Interleukin-5-mediated Allergic Airway Inflammation Inhibits the Human Surfactant Protein C Promoter in Transgenic Mice*

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Allergen challenge in the lung of humans and animals is associated with surfactant dysfunction, but the mechanism of this effect has not been established. By using a murine model of asthma we now report the effect of allergen-induced airway inflammation on the expression of transgenes regulated by the human surfactant protein (hSP)-C promoter. The hSP-C 3.7-kilobase pair promoter was used to direct the expression of eotaxin, an eosinophil-selective chemokine, into the lungs of several transgenic lines. As expected, the transgenic mice expressed increased amounts of eotaxin mRNA and protein compared with wild-type mice. Surprisingly, following allergen challenge, there was a marked down-regulation of transgene mRNA in three independent transgenic lines. The down-regulation was in contrast to other related proteins such as endogenous eotaxin and surfactant protein D levels, which were both increased following allergen challenge. Consistent with specific down-regulation of the eotaxin transgene, there was no increase in pulmonary eosinophil levels in the transgenic mice above that found in wild-type mice. Analysis of hSP-C transgenic mice with distinct reporter genes and 3′-untranslated regions revealed that allergen challenge was directly affecting the hSP-C promoter. We hypothesized that allergen-induced down-regulation of the hSP-C promoter was related to the eosinophilic inflammation. To test this, we blocked eosinophilic inflammation in the lungs by treating mice with neutralizing antiserum against interleukin-5. Interestingly, this treatment also blocked allergen-induced inhibition of the hSP-C promoter. These results establish that allergic airway inflammation is associated with up-regulation of the surfactant proteins primarily involved in immunity, whereas down-regulation of the surfactant protein primarily involved in maintaining airway patency. Furthermore, the marked down-regulation of the hSP-C promoter is interleukin-5-dependent, implying a critical role for eosinophilic inflammation. These results suggest that alterations in surfactant protein levels may contribute to immune and airway dysfunction in asthma.

The surfactant proteins compose a group of molecules with diverse biological functions. Surfactant protein (SP)3-L-A and SP-D are calcium-dependent lectins that have primary roles in host defense by serving as regulators for innate immune responses (1, 2). SP-B and SP-C are primarily involved in maintaining physiological responses specifically in the lung by reducing lung surface tension thereby promoting proper respiration. In support of the critical function of surfactant proteins, a deficiency or dysregulation of surfactant proteins can have profound effects. For example, surfactant deficiency is responsible for the respiratory distress syndrome of infancy, and surfactant replacement is an effective therapy for this disorder. Furthermore, reduced levels of surfactant constituents are found in the lungs of patients with adult respiratory distress syndrome. Experimental disruption of SP-A, SP-B, or SP-D in mice results in various lung-specific problems including impairment in pulmonary host responses to infection (SP-A and SP-D) and neonatal respiratory failure (SP-B) (3–6). Hence, normal pulmonary function depends upon proper expression and function of surfactant proteins. The pleiotropic and fundamental properties of surfactant proteins underscore the importance of elucidating the role of surfactant proteins in various lung disorders.

Asthma is an airway inflammatory disorder whose prevalence is on the rise worldwide despite increased availability of therapeutic options (7). An extensive amount of experimentation in humans and rodents using models of allergen-induced airway inflammation has demonstrated an essential role for Th2 lymphocytes, eosinophils, and various cytokines (e.g. IL-4, IL-5, and IL-13) (8, 9), but the specific role of surfactant proteins has not been extensively examined. Because surfactant lipids and proteins are primary molecules involved in maintaining airway patency, an impaired process in patients with asthma, they are likely to be involved, at least in part, in the pathophysiology of asthma. Consistent with this, segmental allergen challenge in sensitized animals and humans results in loss of surfactant activity (10–12). Furthermore, although no difference in bronchoalveolar lavage fluid (BALF) phospholipid levels are seen between patients with asthma and controls, following allergen challenge, only patients with asthma have a change in the ratio between phosphatidylcholine and phosphatidylglycerol, the major lipid constituents of surfactant (13).

