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Article

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Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase– and cathepsin-dependent emphysema

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Cigarette smoke exposure is the major cause of chronic obstructive pulmonary disease (COPD). However, only a minority of smokers develop significant COPD, and patients with asthma or asthma-like airway hyperresponsiveness or eosinophilia experience accelerated loss of lung function after cigarette smoke exposure. Pulmonary inflammation is a characteristic feature of lungs from patients with COPD. Surprisingly, the mediators of this inflammation and their contributions to the pathogenesis and varied natural history of COPD are not well defined. Here we show that IL-13, a critical cytokine in asthma, causes emphysema with enhanced lung volumes and compliance, mucus metaplasia, and inflammation, when inducibly overexpressed in the adult murine lung. MMP-2, -9, -12, -13, and -14 and cathepsins B, S, L, H, and K were induced by IL-13 in this setting. In addition, treatment with MMP or cysteine proteinase antagonists significantly decreased the emphysema and inflammation, but not the mucus in these animals. These studies demonstrate that IL-13 is a potent stimulator of MMP and cathepsin-based proteolytic pathways in the lung. They also demonstrate that IL-13 causes emphysema via a MMP- and cathepsin-dependent mechanism(s) and highlight common mechanisms that may underlie COPD and asthma.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a generic term that encompasses chronic bronchitis (CB), small airway disease, and emphysema. Patients with CB have airway mucus metaplasia and mucus gland hypertrophy, and emphysema is characterized by tissue destruction with resulting alveolar enlargement. COPD affects 16 million people in the United States alone and is the fourth leading cause of death worldwide (1).

COPD occurs predominately in cigarette smokers (1). However, the relationship between cigarette smoking and COPD is complex, with only 10–15% of active smokers developing clinical COPD (2, 3) and estimates of cumulative smoking exposure explaining only 10–15% of the variation in pulmonary function seen in population-based investigations (2, 4, 5). Studies of this heterogeneity have also demonstrated that the presence of concurrent asthma or asthma-like airways hyperresponsiveness (AHR) or eosinophilia correlates with the development of or acquisition of a COPD phenotype characterized by accelerated loss of pulmonary function and chronic symptomatology (2, 6–9). They also highlighted the large number of patients that manifest features of COPD and asthma (1). These observations led, in 1961, to the formulation of the "Dutch Hypothesis." This hypothesis proposes that the distinctions between COPD and asthma are not absolute and that similar mechanisms can contribute to the pathogenesis of both disorders (10, 11). They also led Burrows et al. to suggest, over a decade ago, that chronic airflow obstruction in adults should be differentiated on the basis of whether or not there are accompanying asthmatic features (12, 13). To date, however, the mechanisms responsible for the AHR and eosinophilia seen in COPD, and the existence of and nature of the biologic responses that might be common to COPD and asthma have not been elucidated.

The protease/antiprotease hypothesis has dominated pathogenetic thinking in COPD for more than 35 years. It proposes that an antiprotease "shield" protects the normal lung from locally elaborated proteases and that emphysema is the result of an abnormal increase

in proteases and/or reduction in pulmonary antiproteases (1). Inflammation, characterized by increased numbers of macrophages, lymphocytes, neutrophils, and/or eosinophils is a characteristic feature of lungs from patients with COPD (1, 14–19). However, the nature of the mediators involved in this inflammation and the ability of these mediators to generate the emphysema and mucus changes, protease/antiprotease alterations, and varied natural history of COPD have not been investigated.

Because Th2-dominated inflammation underlies the pathogenesis of asthma and generates AHR and eosinophilia (20–22), we hypothesized that Th2 cytokines can also activate proteolytic pathways that could contribute to the pathogenesis of COPD. To test this hypothesis, we used an inducible overexpression transgenic modeling system to target IL-13, a Th2 cytokine that is strongly implicated in the pathogenesis of asthma and causes AHR and eosinophilia (20, 23), to the adult murine lung. These studies demonstrate that IL-13 causes a phenotype that mirrors human COPD including emphysema with enhanced lung volumes and pulmonary compliance; mucus metaplasia; and macrophage-, lymphocyte-, and eosinophil-rich inflammation. They also define the MMP and cathepsin abnormalities that generate the emphysema and demonstrate the efficacy of proteolytic blockade in ameliorating this response.

Methods

Transgenic mice. These experiments were undertaken with CC10-rtTA-IL-13 mice in which the Clara cell 10-kDa (*CC10*) protein promoter and two transgenic constructs target IL-13 to the murine lung in an externally regulatable fashion. The CC10-rtTA transgenic system and the constructs that were used have been described previously by our laboratory (24). Construct 1, CC10-rtTA-hGH, contains the CC10 promoter, the reverse tetracycline transactivator (rtTA), and human growth hormone (hGH) intronic and polyadenylation sequences (Figure 1). The rtTA is a fusion protein made up of a mutated tetracycline repressor (rtet-R) and the Herpes virus VP-16 transactivator. Construct 2, tet-O-CMV-IL-13-hGH, contains a polymeric tetracycline operator (*tet-O*), minimal cytomegalovirus (CMV) promoter, murine IL-13 cDNA, and hGH intronic and polyadenylation signals (Figure 1). In this system, the *CC10* promoter directs the expression of rtTA to the lung. In the presence of doxycycline (dox), rtTA is able to bind in trans to the *tet-O* and the VP-16 transactivator activates IL-13 gene transcription. In the absence of dox, rtTA binding occurs at very low levels and only low-level gene transcription is noted. The preparation of the CC10-rtTA construct has been described previously (24). The tet-O-CMV-IL-13 construct was prepared by replacing the IL-11 cDNA in the construct tet-O-CMV-hIL-11 described by our laboratory previously (24) with the murine IL-13 cDNA. This construct was checked for correct insert orientation by restriction enzyme digestion and sequencing. Both constructs were purified, linearized, separated by electrophoresis through agarose and purified as described previously (24). Transgenic mice were prepared in (CBA X C57BL/6) F_2 eggs by mixing and simultaneously injecting the constructs into pronuclei as described previously (24).

Documentation of transgene status. The presence or absence of the transgenes was initially evaluated using Southern blot analysis and later by PCR. Southern analysis was performed as described previously using cDNA encoding murine IL-13 or rtTA (20, 24). PCR for rtTA and IL-13 were also performed using protocols described by our laboratory (20, 24). All CC10-rtTA-IL-13 lineage animals were evaluated for the presence of both the rtTA and IL-13 containing transgenic constructs.

Dox water administration. CC10-rtTA-IL-13 animals were maintained on normal water until 1 month of age. At that time, they were randomized to normal water or water containing dox (0.5 mg/ml) as described previously (24).

Bronchoalveolar lavage and quantification of IL-13 levels. Mice were euthanized, the trachea was isolated by blunt dissection, and small caliber tubing was inserted and secured in the airway. Two volumes of 1.0 ml of PBS with 0.1% BSA were then instilled and gently aspirated and pooled. Each BAL fluid sample was centrifuged, and the supernatants were stored in –70°C until used. The levels of IL-13 were determined immunologically using a commercial ELISA (R&D Systems Inc., Minneapolis, Minnesota, USA) as per the manufacturer's instructions.

Lung volume and compliance assessment. The animals were anesthetized, the trachea was cannulated, and the lungs were ventilated with 100% O_2 via a "T" piece attachment. The trachea was then clamped and oxygen absorbed in the face of ongoing pulmonary perfusion. At the end of this degassing, the lungs and heart were removed en bloc and inflated with PBS at gradually increasing pressures from 0 to 30 cm. The size of the lung at each 5-cm interval was evaluated via volume displacement.

