Regulation of SLPI and elafin release from bronchial epithelial cells by neutrophil defensins

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secretory leukocyte proteinase inhibitor; inflammation; proteinase inhibitors

PROTEINASE INHIBITORS IN THE LUNG provide protection against the extracellular activity of serine proteinases such as neutrophil elastase (NE). The extracellular activity of NE is restricted by inhibitors that include α1-proteinase inhibitor (α1-PI), secretory leukocyte proteinase inhibitor (SLPI) (13), and elafin/skin-derived antileukoproteinase (SKALP) (23). Whereas α1-PI is mainly produced by the liver and reaches the lung via passive diffusion (27), SLPI and elafin/SKALP are locally produced in the lung. SLPI, also known as antileukoprotease, is a 12-kDa nonglycosylated, cat-ionic protein that is produced by serous cells of the submucosal bronchial glands, by nonciliated cells of the bronchial epithelium, and by neutrophils (for a recent review, see Ref. 28). Its major physiological function is considered to be the inhibition of NE, but it is also a potent inhibitor of a variety of other proteinases including cathepsin G and tryptase. Besides this antiproteinase activity, SLPI also displays several other activities. Antibacterial and antifungal activities of SLPI have been described (28), and SLPI was shown to restrict the replication of human immunodeficiency virus-1 (17). Furthermore, SLPI may have anti-inflammatory activities as demonstrated by the observation that it inhibits monocyte/macrophage proinflammatory activities (9, 35).

Whereas SLPI is well characterized at both the gene and protein levels, little is known about the regulation of SLPI expression in the lung. Studies (1, 2, 16, 24) with both airway epithelial cell lines and primary cultures have indicated that the SLPI gene is constitutively expressed in these cells. This expression can be increased by proinflammatory stimuli such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β (16, 24). In addition to SLPI, the presence of elafin/SKALP has been reported in airway epithelial cells (23, 25) and bronchial secretions (30). Elafin/SKALP is a serine proteinase inhibitor with a structural homology to SLPI. Whereas SLPI is a potent inhibitor of elastase and cathepsin G, elafin/SKALP inhibits elastase and proteinase-3. Like SLPI, the expression of elafin/SKALP can also be regulated by TNF-α and IL-1β (24). These findings suggest that epithelial cells respond to inflammatory stimuli by increasing their antiproteinase shield.

Interestingly, the regulation of SLPI may also be influenced by the neutrophils themselves. During inflammation, neutrophils release cytotoxic compounds including reactive oxygen intermediates and serine proteinases. These reactive oxygen intermediates are able to inactivate the proteinase inhibitory activity of...
released SLPI, thereby reducing the antiproteinase screen (14). NE has been shown to increase SLPI mRNA expression in lung epithelial cells in vitro. This increase was accompanied by a decrease in SLPI protein release (1). Furthermore, it was shown that glucocorticoids enhance both SLPI mRNA expression and protein release and synergistically enhance the NE-induced increase in SLPI mRNA expression (2). The effect of neutrophils or their products on elafin/SKALP production is unknown.

In addition to proteinases and reactive oxygen intermediates, stimulated neutrophils also release defensins. Neutrophil defensins (human neutrophil peptide-1 to -4) belong to the subfamily of α-defensins and are small, cationic, and antimicrobial peptides that display cytotoxic activity against various mammalian cell types in vitro, including airway epithelial cells (32). Evidence for in vivo release of neutrophil defensins is derived from observations showing high concentrations of defensins in purulent sputum from patients with chronic airway inflammation (20, 26) and in the epithelial lining fluid of α1-PI-deficient patients (21). In addition, neutrophil defensin deposits on the epithelial surface were observed in lung tissue from patients with a diffuse panbronchiolitis (4). In vitro studies have shown that defensins induce the expression of IL-8 in airway epithelial cells (33) as previously reported for NE (5). The effects of defensins on airway epithelial cells, i.e., cell lysis and chemokine synthesis, are inhibited by α1-PI (20, 33). Furthermore, defensin-induced chemokine synthesis is decreased by the glucocorticoid dexamethasone (31). In the present study, we investigated the effect of neutrophil defensins on SLPI and elafin/SKALP production in cultured bronchial epithelial cells.