In the present investigation, we were interested in testing the hypothesis that allergic airway inflammation regulates the expression of hSP-C, a major gene product of alveolar type II epithelial cells. We chose to utilize an established murine model of asthma and an established transgenic system to facilitate direct analysis of human SP-C expression. Specific transcription of the hSP-C gene in pulmonary epithelial cells is surfactant protein; kb, kilobase pair; IL, interleukin; OVA, ovalbumin; PBS, phosphate-buffered saline; BALF, bronchoalveolar lavage fluid; ELISA, enzyme-linked immunosorbent assay; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; UTR, untranslated region.
regulated by the first 3.7 kb of the SP-C promoter (14). This region confers all of the necessary elements to direct specific expression of transgenes in the distal respiratory epithelium and has been instrumental for experimental analysis of lung development and disease (15). We therefore examined the effect of allergic airway inflammation on the expression of transgenes under the control of the hSP-C 3.7-kb promoter.

MATERIALS AND METHODS

Mice—Specific pathogen-free FVB mice, 8–10 weeks old were obtained from the NCI, Frederick, MD. Eotaxin or SP-B transgenic mice were generated by introducing the murine eotaxin cDNA construct (20–328 base pairs) or the coding region of the human SP-B cDNA (10–837 base pairs) (16) into the BarnHI/EcoRI site of the hSP-C 3.7 transgenic cassette (construct I and II, respectively) in Fig. 1. This transgenic construct uses the simian virus 40 (SV40) polyadenylation signal that is used as a probe for identifying transgenic mice by Southern blot analysis of tail DNA. In other experiments, transgenic mice containing a “mammalianized” SP-C 3.7 promoter followed by noncoding 5′ exons from the rabbit β-globin gene, the hSP-B cDNA, and the 3′ polyadenylation signal from bovine growth hormone were examined (construct III in Fig. 1) (17). All mice (FVB/N) were housed under pathogen-free conditions and examined between 6 and 16 weeks of age.

Allergic Triangle of Mice—A model of allergic inflammation was established using methods described previously (18). In brief, mice were lightly anesthetized with Metofane inhalation (methoxyfluorane; Bayer Pharmaceuticals, Spokane, WA) or 50 mg) of anti-IL-5 (TRFK-5) or control IgG1 (GL113 (anti-IL-5) of streptavidin-horseradish peroxidase (Immunotech, Westbrook, ME)) were applied to the nares using a micropipette with CO2 inhalation and exsanguinated, and the trachea was cannulated and lavaged with two replicates volumes (0.8 ml) of normal saline containing 0.2 mM EDTA to obtain BALF. Lavage fluid was centrifuged at 250 x g for 20 min at 4 °C, and the cell pellet was resuspended in PBS and analyzed for total cell count and differential analysis. Aliquots of the BALF supernatant were taken for total protein analysis by bicinchoninic acid analysis (Pierce). The 32P-labeled probes were prepared for mouse eotaxin cDNA (19), SV40 polyadenylation signal sequence, and mouse surfactant protein cDNAs (SP-A, SP-B, SP-C, and SP-D) (kindly provided by Dr. Jeffrey Whitsett) as described previously (20, 21) and hybridized overnight at 42 °C using 1–2 × 106 dpm/ml of the respective probes (22, 23). The membranes were washed for 20 min at 42 °C, 20 min at 50 °C, 20 min at 60 °C in 2× SSC, 0.1% SDS and 20 min in 0.1× SSC, 0.1% SDS.