Histological analysis. Animals were sacrificed via cervical dislocation, a median sternotomy was performed, and right heart perfusion was accomplished with calcium- and magnesium-free PBS to clear the pulmonary intravascular space. The lungs were then fixed to pressure (25 cm) with neutral buffered 10% forma-

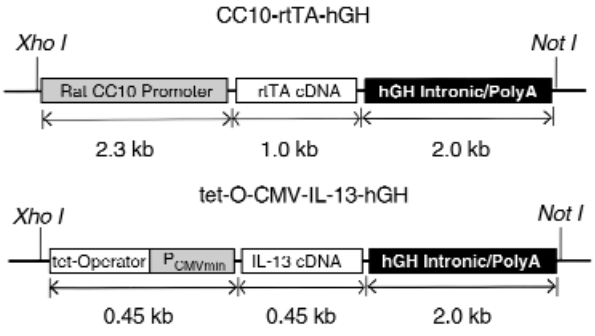

Figure 1
Constructs utilized in the generation of CC10-rtTA-IL-13 mice.

Table 1
RT-PCR primers

mRNA of interest	Sequences	Product Length (bp)
Gelatinase A (MMP-2)	(UP) 5′-TCT GCG GGT TCT CTG CGT CCT GTG C-3′ (LO) 5′-GTG CCC TGG AAG CGG AAC GGA AAC T-3′	(861)
Matrilysin (MMP-7)	(UP) 5′-ACA TCA GTG GGA ACA GGC TCA G-3′ (LO) 5′-ACA GTA CCG GGA ACA GAA GAG T-3′	(606)
Gelatinase B (MMP-9)	(UP) 5′-CAA AGA CCT GAA AAC CTC CAA CCT C-3′ (LO) 5′-TTC TCC GTT GCC GTG CTC CGT GTA G-3′	(757)
Macrophage Metalloelastase (MMP-12)	(UP) 5′-AAG CAA CTG GGC AAC TGG ACA ACT C-3′ (LO) 5′-TGG TGA CAG AAA GTT GAT GGT GGA C-3′	(631)
Collagenase (MMP-13)	(UP) 5′-CTT CTG GCA CAC GCT TTT CCT C-3′ (LO) 5′-CGC AGC GCT CAG TCT CTT CAC C-3′	(606)
MT-MMP1 (MMP-14)	(UP) 5′-GTC TCC TGC TCC CCC TGC TCA C-3′ (LO) 5′-CAT GCA CAG CCA CCA AGA AGA T-3′	(690)
TIMP-1	(UP) 5′-GCA TCT GGC ATC CTC TTG TTG-3′ (LO) 5′-GAC AGT GTT CAG GCT TCA GTT TTT C-3′	(637)
TIMP-2	(UP) 5′-GCA ACA GGC GTT TTG CAA TGC AGA C-3′ (LO) 5′-GCT TTT CAA TTG GCC ACA GGG GCT C-3′	(598)
TIMP-3	(UP) 5′-CTG GCT TGG GCT TGT CGT GCT CCT GA-3′ (LO) 5′-GGG AAG GAG GTG AGG TGG GGC AGG TC-3′	(659)
TIMP-4	(UP) 5′-CAC GCC ATT TGA CTC TTC CCT-3′ (LO) 5′-CCA GCA GCC AGT CCG TCC AGA-3′	(269)
Cathepsin B	(UP) 5′-CTT GAT CCT TCT TTC TTG CCT GCT G-3′ (LO) 5′-GAA TCG TAG ACT CCA CCT GAA ACC A-3′	(513)
Cathepsin L	(UP) 5′-CTA CAC AAC GGG GAA TAC AGC AAC G-3′ (LO) 5′-ACT ATA GAA CTG GAG AGA CGG ATG G-3′	(600)
Cathepsin K	(UP) 5′-GGG AGA CAT GAC CAG TGA AGA AGT G-3′ (LO) 5′-TGC TCT CTT CAG GGC TTT CTC GTT C-3′	(481)
Cathepsin H	(UP) 5′-CGA GCT GAC CGT GAA CGC CAT AG-3′ (LO) 5′-AGC TTT TTG GGG GTT GAA TCT GC-3′	(595)
Cathepsin S	(UP) 5′-GGG TTC TTG TGG TGC CTG TTG G-3′ (LO) 5′-CCG TAC AGG AGG GGT CAT CAT A-3′	(425)
SLPI	(UP) 5′-CTG GAC TGT GGA AGG AGG CAA AAA TG-3′ (LO) 5′-AGT AGT TTC CAG AGC ACA CCG AGC AC-3′	(398)
Cystatin C	(UP) 5′-GCG AGT ACA ACA AGG GCA GCA A-3′ (LO) 5′-TAG GGA AGG AGC ACA AGT AAG G-3′	(323)
α_1-Antitrypsin	(UP) 5′-TGC CCA TGA TGA CCC TCT C-3′ (LO) 5′-TGG GGC TCT GAG TGT GTT CT-3′	(498)

Primers used for RT-PCR evaluation in these studies. The sequence of the upper (UP) and lower (LO) primers and the size of the expected RT-PCR product are illustrated.

lin, fixed overnight in 10% formalin, embedded in paraffin, sectioned at 5 μm and stained. Hematoxylin and eosin (H&E) and periodic acid-Schiff with diastase (D-PAS) stains were performed in the Research Histology Laboratory of the Department of Pathology at Yale University School of Medicine.

Calculation of histological mucus index. The histological mucus index (HMI) provides a measurement of the percentage of epithelial cells that are D-PAS$^+$ per unit airway basement membrane. It was calculated from D-PAS–stained sections as described previously by our laboratories (25).

mRNA analysis. mRNA levels were assessed using Northern blot and RT-PCR as described previously by our laboratories (26, 27). In these experiments, total cellular RNA from lungs or a variety of other mouse tissues were obtained using Trizol reagent (GIBCO BRL, Grand Island, New York, USA) as per the manufacturer's instructions. When Northern blots were being performed, the RNA was fractionated by formaldehyde-agarose gel electrophoresis, transferred to a nylon membrane, and probed with ^{32}P-labeled cDNA encoding the moiety of interest. The cDNA probes were generated using RT-PCR with the primers in Table 1 and whole lung RNA from CC10-IL-13 mice (20). All cDNA were sequenced before utilization. Equity of sample loading and the efficacy of transfer were assessed by stripping and reprobing the membrane with cDNA encoding β-actin. In the RT-PCR assays, RNA samples were reverse transcribed, and gene-specific primers (Table 1) were used to amplify selected regions of each target moiety. For each cytokine, the optimal numbers of cycles that will produce a quantity of cytokine product that is directly proportional to the quantity of input mRNA was determined experimentally. To verify that equal amounts of undegraded RNA were added in each RT-PCR reaction, β-actin was used as an internal standard. Amplified PCR products were detected using ethidium bromide gel electrophoresis, quantitated electronically, and confirmed by nucleotide sequencing.

Western blot analysis. Western blot analysis of bronchoalveolar lavage (BAL) fluid was performed using antibodies to MMP-12 (28) and antibodies to MMP-2 and -13 (NeoMarkers, Fremont, California, USA) and MMP-14 (RDI Inc., Flanders, New Jersey, USA) as described previously by our laboratory (26).

Zymography. Gelatin and casein zymography were performed using BAL fluid as described previously by our laboratory (28, 29).

Immunohistochemistry. Immunohistochemistry was undertaken to localize MMP-12 and MMP-9 using techniques and reagents described previously by our laboratories (28, 29).

Active site probe analysis of cysteine proteases. Active site probe analyses were performed as described previously by our laboratories (30). Lung lysates or cell-

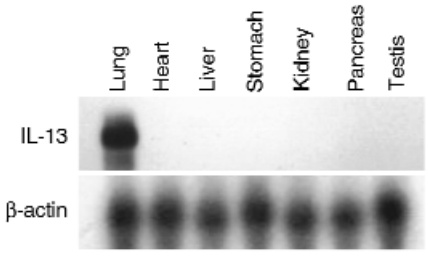

Figure 2
Organ specificity of IL-13 expression. CC10-rtTA-IL-13 mice were given dox water for 2 weeks. RNA was then isolated from the lung and other organs and IL-13 mRNA quantitated by Northern analysis.

