Secretory Leukocyte Protease Inhibitor Prevents Allergen-Induced Pulmonary Responses in Animal Models of Asthma

CLIFFORD D. WRIGHT, ANDREW M. HAVILL, SCOT C. MIDDLETON, MOHAMMED A. KASHEM, PATRICE A. LEE, DAVID J. DRIPPS, THOMAS G. O’RIORDAN, MICHAEL P. BEVILACQUA, and WILLIAM M. ABRAHAM

Department of Inflammation Research, Amgen, Inc., Boulder, Colorado (C.D.W., M.A.K., P.A.L., D.J.D., M.P.B.); Department of Pharmacology, Amgen, Inc., Thousand Oaks, California (A.M.H., S.C.M.); and Pulmonary Division, University of Miami at Mount Sinai Medical Center, Miami Beach, Florida (T.G.O., W.M.A.)

Accepted for publication January 5, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

Secretory leukocyte protease inhibitor (SLPI) is a naturally occurring protein of human airways that exhibits broad spectrum inhibitory activity against mast cell and leukocyte serine proteases implicated in asthma pathology. To assess the potential therapeutic utility of SLPI in this disorder, its effects on antigen-induced pulmonary responses were evaluated. In Ascaris-sensitized sheep, SLPI (3 mg) administered by aerosol daily for 4 days, with the final dose 0.5 h before antigen challenge, reduced the areas under the curve for early- and late-phase bronchoconstriction (73 and 95%, respectively; *p < .05 versus control responses). SLPI also inhibited the development of airway hyperresponsiveness to carbocell (84%, *p < .05 versus control response) measured 24 h after antigen challenge. In ovalbumin-sensitized guinea pigs, intratracheal administration of SLPI daily for 3 days, with the final dose 1 h before antigen challenge, inhibited the development of airway hyperresponsiveness to histamine with an ED50 of <0.05 mg/kg. Prolonged pharmacodynamic activity of SLPI was observed in both species. In a murine model of atopic asthma, SLPI inhibited leukocyte influx into the airways after chronic antigen challenge. SLPI administered to sheep by the predosing protocol described above also prevented the antigen-induced decrease of tracheal mucus velocity (*p < .05). In addition, a single aerosol administration of SLPI (30 mg) to sheep 1 h after antigen challenge inhibited the subsequent late-phase bronchoconstriction and development of hyperresponsiveness and reversed the stimulated decrease in tracheal mucus velocity. These results suggest that SLPI may provide therapeutic intervention against the pathophysiology of asthma and its underlying pathology.

Asthma is a chronic pulmonary disorder characterized by two key pathophysiologic components: recurrent bronchoconstriction and development of airway hyperresponsiveness to allergenic and environmental stimuli. These physiologic responses are manifested as cough, wheezing, and shortness of breath. Although there has been great success in the development of symptomatic therapies for asthma, such agents fail to treat the underlying pathophysiologic responses that occur within asthmatic airways, including bronchial infiltration of inflammatory cells, mucus gland hypertrophy and mucus hypersecretion, epithelial cell desquamation, fibrosis, edema, and smooth muscle hypertrophy (Dunnill, 1960).

Emerging evidence suggests that serine proteases play a key role in the pathophysiology of asthma. Mast cell and leukocyte serine proteases are elevated in the airways of asthmatic patients (Wenzel et al., 1988; Fahy et al., 1995). In addition, patients with reduced antiprotease activity as a result of α1-proteinase inhibitor deficiency have an increased propensity to develop asthma (Eden et al., 1997). In animal studies, inhalation of elastase (Suzuki et al., 1996) or trypsin (Molinari et al., 1996) promotes bronchoconstriction and development of airway hyperresponsiveness, whereas specific inhibitors of these proteases reduce antigen-induced airway responses in vivo (Clark et al., 1995; Fujimoto et al., 1995). Serine proteases, including cathepsin G (Venaille et al., 1995), elastase (Mendis et al., 1990), and tryptase (Ross et al., 1991; Walls et al., 1995; Imaura et al., 1996), have also been implicated in promoting airway pathology associated with asthma. In addition, tryptase has been shown to stimulate allergic mediator release from mast cells both in vitro (He and Walls, 1997) and in vivo (Molinari et al., 1996).