MATERIALS AND METHODS

Isolation of defensins. Defensins were isolated as human neutrophil peptide-1 alone from neutrophils as previously described (33). The purity of the defensin preparations was assessed by SDS-PAGE, acid urea-PAGE, and NH2-terminal sequence analysis. Human NE was isolated from purulent sputum and purified as previously described (32).

Cell cultures. Bronchial tissues were obtained from patients who underwent a lobectomy or pneumectomy for lung cancer at the Leiden University Medical Center (Leiden, The Netherlands). After excision, the tissues were trimmed, washed, and incubated overnight at 4°C with 0.1% (wt/vol) proteinase type IV in Ca2+- and Mg2+-free Hanks’ balanced salt solution (both from Sigma, St. Louis, MO). Epithelial cells were gently scraped off the luminal surface, washed once, and subsequently cultured in keratinocyte serum-free medium in Vitrogen-fibronectin-BSA-coated six-well plates (Costar) that were precoated for 2–6 h with a combination of Vitrogen (30 µg/ml; Celtrix Laboratories, Palo Alto, CA), fibronectin (10 µg/ml; isolated from human plasma), and bovine serum albumin (BSA; 10 µg/ml; Boehringer Mannheim, Mannheim, Germany) in PBS. Keratinocyte serum-free medium was supplemented with epidermal growth factor (0.2 ng/ml; Gibco BRL), bovine pituitary extract (25 µg/ml; Gibco BRL), isoproterenol (1 µM; Sigma), 200 U/ml of penicillin, and 200 µg/ml of streptomycin. During the first week of culture, the antimycoplasma agent ciproxyn (2.5 µg/ml; Bayer) was added to the medium. After near confluency was reached, the cells were trypsinized (0.03% [wt/vol] trypsin, 0.01% [wt/vol] EDTA, and 0.1% [wt/vol] glucose in PBS, pH 7.5) and either subcultured for a maximum of two subsequent passages or stored in liquid nitrogen. The cultures were devoid of connective tissue and inflammatory cells, and the epithelial origin was established by demonstrating cytokeratin expression (cytokeratins 5, 8, and 19). All cells were cultured at 37°C in 5% CO2 humidified air.

Stimulation of subcultures of primary bronchial epithelial cells. Subcultures of primary bronchial epithelial cells (PBECs) were plated in Vitrogen-fibronectin-BSA-coated 24-well plates (Costar) at a concentration of 2 × 105 cells/well. Thirty-six hours before onset of the experiments, when cells had reached a confluence of ~70–80%, the medium was replaced by keratinocyte serum-free medium containing epidermal growth factor and bovine pituitary extract, penicillin, streptomycin, and 1 mM CaCl2 (referred to as high-calcium medium). The cells were incubated for an additional 36 h to allow for cellular differentiation and were next stimulated for various time periods in this medium with and without defensins (100 µg/ml).

The effect of α1-PI (Cutter Biological, Berkeley, CA) on defensin-mediated SLPI synthesis was examined by preincubating defensins for 1 h at 37°C with an equimolar concentration of α1-PI and subsequently adding the mixture to the culture. To study the effect of dexamethasone on defensin-induced SLPI release, PBECs were cultured as described above to 70–80% confluence, and then the medium was changed to hydrocortisone-free, high-calcium medium (GIBCO BRL). Twenty hours later (i.e., 16 h before the addition of a stimulus), the cells were preincubated overnight with dexamethasone (10–6 M) or hydrocortisone-free medium alone. Next, the cells were stimulated as described above in the presence and absence of dexamethasone.

To analyze mRNA levels, PBECs were plated in Vitrogen-fibronectin-BSA-coated six-well plates (Costar) and subsequently cultured and treated as described above.