Western Blot Analysis—Surfactant proteins (SP-A, SP-B, SP-C, and SP-D) in the BALF were examined by Western blot analysis. Mice were lavaged with five 1.0-ml washes of PBS (pH 7.4 containing 1% FBS and 0.2 mM EDTA). The recovered BALF (~4.6 ml) was centrifuged at 18,000 × g for 20 min, and the pellet was resuspended in 100 µl of distilled water. Total protein in each samples was determined by bicinchoninic acid assay (Life Technologies, Inc). Quantitation of Eotaxin in BALF—A sandwich enzyme-linked immunosorbent assay (ELISA) was performed to measure eotaxin protein levels in BALF. The wells of ELISA plates (DYNEX Technologies, Chantilly, VA) were first coated with 40 ng (100 µl) of diluted mouse eotaxin-specific goat IgG (AF-420NA; R&D Systems, Minneapolis, MN) in PBS and incubated overnight at 4 °C. Following the incubation, the plate was washed with phosphate-buffered saline with 0.05% Tween 20 (PBST). Nonspecific binding sites were blocked with 300 µl of PBS containing 1% bovine serum albumin, 5% sucrose, and 0.05% Na2SO4 and incubated at room temperature for 1 h. Mouse eotaxin (kindly provided by PeproTech, Rocky Hill, NJ) was used as a quantitation standard. The eotaxin standard and BALF samples were diluted in the blocking solution and added to each well and incubated for 2 h at room temperature. Wells were washed three times with PBST and then further incubated with 20 ng (100 µl) of mouse eotaxin affinity-purified polyclonal secondary antibody (BAF-420, R & D Systems) for 2 h at room temperature followed by three washes with PBST. Fifty nanograms (100 µl) of streptavidin-horseradish peroxidase (Immunotech, Marseilles, France) was added and incubated for 1 h at room temperature. horseradish peroxidase substrate solution that contains 0.3 g/liter in a glycine/citric acid buffer and 0.01% H2O2 (Kirkgaard & Perry, Gaithersburg, MD) was used to complete the reaction. The substrate reaction was stopped by adding 50 µl/well of 1 M H2SO4 and quantitated by an ELISA plate reader at 495 nm.

Statistical Analysis—Data are expressed as mean ± S.E. Statistical significance comparing different sets of mice was determined by Student’s t test.

RESULTS

Generation of hSP-C Eotaxin Transgenic Mice—The 3.7-kb 5′-flanking region of the hSP-C gene contains all of the necessary elements to promote specific expression of transgenes into pulmonary type II cells (27). We initially generated transgenic mice that utilized the hSP-C promoter to direct expression of eotaxin, an eosinophil-selective chemokine (28, 29), in type II cells of the respiratory epithelium. The hSP-C promoter contains 5′ of all constructs and is illustrated in the shaded rectangles. The inserted cDNAs are murine eotaxin (designated mEotaxin) in construct I and hSP-B in constructs II and III, respectively. The polyadenylation signal (3′-polyadenylation-Exon) is derived from the SV40 small intron and exon (designated I/E) (constructs I and II) or from the bovine growth hormone exon (construct III). In construct III, noncoding exons 2 and 3 from the rabbit β-globin gene (designated rGlob E2/3) have been inserted between the hSP-C promoter and the hSP-B cDNA. The relative proportions of the DNA components are not drawn to scale.

Northern Blot Analysis—RNA was extracted from the lung tissue using the Trizol reagent (Life Technologies, Inc) following the manufacturer’s protocol. Twenty micrograms of total RNA from each sample were electrophoresed on 1.5% formaldehyde agarose gels and transferred to GeneScreen hybridization membrane (PerkinElmer Life Sciences) with 10× SSC. The membrane was UV cross-linked and prehybridized at 42 °C for 1 h in a 50% formamide buffer (pH 7.5), containing 10% dextran sulfate, 5× SSC, 1× Denhardt’s solution, 1% SDS, 100 µg/ml of herring sperm DNA, and 20 µl Tris. The 32P-labeled probes were prepared for mouse eotaxin cDNA (19), SV40 polyadenylation signal sequence, and mouse surfactant protein cDNAs (SP-A, SP-B, SP-C, and SP-D) (kindly provided by Dr. Jeffrey Whitsett) as described previously (20, 21) and hybridized overnight at 42 °C using 1–2 × 106 dpm/ml of the respective probes (22, 23). The membranes were washed for 20 min at 42 °C, 20 min at 50 °C, 20 min at 60 °C in 2× SSC, 0.1% SDS and 20 min in 0.1× SSC, 0.1% SDS.

