Therapeutic Dosing with Anti-Interleukin-13 Monoclonal Antibody Inhibits Asthma Progression in Mice

Gaoyun Yang, Li Li, Amy Volk, Eva Emmell, Ted Petley, Jill Giles-Komar, Patricia Rafferty, Mani Lakshminarayanan, Don E. Griswold, Peter J. Bugelski, and Anuk M. Das


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ABSTRACT

In vivo models have demonstrated that interleukin-13 (IL-13) plays an important role in asthma; however, few studies have evaluated the effect of inhibition of IL-13 on established and persistent disease. In the present study, we have investigated the effect of a therapeutic dosing regimen with an anti-IL-13 monoclonal antibody (mAb) in a chronic mouse model of persistent asthma. BALB/c mice were sensitized to allergen [ovalbumin (OVA); on days 1 and 8] and challenged with OVA weekly from day 22. Anti-IL-13 mAb or vehicle dosing was initiated following two OVA challenges when disease was established. At this time, mice exhibited airway hyperresponsive-ness (AHR), increased mucus production, inflammation, and initiation of subepithelial fibrosis compared with saline-challenged mice. Mice received four additional OVA challenges. Treatment with anti-IL-13 mAb inhibited AHR and prevented the further development of subepithelial fibrosis and progression of inflammation. Furthermore, mAb treatment reversed the mucus hyperplasia to basal levels. These effects were associated with an inhibition of cytokines, chemokines, and matrix metalloproteinase-9. These data demonstrate that neutralization of IL-13 can inhibit the progression of established disease in the presence of repeated allergen exposures.

Asthma is characterized by the presence of reversible bronchoconstriction, increased sensitivity to specific and nonspecific bronchospastic agents, and excessive mucus production. These clinical features are accompanied by an underlying pathology of inflammation and airway remodeling. The pathologic changes are thought to contribute to the clinical symptoms of the disease (Fireman, 2003). Current therapies for asthma provide symptomatic control but do not halt the underlying disease, highlighting the unmet medical need. Patients are continually exposed to allergens or other bronchospastic agents that may contribute to the maintenance or progression of persistent disease. Evidence to date suggests that interleukin-13 (IL-13) has an important role in asthma; it is associated with human disease, and preclinical models have demonstrated that it induces many of the features associated with human asthma.

Elevated levels of IL-13 mRNA and protein (Huang et al., 1995) have been described in human disease pathogenesis, and polymorphisms in the IL-13 gene have been associated with asthma (Wills-Karp, 2000). Multiple studies have demonstrated that administration of recombinant murine (rm) IL-13 to the lungs of mice induces airway mucus hyperplasia, eosinophilia, and airway hyperresponsiveness (AHR) (Grunig et al., 1998; Wills-Karp et al., 1998; Singer et al., 2002; Kibe et al., 2003; Vargaftig and Singer, 2003a,b). These effects of IL-13 are reproduced in transgenic mouse systems where IL-13 overexpression is induced in a constitutive or inducible manner (Zhu et al., 1999, 2001; Lanone et al., 2002). Chronic transgenic overexpression of IL-13 also induces subepithelial fibrosis and emphysema. Mice deficient in the IL-13 (and IL-4) signaling molecule signal transducer and activator of transcription 6 (STAT6) fail to develop allergen-induced AHR and mucus hyperplasia (Kuperman et al., 2002). Finally, utilization of an IL-13-specific neutralization strategy with soluble IL-13 receptor fusion protein (sIL-13Ra2Fc) has demonstrated the pivotal role of this cytokine in experimental allergen ovalbumin (OVA)-induced airway disease (Grunig et al., 1998; Wills-Karp et al., 1998; Taube et al., 2002).
Clinically, therapeutic interventions occur in the presence of established disease. To date, experimental therapeutic intervention studies have not investigated the effects of IL-13 neutralization in the presence of chronic, persistent allergen challenges. In the present study, we used a rat monoclonal antibody (mAb) to neutralize the actions of mouse IL-13 specifically and initiated treatment with the mAb once disease was established. Furthermore, we continued mAb treatment in the presence of further allergen challenges. Our data demonstrate that anti-IL-13 mAb treatment inhibits AHR, chronic inflammation, subepithelial fibrosis, and reverses mucus hyperplasia. Associated with these effects, neutralization of IL-13 inhibited the production of multiple cytokines, chemokines, and matrix metalloproteinase-9 (MMP-9). These data support the hypothesis that IL-13 is an important upstream mediator involved in asthma pathogenesis and disease progression.