Northern blot analysis. Total RNA was extracted from epithelial cells according to the acid guanidinium-thiocyanate method, and Northern blot analysis was performed as previously described (33). SLPI mRNA was detected by using a 510-bp I fragment of full-length SLPI cDNA probe (kindly provided by Dr. R. Heinzel-Wieland, Grunenthal, Aachen, Germany). As an IL-8 probe, a 1.2-kb EcoRI fragment of full-length IL-8 cDNA (kindly provided by Dr. J. Oppenheim, Laboratory of Molecular Immunoregulation, National Cancer Institute, Frederick, MD) was used. To assess equal loading, filters were probed with a glyceraldehyde-3-phosphate dehydrogenase cDNA probe (American Type Culture Collection). Autoradiographs were quantified with light densitometry (densitometer CD 60, Desaga, Heidelberg, Germany). The SLPI and IL-8 mRNA levels were calculated as a ratio of the mRNA glyceraldehyde-3-phosphate dehydrogenase level, and mRNA levels in stimulated cells are expressed as the multiple of increase compared with that in control cells.

SLPI, elafin/SKALP, and IL-8 ELISA. SLPI (12) and elafin/SKALP (15) protein levels were detected by ELISA in cell-free supernatants as previously described. Before the SLPI ELISA, the samples were boiled for 2 min to dissociate any putative elastase-SLPI or elastase-elafin/SKALP complexes and to inactivate possible proteinase activity.

IL-8 was measured in cell-free supernatants with a sandwich ELISA with a monoclonal anti-IL-8 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) as the capturing antibody and
RESULTS

Effect of defensins on SLPI protein production by PBECs. To study the effect of defensins on SLPI production by PBECs, the cells were incubated with defensins (100 µg/ml) or medium alone for several time periods. As shown in Table 1, PBECs constitutively secreted SLPI protein that was time dependently increased on incubation with defensins. A significant increase in defensin-induced SLPI protein release was observed after 24 and 48 h. No significant difference was observed between the level of SLPI detected after 48 and 72 h of stimulation. We next studied whether the effect of defensins on SLPI release was dose dependent. After 48 h of incubation, defensins increased SLPI protein levels in a concentration range between 50 and 200 µg/ml. The minimal stimulatory defensin concentration was 50 µg/ml (487 ± 194 ng/ml of SLPI for defensin-treated cells vs. 305 ± 127 ng/ml of SLPI for control cells; n = 3 individual experiments), whereas the largest increase in SLPI release was observed with a defensin concentration of 100 µg/ml (503 ± 209 ng/ml of SLPI for defensin-treated cells vs. 305 ± 127 ng/ml of SLPI for control cells; n = 3 individual experiments). A higher concentration of defensins (200 µg/ml) also increased SLPI release, but this increase was to a smaller extent compared with that with the lower defensin concentration (423 ± 192 ng/ml of SLPI for defensin-treated cells vs. 305 ± 127 ng/ml of SLPI for control cells; n = 3 individual experiments). At this concentration, we observed marked morphological changes such as nuclear condensation and swollen cytoplasm, suggesting that at 200 µg/ml, defensins are most likely toxic.

In addition to SLPI, control PBECs release the closely related proteinase inhibitor elafin/SKALP (Table 1). In contrast to what was observed for SLPI, defensins did not enhance the release of this proteinase inhibitor. Defensins even tended to decrease the release of elafin/SKALP, but this decrease did not reach a significant difference.

As previously shown, the release of IL-8 was assessed and shown to be increased by defensins. The stimulatory effect of defensins on IL-8 release was maximal after 24 h of incubation, and no further increase was observed at later time points (Table 1). This is in contrast to the stimulatory effect of defensins on SLPI release that continues beyond 24 h of incubation.