ABBREVIATIONS: SLPI, secretory leukocyte protease inhibitor; pNA, p-nitroanilide; BSA, bovine serum albumin; Rl, mean pulmonary flow resistance; Vtg, thoracic gas volume; SR, specific lung resistance; PBS, phosphate-buffered saline; Fpeh, pause enhanced; Te, total expiratory time; RT, relaxation time; PEF, peak expiratory flow; PIF, peak inspiratory flow; i.t., intratracheal.
These observations implicate serine proteases in both the pathophysiology and airway pathology of asthma.

Secretory leukocyte protease inhibitor (SLPI) is a naturally occurring protease inhibitor produced by mucosal epithelial cells, serous cells, and bronchiolar goblet cells in human airways (Thompson and Ohlsson, 1986; Eisenberg et al., 1990). SLPI has been characterized as an 11.7 kDa, nonglycosylated protein comprised of two homologous domains. The COOH-terminal domain of SLPI is primarily responsible for the potent broad spectrum inhibition of mast cell and leukocyte serine proteases, whereas the NH2-terminal domain mediates an interaction with heparin, which accelerates binding of the inhibitor to serine proteases (Faller et al., 1992). Physical properties of SLPI enhance its potential as a treatment of pulmonary diseases (Vogelmeier et al., 1990). Acid stability of SLPI allows the inhibitor to remain functionally active under acidic inflammatory conditions. With a pI > 9, SLPI may also bind tissue sites favored by proteases, thus facilitating prolonged inhibition of protease activity in the airways. The emerging role of proteases in asthma, as well as the functional and physical properties of SLPI, suggests that this molecule may become an important new mechanism-based therapy for asthma. To assess this possibility, we have conducted studies in animal models to evaluate the efficacy of SLPI against pathophysiology and pathology associated with asthma.

## Materials and Methods

### SLPI

Recombinant SLPI was expressed and purified as described previously (Eisenberg et al., 1990). The recombinant protein was >99% pure as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and high performance liquid chromatography. The purified protein contained 0.72 EU lipopolysaccharide/mg protein.

### Biochemical Assays

Human lung tryptase (Cortex Biochem, Inc., San Leandro, CA) activity was assessed using vasoactive intestinal peptide (Sigma Chemical Co., St. Louis, MO) as a substrate in 100 mM Tris-HCl (pH 8.0) with 1 μg/ml heparin and 0.02% Triton X-100. Tryptase was incubated with various concentrations of SLPI for 1 h at 37°C. Vasoactive intestinal peptide cleavage was assayed by reversed phase high performance liquid chromatography (Delaria and Muller, 1996). The Ki value was determined from measurements of fractional activity of tryptase at various SLPI concentrations.

Other serine proteases were assayed using specific chromogenic peptide p-nitroanilide (pNA) substrates in a 96-well microtiter plate format. Each protease was incubated with various concentrations of SLPI for 15 min at 37°C in specific assay buffer. The residual protease activity was measured following the addition of the respective substrate. The p-nitroanilide product of proteolysis was quantified at 405 nm on a SpectraMAX 340 plate reader (Molecular Devices, Sunnyvale, CA). Human neutrophil elastase (Calbiochem-Novabiochem International, San Diego, CA) was assayed using pyroGlu-Pro-Sunyvale, CA). Human neutrophil elastase (Calbiochem-Novabiochem) was assayed using N-Benzoyl-Ile-Glu-Gly-Arg-pNA (Calbiochem-Novabiochem) and tissue kallikrein (prepared at Amgen) activities were assessed in 50 mM Tris-HCl (pH 7.8), 200 mM NaCl, and 0.05% bovine serum albumin (BSA; Lottenberg et al., 1981). Human plasma thrombin (Boehringer Mannheim) was assayed using H-o-Phe-Pip-Arg-pNA (Pharmacia Hepar) and t-Val-Leu-Arg-pNA (Sigma), respectively (Lottenberg et al., 1981). The inhibition constants (Kis) of human SLPI against each proteolytic enzyme were determined as described previously (Zitnik et al., 1997).