Materials and Methods

Mice. BALB/c female mice (6–8 weeks old, weighing 20–25 g) were from Charles River Laboratories (Raleigh, NC). All mice were maintained under specific pathogen-free conditions and maintained on an OVA-free diet with free access to food and water at Centocor Inc. Sprague-Dawley rats (20 weeks old) were used for the generation of anti-IL-13 mAb and housed and cared for at Covance Research Products Inc. (Denver, PA). All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of Centocor Inc. or Covance Research Products Inc. as appropriate.

Generation of Rat Anti-Mouse IL-13 mAb (CNTO 134). Rat-anti-mouse IL-13 mAb (CNTO 134; IgG2a isotype) was generated at Centocor Inc. with 50 µg of rm IL-13 (R&D Systems, Minneapolis, MN) using conventional immunization protocols and hybridoma technology. Solid-phase enzyme immunoassay was used to screen for antibodies specific for rm IL-13. Briefly, plates were coated overnight with the IL-13 wells and washed. The wells were probed with horseradish peroxidase-labeled goat anti-rat IgG. Plates were washed then incubated with a final concentration of 5 ng/ml mouse IL-13 together with different concentrations of CNTO 134 for 3 days at 37°C and 5% CO2. The IL-13-dependent cell proliferation was measured using a luminescent ATP detection assay kit (PerkinElmer Life and Analytical Sciences, Boston, MA). Inhibition of IL-13 resulted in lower proliferation of B9 cells that could be measured based on the amount of ATP per well. The concentration of CNTO 134 that inhibited B9 cell proliferation by 50% (IC50) was 17 ng/ml (data not shown).

In Vivo OVA Model Protocol. Mice were immunized intraperitoneally (i.p.) with 10 µg of OVA (Sigma-Aldrich, St. Louis, MO) in 100 µl of PBS mixed with the same volume of Injet Alum (Pierce, Rockford, IL) on day 1 and boosted in the same way on day 8. On day 22, 29, 36, 43, 50, and 52, mice received an intranasal (i.n.) challenge with 50 µl of PBS or 100 µg of OVA (2 mg/ml) under ketamine/xylazine anesthesia (90 and 10 mg/kg i.p., respectively). Rat anti-mouse IL-13 mAb (CNTO 134; 500 µg/mouse) or vehicle (PBS; 200 µl/mouse) treatment was initiated on day 36, and agents were administered intravenously (i.v.) 1 h prior to each i.n. challenge. Our previous in-house studies have established the appropriateness of using the vehicle (PBS) as the control for CNTO 134 since no biological differences are observed in the OVA model between mice treated with vehicle and those treated with control IgG (data not shown). A subset of mice was sacrificed on day 36; these mice did not receive day 36 i.n. challenge or i.v. treatment (Fig. 1).

AHR. AHR was measured in mice using whole-body plethysmography on day 36 and 53 (Buxco, Sharon, CT). AHR was measured following aerosolization of PBS followed by increasing concentrations of methacholine (10–40 mg/ml; Sigma-Aldrich) for 2 min into the chamber. AHR was expressed as the average enhanced pause (Penh) (Hamelmann et al., 1997) that was measured over a 5-min period following aerosol exposure to PBS or methacholine. All mice were exposed to PBS and subsequently exposed to each methacholine dose. There was approximately an interval of 60 min between each aerosol exposure, and within this period of time the Penh values had returned to baseline.