Modulation of the defensin-induced increase in SLPI protein release by α1-PI. We next examined the effect of α1-PI on the defensin-induced increase in SLPI protein release by PBECs. Cells were stimulated for 24 and 48 h with defensins (100 µg/ml) that had been preincubated with α1-PI in a 1:1 molar ratio. At this ratio, α1-PI was previously shown to restrict defensin-induced IL-8 production and cell lysis (20, 33). After 24 h of stimulation, α1-PI did not prevent defensin-induced SLPI synthesis but enhanced the defensin-induced increase in SLPI protein (Fig. 1) by 27%. This effect was more pronounced after 48 h of incubation when α1-PI significantly enhanced the defensin-induced increase in SLPI protein twofold (281 ± 16 and 612 ± 51 ng/ml for cells stimulated with defensins alone and in presence of α1-PI, respectively; P < 0.01). This increase is likely due to an additive effect of α1-PI because α1-PI alone also had a marked stimulatory effect on SLPI protein release. In contrast, no effect of α1-PI alone or in combination with defensins was observed on elafin/SKALP release (data not shown), whereas in the presence of α1-PI, the defensin-induced IL-8 levels in PBECs were significantly decreased at both 24 and 48 h (Fig. 1).

Table 1. Effect of defensins on SLPI, IL-8, and elafin/SKALP production in primary bronchial epithelial cells

<table>
<thead>
<tr>
<th>Protein</th>
<th>Stimulus</th>
<th>Time 24 h</th>
<th>Time 48 h</th>
<th>Time 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLPI, ng/ml</td>
<td>Control</td>
<td>135 ± 26</td>
<td>205 ± 76</td>
<td>442 ± 163</td>
</tr>
<tr>
<td></td>
<td>Defensins</td>
<td>195 ± 23†</td>
<td>364 ± 118*</td>
<td>615 ± 254</td>
</tr>
<tr>
<td>IL-8, ng/ml</td>
<td>Control</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td></td>
<td>Defensins</td>
<td>11 ± 3†</td>
<td>11 ± 2†</td>
<td>10 ± 1*</td>
</tr>
<tr>
<td>Elafin/SKALP, ng/ml</td>
<td>Control</td>
<td>163 ± 59</td>
<td>195 ± 76</td>
<td>233 ± 118</td>
</tr>
<tr>
<td></td>
<td>Defensins</td>
<td>118 ± 36</td>
<td>179 ± 80</td>
<td>250 ± 125</td>
</tr>
</tbody>
</table>

Values are means ± SE; n > 4 separate experiments except n = 3 separate experiments for 72-h interleukin (IL)-8. Each experiment was performed with cells from a different donor, and within an experiment, each test condition was performed in triplicate. Subcultures of primary bronchial epithelial cells were incubated with defensins (100 µg/ml) or medium alone (control) for indicated time periods. Supernatants were harvested and analyzed for secretory leukocyte proteinase inhibitor (SLPI), IL-8 and elafin/SKALP protein by ELISA. Significant difference from control cells: *P < 0.05; †P < 0.01.
As a control, we analyzed IL-8 mRNA. As shown in Fig. 3C, defensins markedly increased IL-8 mRNA that was completely reduced in the presence of α1-PI.

Effect of combinations of defensins and NE on SLPI release by PBECs. Previously, van Wetering et al. (32) have shown that NE prevents defensin-induced cell lysis and IL-8 synthesis in airway epithelial cells. Vice versa, we observed that defensins limit NE-mediated cellular detachment. Therefore, we examined the effect of NE on the defensin-induced increase in SLPI synthesis. Whereas defensins induced a significant increase in SLPI protein release after incubation of PBECs for 24 h, NE significantly reduced SLPI protein release by PBECs (Fig. 4). Incubation of PBECs for 24 h with both NE and defensins resulted in SLPI levels that were lower than in both control and defensin-treated cells. This indicates that NE decreases SLPI release irrespective of the stimulatory effect of defensins.

Effect of dexamethasone on defensin-induced increase in SLPI protein and mRNA synthesis. Recently, van Wetering et al. (31) demonstrated that the glucocorticoid dexamethasone reduces defensin-induced cytokine synthesis in airway epithelial cells. Therefore, we studied the effect of dexamethasone on the defensin-induced increase in SLPI protein synthesis. PBECs were preincubated overnight with defensins (100 µg/ml) in the presence and absence of dexamethasone. Although dexamethasone tended to decrease defensin-enhanced SLPI secretion, this effect was not significant. Neither did dexamethasone affect the basal release of SLPI protein by PBECs (Table 2). Similarly, although dexamethasone tended to reduce the elafin/SKLAP release in defensin-treated cells, this effect was not significant. In contrast, dexamethasone significantly inhibited IL-8 release by defensin-treated and control cells. Finally, we could not detect any effect of dexamethasone on SLPI mRNA levels (data not shown), whereas dexamethasone markedly reduced IL-8 mRNA levels (31).

