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Suppression of macrophage responses to bacterial lipopolysaccharide (LPS) by secretory leukocyte protease inhibitor (SLPI) is independent of its anti-protease function

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Abstract

Secretory leukocyte protease inhibitor (SLPI), a potent serine protease inhibitor, has been shown to suppress macrophage responses to bacterial lipopolysaccharide (LPS). SLPI contains two topologically superimposable domains. Its C-terminal domain binds and inhibits target proteases. It is not clear whether SLPI's anti-protease function plays a role in the LPS-inhibitory action of SLPI. Four single amino acid substitution mutants of SLPI, M73G, M73F, M73E and M73K, were generated. Wild type SLPI is a potent inhibitor of chymotrypsin and elastase. Mutants M73G and M73F selectively lost inhibitory function towards chymotrypsin and elastase, respectively, whereas mutants M73K and M73E inhibited neither elastase nor chymotrypsin. Macrophage cell lines were established from RAW264.7 cells to stably express each SLPI mutant. Expression of the SLPI protease inhibition mutants suppressed NO and TNF production in response to LPS in a similar fashion as wild type SLPI. Expression of truncated forms of SLPI, containing only its N-terminus or its C-terminus, was similarly sufficient to confer inhibition of LPS responses. Thus, the LPS-inhibitory action of SLPI is independent of its anti-protease function.

Keywords: Macrophage; SLPI mutant; Lipopolysaccharide; Inflammation

1. Introduction

Human SLPI is a 12-kDa non-glycosylated single chain protein found in blood [1,2] and mucosal secretions including saliva [3], seminal plasma [4] and cervical [5], nasal and bronchial mucus [6,7]. SLPI is a potent inhibitor of the neutrophil-derived serine proteases elastase and cathepsin G [3,8,9] and accounts for 80–90% of the elastase-inhibitory capacity of bronchial secretions [7] and 97% of that of nasal secretions [10]. SLPI is produced by epithelia, macrophages and neutrophils. SLPI expression is up-regulated in vivo in a number of pathological situations such as septic shock [1], pneumonia [11], emphysema [12] and chronic Chagasic cardiomyopathy [13]. SLPI is thought to protect the host from excessive tissue damage by leukocyte proteases released during inflammation.

Mouse and human SLPI are 68% identical at the nucleotide level and 60% identical at the amino acid level, with positional conservation of all 16 cysteine residues [14]. Besides functioning as potent protease inhibitors, human and mouse SLPI also act as modulators of innate immune responses of macrophages [14–16], neutrophils [1,17] and endothelial cells [18]. For example, using differential display analysis, we cloned mouse SLPI as a macrophage product that is preferentially expressed in a cell line refractory to LPS stimulation [14]. Anti-inflammatory effects of SLPI have been observed using either exoge-

Abbreviations: IkB, inhibitor for NF-κB; IKK, IkB kinase; IRAK, IL-1 receptor associated kinase; MyD88, myeloid differentiation antigen 88; SLPI, secretory leukocyte protease inhibitor; TIR, toll IL-1 receptor; TIRAP, TIR domain-containing adapter protein; TRAF, TNF receptor associated factor; TRAM, TRIF-related adapter molecule; TRIF, TIR containing adapter inducing IFNβ

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nously administered recombinant proteins or endogenous gene delivery systems. Forced expression of SLPI in macrophages confers an LPS-hyporesponsive phenotype [14–16]. It is not clear how, if at all, SLPI's anti-protease function contributes to its LPS-antagonistic activity.

In this paper, we report the generation of macrophage cell lines that stably produce full-length, truncated or anti-protease active-site mutated SLPI. Compared to mock transfectants, cells expressing anti-protease inactive mutants of SLPI or the N-terminal domain of SLPI, which possesses no anti-protease activity, are as hyporesponsive to LPS stimulation as macrophages expressing wild type SLPI. This result demonstrates that the LPS suppressive function of SLPI is independent of its known anti-protease functions.