Bronchoalveolar Lavage (BAL). After AHR measurements, mice were euthanized (CO2 asphyxiation); the trachea was cannulated, BAL was performed by slowly injecting 1 ml of PBS (once) into the trachea, and the lavage was retrieved. Supernatants were collected after centrifugation (10 min; 1500 rpm) for further analyses. BAL cells were resuspended in 1 ml of PBS (containing 2% fetal calf serum) for total and differential cell counts.

Histology and Morphometric Analyses. Following BAL, the right lung was clamped off and removed, and the left lung was fixed with 10% buffered formalin under constant pressure of 15-cm water. After fixation, lungs were dehydrated and embedded in paraffin by routine methods. Lungs were oriented in the blocks so that parabihal sagittal sections were obtained. Five micron serial sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS) (counterstained with hematoxylin), or picric-Sirius Red (SR). H&E-stained sections were used for general evaluation of histopathologic change; PAS was used for morphometric analysis of mucus inclusions and inflammatory infiltrates. SR, viewed and photographed under cross-polarized light, was used for morphometric analysis of mature collagen fibers (Dolhnikoff et al., 1999). For semi-quantitative analysis of mucus inclusions, cellularity, and mature collagen, sections were analyzed morphometrically using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). PAS-stained sections were thresholded to measure only the area of mucus; H&E sections were thresholded to measure all nuclei. This can be taken as a measure of cellularity. To calculate the percentage of lung sections were thresholded to measure all nuclei. This can be taken as a measure of cellularity. To calculate the percentage of lung

Fig. 1. Schematic diagram illustrating the protocol for OVA sensitization, challenges, and mAb treatment. All mice were sensitized to OVA. Mice were challenged weekly with OVA or PBS on days 22, 29, 36, 43, 50, and 52. CNTO 134 or vehicle treatment (weekly) was initiated on day 36. On day 36, a subset of mice was sacrificed and did not receive OVA or PBS challenge. The remaining mice were sacrificed on day 53.
occupied by mucus or nuclei, the stained area for each constituent was divided by the total area of lung (including blood vessels and air space) included in the 4x field: % lung = stained area/total area × 100. To measure mature collagen, Sirius red-stained sections were photographed with cross-polarized light, and the images were converted from red-green-blue to hue-saturation-intensity. These images were thresholded to measure only mature, i.e., birefringent, collagen, and the integrated optical density for collagen was measured for each 4x field. A random, low-magnification 4x objective field of the parahilar region, including the mainstem bronchus and its primary and secondary branches, was photographed for each lung using a Nikon E800 equipped with plan apochromatic lenses and a Nikon DXM1200 digital camera. Images were stored as red-green-blue.tif files for morphometric analysis.

Chemokine, Cytokine, and MMP-9 Detection by Enzyme-Linked Immunosorbent Assay. The right lungs from some mice were homogenized in PBS, and supernatants were assayed for chemokines, cytokines (R&D Systems), and total MMP-9 (Amersham Biosciences Inc., Piscataway, NJ).

Statistical Analysis. Data are summarized using mean ± S.E.M. Statistical differences between groups were tested based on analysis of variance with two-tailed tests. For day 36, AHR data analyses for significant differences within the entire data set were performed on means and S.E.M. across both days 36 and 53. Comparisons between the groups of animals (both PBS groups versus both OVA groups; group 1 and 3 versus group 2 and 4) were made at all three methacholine concentrations (10–40 mg/ml) after fitting a mixed-effect linear model with day as a random effect and baseline and PBS as covariates. Statistical testing was performed based on two-tailed tests, and significance was claimed if the p value was less than 0.05.