DISCUSSION

Proinflammatory cytokines and neutrophil-derived serine proteinases are thought to be involved in the regulation of the expression of SLPI in the lung. The results of the present study show that neutrophil defensins also enhance the release of SLPI protein in airway epithelial cells without having a significant stimulatory effect on SLPI mRNA synthesis. Defensins increased SLPI protein release in a time- and dose-dependent fashion, with the highest increase observed after 48 h of stimulation with a defensin concentration of 100 µg/ml. The defensin-mediated increase in SLPI protein release was even further enhanced in the presence of α1-PI. Again, there was a small stimulatory but nonsignificant effect on SLPI mRNA synthesis. In contrast, in the presence of α1-PI, both defensin-induced IL-8 protein and mRNA synthesis were completely prevented, suggesting that different mechanisms account for the effect of defensins on IL-8 and SLPI synthesis.
SLPI release. As observed earlier for defensin-induced IL-8 synthesis, NE also abolishes defensin-induced SLPI release (32). In contrast to SLPI, defensins either alone or in combination with α-PI did not affect elafin/SKALP protein secretion. Finally, dexamethasone did not affect spontaneous or defensin-induced SLPI secretion but did inhibit IL-8 release.

The results of the present study demonstrate a specific effect of defensins on SLPI synthesis without affecting other constitutively released proteins such as elafin/SKALP. Whereas the stimulatory effect of defensins on IL-8 synthesis was maximal in the first 24 h, the effect of defensins on SLPI release continued until 48 h. At a concentration of 200 µg/ml, defensins were cytotoxic for PBECs, which most likely is reflected by the decrease in SLPI synthesis at this concentration.

Regulation of SLPI production in airway epithelial cells in vitro has been reported in various studies (1, 2, 16, 24). In line with these studies, we observed a constitutive expression of SLPI in cultured bronchial epithelial cells. Our study extends these previous observations by demonstrating that defensins, a major neutrophil product, increase SLPI protein release without clearly affecting SLPI mRNA levels. Although defensins tended to increase SLPI mRNA levels 1.5- to 2-fold, this effect was nonsignificant. This is in line with

Table 2. Effect of dexamethasone on defensin-induced SLPI, IL-8, and elafin/SKALP production in primary human bronchial epithelial cells

<table>
<thead>
<tr>
<th>Protein</th>
<th>Stimulus</th>
<th>Dexamethasone</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Presence</td>
</tr>
<tr>
<td>SLPI, ng/ml</td>
<td>Control</td>
<td>256 ± 78</td>
</tr>
<tr>
<td></td>
<td>Defensins</td>
<td>329 ± 100°</td>
</tr>
<tr>
<td>IL-8, ng/ml</td>
<td>Control</td>
<td>4 ± 1</td>
</tr>
<tr>
<td></td>
<td>Defensins</td>
<td>24 ± 3t</td>
</tr>
<tr>
<td>Elafin/SKALP, ng/ml</td>
<td>Control</td>
<td>400 ± 168</td>
</tr>
<tr>
<td></td>
<td>Defensins</td>
<td>274 ± 127</td>
</tr>
</tbody>
</table>

Values are means ± SE of at least 3 separate experiments. Each experiment was performed with cells from a different donor, and within an experiment, each test condition was performed in triplicate.

Fig. 3. Effect of defensins and α-PI on SLPI and IL-8 mRNA synthesis in PBECs. A: representative Northern blot analysis showing SLPI and corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA of PBECs that were stimulated with defensins (100 µg/ml) or α-PI (1.5 mg/ml) alone or in combination for 48 h. B: results of densitometric analysis of Northern blot in A. Results are expressed as multiple of increase compared with control cells. C: as in A, IL-8 and corresponding GAPDH mRNA.