The hSP-C promoter transgenic constructs. The hSP-C promoter containing transgenic constructs employed in this study are schematically diagrammed. The hSP-C promoter is located 5′ of all constructs and is illustrated in the shaded rectangles. The inserted cDNAs are murine eotaxin (designated mEotaxin) in construct I and hSP-B in constructs II and III, respectively. The polyadenylation signal (3′-polyadenylation-Exon) is derived from the SV40 small intron and exon (designated I/E) (constructs I and II) or from the bovine growth hormone exon (construct III). In construct III, noncoding exons 2 and 3 from the rabbit β-globin gene (designated rGlob E2/3) have been inserted between the hSP-C promoter and the hSP-B cDNA. The relative proportions of the DNA components are not drawn to scale.
cells of mice to examine the consequences of eotaxin overexpression in the lungs (see transgenic construct I in Fig. 1). Three different eotaxin transgenic founder lines were established that had variable but increased expression of eotaxin lung mRNA (Fig. 2A) and protein in the BALF (Fig. 2B). For example, wild-type mice had $345 \pm 6205$ pg/ml eotaxin in the BALF, and the transgenic lines had increased levels ranging from $687 \pm 61$ to $3587 \pm 223$ pg/ml. The specificity of the ELISA for eotaxin was determined by demonstrating undetectable eotaxin in mice genetically deficient in eotaxin (Fig. 2B). Interestingly, the baseline level of eosinophils in the BALF and lungs was not different between wild-type and eotaxin transgenic mice (data not shown). This prompted investigation of the effect of the eotaxin transgene during allergic airway inflammation, since it was hypothesized that eotaxin would cooperate with other signals generated during allergic airway inflammation (e.g., IL-5) to promote eosinophil recruitment into the lung.

**FIG. 2.** Transgene expression in hSP-C-driven eotaxin transgenic mice. A, the level of transgene mRNA was determined by Northern blot analysis of total RNA isolated from wild-type (WT) and three different eotaxin transgenes lines, designated ET 1.2, ET 1.5, and ET 2.4. The expression of transgene was determined by hybridization to the transgene-specific probe from the SV40 polyadenylation sequence. The total amount of RNA was visualized by ethidium bromide (EtBr) staining of the gel. Each lane represents a separate mouse. B, the level of eotaxin protein in the BALF was determined by ELISA. The specificity of the ELISA was determined by analysis of BALF from eotaxin-deficient mice (Eotaxin −/−). The results of this experiment are expressed as mean ± S.E., n = 7–8 mice.

**FIG. 3.** Eosinophil levels in the BALF following allergen or saline challenge. Wild-type and hSP-C eotaxin-transgenic mice were challenged with saline or *A. fumigatus* allergen, and the level of eosinophils in the BALF was determined. Each circle represents a separate animal. The horizontal line is the mean.

Aspergillus-induced Airway Inflammation in Eotaxin Transgenic Mice—Allergic airway inflammation (asthma) can be triggered in mice by repeated exposure of the airways to antigens. For example, exposure of naive mice to *A. fumigatus* antigens 3 doses/week for 3 weeks results in marked inflammatory responses. We therefore subjected eotaxin transgenic mice to Aspergillus-induced allergic airway inflammation to determine if the eotaxin transgene would enhance eosinophil recruitment. In wild-type mice, 18 h after the last allergen exposure, there was ~100-fold increase in the total BALF cells with the largest increase being in the eosinophil lineage ($4 \pm 2 \times 10^5$ to $1.9 \pm 0.16 \times 10^6$) followed by the neutrophil lineage ($1.3 \pm 1.2 \times 10^4$ to $5.6 \pm 2.4 \times 10^5$). We next examined the effect of the airway challenge on the number of eosinophils in the eotaxin transgenic mice. Surprisingly, we did not observe a significant difference in eosinophil levels between wild-type and eotaxin transgenic mice (Fig. 3). Although a highly significant increase in eosinophil numbers was observed in both groups when compared with their respective saline control mice, the number of eosinophils in the allergen-challenged wild-type and eotaxin
transgenic mice was comparable \((1.39 \pm 1.06 \times 10^6\) and \(3.2 \pm 1.5 \times 10^6\), respectively). Similarly, levels of eosinophils in the lung tissue did not differ between wild-type and transgenic mice (data not shown).