### Antigen-Induced Airway Responses in Sheep

#### Airway Mechanics

Adult ewes (median weight, 30 kg) were instrumented as described previously (Abraham et al., 1992). Mean pulmonary flow resistance (Rm) was calculated from an analysis of 5 to 10 breaths by dividing the change in transpulmonary pressure by the change in flow at midtidal volume. Immediately after Rm determination, thoracic gas volume (Vt) was measured in a constant volume body plethysmograph to calculate specific lung resistance (SRm) by the equation SRm = Rm × Vt.

A Raindrop jet nebulizer (Puritan-Benett, Lenexa, KS), operated at a flow rate of 6 liters/min, was used to generate droplets with a mass median aerodynamic diameter of 3.6 ± 1.9 μm. Aerosol delivery was controlled using a dosimetry system (Abraham et al., 1992), which was activated for 1 s at the onset of the inspiratory cycle of a piston respirator (Harvard Apparatus Co., South Natick, MA). Aerosols were delivered at a tidal volume of 500 ml and a respiratory rate of 20 breaths/min. SLPI was delivered at physiologic pH in PBS. Dosing regimens described below were designed to compare the efficacy of acute and prophylactic administration.

Sheep sensitive to Ascaris suum antigen, which exhibited both early- and late-phase bronchoconstriction, were used for these studies. Sheep were challenged with A. suum extract (82,000 protein nitrogen units/ml in phosphate-buffered saline (PBS; Greer Diagnostics, Lenoir, NC) delivered as an aerosol at a rate of 20 breaths/min for 20 min. Changes in SRm were monitored for 8 h after antigen challenge.

### Airway Hyperresponsiveness

Baseline airway responsiveness was determined by measuring the SRm immediately after saline inhalation and consecutive administration of 10 breaths of increasing concentrations of carbachol (0.25, 0.5, 1.0, 2.0, and 4.0% w/v). Airway responsiveness was estimated by determining the cumulative carbachol breath units required to increase SRm by 400% over the postsaline value (PC400). One breath unit was defined as 1 breath of an aerosol containing 1% w/v carbachol (Abraham et al., 1992). Antigen-induced airway hyperresponsiveness was determined by repeating the carbachol dose response 24 h after antigen challenge.

### Guinea Pig Airway Hyperresponsiveness

Male Hartley guinea pigs (Charles River Laboratories, Inc., Wilmington, MA) were sensitized to ovalbumin by i.p. injection with a 0.5-ml solution of 10 μg of ovalbumin and 10 mg of aluminum hydroxide in PBS. Booster injections were administered on weeks three and five to ensure high titers of IgE and IgG1 (Andersson, 1981). Seven to nine weeks after the initial injection, the animals were used to evaluate antigen-induced guinea pig airway responses.

To evaluate antigen-induced airway hyperresponsiveness in guinea pig, a baseline histamine bronchoprovocation was initially conducted in unrestrained animals. Guinea pigs (450–600 g) were
placed in a whole-body plethysmograph (Buxco Electronics, Troy, NY). The animals were exposed to 5-s bursts of histamine aerosol generated by a DeVilbiss ultrasonic nebulizer (Somerset, PA). Bronchoconstriction was assessed as $P_{\mathrm{enh}}$ (Chand et al., 1993). $P_{\mathrm{enh}}$ can be conceptualized as the phase shift of the thoracic flow and the nasal flow curves. Increased phase shift correlates with increased respiratory system resistance. $P_{\mathrm{enh}}$ is calculated by the formula: $P_{\mathrm{enh}} = \left[ \frac{T_e}{RT} - 1 \right] \times \left[ \frac{P_{\mathrm{EF}} / P_{\mathrm{IF}}}{1} \right]$, where $T_e$ is the total expiratory time, $RT$ is relaxation time, $P_{\mathrm{EF}}$ is peak expiratory flow, and $P_{\mathrm{IF}}$ is peak inspiratory flow. The peak bronchoconstrictor response in response to rising histamine concentrations of 0, 25, 50, 100, and 200 mg/ml in PBS administered at 10-min intervals was determined. Three days after the histamine baseline determination, the guinea pigs were again placed in the whole-body plethysmograph and exposed to ovalbumin for 30 min after a 3-s aerosolized burst of 0.1% ovalbumin in PBS. Six hours after antigen exposure, the development of hyperresponsiveness was evaluated by repeating the histamine bronchoprovocation. Administration of saline or SLPI had no effect on baseline airway responsiveness to histamine (data not shown).