2. Materials and methods

2.1. Purification of mSLPI and its mutants

The rSLPI cDNA encoding the mature secreted protein was generated by PCR from a macrophage cDNA library [14] and subcloned into pT7-blue (Novagen). Mutant constructs (Fig. 1A) in the same vector were generated using the QuikChange™ Site-Directed Mutagenesis Kit (Stratagene) according to manufacturer's instructions. Wild type and mutant SLPI coding sequences were cloned into pTriEx-3Neo (Novagen) with the addition of a hexahistidine-tag to its COOH-terminus. To produce recombinant SLPI and its mutants, *Escherichia coli* strain Origami™ (DE3)pLacI B (Novagen) was transformed with a respective construct and grown in LB medium at 37 °C in the presence of ampicillin (100 μg/ml), kanamycin (15 μg/ml), tetracycline (12.5 μg/ml) and chloramphenicol (30 μg/ml) to an OD of 0.5. Expression of recombinant SLPI and its mutants was induced by addition of 1 mM IPTG for 5 h at 27 °C. The cells were then pelleted and resuspended in 50 mM sodium phosphate buffer (pH 8.0) containing 300 mM NaCl and 10 mM imidazole. After sonication of bacteria, sequential 15,000×*g* and 50,000×*g* centrifugations were performed. The recovered supernatants were incubated with Ni-NTA resin at 4 °C for 1 h. Recombinant SLPI and its mutants were eluted with 200 mM imidazole, dialyzed against PBS buffer (10 mM sodium phosphate, 0.9% NaCl, pH 7.4), concentrated and stored in aliquots at −80 °C until use.

2.2. Protease inhibition assays

In all protease assays, the enzymes were preincubated with or without recombinant SLPI or its mutants at 37 °C for 20 min before addition of the respective substrate. The concentrations of SLPI and its mutants used were 120 nM (elastase and chymotrypsin) or 1.5 μM (trypsin and cathepsin G). The enzymatic activity was measured by the initial rate of absorbance changes at 405 nm. Human

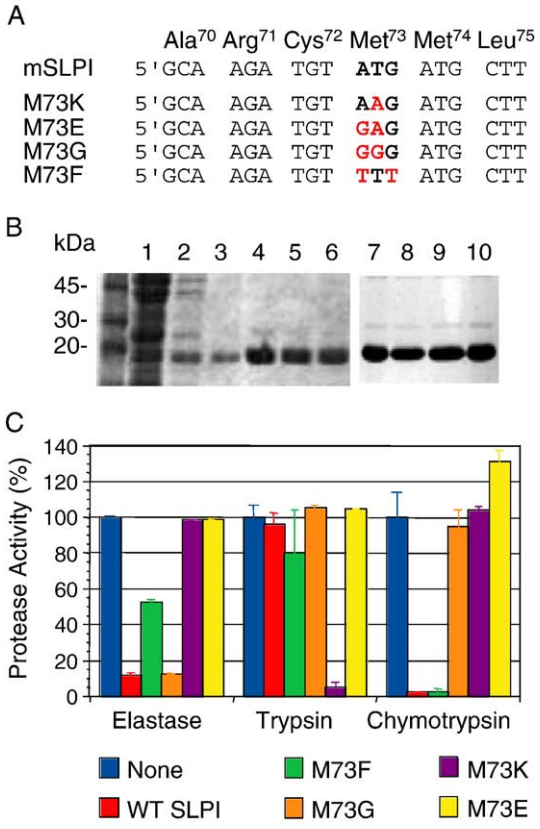

Fig. 1. Generation of SLPI mutants. (A) Sequences of wild type mouse (m) SLPI and 4 active site mutants. (B) Purification of recombinant SLPI mutants by Ni-NTA beads. Coomassie blue staining of elution fractions of wild type SLPI (1, input bacterial lysate; 2, flow through; 3–6, elution fractions) and purified mutants (7, M73K SLPI; 8, M73G SLPI; 9, M73E SLPI; 10, M73F SLPI). (C) Differential inhibitory activities of SLPI mutants on elastase, trypsin and chymotrypsin. Protease assays were performed by measuring absorption changes of colorimetric substrates with the appropriate protease in the presence of SLPI or its mutants as described in the Materials and methods. The results are means ± S.D. of duplicates in 1 of 4 experiments, expressed as the percentage of the protease activity in the presence vs. in the absence of inhibitors.