Mean and S.E.M. across both days 36 and 53. Comparisons between the groups of animals (both PBS groups versus both OVA groups; group 1 and 3 versus group 2 and 4) were made at all three methacholine concentrations (10–40 mg/ml) after fitting a mixed-effect linear model with day as a random effect and baseline and PBS as covariates. Statistical testing was performed based on two-tailed tests, and significance was claimed if the p value was less than 0.05. Day 53 AHR data were analyzed by analysis of variance. Statistical significance was claimed with p values less than 0.05.

Results

Neutralization of IL-13 Reverses Established AHR. It was important to establish the presence of airway disease at the time CNTO 134 treatment was initiated. Disease was induced in mice, following OVA sensitization, with two allergen challenges a week apart (outlined in Fig. 1). One week after the second allergen challenge (day 36), a small subset of mice was analyzed for the presence of allergic disease. AHR to methacholine challenge was observed in mice challenged with OVA compared with PBS-challenged mice (Table 1).

Anti-IL-13 treatment was initiated on day 36, and mice continued to receive multiple allergen challenges. Treatment with CNTO 134 inhibited AHR on day 53 (Fig. 2).

Neutralization of IL-13 Inhibits the Progressive Increase in BAL Inflammation and Cellularity in Lung Tissue. Two OVA challenges also induced an increase in the numbers of eosinophils, mononuclear cells, and neutrophils in the BAL on day 36 (Fig. 3, a, b, and c, respectively). The BAL cell numbers revealed that the airway inflammation had progressed in day 53 OVA-challenged mice compared with day 36 mice. The increased airway inflammation was supported by the increased cellularity observed by morphometric analysis of the lung histology (Fig. 4, a and c; Fig. 5a). Histopathologic examination of the lung sections of day 36 OVA-challenged mice revealed increased cellularity characterized by perivascular inflammatory cell cuffing, airway smooth muscle cell hypertrophy, and goblet cell hypertrophy and hyperplasia compared with PBS-challenged mice. All these changes progressed between days 36 and 53, and the inflammatory infiltrates overwhelmingly contributed to the increase in cellularity. The perivascular cuffing extended from the primary branches of the pulmonary artery to the level of arterioles and was composed of a mixed inflammatory infiltrate in which eosinophils were prominent. In the more severely affected mice, inflammatory cell infiltrates extended to involve the mainstem bronchi and primary, secondary, and respiratory bronchioles. The infiltrates occasionally formed follicular structures. Airway smooth muscle cell hypertrophy was confined mainly to the bronchi and primary bronchioles. The epithelium lining the mainstem bronchi and primary and secondary bronchioles was markedly hypertrophied and hyperplastic. The epithelium of the respiratory bronchioles was also hypertrophied, but much less so.

Treatment with CNTO 134 significantly inhibited the progressive increase in BAL eosinophilia (Fig. 3a). Increased numbers of BAL mononuclear cells were observed on day 53 compared with day 36 OVA-challenged mice, and treatment of mice with CNTO 134 resulted in significantly fewer mononuclear cells in the BAL (Fig. 3b). Allergen challenge stimulated a BAL neutrophilia that was not significantly modulated by CNTO 134 treatment (Fig. 3c).

### TABLE 1

<table>
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<th>Time</th>
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n/a, not applicable.
inflammation in the airways with CNTO 134 treatment was supported by the morphometric analyses of the lung histology. A significant increase in the cellularity was observed in the lung tissue in OVA-challenged mice on day 53 compared with day 36 mice (Fig. 4, c and e; Fig. 5a). CNTO 134 treatment prevented this progressive increase in lung cellularity (Fig. 4, e and g; Fig. 5a). Histologically, a mixed inflammatory cell infiltrate, rich in eosinophils, was still present but was more sparse and less well organized than observed in the PBS-treated mice. Similarly, there was still evidence of airway smooth muscle cell hypertrophy. As was seen in the PBS-treated OVA-challenged mice, the inflammatory infiltrates accounted for the majority of lung cellularity.