SLPI was administered by intratracheal (i.t.) instillation in PBS (pH 7.2). After anesthetizing a guinea pig with inhaled methoxyflurane, an endotracheal tube (18-gauge Teflon sheath) was passed into the trachea with the aid of a fiberoptic light source. SLPI (or PBS for control animals) was dosed through the tube, followed by a bolus of air to facilitate dispersion. No overt side effects of SLPI administration were observed. In addition, heat-inactivated SLPI exhibited no pharmacologic activity in vivo.

**Airway Inflammation in Mice**

Airway inflammation was assessed in a murine model of atopic asthma (Blyth et al., 1996). Balb/c mice (15–18 g; Charles River Laboratories Inc., Wilmington, MA) received i.p. injections of either saline (0.9%) or PBS for control animals) was dosed through the tube, followed by a bolus of air to facilitate dispersion. No overt side effects of SLPI administration were observed. In addition, heat-inactivated SLPI exhibited no pharmacologic activity in vivo.

**Tracheal Mucus Velocity in Sheep**

Restrained adult ewes were nasally intubated with an endotracheal tube (inside diameter, 7.5 cm; Mallinckrodt Medical, Inc., St. Louis, MO) shortened by 6 cm. The cuff of the tube was placed immediately below the vocal cords, as verified by fluoroscopy, to allow for maximal exposure of the tracheal surface. The inspired air was warmed and humidified using a Bennett humidifier (Puritan-Bennett, Lenexa, KS). The endotracheal tube cuff was inflated only during antigen and drug exposure to minimize physical impairment of tracheal mucus velocity.

Tracheal mucus velocity was quantified by fluoroscopy as described previously (O’Riordan et al., 1997). Five to 10 radiopaque Teflon particles (1-mm diameter, 0.6-mm thick; 1.5 to 2.0 mg) were insufflated into the trachea using a modified suction catheter connected to a source of compressed air at a flow rate of 3 to 5 liters/min. Particle movement over a 1-min period, detected by fluoroscopy, was recorded on videotape. The actual distance of particle movement was determined to comparison with spaced radiopaque markers in an external collar. SLPI was delivered by nebulizer in PBS at physiologic pH according to regimens described below. No overt side effects of SLPI administration were observed.

**Statistical Analysis**

Two-way ANOVA, followed by the Newman-Keuls test, was used to evaluate areas under the curve for histamine dose responses in guinea pigs and $SR_L$ and tracheal mucus velocity in sheep. A paired $t$ test (two-tailed) was used to evaluate changes in airway hyperresponsiveness in sheep and airway inflammation in mice. $ED_{50}$ values were determined by linear regression analysis of dose-response data.

**Results**

**SLPI Inhibits Selected Serine Proteases**

SLPI exhibits potent broad spectrum inhibition of leukocyte and mast cell serine proteases implicated in asthma pathology. As shown in Table 1, SLPI blocks the activities of elastase as well as chymotrypsin- and trypsin-like serine proteases. Of particular note, SLPI is a potent inhibitor of cathepsin G, elastase, and mast cell tryptase. In contrast, factor Xa, kallikreins, thrombin, and plasmin are unaffected by SLPI at concentrations up to 83 to 100 μM.