neutrophil elastase (15 nM, Calbiochem) activity was measured in 100 mM Tris–HCl, pH 6.5, 960 mM NaCl and 0.1% BSA using the substrate pyro-Glu-Pro-Val-pNA (0.5 mM; Chromogenix) [19]. Bovine pancreatic trypsin (100 nM, Sigma) was treated with L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK) (Sigma), and its activity determined in 50 mM Tris–HCl, pH 7.8, 20 mM $CaCl_2$ using *N*-benzoyl-L-Arg-pNA (Boehringer Mannheim, Inc.) as a substrate [20]. Bovine pancreatic α-chymotrypsin (12.5 nM, Sigma) activity was measured in 100 mM Tris–HCl, pH 7.5, 10 mM $CaCl_2$ using *N*-suc-Ala-Ala-Pro-Phe-pNA (Sigma) as a substrate [21]. Human neutrophil cathepsin G (0.1 U/ml, Calbiochem-Novabiochem) activity was measured in 100 mM Tris–HCl, pH 7.5, 20 mM $MgCl_2$ and 1% DMSO using 2-aminobenzoyl-threonyl-prolyl-phenylalanyl-seryl-alanyl-leucyl-glutaminyl-N-(2,4-dinitrophenyl) ethylenediamine dinitrobenzene as a substrate [22].

2.3. Generation of macrophage cell lines stably expressing wild type, mutant or truncated SLPI

For mutant SLPI expressing cells, the entire open reading frame for wild type SLPI and its mutants were cloned into pIRES-3Neo (Clonetech). Coding sequences for Gly^{1}–Pro^{54} (SLPI-N) or Lys^{56}–Met^{106} (SLPI-C) were cloned into an expression vector p463-neo [14] downstream of SLPI's secretory signal. Plasmids were electroporated into RAW 264.7 cells (for mutant SLPI) or HeNC2 (for truncated SLPI) using a Gene Pulsar (Bio-Rad). Stable transfectants were selected in neomycin-containing medium, cloned by limiting dilution and confirmed by Northern and Western analyses for elevated expression levels of SLPI. Two to five clones from each transfection were analyzed for their response to LPS.

2.4. Expression of SLPI-C NS SLPI-C (M73E) via retroviral transfection

cDNAs coding for SLPI-C and SLPI-C (M73E) were inserted into retroviral vector pMSCV IREs GFP (Clontech) at *Hpa*1 and *Eco*R1 sites. Viral particles were generated using GP2 −293 packaging cells (Clontech), maintained in DMEM supplemented with 200 U/ml penicillin, 200 μg/ml streptomycin and 10% fetal bovine serum. Cells at 50% confluency in 100-mm culture plates were co-transfected with a viral envelope expressing vector pVSV-G (Clontech) and pMSCV encoding SLPI-C, SLPI-C (M73E), or GFP only using FuGENE 6 (Roche Applied Science). Two days after transfection, 2.5 ml supernatant containing virus were cleaned by centrifugation an added to RAW cells in 6-well plates (3×10^5 cells/well) with 4 μg/ml of polybrene.

2.5. Western blot

Procedures were similar to those described [16]. Polyclonal anti-SLPI raised in rabbit against recombinant SLPI [14,23] was used at 0.1 μg/ml in the blots.

2.6. Northern blot

Total RNA (20 μg) from stable cells was electophoresed in 1% agarose gel, transferred to nylon membranes (PerkinElmer Life Science), and hybridized with ^{32}P-α-dCTP labeled SLPI and β-actin probes sequentially.

2.7. Assay for secretion of nitrite and TNF

The mock transfectant and the stably transfected macrophages producing wild type, mutated or truncated SLPI were seeded in 96-well plates (3×10^4 cells/well in 100 μl medium) and treated with indicated concentrations of LPS at 37 °C for 48 h. For transient transfected cells, 24 h after viral infection, cells were changed with new medium and treated with different concentrations of LPS. Nitrite and TNF levels in conditioned media were determined by the Griess reaction [23] and ELISA (Duoset, R&D Systems), respectively.

2.8. Statistical analysis

Results are expressed as the average and S.D. or S.E.M. in the figures. Data were analyzed by the Student's *t*-test for independent samples. Significant differences between testing samples and the control are indicated.