**Allergen Exposure Induces Down-regulation of the hSP-C Transgene**—Since we expected the eotaxin transgene to cooperate with allergen-induced signals in the lung thereby promoting increased eosinophil levels, we hypothesized that allergen challenge might be inhibiting the expression of the transgene. We therefore examined the effect of airway inflammation on the transgene expression. Interestingly, whereas eotaxin transgenic mice had readily detectable transgene expression following saline treatment, the transgene mRNA was barely detectable following allergen challenge (Fig. 4A). The decreased expression of the transgene was seen in all hSP-C transgenic lines (data not shown). We also subjected sensitized mice to allergen challenge with OVA to determine if the allergen-induced SP-C down-regulation was applicable to other allergens. Similar to the effects of challenge with *A. fumigatus*, OVA exposure also down-regulated the level of the eotaxin transgene (Fig. 4B).

**Effect of Allergic Airway Inflammation on the Expression of Other Related Genes**—We were next interested in determining if allergic airway inflammation was a nonspecific phenomenon associated with the down-regulation of other epithelial genes. We therefore examined the expression of the endogenous murine surfactant proteins and eotaxin. Of note, the mRNA for SP-D, and to a lesser extent SP-A, was increased following allergen challenge compared with saline challenge (Fig. 5A). Additionally, the protein level of SP-D, and to a lesser extent SP-A, was also increased. Interestingly, whereas the endogenous SP-C mRNA was not altered at this time point, the level of the SP-C protein was substantially reduced (Fig. 5B). We also examined the expression of the endogenous eotaxin mRNA. As expected, allergen challenge markedly increased the level of eotaxin in the lung. Taken together, these results.
Surfactant proteins have been recognized as essential molecules involved in two main critical processes in the lung, maintenance of alveolar surfactant function and host defense. As such, they have the potential to be critically involved in the pathogenesis of asthma, an allergic inflammatory disorder of the lungs characterized by airway obstruction. To examine this process, we have subjected mice to experimental models of asthma (antigen-induced allergic airway inflammation) and examined the effect on transgenes regulated by the hSP-C promoter, as well as on the level of endogenous surfactant protein RNA signal and revealed 12.0 ± 2.1 (mean ± S.D.)-fold decrease following allergen challenge.

**FIG. 7.** The effect of allergen challenge on the expression of the hSP-C transgene containing the bovine growth hormone 3′-UTR. The level of hSP-C driven-SP-B transgene mRNA was determined by Northern blot analysis of total RNA isolated from mice challenged with saline or *A. fumigatus* allergen. The expression of the transgene was determined by hybridization to the transgene-specific cDNA probe from the bovine growth hormone polyadenylation sequence. The total amount of RNA was visualized with ethidium bromide (EtBr) staining of the gel. Each lane represents a separate mouse. Quantitative analysis of the relative mRNA signal was performed by comparison of the intensity of the transgene mRNA to the 28 S ribosomal protein RNA signal and revealed 12.0 ± 2.1 (mean ± S.D.)-fold decrease following allergen challenge.