**SLPI Inhibits Early- and Late-Phase Bronchoconstriction and Development of Airway Hyperresponsiveness in Antigen-Challenged Models**

The effects of SLPI against antigen-induced early and late bronchoconstriction and development of airway hyperresponsiveness were evaluated in a sheep bronchoprovocation model. In the control trial, antigen challenge stimulated an $SR_L$ increase from a baseline of 1.04 ± 0.04 liters/cm H$_2$O/liter/s to peak early- and late-phase responses of 3.95 ± 1.37 and 2.23 ± 0.13 liters/cm H$_2$O/liter/s. The development airway hyperreactivity was characterized as the reduction of the amount of carbocochol required to increase $SR_L$ by 400% from a baseline of 20.9 ± 4.16 breath units to 9.37 ± 2.69 breath units 24 h after antigen challenge. SLPI (3-mg dose) preadministered daily for 4 days, with the final dose 0.5 h before antigen challenge, provided 48 and 100% inhibition of peak early- and late-phase bronchoconstriction ($n = 4$) (Fig. 1A), respectively. Areas under the curve for the early- and late-phase responses were reduced by 73 and 95% ($p < .05$ versus antigen-stimulated responses), respectively. In addition, 84% inhibition of the development of hyperresponsiveness was observed 24 h after antigen challenge ($p < .05$ versus antigen-stimulated response) (Fig. 1B). In comparison, SLPI administered as a single dose from 10 to 100 mg 0.5 h before antigen challenge inhibited early- and late-phase responses with $ED_{50}$s of 76 and 48 mg, respectively, with a no
effect dose of 10 mg (data not shown). The prophylactic regimen provided inhibitory activity equivalent to that achieved with a single 100-mg aerosol dose of SLPI administered 0.5 h before antigen challenge.

SLPI was also evaluated for its effect on antigen-induced development of airway hyperresponsiveness in guinea pigs (Fig. 2). Six hours after antigen challenge, an increased pulmonary response to histamine bronchoprovocation was observed. Buffer or SLPI had no effect on baseline airway responsiveness to histamine (data not shown). Six hours after antigen challenge, airway hyperresponsiveness, assessed as the area under the histamine dose-response curve, was increased 143% above control (p < .05 versus baseline histamine response). Comparisons between treatment groups were based on areas under the histamine dose-response curves. i.t. instillation of SLPI daily for 3 days with the final dose administered 1 h before antigen challenge increased the potency for inhibition of the development of hyperresponsiveness, with an ED_{50} of 0.05 mg/kg (mean ± S.E.M., n = 6–8) (p < .05 for all treatment groups versus antigen-stimulated control response).

**TABLE 2**

<table>
<thead>
<tr>
<th>Sensitization</th>
<th>Challenge</th>
<th>Treatment</th>
<th>n</th>
<th>Eosinophils cells/ml</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Macrophages</th>
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<td>Saline</td>
<td>7</td>
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<td>260 ± 7</td>
<td>49 ± 6</td>
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<td>49 ± 3</td>
<td>2570 ± 282</td>
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<tr>
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<td>Ovalbumin</td>
<td>Saline</td>
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<td>1300 ± 346</td>
<td>2440 ± 1030</td>
<td>5100 ± 2040</td>
</tr>
<tr>
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<td>Ovalbumin</td>
<td>SLPI (1 mg/kg)</td>
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<td>270 ± 97</td>
<td>269 ± 70</td>
<td>86 ± 8</td>
<td>3950 ± 1000</td>
</tr>
</tbody>
</table>

*p < .05 versus saline-sensitized, saline-challenged control.

*p < .1 versus saline-sensitized, saline-challenged control.

*p < .05 versus ovalbumin-sensitized, ovalbumin-challenged control.

*p < .05 versus ovalbumin-sensitized, ovalbumin-challenged control.