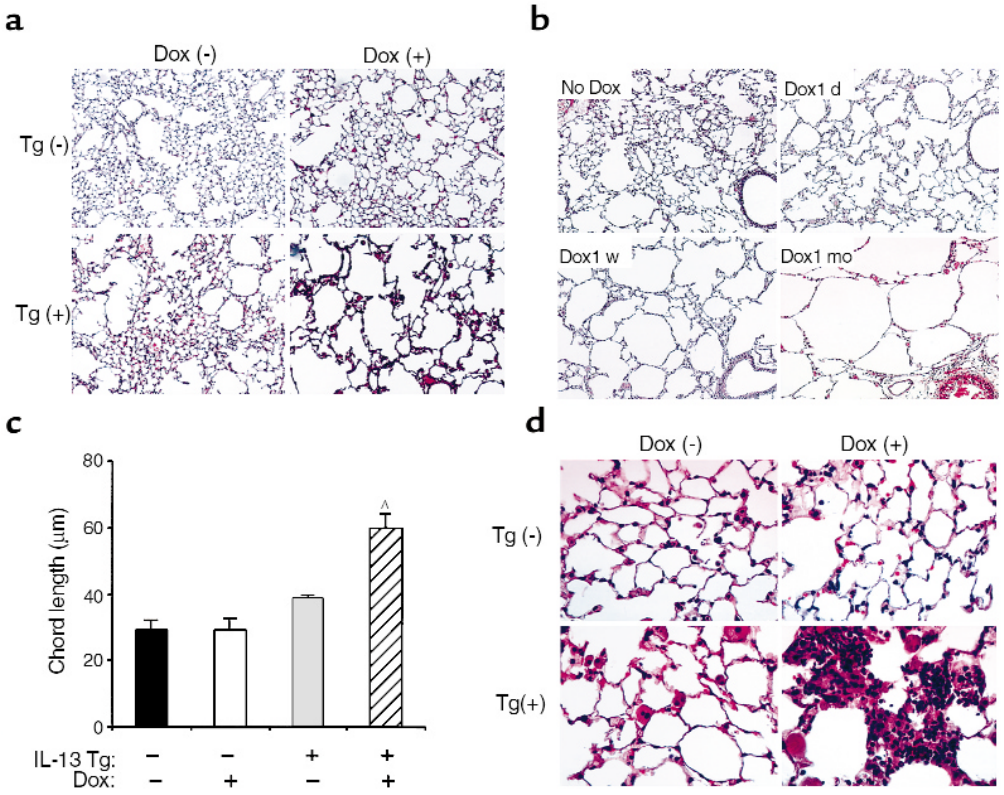

Figure 3
Characteristics of CC10-rtTA-IL-13 mice. (**a**) Compares H&E-stained lungs from 6-week-old nontransgenic [Tg (–)] and double transgenic [Tg (+)] mice that received normal water [Dox (–)] or dox water [Dox (+)] for the last 2 weeks of their lives. Emphysema is seen only in double transgenic animals receiving dox water. Unless otherwise stated, all photos are at ×25 original magnification. (**b**) Illustrates the kinetics of this response. Alveolar enlargement was seen after 1 week and was more pronounced at 1 month. (**c**) Illustrates the chord lengths of nontransgenic and double transgenic mice on normal or dox water for 4 weeks. $^{A}P < 0.01$ versus all other conditions. (**d**) Higher-power (×100) view comparing the histological features (H&E) of nontransgenic and double transgenic mice that had received normal or dox water for 1 month.

free BAL, normalized to total protein, were incubated with an ^{125}I-JPM analog of E-64 and resolved by SDS-PAGE and autoradiography.

Morphometric analysis. Alveolar size was estimated from the mean cord length of the airspace as described previously by our laboratory (24). This measurement is similar to the mean linear intercept, a standard measure of air space size, but has the advantage that it is independent of alveolar septal thickness. Sections were prepared as already described here. To obtain images at random for analysis, each glass slide was placed on a printed rectangular grid and a series of dots placed on the coverglass at the intersection of the grid lines, i.e., at intervals of approximately 1 mm. Fields as close as possible to each dot were acquired by systematically scanning at 2-mm intervals. Fields containing identifiable artifacts or nonalveolated structures such as bronchovascular bundles or pleura were discarded.

A minimum of ten fields from each mouse lung were acquired into a Macintosh G3 computer (Apple Computer Inc., Cupertino, California, USA) through a framegrabber board. Images were acquired in 8-bit gray-scale at a final magnification of 1.5 pixels per micron. The images were analyzed on a Macintosh computer using the public domain NIH Image program written by Wayne Rasband at NIH (31) using a custom-written macro available from the web site (31). Images were manually thresholded and then smoothed and inverted. The image was then subject to sequential logical image match "and" operations with a horizontal and then vertical grid. At least 200 measurements per field were made in transgene-positive animals and 400 measurements per field were made in the transgene-negative animals. The length of the lines overlying air space air was averaged as the mean chord length. At least four animals were studied at each time point in the presence and absence of dox water. Chord length increases with alveolar enlargement.

Pharmacological intervention. Inhibitor studies were performed to elucidate the importance of MMPs and cysteine proteinases in the IL-13–induced phenotype. In the studies looking at the MMPs, 4- to 6-week-old CC10-rtTA-IL-13 animals and nontransgenic littermate controls were treated with GM-6001 (AMS Scientific, Pleasant Hill, California, USA), an inhibitor of a variety of MMP moieties (32, 33) at a dose of 200 mg/kg. Other animals received the inactive peptide control (C1006) or the vehicle control (DMSO). All treatments were admin-

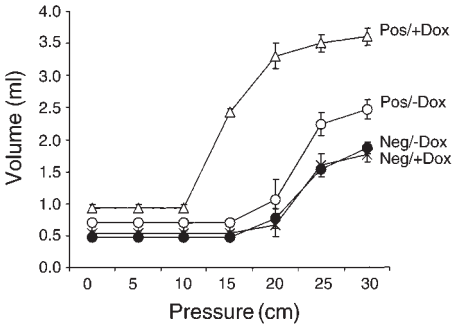

Figure 4
Effect of IL-13 on lung compliance. In these experiments, 1-month-old transgene negative (Neg) and dual transgene positive (Pos) mice were randomized to water with (+) or without (–) dox for 4 weeks before evaluation. Pressure-volume relationships were evaluated as described in Methods.

istered intraperitoneally daily starting on day 1. Dox was added to the animal's drinking water starting on day 2. Animals were sacrificed on day 12, at which time BAL was performed, lung volumes were assessed, histologies were evaluated, and morphometric evaluations were undertaken. Studies evaluating the role(s) of cysteine proteases were performed in an identical fashion except that comparisons were made of the cysteine protease inhibitors E-64 (7.5 mg/kg) and leupeptin (15 mg/kg) and saline vehicle controls (34, 35).

Statistical analysis. Values are expressed as means ± SEM. As appropriate, groups were compared by ANOVA with Scheffe's procedure post hoc analysis, with the Student's two-tailed unpaired *t* test or with nonparametric assessments (Wilcoxon's rank sum, Mann-Whitney *U* test) using the Stat View software for the Macintosh (Abacus Concepts Inc., Berkeley, California, USA).

Results

Rationale for and generation of inducible transgenic mice. Mice and humans are born with immature lungs that contain large sack-like structures. Normal alveolar size and number are acquired only after a growth and septation process that occurs during the first month and years of life, respectively (36). Thus, enlarged alveoli in adult mice or humans can be caused by processes that alter alveolar development or processes that destroy otherwise normal adult alveolar septae (1, 24, 37). The emphysema in COPD is caused by the latter (1). The need to differentiate development-dependent and adult-onset phenotypes confounds the use of transgenic modeling to study the pathogenesis of COPD. Specifically, any overexpression system in which a protein is expressed during lung development cannot be appropriately interpreted to represent destructive emphysema unless it is shown that appropriate lung development occurs before the emphysema is seen. To our knowledge, this has not been accomplished to date using conventional overexpression modeling systems.