**FIG. 8.** The effect of anti-IL-5 on allergen-induced inhibition of the hSP-C eotaxin transgene. *A.* mice were subjected to treatment with saline (−) or allergen (+) (*A. fumigatus*), and the level of the hSP-C eotaxin transgene was determined. Allergen-challenged mice were pretreated with anti-IL-5 or isotype-matched control antiserum. The level of the mRNA was determined by Northern blot analysis using a probe encoding for the SV40 polyadenylation sequence. Each lane represents a separate mouse. *B.* Quantitative analysis (by PhosphorImager computation) of the relative mRNA signal from the bands in *A* was performed by comparison of the intensity of the transgene mRNA to the 28 S ribosomal protein mRNA signals. The results of this experiment are expressed as mean ± S.E.

establish that the down-regulation of the hSP-C transgene by allergen was a specific event, not likely related to generalized epithelial cell toxicity.

Allergen-induced Down-regulation of the hSP-C Transgene Is Independent of Eotaxin—We were next interested in ruling out the possibility that the mechanism for the down-regulation of the transgene by allergen was dependent upon the presence of the eotaxin cDNA in the genetic construct. Although unlikely, it remained possible that the inhibition of the transgene was dependent upon sequences in the eotaxin cDNA or that the overexpressed eotaxin protein was inhibiting the transgene. To test these possibilities, we examined the effect of allergen challenge on an independent hSP-C transgenic mouse line. We examined transgenic mice that contained the hSP-B cDNA under the control of the same 3.7-kb hSP-C promoter used in the eotaxin transgenic mice (*construct II* in Fig. 1) (16). Interestingly, down-regulation of the hSP-C-SP-B transgene in response to allergen was observed in all mice when compared with their respective saline-treated controls (Fig. 6).

Allergen-induced Down-regulation of the hSP-C Transgene Is Independent of the 3′-Untranslated Region (UTR) of the Construct—It remained possible that the ability of allergen exposure to decrease the level of transgenes regulated by the hSP-C promoter was dependent upon the 3′-UTR of the transgenic construct, since 3′-UTR have been shown to be involved in mRNA stability (30). Since the hSP-C transgenic construct contained a viral polyadenylation signal (from SV40) (14), we were interested in examining the effect of allergen challenge in hSP-C transgenic mice that utilized the same 3.7-kb hSP-C promoter but a different 3′-UTR. To test this, we employed transgenic mice that contained a mammalian-derived 3′-UTR composed of the bovine growth hormone polyadenylation sequence (*construct III* in Fig. 1) (17). Interestingly, we observed down-regulation of the hSP-C-transgene in these mice in response to allergen when compared with saline-treated controls (Fig. 7). Taken together, these results demonstrate that allergen-induced inflammation down-regulates the hSP-C transgene by directly affecting the hSP-C promoter.

Allergen-induced Down-regulation of the hSP-C Transgene Is Blocked by Anti-IL-5—We hypothesized that the influx of inflammatory cells during allergic airway inflammation was involved in down-regulating the SP-C transgene. Since eosinophils are the predominant inflammatory cell in *A. fumigatus*-induced allergic airway inflammation, we were interested in determining if these cells could directly down-regulate the SP-C transgene. To test this hypothesis, we treated mice with neutralizing doses of anti-IL-5, a well accepted approach for attenuating allergen-induced eosinophil recruitment to the lungs (31). For example, eosinophils were 1.8 ± 0.4 × 10⁶ and 1.0 ± 0.3 × 10⁷ in the BALF of allergen-challenged mice following nonimmune control and anti-IL-5 serum, respectively. Interestingly, mice treated with anti-IL-5 (but not isotype matched control antiserum) were resistant to allergen-induced SP-C inhibition. Northern blot analysis of lung RNA revealed readily detectable transgene mRNA levels in mice treated with saline and in allergen-challenged mice pretreated with anti-IL-5 (Fig. 8A). In contrast, allergen-challenged mice treated with control antiserum had a markedly reduced level of transgene mRNA. Quantitative analysis of the transgene mRNA level, performed by normalization of the mRNA signal to the 28 S ribosomal protein mRNA, revealed no significant inhibition of transgene mRNA in mice treated with anti-IL-5 (Fig. 8B). These data suggest that the allergen-induced eosinophilic inflammation was responsible for the inhibition of the hSP-C promoter.