**SLPI Inhibits Antigen-Induced Airway Inflammation in Mice**

The effect of SLPI against antigen-induced airway inflammation was evaluated in a murine model of atopic asthma (Blyth et al., 1996) (Table 2). Ovalbumin-sensitized mice challenged i.t. three times with antigen, each challenge ad-
ministered 3 days apart, exhibited increased numbers of eosinophils (saline control: 22 ± 4 cells/ml; postchallenge: 5310 ± 2000, p < .05 versus control), lymphocytes (saline control: 260 ± 7 cells/ml; postchallenge: 1300 ± 346, p < .05 versus control), and neutrophils (saline control: 49 ± 6 cells/ml; postchallenge: 2440 ± 1030, p < .1 versus control) in bronchoalveolar lavage fluid. Treatment with SLPI (1 mg/kg, i.t.) 30 min before each antigen challenge inhibited eosinophil, lymphocyte, and neutrophil influx by 95.3 (p < .05 versus antigen-stimulated control), 99.1 (p < .05 versus antigen-stimulated control), and 98.5% (p < .1 versus antigen-stimulated control), respectively. No significant inhibition of macrophage influx by SLPI was observed. These results demonstrate the ability of SLPI to inhibit antigen-induced airway inflammation.

**SLPI Prevents Antigen-Induced Reduction of Tracheal Mucus Velocity in Sheep**

Antigen-induced effects on mucociliary function in sheep were assessed by measuring tracheal mucus velocity (Fig. 3). Beginning 2 h after *Ascaris* challenge, significant reductions of tracheal mucus velocity from a baseline of 10.4 ± 1.12 mm/min were observed (n = 3) (#p < .05). After 6 h, tracheal mucus velocity had decreased to 42% of the baseline response. SLPI (30 mg) alone had no effect on baseline velocity (data not shown). SLPI (3-mg dose) predistributed daily for 4 days, with the final dose 0.5 h before antigen challenge, prevented the antigen-induced decrease in tracheal mucus velocity (n = 3) (*p < .05). This prophylactic regimen provided inhibitory activity equivalent to that achieved with a single 30-mg aerosol dose of SLPI administered 0.5 h before antigen challenge. The single administration, no-effect dose was 10 mg (data not shown).

**SLPI Exhibits Prolonged Pharmacodynamic Activity in Sheep and Guinea Pigs**

The pharmacodynamic activity of SLPI in the sheep model was evaluated using a modified prophylactic dosing regimen. As described above, SLPI (3 mg) was administered daily for 3 days before antigen challenge. The protocol was modified such that the final dose of SLPI was administered 24 h before antigen challenge (n = 3) (Fig. 4A). Despite the interval between the final dose and antigen challenge, SLPI retained significant inhibition of the area under the curve for antigen-induced change in SRL during the period 5 to 8 h after antigen challenge (p = 0.021 versus antigen-stimulated response). Development of airway hyperresponsiveness was also inhibited (data not shown). In contrast, the immediate bronchoconstriction was no longer inhibited.

The duration of action of SLPI was further examined in a guinea pig model of airway hyperresponsiveness (Fig. 4B). Hyperresponsiveness was evaluated as the change in the histamine dose required to induce a 100% change in airway resistance (PC100) 24 h after antigen challenge. SLPI was administered as a single 5-mg i.t. dose at various times before antigen challenge. Treatment with SLPI as long as 48 h before antigen challenge inhibited the subsequent development of airway hyperresponsiveness (n = 4 to 10) (*p < .05). In contrast, no inhibitory effect was observed for SLPI administered 72 h before antigen challenge. These results demonstrate a prolonged pharmacodynamic effect of SLPI against antigen-induced airway hyperresponsiveness.

**SLPI Exhibits Rapid Onset of Pharmacologic Activity**

SLPI was shown to have a rapid onset of pharmacologic efficacy in sheep when administered after antigen challenge (Fig. 5). SRL increased from a baseline of 1.03 ± 0.02 liters × cm H2O/liter/s to a peak early-phase response of 3.86 ± 0.32 liters × cm H2O/liter/s following antigen challenge. SLPI (30 mg) administered by aerosol 1 h after antigen challenge and the resultant peak of early-phase bronchoconstriction is effective in inhibiting the subsequent late-phase bronchoconstriction (n = 5) (*p < .05 versus antigen-stimulated bronchoconstriction) (Fig. 5A) and development of airway hyperresponsiveness (n = 5) (*p < .05 versus antigen-stimulated hyperresponsiveness) (Fig. 5B). A similar dosing protocol rapidly reversed the decrease in tracheal mucus velocity observed after antigen challenge (n = 6) (*p < .05) (Fig. 5C).