3. Results

3.1. Generation of SLPI mutants

We previously demonstrated that macrophage cell lines expressing elevated levels of SLPI are refractory to LPS stimulation [14]. To address whether the anti-protease function of SLPI contributes to its ability to suppress the LPS response, we generated anti-protease inactive mutants of mouse SLPI. Mutation of mouse SLPI was targeted at Met^{73} because this residue corresponds to the active site Leu^{72} of human SLPI [14,24]. The 396-bp coding sequence of mouse SLPI gene was cloned into pT7-Blue to yield pT7-mSLPI, which was used as the template for the subsequent site-directed mutagenesis. Four mutants with substitutions of Met^{73} by Gly, Phe, Glu or Lys were generated (Fig. 1A). It was expected that changes in the size (M73G), charge (M73K and M73E) or hydrophobicity (M73F) in the Met^{73} side chain could eliminate some, if not all, of SLPI's anti-protease function.

3.2. Differential anti-protease activities of SLPI mutants

To verify that the mutants possess an altered anti-protease function, the coding sequences of wild type SLPI and its four mutants were cloned into the expression vector pTriEx-3Neo, which introduced a His_6-tag at the COOH-terminus of each clone. *E. coli* was transformed with the resulting pTriEx3 plasmids. Recombinant SLPI and its mutants were induced by IPTG, and affinity-purified with Ni-NTA agarose beads. The purity of recombinant wild type and mutant SLPI was confirmed by Coomassie blue-stained SDS-PAGE (Fig. 1B). Human neutrophil cathepsin G activity was not inhibited by mouse SLPI or any of its mutants at the concentrations tested (not shown). Wild type SLPI is a potent inhibitor of elastase and chymotrypsin, but a poor inhibitor of trypsin (Fig. 1C). The four single amino acid substitution mutants of SLPI, on the other hand, showed different inhibition patterns. M73F partially lost anti-elastase activity, M73G lost anti-chymotrypsin activity, M73K lost both anti-elastase and anti-chemotrypsin activity but became a potent inhibitor of trypsin, and M73E exhibited no anti-protease activity against any of the three proteases (Fig. 1C). Thus, these SLPI mutants comprise a

useful panel of reagents with which to dissect the role of anti-elastase, anti-chemotrypsin or anti-trypsin functions of SLPI.

3.3. Generation of macrophage cell line expressing different SLPI mutants

The suppressive effect of SLPI on LPS responsiveness was convincingly demonstrated with macrophage cell lines expressing an elevated level of SLPI [14,15]. We next generated a series of macrophage cell lines stably expressing each of the SLPI mutants from RAW 264.7 cells. Transfections with wild type SLPI or vector alone were included as controls. Transfected cells were selected in G418-containing medium, and individual clones were generated by limiting dilution. Clones with elevated expression of SLPI or its mutants were chosen for the subsequent experiments (Fig. 2A). These clones also secreted increased amounts of SLPI protein or its mutants into the conditioned media (Fig. 2B).

3.4. Suppression of LPS response by macrophages expressing SLPI mutants

Comparisons of the LPS response were made between macrophages stably producing wild type and each of the SLPI mutants. Macrophages were incubated with or without of LPS (100 ng/ml) for 48 h, conditioned media collected to assay nitrite and TNF (Fig. 3). Mock transfectants responded to LPS by producing increased amounts of nitrite and TNF in a manner indistinguishable from the parental RAW264.7 cells (not shown). Macrophages expressing wild type SLPI produced a reduced level of nitrite and TNF in response to LPS, as compared with the mock transfectants. This LPS-hyporesponsive phenotype is reminiscent of that of the SLPI expressing macrophage cell line HeNC2 used in an early study [14]. Cells expressing each of SLPI mutants were also hyporesponsive to LPS (Fig. 3). Among them, the cell line expressing M73E SLPI, which had lost activity against all three serine proteases tested, responded even less to LPS than cells expressing wild type SLPI. Thus, the anti-protease activity of SLPI is not required for SLPI's LPS-suppressive function in macrophages.

