SLPI and elafin: multifunctional antiproteases of the WFDC family

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Abstract
SLPI (secretory leucoprotease inhibitor) and elafin represent the archetypal members of the WFDC [WAP (whey acidic protein) four disulfide core] family of proteins, and were originally characterized as protease inhibitors but have since been shown to possess a wider repertoire of activities. These functions include antimicrobial and immunomodulatory properties, suggesting that these proteins may play key roles in the innate immune response, and indicate the potential to develop some of these proteins as novel therapeutics. Susceptibility to host and bacterial protease cleavage may, however, limit the efficacy of recombinant protein therapies in diseases with a high protease burden such as CF (cystic fibrosis) lung disease. To overcome this problem, further refinement of the native proteins will be required to provide effective treatment strategies.

Introduction
WFDC [WAP (whey acidic protein) four-disulfide core] motifs are composed of approximately 50 amino acids including eight conserved cysteine residues at defined positions, which form four disulfide bonds [1]. This motif is present in a number of other small, secreted proteins, including elafin and eppin, which form the WFDC family of proteins. The majority of corresponding genes for these proteins are found localized on human chromosome 20q12-13; however, some examples of non-chromosome 20 WFDC proteins have been identified including WFIKKN and ps20 on chromosome 16 and WFIKKKN2 on chromosome 17 [2,3]. Although a total of 18 WFDC proteins have been identified to date [3], SLPI (secretory leucoprotease inhibitor) and elafin remain the best characterized of the WFDC family of proteins. Initially, it was thought that the biological role of this locus on chromosome 20 was the regulated inhibition of a wide spectrum of microbial and leucocyte proteolytic enzymes. However, although SLPI and elafin have been characterized as potent antiproteases, subsequent studies have found that the protease inhibitory domain of some WFDC proteins is very small, raising doubts about their actual antiprotease potential [3]. In addition to the antiprotease activity, there is increasing published data demonstrating that WFDC domains may also possess other properties such as antibacterial, anti-fungal, antiviral and anti-inflammatory functions.

SLPI and elafin
Human SLPI was first identified as an 11.7 kDa cationic protein isolated from parotid secretions by Thompson and Ohlsson in 1986 [4]. In this work, SLPI was isolated, its amino acid sequence determined and its properties characterized with a noted high affinity for leucocyte elastase leading to its name being leucoprotease inhibitor [4]. SLPI expression has also been identified in the lung of chronically infected patients [5–8]. SLPI consists of 107 amino acids possessing two homologous WFDC domains [4,9,10].

The elafin precursor protein trappin-2 (pre-elafin) is cleaved to form the mature 6 kDa protein elafin. Trappin-2 is 117 amino acids in length, comprising a 22-residue signal peptide [11], followed by a cementoin domain and a C-terminal WFDC domain homologous with the C-terminal WFDC domain of SLPI. The cementoin domain contains multiple GQDPVK sequences that act as a transglutaminase substrate, which permits cross-linking of the inhibitor to ECM (extracellular matrix) proteins [10,14]. Elafin expression has been identified in bronchial secretions and in the skin [11–14].

Antiprotease function
Despite the multifaceted nature of SLPI and elafin, both proteins are regarded primarily as protease inhibitors, showing potent but differential inhibitory specificities. Both proteins are physiologically important NE (neutrophil elastase) inhibitors. In addition, SLPI has also been shown to inhibit cathepsin G but not proteinase 3 [15,16], yet elafin is a known proteinase-3 inhibitor but cannot inhibit cathepsin G [17,18]. Eisenberg et al. [15] demonstrated that SLPI’s antiprotease activity was exclusive to the C-terminal WAP domain, with Leu22 identified as the P1 active site residue responsible for both elastase and chymotrypsin inhibitory activity.

Antibacterial activity
In addition to their antiprotease activity, both SLPI and elafin have a broad range of antibacterial activity. It is thought that this activity of both SLPI and elafin is mediated via their cationic charge (+12 and +3 respectively), which,
like many cationic antimicrobial proteins, allows them to destabilize bacterial membranes [19]. SLPI is the third most abundant antimicrobial protein of the upper airways with levels in healthy lung epithelial lining fluid as high as 10 μg/ml [20,21]. Both SLPI and elafin have been shown to have antibacterial activity against Gram-positive and Gram-negative species. SLPI has been found to be effective against pathogenic species common in the upper airways such as Pseudomonas aeruginosa and Staphylococcus aureus, in addition to Staphylococcus epidermidis and Escherichia coli [19,22,23]. While elafin also exhibits bactericidal activity against Ps. aeruginosa and S. aureus [24,25], no activity was observed against E. coli [26]. The N-terminal domain of SLPI was shown to possess greater antibacterial activity against both Gram-positive and Gram-negative species in comparison with the C-terminal domain; however, maximal bactericidal activity required the presence of both domains [22]. Although mature elafin contains only one WFDC domain, the precursor protein trappin-2 shows greater antibacterial activity than the mature form [26]. It was further demonstrated that both the cementoin and WFDC domains possess significant independent antibacterial activity [26].