To successfully use overexpression modeling to characterize the effects of IL-13 in the adult lung, we used a novel, externally regulable, dual-construct overexpression transgenic system developed in our laboratory (24). The constructs required for these transgenics were prepared, purified, and microinjected (Figure 1). Four dual transgene–positive CC10-rtTA-IL-13 founder mice were identified, and lines were obtained after breeding with C57BL/6 animals. These mice were kept on normal water until they were 1 month of age. They were then randomized to normal water or dox water. In the absence of dox administration, BAL IL-13 levels were less than or equal to 75 pg/ml. Increased levels of BAL IL-13 were noted within 48 hours, and steady-state levels between 0.5 and 1.5 ng/ml were seen within 96 hours of dox administration (data not shown). In all cases, organ blot analysis demonstrated that IL-13 was produced in a lung-specific fashion (Figure 2). Qualitatively, similar phenotypes were seen in all transgenic lines. They are summarized as follows:

Histological, structural, and physiological evaluation. Lungs from nontransgenic mice on normal or dox water did not show any histological abnormalities. In addition, they could not be distinguished on H&E evaluation from lungs from double transgenic mice on normal water (Figure 3a). In contrast, dox-induced IL-13 caused inflammatory cell infiltration and pronounced emphysema in transgenic animals. The emphysema manifested as histological (Figure 3, a and b) and morphometrically obvious (Figure 3c) alveolar enlargement with loss of the orderly appearance of the acinus (38). It was prominent after 7 days and continued to progress throughout the first month of dox administration (Figure 3b). The inflammation contained increased numbers of macrophages, lymphocytes, and eosinophils in peribronchial and parenchymal tissues

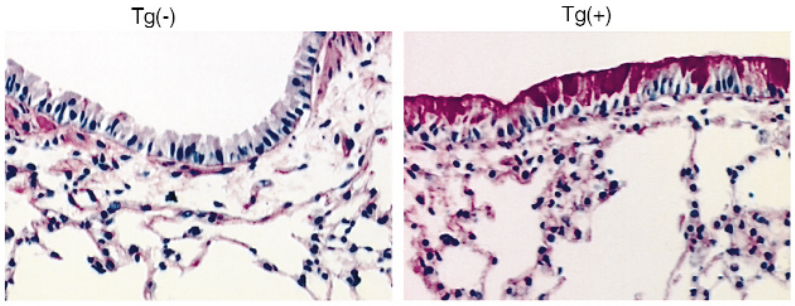

Figure 5
Mucus responses in nontransgenic and transgenic mice. The mucus metaplasia in nontransgenic (left) and transgenic mice (right) that received dox for 2 weeks are compared using PAS stains.

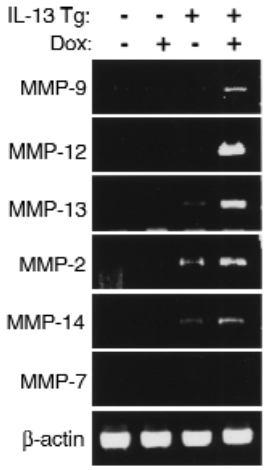

Figure 6
Effect of IL-13 on respiratory MMP mRNA. RT-PCR was used to compare the levels of mRNA encoding a variety of MMPs in lungs from 2-month-old nontransgenic and transgenic CC10-rtTA-IL-13 mice on normal water (–) or dox water (+) from 1 to 2 months of age.

and BAL. It could be seen within 7 days and was more prominent after 1 month of dox administration (Figure 3d, and data not shown).

Enhanced lung volumes and increased pulmonary compliance are characteristic features of human emphysema (1). To determine whether this murine model recapitulated these important clinical features of COPD, we compared the lung volumes and compliances of nontransgenic and transgenic mice before and after dox-induced IL-13 production. As seen in Figure 4, similar pressure-volume relationships were seen in lungs from nontransgenic mice that received normal water and dox water. A slight increase in compliance was seen in transgenic mice on normal water. In contrast, marked increases in pulmonary compliance and lung volumes were seen in transgenic mice in which IL-13 was induced with dox water (Figure 4, and data not shown). When viewed in combination, these studies demonstrate that IL-13 causes emphysema with lung volume enlargement and enhanced pulmonary compliance analogous to that seen in human COPD.

Mucus evaluation. To determine whether IL-13 caused mucus abnormalities comparable to those in COPD, we compared the goblet cells in nontransgenic and transgenic mice receiving normal and dox water using D-PAS staining and calculated HMIs. Lungs from nontransgenic animals receiving normal water or dox water did not contain D-PAS$^+$ cells. In accord with their low-level IL-13 production, small numbers of D-PAS–positive cells were noted in transgenic animals receiving normal water (HMI = 10–20). In contrast, impressive increases in the levels of D-PAS staining were appreciated with dox-induced IL-13 production (HMI = 60–80) (Figure 5). Given that mice do not have intrapulmonary submucosal mucus glands (39), IL-13–induced alterations in these structures could not be assessed. These studies demonstrate that IL-13 is a potent inducer of airway mucus metaplasia.

Effect on MMPs. To define the mechanism(s) of IL-13–induced alveolar septal destruction, we compared the levels of mRNA encoding COPD-relevant MMPs in 2-month-old inducible CC10-rtTA-IL-13 mice and their nontransgenic littermate controls that had been randomized to normal water or dox water for 1 month. These studies demonstrated that IL-13 is a potent stimulator of MMP-9 and -12 mRNA accumulation (Figure 6). MMP-2, MMP-13, and MMP-14 were also induced, whereas comparable alterations in MMP-7 were not appreciated (Figure 6). Kinetic evaluations demonstrated that the increases in the levels of mRNA encoding MMP-12; MMPs -2, -13, and -14; and MMP-9 were seen after 1 day, 10 days, and 10–30 days of dox administration, respectively (data not shown). Western blot analysis confirmed the induction of MMP-2, MMP-12, MMP-13, and MMP-14 (Figure 7a, and data not shown) and immunohistochemistry demonstrated that MMP-12 was seen predominantly in macrophages, and MMP-9 was seen in parenchymal locations in epithelial cells and interstitial fibroblast-like cells (Figure 7, b–f, and data not shown). Zymography also demonstrated IL-13–induced BAL moieties that comi-

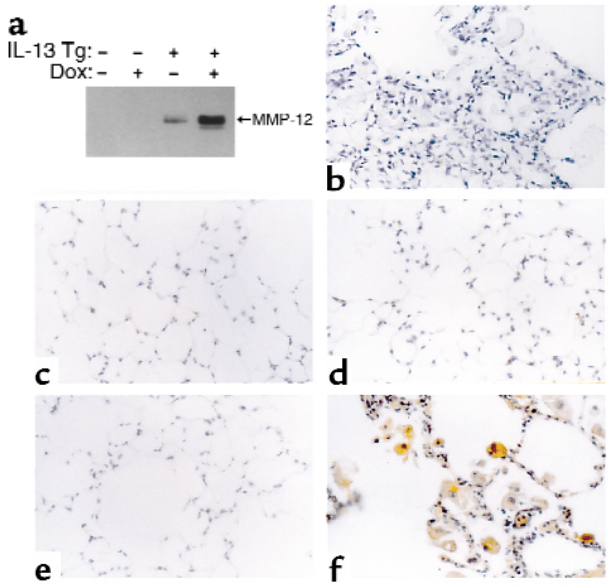

Figure 7
Western analysis and immunohistochemistry of BAL fluids and tissues from CC10-rtTA-IL-13 mice. BAL fluids and tissues were obtained from nontransgenic and transgenic CC10-rtTA-IL-13 animals on normal or dox water from 1–2 months of age. (**a**) Immunoblots were performed on BAL fluids using antibodies against MMP-12. (**b**–**f**) Immunohistochemistry was used to localize MMP-12 in the lung. In the immunohistochemical evaluation, photomicrograph **b** is from double transgenic mice treated with dox and stained with preimmune antiserum; **c** and **d** are from nontransgenic mice treated with normal and dox water, respectively, and stained with antiserum against MMP-12, and **e** and **f** are from double transgenic mice treated with normal and dox water, respectively, and stained with anti–MMP-12.