Neutralization of IL-13 Reverses Excessive Mucus Production. Lung histology revealed a significant increase in mucus staining in allergen-challenged mice on day 36 (Fig. 4, a and c; Fig. 5b). Mucus was another pathological feature that progressed between days 36 and 53 (Fig. 4, c and e; Fig. 5b). The fraction of the epithelium lining the mainstem bronchus that was formed by goblet cells in some cases approached 100% and was also increased in the primary and secondary bronchioles. Goblet cells were observed only very rarely in the respiratory bronchioles. Neutralization of IL-13 with CNTO 134 significantly inhibited not only the progressive increase in mucus from day 36 to day 53 (Fig. 4, c, e, and g; Fig. 5b), but it also reversed the mucus levels to near-background levels when compared with OVA-challenged day 36 mice (Fig. 4, a, c, and g; Fig. 5b). Histologically, the fraction of airway epithelium occupied by goblet cells was less than what was observed on day 36 in the OVA-sensitized and -challenged mice and, in some mice, almost normal.

Neutralization of IL-13 Inhibits the Development of Fibrosis. Subepithelial fibrosis was assessed by staining the lung sections with Sirius red and measuring birefringence, a measurement of the amount of mature collagen. Although significant subepithelial fibrosis was not apparent on day 36 (Fig. 4, b and d; Fig. 5c), subepithelial fibrosis had developed significantly in the airway interstitium by day 53 in the lungs of mice challenged with OVA (Figs. 4f and 5c). Treatment with CNTO 134 prevented the development of fibrosis (Figs. 4h and 5c).

IL-13 Regulates MMP-9 Activity and Production of Proinflammatory Mediators. Airway remodeling is associated with MMP activity. To address whether IL-13 was
involved in MMP activation, total MMP-9 protein levels were measured in the lung tissue of mice (Fig. 6). Significant increases in MMP-9 were observed in OVA-challenged mice on day 53. Neutralization of IL-13 resulted in a reduction in MMP-9. A number of cytokines and chemokines was also modulated with anti-IL-13 treatment. Allergen challenges induced significant increases in the levels of IL-4, -5, and -13, tumor necrosis factor (TNF\(\alpha\)), KC, and eotaxin by day 36, and these levels remained elevated on day 53 together with significant increases in JE levels (Table 2). Eotaxin levels increased further on day 53 compared with levels present on day 36. This may reflect the progressive increase in eosinophils in the BAL between the two time points. Neutralization of IL-13 inhibited the levels of all cytokines and chemokines. The effect of CNTO 134 on IL-13 protein levels was not assessed since CNTO 134 interfered with the enzyme-linked immunosorbent assay.

### Discussion

The current study has demonstrated that an IL-13-specific neutralization approach with an mAb may be an effective therapy for the treatment of asthma. To our knowledge, this
is the first demonstration that therapeutic dosing with an anti-IL-13 mAb in established, persistent, progressive disease in mice modulated lung function as well as the underlying pathologic features associated with the disease. Furthermore, our data show that anti-IL-13 treatment halts disease progression and even reverses certain features associated with experimental asthma.