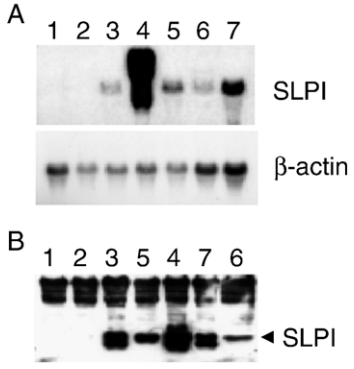

Fig. 2. Enhanced expression levels of SLPI and its mutants in macrophage cell lines. Expression of SLPI mRNA by RAW264.7 (1) or clones stably transfected with the vector alone (2), wild type SLPI (3) or its mutants (4–7). (A) Northern blot with SLPI cDNA and β-actin as probes. (B) Secreted SLPI and its mutants were TCA precipitated from the culture medium and immunoblotted with polyclonal anti-SLPI antibody. Macrophage cell lines expressing M72F SLPI (lane 4), M72G SLPI (lane 5), M72K SLPI (lane 6) and M72E SLPI (lane 7).

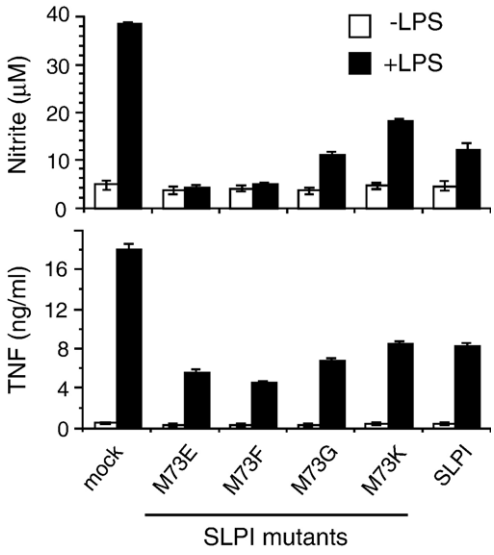

Fig. 3. Suppression of LPS response in RAW265.7 cells expressing mutant SLPI. LPS-induced release of nitrite or TNF were measured from conditioned media of cell lines expressing indicated proteins. Results are means ± S.E.M. of triplicates. One of four similar experiments is shown. Student's *t*-test, $P < 0.05$ for all mutants and wild type SLPI vs. mock control.

3.5. Either N- or C-terminal half of SLPI is sufficient in suppressing LPS response in macrophages

Crystallographic evaluation revealed that SLPI is a boomerang-shaped molecule comprised of two topologically superimposable domains [25], with its antiprotease activity localized to its C-terminal domain [26]. The N-terminal domain of SLPI does not contribute to protease inhibition directly. However, comparison of kinetic constants of inhibitory reactions of elastase and chymotrypsin by full-length SLPI and its C-terminal domain suggested that the N-terminal domain of SLPI binds to heparin and helps stabilize the protease/SLPI complex [27]. We next asked whether a single domain or both domains of SLPI are required for LPS-suppressive activity. Stably transfected macrophages producing either a C-terminal or N-terminal truncated form of SLPI were established from HeNC2 [14]. Parental cells transfected with the full-length SLPI or the vector alone were included as controls. Individual SLPI producing clones were selected and the expression of full- or half-length SLPI confirmed by Northern and Western analyses (not shown). We have previously shown that stable transfection of full-length SLPI renders HeNC2 completely unresponsive to LPS [14]. As compared to identically

treated mock transfectants, cells that express either the N-terminus (SLPI-N) or C-terminus (SLPI-C) of SLPI produce little nitrite or TNF in response to LPS (Fig. 4). The inhibition of LPS response afforded by truncated SLPI was as effective as full length wild type SLPI. Because SLPI's anti-protease function is located in its C-terminal domain, the ability of N-terminal SLPI to suppress the LPS response is consistent with the finding that anti-protease function is not required for the LPS-suppressive action of full-length SLPI. This result also indicates that either half of SLPI is sufficient for its LPS-suppressive action.

3.6. Mutant C-terminal half of SLPI is capable of suppressing LPS response of macrophages

To address whether mutated SLPI-C also possessed LPS-antagonistic activity, we transfected RAW cells with SLPI-C or its mutant (M73E) using a retroviral vector pMSCV (Clontech). This transient transfection resulted in more than 80% transfection efficiency as judged by expression of GFP that was present in the same vector with SLPI-C. Control cells were transfected with GFP only. Twenty-four hours after transfection, cells were treated with different concentrations of LPS, conditioned media (24 h) were collected and TNF contents determined by ELISA. Fig. 5 shows that mutant SLPI-C (M73E) was capable of inhibiting LPS-induced TNF release as its wild type.