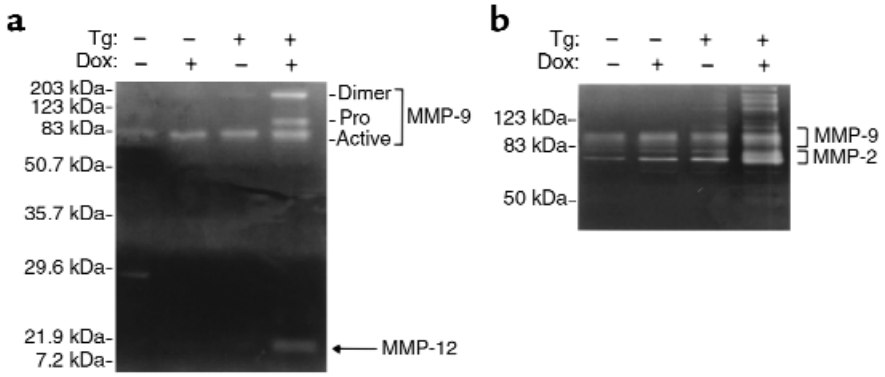

Figure 8
Zymography of BAL fluid from IL-13–overexpressing mice. BAL fluids were obtained from nontransgenic and transgenic CC10-rtTA-IL-13 animals on normal or dox water at 1–2 months of age. Casein zymography (**a**) and gelatin zymography (**b**) were undertaken. The presence of MMP-9, -12, and -2 can be appreciated in the zymograms.

grated with the unprocessed and/or fully processed forms of MMP-9, -12, and -2 (Figure 8, a and b). The activities in these bands were inhibited by EDTA, which establishes them as metalloproteinases. Thus, IL-13 is a potent inducer of a variety of MMP moieties that collectively have potent elastolytic and collagenolytic activities.

Effects of MMP antagonist. Studies were next undertaken to define the importance of the induced MMPs in the pathogenesis of the lesions in our animals. This was done by determining whether the broad-spectrum MMP antagonist GM-6001 (32, 33) altered the IL-13–induced phenotype in our transgenic mice. GM-6001 did not alter the lung or alveolar size of nontransgenic animals receiving normal or dox water. It did, however, abrogate the IL-13–induced increase in lung volume by greater or equal to 70% (Figure 9a) and caused comparable decreases in lung and alveolar size assessed visually, histologically, and via chord length measurements ($P < 0.001$ for all comparisons) (Figure 9, b and c, and data not shown). GM-6001 also caused impressive alterations in pulmonary inflammation, decreasing BAL cell recovery and eosinophil and lymphocyte numbers by more than 90% ($P < 0.001$ for all comparisons). C1006, an inactive peptide control, and DMSO, the vehicle control, did not cause similar alterations (Figure 9, a–c, and data not shown). GM-6001 also did not inhibit IL-13–induced mucus metaplasia (data not shown). In these experiments, GM-6001 also did not alter the levels of IL-13 in the BAL fluids from double transgenic animals on dox water. This demonstrates that the effects of GM-6001 were not due to an inhibitory interaction with the CC10 promoter or drug-induced anorexia with a decrease in dox water intake by our animals (data not shown). Lastly, GM-6001 did not inhibit the levels of MMP-2, -12, -13, and -14 gene expression in lungs from nontransgenic or double transgenic mice on normal or dox water (Figure 10, and data not shown). This demonstrates that although GM-6001 decreases inflammatory cell influx by more than 90%, these inflammatory cells do not contribute, in a major way, to the induction of these MMP moieties in IL-13–treated lungs. When viewed in combination, these studies demonstrate that IL-13 induces emphysema and tissue inflammation via an MMP-dependent pathway. They also demonstrate that IL-13 utilizes a different MMP-independent pathway to induce airway mucus metaplasia.

IL-13 regulation of cysteine proteinases. Studies were also undertaken to determine whether cysteine proteases were induced by IL-13 in our modeling system. This was done by comparing the levels of mRNA encoding a number of airway-relevant cysteine proteases in lungs from CC10-rtTA-IL-13 animals and littermate controls. As shown in Figure 11a, IL-13 caused impressive increases in the levels of mRNA encoding cathepsins B, H, K, S, and L. This increase occurred predominantly in resident lung cells because GM-6001, which caused a greater than 90% decrease in inflammatory cell influx, did not alter the ability of IL-13 to induce these cathepsin moieties (data not shown). Active site probe analysis, which identifies bioactive cathepsin moieties (30), also demonstrated increased levels of these cathepsins in lung lysates from IL-13–expressing mice and free cathepsin S in BAL fluids from these animals (Figure 11, b and c). Thus, IL-13 is a potent inducer of cysteine proteinase production in the lung.

Effects of cysteine proteinase antagonists. To define further the role(s) of the induced cysteine proteinases, we determined whether the cysteine protease inhibitors E-64 and leupeptin altered the phenotype of double transgenic CC10-rtTA-IL-13 mice. In these studies, neither E-64 nor leupeptin altered the size of the lungs or alveoli of nontransgenic mice on normal water or dox water. In contrast, they were potent inhibitors of IL-13-induced emphysema, causing a 65% or greater decrease in the IL-13–induced increase in lung volume (Figure 12a) and comparable decreases in lung and alveolar size assessed visually, histologically, or morphometrically ($P < 0.01$ for all comparisons) (Figure 12, b and c, and data not shown). E-64 and leupeptin also caused an approximately 60% decrease in BAL cell recovery and an 80% or greater decrease in BAL eosinophils and lymphocytes ($P < 0.001$ for all comparisons). Importantly, they did not alter IL-13–induced mucus metaplasia, IL-13–induced MMP gene expression, or the levels of BAL IL-13 (data not shown). These studies demonstrate that cysteine proteases play a major role in the pathogenesis of IL-13–induced emphysema and tissue inflammation, but not in IL-13–induced mucus metaplasia.

Effects of cysteine proteinase antagonists and MMP antagonists in combination. As noted above, IL-13–induced alveolar enlargement was partially abrogated by treatment