SLPI and elafin have also been shown to possess antifungal activity against Aspergillus fumigatus, a common airway pathogen [27], and Candida albicans, which is most commonly associated with the epidermis [28]. This activity was found to be attenuated by both high salt and heparin, suggesting this activity is attributable to cationic charge [27].

**Antiviral activity**

Another potentially important antimicrobial activity of SLPI was identified by McNeely et al. [20], who found that SLPI inhibited the ability of HIV-1 to infect macrophages. This was characterized further by Ma et al. [29], who found SLPI could competitively bind to annexin II, an important cellular co-factor that facilitates HIV infection. This is supported by observational studies showing that heightened SLPI expression is associated with decreased HIV-1 infection [30,31]. In comparison with SLPI, much less is known about the antiviral activity of elafin; however, elevated levels of elafin in the vaginal tract have recently been associated with increased resistance to HIV infection [32]. Given the structural conservation between elafin’s WFDC domain and the second C-terminal WFDC domain of SLPI it has been suggested that elafin is also capable of interacting with key cell surface cofactors to prevent cellular invasion by HIV rather than a direct interaction with the virus [32].

**Immunomodulatory activity**

Both SLPI and elafin have been shown to inhibit the inflammatory response both in vitro and in vivo. In vitro studies using human monocytes found that LPS (lipopolysaccharide) and LTA (lipoteichoic acid)-induced activation of NF-κB (nuclear factor κB) was inhibited by both SLPI and elafin [33]. SLPI mediates its anti-inflammatory activity via both intracellular and extracellular mechanisms [18,34,35]. Outside of the cell, SLPI can bind and neutralize LPS thereby preventing TLR (Toll-like receptor) activation [36]. Intracellularly, SLPI can affect NF-κB activity in the nucleus of monocytes, where it competes with p65 for binding to NF-κB binding sites in the promoter region of genes such as IL-8 (interleukin 8) and TNFα (tumour necrosis factor α) [37]. Consequently, binding of p65 is prevented, and transcription and subsequent production of these pro-inflammatory cytokines are inhibited. In addition, SLPI also exerts effects in the cytoplasm of treated cells by preventing degradation of key regulatory proteins such as IκB (inhibitor of NF-κB) α, IκBβ and IRAK (IL-1 receptor-associated kinase), thereby preventing subsequent NF-κB activation [34,35]. An SLPI variant in which the scissile bond methionine residue (Leu72–Met73) was oxidized showed significantly attenuated anti-inflammatory and antiprotease activities suggesting that the anti-inflammatory activity exerted by SLPI in the cytoplasm may be mediated via its antiprotease activity [34,38,39].

As with SLPI, both extracellular and intracellular mechanisms of action are known to contribute to the overall anti-inflammatory activity exerted by elafin. In the extracellular environment, elafin can bind and neutralize LPS in a charge-based interaction similar to SLPI [40]. Interestingly elafin has also been shown to play a role in the resolution of inflammation by inhibiting NE-mediated cleavage of CD14 [18,19]. Intracellularly, Butler et al. [41] found that elafin exerts an inhibitory effect on the cytoplasmic ubiquitin–proteasome pathway, as was evident from the accumulation of ubiquitinated IRAK-1 and IκBα/β in LPS-activated monocyctic cells. Furthermore, activation of the AP-1 (activator protein 1) pathway components c-Jun and JNK (c-Jun N-terminal kinase) by LPS was also inhibited by elafin [41].

The immunomodulatory activity of SLPI has also been demonstrated in an in vivo model of SLPI deficiency. Nakamura et al. [42] demonstrated that SLPI knockout mice were more susceptible to LPS-induced endotoxin shock and showed a higher mortality than wild-type mice. In addition, a clinical study carried out by McElvaney et al. [43,44] in which aerosolized recombinant human SLPI was given to CF (cystic fibrosis) patients found that SLPI effectively inhibited NE activity while also retaining its immunoregulatory function by reducing both IL-8 and neutrophil levels in CF epithelial lining fluid.