4. Discussion

The physiological role of SLPI was originally believed to center on its inhibition of neutrophil elastase and cathepsin G at sites of inflammation [3,28]. Recent studies have discovered multifaceted roles of SLPI in pathological

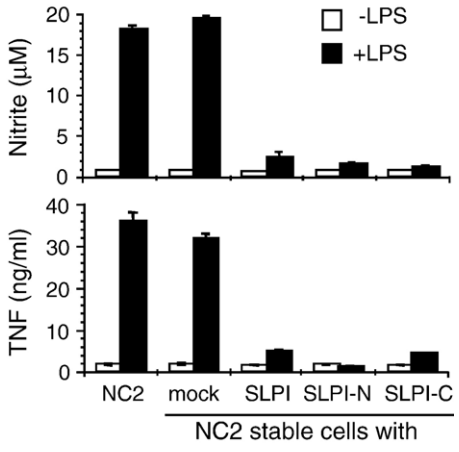

Fig. 4. Suppression of LPS response in HeNC2 cells expressing full-length or truncated SLPI. LPS-induced release of nitrite or TNF were measured from conditioned media of cell lines expressing the full-length or truncated forms of SLPI. Results are expressed as means ± S.E.M. of triplicates. One of five similar experiments is shown. Student's *t*-test, $P < 0.02$ for truncated and wild type SLPI vs. mock control.

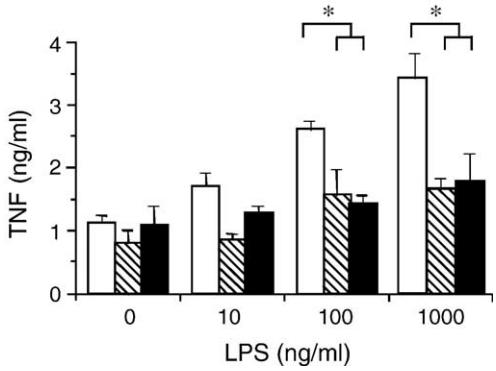

Fig. 5. Suppression of LPS-induced TNF by mutated SLPI-C (M73E). RAW cells were transiently transfected with vector (mock, open), SLPI-C (hetched) or SLPI-C (M73E) (solid) for 24 h, then treated with indicated concentrations of LPS for another 24 h. TNF contents in the conditioned media were determined by ELISA. Results are means ± S.E.M. of triplicates from one of three similar experiments. *Student's *t*-test, $P < 0.05$.

settings. These include promotion of wound healing [28], prevention of HIV infection [29], protection of lung damage in cystic fibrosis [30–33], inhibition of cartilage resorption in acute and chronic arthritis [34], prevention of acute alveolitis by immune complex deposition [35] and protection of mice from endotoxic shock [36]. Additional functions of SLPI in vitro include bactericidal activity [37,38], suppression of the LPS response of macrophages [14,15], inhibition of TNF-induced oxidative burst of neutrophils [1,17], and suppression of ConA- or LPS-induced COX-2, PGE2 and MMPs production by human monocytes [39]. It has not been completely examined how many of these functions result from SLPI's anti-protease activity.

Anti-HIV activity and monocyte-inhibitory actions of SLPI have been reported to be independent of SLPI's anti-protease functions because anti-protease inactive mutants retain full activity in these two settings [29,39]. Similarly, the bactericidal activity of SLPI against *E. coli* was found to localize to its N-terminal domain, and thus is also independent of its anti-protease function [37]. On the other hand, prevention of acute alveolitis [40] and promotion of wound healing [17] require anti-protease activity of SLPI. The LPS suppressive action of SLPI in macrophages has been repeated demonstrated using macrophages expressing endogenous SLPI [14,15]. By establishing cells that stably express truncated or mutated forms of SLPI, we show here that the anti-protease function of SLPI has no role in its suppression of macrophage LPS responses. In addition, we show that either the N-terminal or the C-terminal half of SLPI is sufficient to attenuate the macrophage response to LPS. This is in contrast to the anti-HIV activity of SLPI, which requires both domains of SLPI, albeit not its anti-protease activity [29].