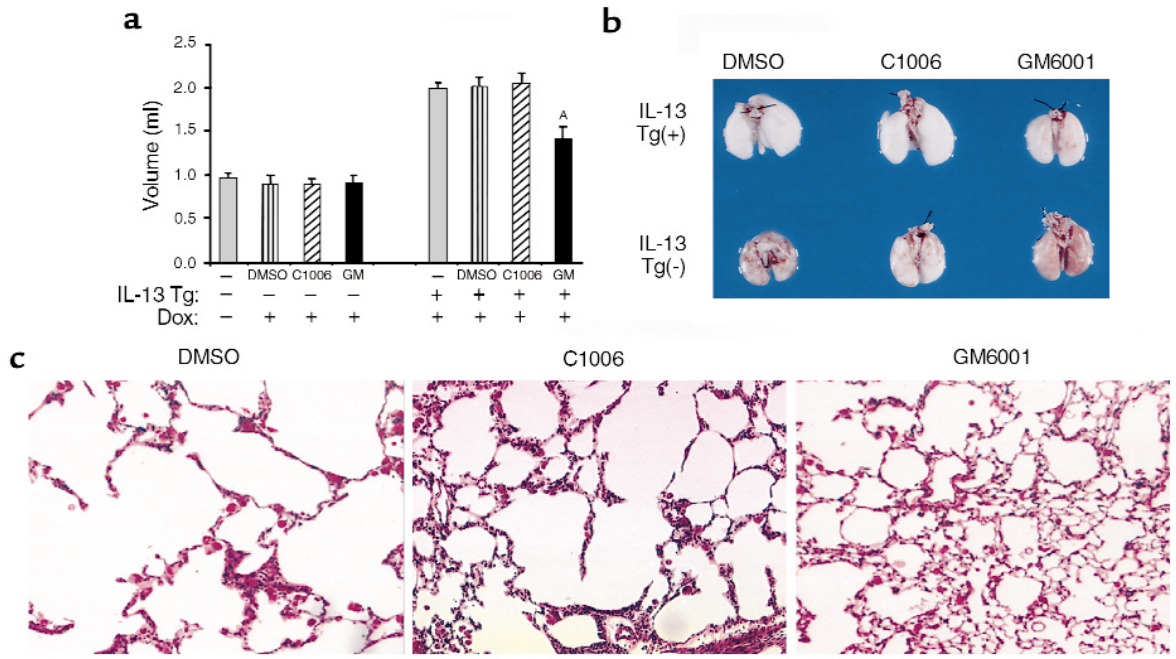

Figure 9
Effect of MMP antagonist on IL-13–induced emphysema. One-month-old nontransgenic and transgenic CC10-rtTA-IL-13 mice on dox water were randomized to receive daily intraperitoneal doses of GM-6001 (200 mg/kg), C1006, and or DMSO. The effects of these agents on lung volume and size (**a** and **b**) and histology (**c**) are illustrated. The ability of GM-6001, but not its controls, to inhibit lung volume, decrease lung size, and decrease alveolar size can be readily appreciated. $^{A}P < 0.05$ versus transgenic mice on dox water that received and did not receive C1006 or DMSO.

with cysteine proteinase antagonists or MMP antagonists individually. Studies were next undertaken to determine whether treatment with these protease inhibitors in combination would alter the IL-13 phenotype in a fashion that was quantitatively and/or qualitatively different than would be seen with the antagonists individually. This was done by comparing the size of the lungs and alveoli from double transgenic mice that received daily doses of GM-6001, leupeptin, or the antagonists in combination starting the day before and throughout a 10-day course of dox water administration. GM-6001 and leupeptin individually decreased lung volume and alveolar size (Table 2, and data not shown). Mice receiving both antagonists manifest a further decrease in alveolar and lung size (Table 2, and data not shown). The lungs from mice that received the antagonists in combination were significantly smaller than the lungs from mice that were treated with the antagonists individually or with the appropriate vehicle controls ($P < 0.02$ for all comparisons). Similar results were obtained with experiments in which GM-6001 and E-64 were used (data not shown). In no case, however, did the proteases in combination restore alveolar volume or lung size to normal (Table 2, and data not shown).

IL-13 regulation of antiproteases in the lung. To understand fully the balance of proteases and antiproteases in the lungs of these mice, the levels of expression of lung-relevant antiproteases in IL-13 overexpressing mice and appropriate controls were also evaluated. IL-13 did not alter the expression of TIMP-2, TIMP-3, TIMP-4, SLPI, or cystatin C. Interestingly, IL-13 did cause an approximately 80% decrease in pulmonary α_1-AT expression (Figure 13). It also caused a significant increase in TIMP-1 expression that was seen after as little as 1 day and was readily apparent with longer periods of dox administration (Figure 13, and data not shown) ($P < 0.05$ for all comparisons).

Discussion

Increases in total cell recovery, macrophages, neutrophils, lymphocytes (often CD8+), and eosinophils are characteristic findings in BAL fluids and/or lung

Table 2
Effects of protease inhibitors in combination on lung volume

Transgene status/Treatment	Lung volume (ml)
(–)/Vehicle control(s)	0.91 ± 0.07
(–)/GM-6001	0.93 ± 0.08
(–)/Leupeptin	0.95 ± 0.07
(–)/GM-6001 + leupeptin	0.94 ± 0.10
(+)/Vehicle control(s)	2.17 ± 0.06
(+)/GM-6001	1.53 ± 0.10
(+)/Leupeptin	1.56 ± 0.16
(+)/GM-6001 + leupeptin	1.32 ± 0.12^{A,B}

Nontransgenic and double transgenic CC10-rtTA-IL-13 mice were placed on dox water for 10 days in the presence or absence of GM-6001 (200 mg/kg), leupeptin (15 mg/kg), or their vehicle control(s) as noted. At the end of the incubation period, lung volume was assessed as described in Methods. The noted values represent the mean ± SEM of a minimum of eight animals in each group. $^{A}P < 0.02$ versus (+)/leupeptin alone. $^{B}P < 0.011$ versus (+)/GM-6001 alone.

biopsies from patients with COPD (15–17, 19, 40, 41). We hypothesized that mediators involved in these inflammatory responses play central roles in the pathogenesis of this disorder(s). We also hypothesized that mediators that generate Th2-type tissue inflammatory responses also activate proteolytic pathways that contribute to these diseases based on evidence that concurrent asthma, AHR, and eosinophilia correlate with accelerated respiratory deterioration in COPD. To test this hypothesis, we used a regulatable overexpression transgenic modeling system to target IL-13 to the adult murine lung. These studies demonstrate, for the first time, that the overexpression of IL-13 causes a phenotype that mirrors human COPD with prominent emphysema, enlarged lungs, enhanced pulmonary compliance, mucus metaplasia, and a mixed tissue inflammatory response. They also demonstrate that MMPs and cathepsins are induced by IL-13 and play important roles in the pathogenesis of the emphysema in these animals. These are the first studies to use inducible overexpression modeling to investigate the cellular and molecular events involved in the pathogenesis of emphysema. They are also the first to link, in a cause and effect fashion, any mediator of tissue inflammation and emphysema and define the protease/antiprotease alterations that generate this response. When viewed in combination, these studies suggest that IL-13 is an important upstream regulator of pulmonary protease/antiprotease balance that can contribute to the pathogenesis of COPD. Because IL-13 is strongly implicated in the pathogenesis of asthma and can also induce AHR and tissue eosinophilia (20, 23), these studies provide molecular support for the Dutch contention that common mechanisms underlie asthma and COPD in some individuals.

IL-13 is a pleiotropic cytokine with remarkable effects in the lung. This was illustrated in previous work from our laboratory that demonstrated that the constitutive overexpression of IL-13 causes a macrophage-, lymphocyte-, and eosinophil-rich pulmonary inflammatory response, mucus metaplasia, crystal deposition, subepithelial fibrosis, alveolar enlargement and AHR (20). In these CC10-IL-13 animals, the transgene was activated by the CC10 promoter in utero, and the phenotype that was induced by IL-13 was evaluated when the mice were 1–3 months of age. Thus, these studies did not enable us to define the early events or the kinetics of the IL-13–induced phenotype. In addition, as the majority of the alveoli in the murine (and human) lung are formed by developmental processes that occur after birth, these studies did not enable us to determine whether the alveolar enlargement was due to an alteration in alveolar development or to the destruction of otherwise normally formed alveoli. To address these issues, we used an externally regulatable, lung-targeted overexpression transgenic modeling system. This system allowed us to define the kinetics of the IL-13–induced phenotype in the adult, versus the developing, lung. The validity of this approach was established in early studies that demonstrated that indistinguishable phenotypes were noted in lungs from CC10-rtTA-IL-13 double transgenic animals that received dox for extended intervals (>2 months) and 1- to 2-month-old CC10-IL-13 mice (Z. Zhu et al., unpublished observation). The studies with the CC10-rtTA-IL-13 mice also demonstrated that IL-13–induced alveolar enlargement is not development dependent because it occurs when IL-13 is produced in adult lungs with normally formed alveoli. These studies also demonstrated that alveolar enlargement and mucus metaplasia are early responses, whereas pulmonary fibrosis and crystal deposition require more extensive periods of IL-13 exposure. When viewed in combination, these studies highlight the remarkable and complementary nature of the mechanistic insights that can be obtained from the combined use of constitutive and externally regulatable lung-targeted overexpression transgenic approaches.