Cellular and humoral lung inflammation are characteristic features of asthma, and in vivo models of asthma mimic this aspect of disease. Our current study demonstrated a progressive increase in lung tissue cellularity and airway inflammation (eosinophils and mononuclear cells) between days 36 and 53 that was inhibited by neutralization of IL-13. A trend toward inhibition of BAL neutrophilia was also observed. The effect of IL-13 inhibition on allergic inflammation is controversial (Grunig et al., 1998; Wills-Karp et al., 1998; Taube et al., 2002), and studies (including the current study) suggest that neutralization of IL-13 may impact the inflammatory response in more chronic settings (Taube et al., 2002). Data also suggest that IL-13 may play a protective role in acute inflammatory settings. IL-13 has been shown to be anti-inflammatory in a guinea pig model of acute allergic inflammation (Watson et al., 1999). Furthermore, neutralization of IL-13 resulted in an exacerbation of inflammation in an experimental model of acute lung injury (Lentsch et al., 1999). The allergen challenge protocol used in the present study differs significantly from previous studies; at the time mAb treatment was initiated, persistent disease and pathology was established, and mice continued to receive multiple allergen challenges to establish a chronic disease state. The discrepancy in data regarding an effect on cell inflammation may be due to differences in the mouse strain used and the time point at which cell numbers were evaluated, together with the chronicity of the model. Since neutralization of IL-13 reduced the levels of the eosinophil-survival cytokine IL-5 and IL-13 itself has been shown to be a survival factor for eosinophils (Horie et al., 1997), it is possible that increased apoptosis and clearance in the absence of IL-13 contributes to the diminished cell numbers. Interestingly, although multiple cytokines (including IL-13) and chemokines were elevated in the airways of OVA-challenged mice from day 36, only the level of eotaxin increased further at day 53, suggesting its association with the progressive increase in inflammation. IL-13 has been shown to induce eotaxin expression in epithelial cells (Li et al., 1999). Anti-IL-13 mAb treatment reduced, but did not completely reverse, the levels of all the cytokines and chemokines measured. Exogenous administration (Grunig et al., 1998; Wills-Karp et al., 1998; Singer et al., 2002; Kibe et al., 2003; Vargafig and Singer, 2003a,b) or transgenic pulmonary overexpression of IL-13 itself (Zhu et al., 1999, 2001; Lanone et al., 2002) induces the infiltration of neutrophils, eosinophils, and mononuclear cells into the lungs of mice together with stimulating the expression of many chemokines, including eotaxin, JE (a mouse homolog of human monocyte chemoattractant protein 1), and KC. Synergistic actions of cytokines such as TNFα with IL-13 (Li et al., 1999; Moore et al., 2002; Kibe et al., 2003), together with positive feedback loops (Vargafig and Singer, 2003b), may explain the incomplete inhibition of the lung inflammation by anti-IL-13 treatment. Interestingly, pulmonary-specific overexpression of IL-13 does not induce IL-4 or IL-5 expression in the lungs (Zhu et al., 1999). Furthermore, lymph node cells from OVA-sensitized and -challenged IL-13 gene-deleted mice release levels of IL-4 and IL-5 that are similar to that released by wild-type-challenged mice (Walter et al., 2001). These data indicate that the inhibition of IL-4 and IL-5 observed in the present study and others (Taube et al., 2002) is secondary to IL-13 neutralization. Indeed, it has been shown that eotaxin stimulates eosinophils to secrete IL-4 (Bandeira-Melo et al., 2002).

Multiple studies, including the present study, demonstrate a dissociation between the cellular (particularly eosinophils) inflammatory component following OVA challenge and AHR (Walter et al., 2001; Taube et al., 2002). Similar to OVA-induced AHR, exogenous administration of IL-13 to mice induces AHR that is dissociated from the inflammation induced by IL-13 (Wills-Karp et al., 1998; Walter et al., 2001; Singer et al., 2002; Venkayya et al., 2002). In agreement with previous studies, we have shown that inhibition of IL-13 can almost completely inhibit AHR with a modest effect on inflammation. Recent data in a chronic OVA-challenge model have demonstrated that AHR and airway remodeling persists for up to 4 weeks following the last OVA challenge in the absence of cellular and humoral inflammation (Leigh et al., 2004). In contrast to the present study, the persistent AHR and remodeling was not modulated by acute inhibition of IL-13 with a soluble IL-13 receptor fusion protein. The Leigh et al. (2004) model also illustrates clearly that AHR, in mice, may occur in the absence of inflammation and is associated with airway remodeling. It will be interesting to establish whether chronic neutralization of IL-13 is required to modulate the remodeling and persistent AHR observed in the model. The mechanisms underlying IL-13-induced AHR are not fully understood. In vitro, IL-13 is able to enhance nerve-stimulated murine jejunal smooth muscle contraction that is STAT6-dependent (Zhao et al., 2003), and IL-13 also increases carbachol-induced murine and human smooth muscle contraction (Tliba et al., 2003). IL-13 mediates its actions...
through a heterodimer receptor consisting of the IL-13 receptor α1 and the IL-4 receptor α chains. Human smooth muscle cells express the IL-4 receptor α and IL-13 receptor α1 receptors (Laporte et al., 2001), and smooth muscle mast cells express IL-13 (Brightling et al., 2003). These in vitro data suggest that IL-13 may have a direct effect on smooth muscle function; however, in vivo evidence suggests an indirect effect of IL-13 on smooth muscle constriction. Reconstitution of STAT6 specifically in lung epithelial cells of STAT6-deficient mice restored IL-13-induced AHR (Kuperman et al., 2002), indicating that IL-13 is able to act on epithelial cells to induce AHR. Furthermore, it has been shown that IL-13-induced AHR is partly mediated by leukotrienes (Varaftig and Singer, 2003a).