One of the reasons for choosing stable cells in this study is that macrophages were exquisitely sensitive to trace amounts of LPS and that purified recombinant SLPI from the bacterial host was often contaminated with traces of

endotoxin. Since in our culture system microbial contaminants were scrupulously monitored and avoided, so the effect of endogenous SLPI on macrophage response to LPS could be determined realistically. There have been reports suggesting that addition of exogenous human SLPI or its C-terminal half could augment mouse macrophage production of TNF [41] or IL-10 and TGFβ in response to LPS [41]. In these studies, the possible endotoxin contamination in the recombinant protein preparation was not addressed. In addition, human SLPI was used on murine cells. It remains to be determined whether the different outcomes between these studies and ours are due to differences in species (mouse vs. human) or testing routes (endogenous vs. exogenous).

The precise mechanism by which SLPI inhibits macrophage responses to LPS remains to be determined. Macrophages use a complex molecular network to detect the presence of traces of microbial products such as LPS and to mount an immediate response to protect the host [42–44]. The most important and consequential outcome of this response is the generation of inflammatory mediators such as cytokines and reactive oxygen intermediates. Much progress has been made recently in understanding the molecular events from surface LPS recognition to the nuclear activation of NF-κB, a transcription factor capable of activating many promoters of genes regulating production of inflammatory mediators [45]. Molecules involved in LPS signaling [42] include membrane proteins CD14, TLR4 and MD2, receptor adapter molecules MyD88, TIRAP, TRIF, TRAM and downstream kinases and signaling molecules such as IRAKs, TRAF6, IKKs and IkB. Among all the known signaling events leading to NF-κB activation, only one involves proteolysis: degradation of the NF-κB inhibitor IkB by the proteasome.

As a potent leukocyte protease inhibitor, it was plausible to suggest that SLPI interfered with the LPS signaling steps involving proteolysis. Taggart et al. [46], showed that SLPI, but not oxidized SLPI, prevented LPS-induced IkB degradation. Since oxidized SLPI is anti-protease inactive, they thus concluded that SLPI's antiprotease activity had to be important for this inhibition. In the same study, however, SLPI failed to inhibit proteasome 20 S peptidase activity. It has been shown that oxidized SLPI also abolished its stimulatory effect on HGF production [47] and its inhibition of TLR-induced cytokine expression [48]. Whether these activities of SLPI are anti-protease related is not clear. It is possible that oxidation of SLPI can affect SLPI's activities beyond its anti-protease function. The findings described in this study argued against the hypothesis that LPS-antagonistic effect of SLPI was antiprotease-dependent and indicated that anti-protease function of SLPI played no role in inhibiting LPS response of macrophages.

Our study indicates that both N-terminal and C-terminal domain of SLPI are capable of suppressing macrophage response to LPS and that the anti-protease sequence of SLPI is dispensable for this function. Both SLPI-N and SLPI-C belong to the whey acidic protein (WAP) family, a motif first identified in milk whey of lactating mice [49]. WAP domain consists of approximately 50 amino acid residues in size with eight cysteine residues at defined positions. Four intracellular disulfide bonds among these cysteines create a tightly packed structure with the peptide chain folded into a central two-stranded β-sheet. In the case of SLPI, its C-terminal protease binding loop projects away from the molecular core [25]. A locus on human chromosome 20 contains 14 genes, including *SLPI*, that have WAP domains [50]. It is possible that the core structure of WAP domain in each domain of SLPI is responsible for its LPS-antagonistic activity. Members of WAP family include protease inhibitor, elafin (elastase-specific inhibitor), caltrin-like protein (a calcium transport inhibitor) and other extracellular proteinase inhibitors [50]. It remains to be determined whether other members of WAP family are also capable of suppressing macrophage activation.

The inflammatory responses of macrophages are subject to positive and negative regulation [51]. Many host-derived anti-inflammatory regulators, such as TGFβ, IL-4, IL-10, prostagladins and glucocorticoids, initiate signaling pathways after interacting with their receptors. It is possible that SLPI also exerts its effect by initiating a negative signal. This is consistent with our observation that both homologous N- and C-terminal domains of SLPI are equally effective in suppressing the LPS response. Recent report on the effect of SLPI on HIV infection suggested that SLPI could bind to surface annexin II on macrophages [52]. Whether this binding transduces a signal remains unclear. More work is needed to determine the nature and the identity of this receptor and whether SLPI is able to act on diverse cell types via its engagement.

Acknowledgments

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