**Discussion**

SLPI, a naturally produced protein in the human airway (Thompson and Ohlsson, 1986; Eisenberg et al., 1990), represents a novel therapeutic approach to the treatment of asthma. Mounting evidence demonstrates the development of a protease-antiprotease imbalance in asthmatic airways. Immediate mast cell responses as well as later leukocyte activation significantly increase the protease load in human airways following antigen exposure (Wenzel et al., 1988; Fahy et al., 1995). The resultant increase in proteolytic activity contributes to airway pathophysiology as well as the airway remodeling associated with asthma. In this report, we have demonstrated that SLPI can provide effective therapy in preventing antigen-induced pathophysiologic airway responses, including early- and late-phase bronchoconstriction and development of airway hyperresponsiveness, mucociliary dysfunction, and airway inflammation in animal models of asthma.

Broad spectrum serine protease inhibitory activity is a key factor in the potential therapeutic utility of SLPI. SLPI provides potent broad spectrum inhibitory activity against mast cell and leukocyte-serine proteases, including cathepsin G, elastase, and trypsin. Previous reports suggest that inhibition of a single serine protease is not sufficient to impact that...
SLPI exhibits prolonged pharmacologic activity. A, sheep were dosed with a 3-mg aerosol dose of daily for 3 days, with the final dose administered 24 h before antigen challenge. Early- and late-phase bronchoconstriction were assessed as the percentage of increase of specific lung resistance (SRₜ) from a baseline of 0.98 ± 0.06 liters × cm H₂O/liter/s after antigen challenge (mean ± S.E.M., n = 3). SLPI inhibited the late-phase response, measured as the area under the curve for antigen-induced change in specific lung resistance during the period 5 to 8 h after antigen challenge (p = 0.021 versus antigen-stimulated response). B, guinea pigs were dosed with a single 5-mg dose of SLPI at different times before antigen challenge. Hyperreactivity was assessed as the change in the histamine dose required to induce a 100% change in airway resistance (PC₁₀₀) 24 h after antigen challenge (mean ± S.E.M., n = 5–10) (*p < 0.05 effect of SLPI versus antigen-stimulated response).

Pathophysiology and pathology associated with asthma. In sheep, α₁-protease inhibitor has been shown to prevent antigen-induced mucociliary dysfunction through inhibition of elastase (O’Riordan et al., 1997) and the development of airway hyperresponsiveness through inhibition of tissue kallikrein (Fortezu et al., 1996) while having no effect on early- or late-phase bronchoconstriction (Fortezu et al., 1996). In additional studies, selective trypsin inhibition was shown to prevent antigen-induced changes in pulmonary mechanics and antigen-induced airway eosinophilia (Clark et al., 1995) while having little impact on tracheal mucus velocity (unpublished data). In comparison, SLPI inhibits early- and late-phase bronchoconstriction, development of hyperresponsiveness, changes in mucociliary clearance, and airway inflammation following antigen challenge. Although SLPI fails to inhibit tissue kallikrein, inhibition of trypsin can prevent activation of prekallikrein as well as the direct release of bradykinin from kininogens (Imamura et al., 1996). In addition, the failure of SLPI to inhibit factor Xa, thrombin, or plasmin suggests that it will not disrupt coagulation or fibrinolysis.

The pharmacologic effect of SLPI is dependent on the antiprotease activity of the inhibitor. Denatured inactivated SLPI had no effect on the development of airway hyperresponsiveness in antigen-challenged guinea pigs. Although denatured SLPI was not evaluated in the sheep airway models, it has been shown that denatured proteins, including α₁-protease inhibitor, have no effect on antigen-induced airway hyperresponsiveness (Fortezu et al., 1996) or antigen-induced decreases in tracheal mucus velocity (O’Riordan et al., 1997). In addition, active SLPI had no effect on baseline airway responses in guinea pigs or sheep.