Fig. 4. Effect of simultaneous incubation of defensins and neutrophil elastase (NE) on SLPI protein release by PBECs. PBECs were stimulated for 24 h with defensins (100 µg/ml) or NE (10 µg/ml) either alone or in combination. Cell-free supernatants were harvested and analyzed by ELISA. Results are means ± SE of 6 independent experiments. Each experiment was performed with cells from a different donor, and within an experiment, each test condition was performed in triplicate.
a recent study (10) showing a similar divergent effect of lipopolysaccharide (LPS) on SLPI protein release and mRNA levels in tracheal gland serous cells. The effect of defensins differs from that of NE because NE decreased SLPI protein release while increasing SLPI mRNA levels (1, 24). We observed that NE inhibits SLPI release from both control and defensin-treated cells.

Recently, van Wetering et al. (31) demonstrated that dexamethasone reduces defensin-induced chemokine synthesis in airway epithelial cells. In addition, it has been suggested that glucocorticoids stimulate SLPI synthesis (2). Based on this information, we investigated the effect of glucocorticoids on both spontaneous and defensin-enhanced SLPI synthesis. The results show that dexamethasone did not affect spontaneous SLPI protein release and mRNA expression. Although dexamethasone tended to slightly reduce the defensin-induced increase in SLPI protein release, this effect was nonsignificant. Others (2) have shown that glucocorticoids are able to increase SLPI mRNA and protein levels in cells of the airway epithelial cell line 9HTEo. Because, in our study, dexamethasone did reduce the defensin-induced IL-8 synthesis in the same primary epithelial cells, our results cannot be explained by an inability of the cells to respond to dexamethasone. Therefore, the apparent contradiction with the studies mentioned is most likely explained by the fact that different cell types, i.e., transformed human airway epithelial cells (2) versus primary bronchial epithelial cells (this study), were used.

In view of the reported stimulatory effects of LPS in concentrations ranging from 10 to 100 µg/ml on SLPI (10) and IL-8 secretion (11), we considered the possibility that our results are explained by contaminating traces of LPS in our defensin or α1-PI preparations. This is, however, unlikely for the following reasons. First, the LPS concentration in our defensin preparations at the concentration tested has been estimated at <100 pg/ml (33). Incubation of PBECs with various concentrations of LPS ranging from 0.1 to 10 µg/ml did not result in increased SLPI or IL-8 protein release (data not shown). Second, α1-PI abolished the defensin-induced IL-8 synthesis, whereas the defensin-induced SLPI synthesis was enhanced. It is also unlikely that our results are explained by the methods used to assess SLPI levels because neither defensins, NE, nor α1-PI interfere in the SLPI ELISA. Finally, we considered the possibility that increased SLPI levels in the supernatants of defensin-treated cells are a result of defensin-induced cell proliferation as previously reported (19). However, this is unlikely because the stimulatory effects of defensins on cell proliferation as described by Murphy et al. (19) were not observed at the concentrations used in the present study but only at much lower defensin concentrations. This is further supported by our observation that defensins do not affect the secretion of other constitutively released proteins such as elafin/SKALP (this study) and complement component C3 (data not shown).

The intracellular mechanism mediating defensin-induced increased SLPI release in airway epithelial cells is unclear. Defensins induced an approximately twofold increase in SLPI protein release without having a clear effect on SLPI transcript levels. This suggests that the effect of defensins may occur at the translational level. Nevertheless, defensins tended to increase SLPI mRNA levels, and because we observed only a limited increase in SLPI release as a result of defensin treatment, it is possible that corresponding small changes in SLPI mRNA synthesis cannot be detected by Northern blot analysis. Because of the cationic character of both SLPI and defensins, we considered the possibility that the defensin-induced increase in SLPI protein is due to competition between SLPI and defensins for putative anionic binding sites on the cells or the extracellular matrix. In this way, binding of defensins to sites on the cells or matrix that bind SLPI may result in increased SLPI protein levels in the supernatant and decreased levels of cell- or matrix-associated SLPI. To evaluate this possibility, lysates were made from the contents of wells after removing the cell supernatant and analyzed for SLPI content (considered to represent matrix- or cell-bound SLPI). Because we observed no difference in SLPI levels in lysates from defensin-treated and control wells (data not shown), this is an unlikely explanation.