**DISCUSSION**

**Surfactant Proteins**

Surfactant proteins have been recognized as essential molecules involved in two main critical processes in the lung, maintenance of alveolar surfactant function and host defense. As such, they have the potential to be critically involved in the pathogenesis of asthma, an allergic inflammatory disorder of the lungs characterized by airway obstruction. To examine this process, we have subjected mice to experimental models of asthma (antigen-induced allergic airway inflammation) and examined the effect on transgenes regulated by the hSP-C promoter, as well as on the level of endogenous surfactant...
proteins. We have found the following: 1) allergen challenge inhibits the human SP-C promoter; 2) allergen-induced inhibition of the hSP-C promoter is dependent on IL-5, suggesting a critical role for eosinophilic inflammation in this process; and 3) that allergen challenge increases the surfactant proteins known to be involved in innate immune responses (SP-A and SP-D). Previous studies on the expression of surfactant proteins in patients with asthma have been limited to analysis of surfactant activity in BALF rather than surfactant protein mRNA levels, since surfactant is synthesized by small airway and alveolar cells that reside in regions that are difficult to sample experimentally in humans. Several recent clinical studies have shown that the BALF of patients with asthma at baseline or following segmental allergen challenge contains dysfunctional surfactant (10, 11). Additionally, experimental dissection in a guinea pig model of asthma has revealed that allergen challenge decreases the surfactant activity in the BALF (32). Additionally, significant changes in surfactant levels have been reported in patients with interstitial pulmonary fibrosis and adult respiratory distress syndrome (1).

In the present study, we have analyzed the effect of experimental allergen challenge on the expression of the hSP-C promoter. We have chosen to focus primarily on transgenic mice expressing reporter genes under the control of the hSP-C 3.7-kb promoter. Although this region may have distinct regulatory elements compared with the mouse or human SP-C promoter, it is sufficient for directing specific gene expression in type II pneumocytes, the cells that normally synthesize SP-C. This promoter construct has been used to generate over 100 different transgenic mouse lines for extensive analysis of lung development and function (15, 27). Our initial results were limited to hSP-C transgenic mice overexpressing murine eotaxin cDNA and demonstrated that allergen challenge markedly inhibited the expression of this transgene. Experimental dissection of hSP-C transgenic mice that were generated with distinct reporter cDNAs (e.g. SP-B instead of eotaxin) and 3′UTRs (e.g. bovine growth hormone instead of the SV40 polyadenylation signal) revealed that allergen challenge was affecting the hSP-C promoter, rather than other elements present in the transgenic construct. Most experimental allergen challenges were conducted utilizing A. fumigatus as an antigen. It should be noted that this antigen was selected because it represents a model antigen that has already been extensively analyzed (33–35). To prove that the results were not strictly related to this allergen, an unrelated antigen (OVA) was used to demonstrate that the effect was not allergen-specific. Additionally, airway inflammation itself, and not allergen challenge, was demonstrated to be responsible for inhibition of the hSP-C promoter. In particular, depletion of allergen-induced airway eosinophilia with anti serum against IL-5 resulted in abatement of the inhibitory effect of allergen challenge. These data suggested that eosinophil influx into the lung was responsible for inhibition of the hSP-C promoter. Eosinophils are known to be a rich source of pleiotropic molecules including lipid mediators (e.g. leukotrienes and platelet-activating factor), cationic granule proteins (e.g. major basic protein), and cytokines (e.g. IL-1 and transforming growth factor-α/β) (36–38). Furthermore, pulmonary epithelial cells express receptors for several of these products including tumor necrosis factor-α and transforming growth factor-α/β. Interestingly, transcription of the SP-C promoter is inhibited by certain proinflammatory triggers such as tumor necrosis factor-α (39). In the mouse SP-C gene, the tumor necrosis factor-α-sensitive elements are located 320 base pairs 5′ from the start of transcription (39). However, it should be pointed out that although the 3.7-kb hSP-C promoter has been extensively characterized and appears to function similar to the endogenous promoter, it remains possible that promoter elements not included in the 3.7-kb region may be differentially affected by allergen challenge. This concern may explain the finding that the endogenous mouse SPC mRNA is not substantially reduced by allergen challenge. However, since the SPC protein level is reduced by allergen challenge, we favor the view that the mouse SPC mRNA is down-regulated at earlier points in the allergen challenge regime. Alternatively, the hSPC promoter may be distinctly inhibited by allergic airway inflammation compared with the mouse SPC promoter. Taken together, our data suggest that allergen exposure induces eosinophilic lung inflammation which inhibits the transcription of the hSP-C promoter.

