a ‘baseline’ shield in resting conditions, elafin might be more important during an inflammatory challenge to the lungs. As for SLPI, trappin-2/elafin mRNA expression is increased by free NE in bronchial epithelial cells that is found at high concentrations (micromolar levels) at inflammatory sites [88,89].

Modulation of inflammation and of the adaptive immune system
Like SLPI, trappin-2 has been shown to be able to modulate maladaptable inflammation [64,90,91]. However, the fact that SLPI and trappin-2 respond differently from prototypical pro-inflammatory cytokines such as IL-1β and TNFα suggested that they are not ‘interchangeable’ and redundant molecules, but may also have more specific functions. Indeed, overexpression of the human trappin-2 gene in the murine lungs can result in an increased influx of inflammatory cells in response to infection/inflammation [91–95], and the interaction of trappin-2 with LPS results in an augmentation of the LPS-induced TNFα response in a murine macrophage cell line [96]. When dissecting this mechanism, Wilkinson et al. [95] suggested that the ‘LPS/bacteria-opsonizing effect’ of trappin-2 may be acting through membrane CD14, both in vitro and in vivo.

In addition, 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 a trappin-2-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 trappin-2 [94]. The Th1 skewing effect of trappin-2 demonstrated is likely to be mediated through the increase in numbers and/or activation status of lung APCs (antigen-presenting cells), as elafin overexpressers exhibited higher numbers of total lung CD11c<sup>+</sup> cells and CD11c<sup>+</sup> MHCII<sup>+</sup> cells [DCs (dendritic cells)], expressing higher levels of the B7 family co-stimulatory molecules CD80 and CD86 (indicative of activated DCs). In accordance with the increase in the number of activated DCs, increased levels of pro-inflammatory cytokines IL-12, TNFα and IFNγ (interferon γ) were observed in bronchoalveolar lavage fluids of trappin-2 overexpressers [94]. Clinical evidence to support a role for trappin-2 in the augmentation of a Th1 phenotype is also available, e.g., 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 [97] and psoriatic skin [98].

More recently, human γδ T-cells have been shown to produce trappin-2/elafin (both mRNA and protein) upon stimulation with supernatant of Pseudomonas aeruginosa grown in culture. Between 2 and 5% of CD3<sup>+</sup> γδ T-cells in the peripheral blood express the γδ TCR (T-cell receptor) instead of the conventional αβ TCR. In contrast with the peripheral blood, γδ T-cells represent a major T-cell population in mucosal surfaces such as the small intestine where 20–30% of local T-cells are γδ T-cells [99,100]. γδ T-cells have the capacity to act as APCs [101] and to secrete antimicrobial effector molecules such as granulysin [102] and the cationic antimicrobial peptide LL37/cathelicidin that is expressed in the peripheral blood, instead of the conventional cationic antimicrobial peptide LL37/cathelicidin that is secreted by keratinocytes [103,104]. Owing 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 and antigen-presenting capacity), γδ T-cells are thought to bridge innate and adaptive immunity [100]. They may, through secretion of mediators such as trappin-2/elafin and LL37/cathelicidin, contribute to the recruitment of neutrophils or the opsonization of the pathogens at mucosal surfaces.

**Conclusion**

Many current studies are uncovering the fact that the WAP-4-DSC (four-disulphide core domain) proteins SLPI and trappin-2/elafin, once thought of as ‘monodimensional’ antiproteases, are in reality pleiotropic molecules with a variety of immunomodulatory functions, and should be considered as important guardians of mucosal surfaces. These molecules can exhibit, depending on the pathogen or the inflammatory milieu, either ‘pro’- or ‘anti’-inflammatory functions. Although what switches one or the other is currently barely known, it will no doubt be the subject of numerous future studies, which should help us in dissecting even further the role of these fascinating WAP-like molecules.

**References**