Airway remodeling is a characteristic underlying feature of asthma and is thought to contribute to the symptoms associated with asthma. The features of airway remodeling assessed in the current study were goblet cell hyperplasia (measured indirectly by staining for mucous-positive cells) and subepithelial fibrosis. Neutralization of IL-13 reversed the increase in mucus-producing cells and inhibited the development of subepithelial fibrosis. IL-13-induced goblet cell hyperplasia is not due to cell proliferation in vivo (Varaftig and Singer, 2003a) but may be due to differentiation of epithelial cells into secretory cells (Zuhdi Alimmam et al., 2000; Laoukili et al., 2001). Previous studies have demonstrated that IL-13-induced goblet cell hyperplasia in vivo partially depends on inflammation (Shim et al., 2001; Singer et al., 2002). Furthermore, IL-13-induced mucus protein expression may be mediated through the epidermal growth factor receptor (Shim et al., 2001). IL-13 has been shown to stimulate fibroblast proliferation in vitro (Ingram et al., 2003; Jakubzick et al., 2003; Saito et al., 2003) and in vivo (Varaftig and Singer, 2003b). IL-13 may activate fibroblast directly through its receptor, which is present on fibroblasts (Doucet et al., 1998; Jakubzick et al., 2003), or indirectly via the stimulation of secondary mediators such as leukotrienes, transforming growth factor-β, and platelet-derived growth factor (Richter et al., 2001; Chibana et al., 2003; Ingram et al., 2003). IL-13 also stimulates collagen deposition from fibroblasts in vitro and in vivo (Doucet et al., 1998; Zhu et al., 1999), which may be mediated through activation of transforming growth factor-β by MMP-9 (Lee et al., 2001). Interestingly, in our study, we observed a progressive increase in MMP-9 levels in the lung tissue between days 36 and 53 that was associated with the development of subepithelial fibrosis and inhibited by anti-IL-13 mAb. Increased levels of MMP-9 have been shown to be elevated in the airways of asthmatics, and immunolocalization studies have demonstrated that MMP-9 expression in the subepithelial basement membrane is associated with fibrosis (Wenzel et al., 2003).

In summary, by using a therapeutic treatment regimen with an anti-IL-13 mAb in a mouse model of persistent disease, we have clearly demonstrated an important role of IL-13 in allergic asthma. Neutralization of IL-13 inhibited and even reversed multiple features of pathophysiology that are inadequately controlled in the clinic at present. We believe that IL-13 neutralization has the potential to be an important therapeutic for the treatment of persistent asthma.

**References**


Inc., Mail Stop: R-4-1, 145 King of Prussia Rd., Radnor, PA 19087. E-mail: ads2@cntus.jnj.com

Address correspondence to: Dr. Anuk M. Das, Immunobiology, Centocor Inc., Mail Stop: R-4-1, 145 King of Prussia Rd., Radnor, PA 19087. E-mail: ads2@cntus.jnj.com