The breadth of pharmacologic activity for SLPI is similar to that reported for corticosteroids. As shown with SLPI, steroid treatment inhibits changes in both pulmonary mechanics (Abraham et al., 1986) and mucociliary function (O’Riordan et al., 1998) in bronchoprovocation models. It is interesting to note that steroids have been reported to increase SLPI transcript levels in airway epithelial cells in vitro (Abbinante-Nissen et al., 1995) and airway levels of SLPI in vivo (Stockley et al., 1986). Although the relative contribution of SLPI elevation to the overall therapeutic activity of steroids is unknown, these observations suggest that SLPI may provide therapeutic activity similar to steroids without the associated systemic adverse effects.

Of particular interest is the ability of a predosing regimen to significantly reduce the amount of SLPI required to provide therapeutic activity. In the guinea pig model of airway hyperresponsiveness, SLPI had an ED₅₀ of 0.56 mg/kg when administered 1 h before antigen challenge. In comparison, the ED₅₀ was reduced to <0.05 mg/kg when administered daily for 3 days before antigen challenge, with final dose administered 1 h before antigen challenge. Similar effects of pretreatment were observed in sheep, where a 3-mg dose of SLPI administered daily for 4 days with the final dose administered 0.5 h before antigen challenge (total dosage of 12 mg) had an inhibitory effect equivalent to single 100- or 30-mg doses administered 0.5 h before antigen challenge in the bronchoconstriction or tracheal mucus velocity models, respectively. In addition, recent evidence suggests that the efficacy of SLPI may also be increased by coadministration with heparin to promote interactions of the inhibitor with target serine proteases (Fath et al., 1998). The extended pharmacodynamic activity of SLPI, as well as the beneficial impact of predosing regimens, may be accounted for, only in part, by its long half-life in the airway. The elimination half-life values of SLPI in the epithelial lining fluid in sheep (Vogelmeier et al., 1990) and humans (McElvany et al., 1993) after aerosol administration are 12 and 6.5 h, respectively, as assessed by immunoassay. SLPI accumulation alone cannot account for the efficacy of predosing, because the total doses given to guinea pigs or sheep approximates only the no-effect doses for single administrations. One explanation may be that predosing reduces the protease tone to ameliorate the subsequent responses to antigen challenge, especially if proteases serve to prime the responses of mast cells and leukocytes (He and Walls, 1997).
Additionally, the predosing period may provide sufficient time for tissue distribution to maximize its inhibitory activity (Dietze et al., 1990). As a result of intracellular compartmentalization of SLPI or distribution to the epithelial surface in the airways, half-life values determined from bronchial fluid may fail to fully quantify SLPI in the airway (Stolk et al., 1995).

Another important pharmacologic characteristic of SLPI is its ability to inhibit responses when administered after the initiation of airway responses. As shown in the sheep models, administration of 30 mg of SLPI 1 h after antigen challenge and the resultant mast cell degranulation is capable of preventing the subsequent late-phase bronchoconstriction, development of airway hyperresponsiveness, as well as reversing the decrease of tracheal mucus velocity. These results demonstrate the potential utility of SLPI as a rescue therapy.

There is increased recognition of the need for agents that prevent airway remodeling to complement symptomatic relief in the treatment of asthma. The ability of SLPI to prevent antigen-induced leukocyte influx and mucociliary dysfunction suggests a potential intervention against critical pathologic changes of the asthmatic airway. The effect of SLPI on tracheal mucus velocity is complemented by its ability to inhibit elastase-induced bronchial secretory cell metaplasia (Lucey et al., 1990). Additional work is required to fully determine the extent of the ability of SLPI to prevent airway pathology associated with asthma.

This study demonstrates the efficacy of SLPI against pathophysiologic responses associated with asthma. SLPI provides broad spectrum inhibition of mast cell and leukocyte serine proteases, which contribute to asthma pathogenesis. In addition, as the predominant protease inhibitor of human airways, SLPI exhibits physical properties that are suited to enhance its potential pharmacologic impact in the lung. However, the ultimate determination of the therapeutic utility of SLPI depends upon its evaluation in human asthma.

**References**