In a murine model of LPS-induced inflammation, recombinant elafin significantly inhibited MIP-2 (macrophage inflammatory protein–2) and KC (keratinocyte chemoattractant) levels, neutrophil influx and protease activity in BAL (broncho-alveolar lavage) fluid of treated mice [45]. In addition, by overexpressing elafin in mice, Sallenave et al. [46] demonstrated further the anti-inflammatory effect of elafin in vivo, with the observation of lower serum-to-BAL ratios of pro-inflammatory cytokines such as TNFα, MIP-2 and monocyte chemoattractant protein 1 than wild-type mice following LPS challenge. However, an increased inflammatory cell influx was detected indicating the possible priming of innate responses by elafin [46].
SLPI and elafin cleavage susceptibility

Overall, the evidence to date suggests that the function of SLPI and elafin is to protect local tissue against the detrimental consequences of inflammation not only as a result of their anti-inflammatory properties but also via their antiprotease and antimicrobial properties. In chronic lung diseases such as CF and COPD (chronic obstructive pulmonary disease), which are characterized by neutrophil influx, there is a significantly increased protease burden in the upper airways. In healthy individuals, the mucosal surface of the airways is held in a delicate protease–antiprotease balance. Host proteases such as NE, cathepsin G and proteinase-3 secreted by neutrophils and, to a lesser extent, mononuclear and epithelial cells, are held in check by serine antiproteases secreted by neutrophils and, to a lesser extent, mononuclear and epithelial cells, are held in check by serine antiproteases such as α1-antitrypsin, SLPI and elafin. However, in CF, this balance is disrupted in favour of the proteases, resulting in deleterious effects such as up-regulated inflammation and tissue remodelling through degradation of the ECM.

Expression of both SLPI and elafin are also up-regulated during inflammation, with in vitro studies showing pro-inflammatory cytokines TNFα and IL-1β significantly increasing expression [3,47]. However, as previously mentioned, SLPI is susceptible to inactivation by oxidation of methionine residues [38]. In addition, cathespins-mediated SLPI cleavage has been implicated in the unregulated protease remodelling in emphysema, with cathespins B, L and S shown to cleave the active site loop of SLPI resulting in diminished antiprotease activity [21]. Dysregulated protease activity in the lungs of patients with community-acquired pneumonia appears to overwhelm the protease–antiprotease balance, also resulting in inactivation of SLPI [39]. Recent work on the status of SLPI in CF BAL fluid found that decreased levels of SLPI present in the Pseudomonas-infected CF lung were due to degradation by NE [48]. Cleavage of SLPI was localized to two sites in the N-terminal WFDC domain and decreased its ability to bind both LPS and NF-κB consensus binding sites [48]. As the antiprotease activity of SLPI is located in the C-terminal domain, N-terminal cleaved SLPI retains serine antiprotease activity against proteases such as cathepsin G [48].

Similar to SLPI, levels of elafin were also found to be significantly decreased as a result of NE-mediated proteolysis in sputum from CF patients with Ps. aeruginosa infection [49]. This is consistent with greater levels of NE also observed in Ps. aeruginosa-infected sputum in comparison with uninfected patients samples [49]. Cleaved fragments of elafin were found to retain antiprotease activity; however, cleavage significantly attenuated LPS-binding capacity and the ability of elafin to act as a substrate for transglutaminase [49]. Elafin is also susceptible to cleavage by the Ps. aeruginosa metalloproteases, pseudolysin and aeruginolysin [50]. Both enzymes cleave the protease–binding loop of elafin; however, the resultant cleavage products retained differential activities [50]. Following pseudolysin treatment, elafin fragments retained antibacterial activity against Ps. aeruginosa but lost their antiprotease activity. In contrast, aeruginolysin failed to inactivate the antiprotease activity of elafin against NE; however, aeruginolysin cleavage prevented elafin binding to ECM components through transglutamination [50].

Conclusions

SLPI and elafin are multifaceted proteins playing important roles in the resolution of inflammation. The potential for development of these proteins as therapeutics has already been demonstrated in studies involving animal models [42,45,46] and in clinical trials [43,44]. While potential recombinant SLPI and elafin therapies hold promise for the future, these efforts may be hampered by the high protease burden characteristic of the diseases where such therapeutics may be of most benefit. Future work may focus on the development of protease resistant variants of SLPI and elafin that retain the important multifunctional activities of these proteins.

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References


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