Previously, van Wetering and colleagues have shown that defensins increase both IL-8 mRNA and protein release in airway epithelial cells (33) and that this was prevented by dexamethasone (31). Because defensins increase cytokine production, it is possible that defensin-induced SLPI synthesis is mediated via endogenous cytokine production. In line with this possibility, it has previously been shown that IL-1β and TNF-α increase SLPI production (24), whereas IL-8 and IL-6 do not (1, 24). We observed that defensin-induced IL-8 production precedes SLPI release. However, whereas in the presence of α1-PI, the defensin-induced increase in SLPI protein secretion is enhanced, defensin-induced IL-8 synthesis is completely prevented. Therefore, the possibility that IL-8 is responsible for the defensin-induced increase in SLPI protein release is unlikely.

α1-PI was found to increase defensin-induced SLPI release. In addition, α1-PI alone also caused an increase in SLPI protein release, which was not observed by Abbinante-Nissen et al. (1). This apparent contradiction is probably due to the fact that in the present study higher concentrations of α1-PI were used. The absence of an inhibitory effect of α1-PI on defensin-induced SLPI secretion is in contrast to its inhibitory effect on defensin-induced IL-8 synthesis, cell lysis (33), and stimulation of bacterial adhesion (8). Because α1-PI is known to form a complex with defensins, it was therefore thought that due to this complex formation, binding of defensins to the cellular membrane is prevented. However, the results of the present study show that in presence of α1-PI, defensins are still able to activate epithelial cells, suggesting that the α1-PI-defensin complex can bind to the epithelial surface. It has previously been shown that cells can be stimulated by complexes formed...
between serpins and their cognate enzymes (22). Whether such a mechanism is involved in the stimulation of epithelial cells by defensin-α1-PI complexes is not known at present.

The concentrations of defensins used in the present study are relevant to the in vivo situation because high concentrations of defensins are present in purulent secretions of patients with chronic bronchitis (20) and cystic fibrosis (26) and in the bronchoalveolar lavage fluid of α1-PI-deficient patients (21). Moreover, high levels of defensins (milligrams per milliliter) are estimated to be present in the sequestered environment between neutrophils and their target cells (6). Studies suggest that in vivo at sites of inflammation, SLPI production may be dynamically regulated. Willems et al. (34) demonstrated an association between the number of SLPI-containing cells and parenchymal destruction in smokers with small-airway disease. Furthermore, a correlation between SLPI-containing cells in bronchial/bronchiolar epithelium and acute inflammatory infiltration in the adjacent alveolar area has been observed in pneumonia (3). Finally, elevated SLPI and α1-PI levels can be detected in the bronchoalveolar lavage fluid of chronic obstructive pulmonary disease patients with chronic bronchitis and emphysema (18, 29). These studies indicate that SLPI expression is actively regulated at the sites of inflammation where defensins can be expected to be present at high levels. It is therefore tempting to speculate that defensins, either free or complexed to α1-PI, are one of the mediators responsible for this increase.

In conclusion, the results from the present study show that defensins released by stimulated neutrophils may enhance the antiproteinase screen in the lung by stimulating the release of SLPI from bronchial epithelial cells. However, because neutrophils are thought to release their products simultaneously and our results show that NE prevents defensin-induced SLPI release, the effect of neutrophils on SLPI synthesis remains to be established. Furthermore, α1-PI appears to reduce the proinflammatory activity of defensins by decreasing defensin-induced epithelial chemokine production and cell lysis but does not prevent the stimulatory effect of defensins on SLPI release. Because α1-PI bound to defensins is unable to inactivate NE (20), this might provide a mechanism by which the antiproteinase screen remains established. In conclusion, the results of the present study indicate that neutrophil-derived products such as defensins and NE are implicated in the mechanisms involved in the dynamic regulation of the antiproteinase screen in the lung at sites of inflammation.

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