In contrast to the inhibition of the hSP-C promoter by allergen challenge, we have found that allergic lung inflammation increases the levels of SP-A and SP-D (but not SP-B). These proteins are known to be critically involved in innate immune responses. Interestingly, the binding of SP-A and SP-D to A. fumigatus enhances the phagocytosis of this organism by human neutrophils and alveolar macrophages (40, 41). Furthermore, these proteins can inhibit specific IgE binding to A. fumigatus allergens and block allergen-induced histamine release from human basophils (42). Thus, the ability of allergen challenge to increase the level of the surfactant proteins involved in immune responses indicates that these proteins are indeed likely to be involved in the host response to allergens.

Finally, our results have several biological conclusions concerning eosinophil recruitment to the lung. We demonstrate that overexpression of eotaxin in the lung is not sufficient for eosinophil recruitment to this organ. This is consistent with the finding that eotaxin is constitutively produced by epithelial cells in the murine lung but not associated with substantial levels of pulmonary eosinophils (43). In contrast, in other organs such as the small intestine, eotaxin expression is critical for base-line eosinophil tissue homing (23). We also demonstrate that overexpression of eotaxin is not associated with eosinophil desensitization, as has been reported with other chemokine transgenic mice (44). In particular, we found no significant difference in the level of allergen-induced eosinophil recruitment into the lung of transgenic mice compared with wild-type mice (Fig. 3).

In conclusion, these findings indicate that attention should be focused on the level of expression of transgenes regulated by the hSP-C promoter when hSP-C transgenic mice are subjected to inflammatory triggers. Additionally, these results suggest that surfactant protein dysfunction in patients with asthma is likely to be caused, at least in part, by allergen-induced and IL-5-dependent decreases in the level of SP-C.

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REFERENCES

1. Mason, R. J., Greene, K., and Voelker, D. R. (1998) Am. J. Physiol. 275, L1–L13
2. LeVine, A. M., Kurak, K. E., Wright, J. R., Watford, W. T., Bruno, M. D., Ross, G. F., Whitsett, J. A., and Korthagen, T. R. (1999) Am. J. Respir. Cell Mol. Biol. 20, 279–286
3. Clark, J. C., Wert, S. E., Bachurski, C. J., Stahlman, M. T., Stripp, B. R., Weaver, T. E., and Whitsett, J. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7784–7788
4. Korthagen, T. R., Bruno, M. D., Ross, G. F., Huelmans, K. M., Igekami, M., Jube, A. H., Wert, S. E., Stripp, B. R., Morris, R. E., Glasser, S. W., Bachurski, C. J., Iwamoto, H. S., and Whitsett, J. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9594–9599
5. Korthagen, T. R., Sheftelyevich, V., Burhans, M. S., Bruno, M. D., Ross, G. F., Wert, S. E., Stahlman, M. T., Jube, A. H., Igekami, M., Whitsett, J. A., and Fisher, J. H. (1998) J. Biol. Chem. 273, 28438–28443
6. Weaver, T. E., and Beck, D. C. (1999) Biol. Neonate 76, Suppl. 1, 15–18
7. Holgate, S. T. (1999) Nature 402, 2–4
8. Drazen, J. M., Arm, J. P., and Austen, K. F. (1996) J. Exp. Med. 183, 1–5