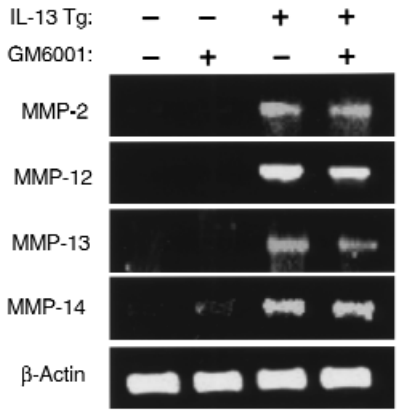

Figure 10
Effect of GM-6001 on IL-13–induced MMP mRNA. One-month-old nontransgenic and transgenic CC10 rtTA-IL-13 mice were randomized to receive daily doses of GM-6001 or DMSO (–) starting 1 day before and continuing for a 10-day cycle of dox administration. The effects of these interventions on the levels of mRNA encoding MMP-2, -12, -13, and -14 are illustrated.

A number of investigators have raised the possibility that MMPs and cathepsins are involved in alveolar destruction in emphysema. These speculations are based on studies from our laboratories and others that demonstrated that MMP-12 is essential for cigarette smoke–induced inflammation and emphysema in the murine lung (28), exaggerated MMP-12 expression in alveolar macrophages from smokers and patients with emphysema (39), and increased levels of MMP-9 and the induction and activation of the MMP-2–MMP-14–TIMP-2 complex in patients with COPD (42, 43). Similarly, macrophages also utilize cathepsins S, L, and K to degrade elastin, and there are increased levels of cathepsin L in BAL cells and fluids from smokers compared with nonsmokers (44–47). With the exception of MMP-12, however, the pathogenetic importance of each of these moieties in animal models of COPD has not been established and the roles of all of these moieties in

human COPD have not been defined. Our studies demonstrate that MMP-2, -9, -12, -13, and -14 and cathepsins B, H, S, K, and L are induced by IL-13 in the murine lung. They also demonstrate that interventions that block MMPs or cysteine proteases have remarkable ameliorative effects on IL-13–induced emphysema and tissue inflammation without significantly altering IL-13–induced mucus metaplasia. The MMP findings are particularly impressive because tetracycline-like compounds, like the doxycycline we used to activate our transgenes, have been reported to inhibit MMP activity in a variety of systems (48, 49). These are the first studies to demonstrate that IL-13 is a potent activator of tissue proteolysis and define the components of this proteolytic pathway. They are also the first studies to demonstrate the efficacy of MMP- or cathepsin-based interventions in an inflammation-based model of emphysema. Thus, these studies demonstrate with certainty that MMPs and cathepsins play major roles in the generation of emphysema in our modeling system. They also raise the possibility that IL-13 or the proteolytic pathways it activates play important roles in other pulmonary proteolytic responses. This could be particularly important in diseases characterized by eosinophilia and tissue destruction or remodeling including pulmonary infections and pulmonary eosinophilic and hypersensitivity syndromes such as invasive and semi-invasive aspergillosis and allergic bronchopulmonary aspergillosis. It is important to point out, however, that these studies do not define the contributions that individual MMPs and/or cathepsins make to the IL-13 phenotype. In addition, they do not address the possibility that proteases, other than those that were studied, contribute in important ways to the generation of the IL-13 phenotype. The latter concern may be particularly relevant to ADAM (a disintegrin and metalloproteinase) family members like TNF-α–converting enzyme (TACE) which are also inhibited by hydroxamic-acid–based MMP antagonists (50, 51).

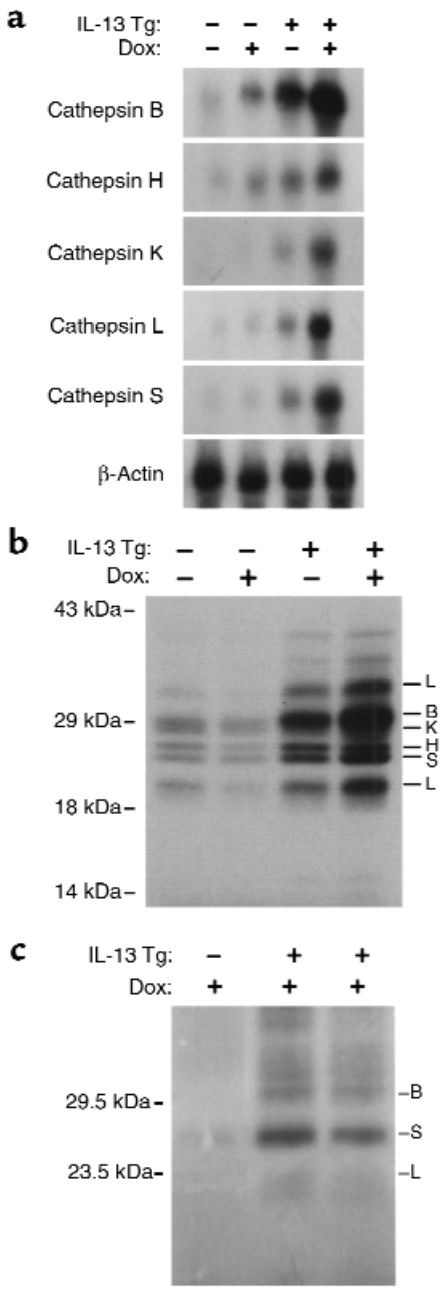

Figure 11
Effect of IL-13 on respiratory cathepsins. (**a**) Northern analysis was used to compare the levels of cathepsin mRNA in lungs from 8-week-old nontransgenic and transgenic CC10-rtTA-IL-13 mice that received normal water or dox water for the 4-week interval before evaluation. (**b** and **c**) Illustration of the levels of active cathepsin proteins (assessed with active site probe analysis) in lung lysates and BAL, respectively, from these animals.

To define the contributions of MMPs and cysteine proteases to the IL-13 phenotype, the effects of MMP and cysteine proteinase inhibitors on IL-13–induced emphysema were evaluated. These studies demonstrated that the MMP antagonists and cysteine proteinase antagonists, individually, partially abrogated the IL-13–induced emphysematous response. They also demonstrated that the protease inhibitors in combination were more effective at reducing lung volume and alveolar size than either inhibitor individually. To our surprise, however, the inhibitors in combination did not totally abrogate IL-13–induced emphysematous alterations. These studies suggest that the MMP and cysteine proteinase pathways contribute in complementary and at least partially separable ways to the pathogenesis of the IL-13–induced emphysematous phenotype. They also suggest that IL-13–regulated proteolytic pathways that are not inhibited by the MMP and cysteine proteinase inhibitors that were used in our studies also contribute to the IL-13 phenotype.

As already noted here, the MMP inhibitor GM-6001 inhibited IL-13–induced emphysema while simultaneously inhibiting IL-13–induced tissue and BAL inflammation. These observations suggest that GM-6001 could mediate its effects by directly inhibiting MMP-induced matrix degradation. Alternatively, inflammatory cells could be the major sources of the MMPs, cathepsins, and

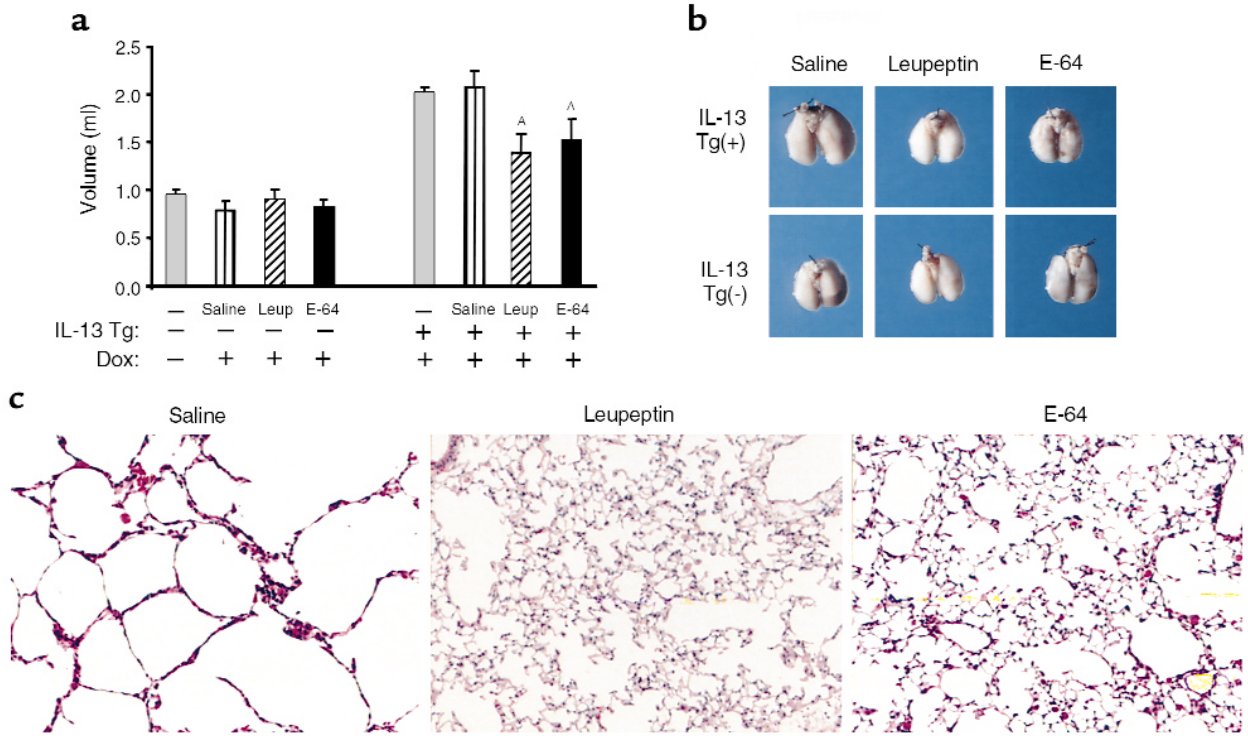

Figure 12
Effects of cysteine protease inhibitors on IL-13–induced emphysema. One-month-old nontransgenic and transgenic CC10-rtTA-IL-13 mice on dox water were given daily intraperitoneal injections of saline, E-64 (7.5 mg/kg), or leupeptin (15 mg/kg). The effects of these interventions on lung volume and size (**a** and **b**) and histology (H&E) (**c**) were evaluated. The ability of E-64 and leupeptin to decrease lung volume, lung size, and alveolar size can be readily appreciated. $^{A}P < 0.05$ versus transgenic mice on dox that received or did not receive saline vehicle control.

other degradative enzymes involved in IL-13–induced pulmonary proteolysis and GM-6001 could mediate its effects solely by decreasing inflammatory cell influx into the lung. In an attempt to address this issue, studies were undertaken to determine whether GM-6001 altered the levels of expression of mRNA encoding IL-13–induced MMPs and cysteine proteinases. These studies demonstrated that GM-6001, although inhibiting emphysema and inflammation, did not significantly decrease the ability of IL-13 to stimulate MMPs -2, -12, -13, and -14 and cathepsin S, H, L, B, and K mRNA accumulation. These observations demonstrate that GM-6001 does not mediate its effects by altering the ability of IL-13 to induce the expression of these MMPs or cathepsins. They also demonstrate that these MMP and cysteine proteinase-stimulatory effects of IL-13 occur predominantly in resident as versus elicited pulmonary cells and that the effects of GM-6001 on the IL-13 phenotype can not be attributed solely to the ability of GM-6001 to alter inflammatory cell recruitment.

To understand fully the protease/antiprotease alterations that lead to emphysema, we also characterized the levels of expression of a variety of important antiproteases in the lungs of IL-13–overexpressing animals. Significant alterations in TIMPs-2-4, SLPI, and cystatin C were not noted. Interestingly, significant stimulation of TIMP-1 was noted. Although this alteration would be expected to inhibit MMP-induced proteolysis, it was not of sufficient magnitude (or in the proper location) to abrogate the MMP-induced emphysema in our animals. In contrast, the expression of pulmonary α_1-AT was decreased in our animals. At first blush, this would be expected to contribute to the pathogenesis of IL-13–induced emphysema. The biologic significance of an isolated decrease in pulmonary α_1-AT production, however, must be evaluated carefully because it is well known that the levels of α_1-AT secreted into the systemic circulation by the liver greatly exceed those produced in pulmonary tissues. In addition, humans with only a single functional *α_1-AT* allele and levels of circulating α_1-AT approximately 50% of normal do not manifest an increased prevalence of emphysema (1).

The Dutch Hypothesis emphasizes the importance of endogenous host factors in the development of COPD and asthma. In this view, AHR is considered a marker of an endogenous predisposition that contributes to the development of both disorders. This contrasts with the "British Hypothesis," which emphasizes the importance of exogenous factors such as cigarette smoke exposure and recurrent infections in the generation of COPD (10). Our studies demonstrate that IL-13, a cytokine that is produced in large quantities by Th2 cells and is strongly implicated in the pathogenesis of asthma, AHR, and tissue eosinophilia (20, 23), causes emphysema when expressed in the adult murine lung.

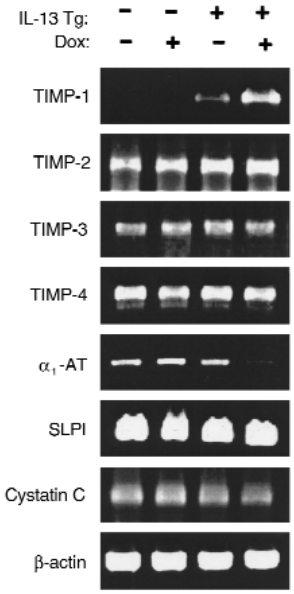

Figure 13
Effect of IL-13 on respiratory antiproteases. RT-PCR was used to compare the levels of mRNA encoding respiratory relevant antiproteases in 2-month-old nontransgenic and transgenic CC10-rtTA-IL-13 mice that received either normal water (–) or dox water (+) from 1 month of age. The lack of effect of IL-13 on TIMPs-2, -3, and-4, SLPI, and cystatin C and the ability of IL-13 to inhibit α_1-AT and stimulate TIMP-1 can be appreciated.

These findings support key features of the Dutch Hypothesis and suggest that at least part of the "basic disturbance" described in the hypothesis is the predisposition to produce exaggerated amounts of IL-13 or activate IL-13–like pulmonary proteolytic pathways. It is important to point out, however, that not all patients with COPD have concurrent asthma or manifest eosinophilia or AHR. This would suggest that the Dutch Hypothesis can account for only a subpopulation of patients with COPD and that the IL-13–based transgenic model described in this report is relevant only to these individuals. Interestingly, it may also be applicable to the emphysema that has recently been described in HIV-infected individuals (52), as evolution from a Th1 to Th2 cytokine profile has been associated with progression to AIDS in HIV-infected individuals (53) and HIV induces the expression of the IL-13 receptor on Kaposi's sarcoma cells (54).

The data in this manuscript demonstrate that IL-13 is a potent stimulator of pulmonary proteolysis. Because IL-13 is produced in large quantities by Th2 and T cell 2 (TC2) cells, these findings support the hypothesis that type II–dominated tissue inflammation can contribute to the pathogenesis of pulmonary emphysema. This interpretation, however, must be viewed with caution because IL-13 can also be produced by type I CD4+ and CD8+ T cells (55), and CD8+ cells are found in large quantities in lungs from patients with COPD (40, 41). Thus, IL-13 may also be produced and activate proteolytic pathways in type I inflammatory responses in the lung. Additional experimentation will be required to determine whether IL-13 is produced in an exaggerated fashion in COPD and if this production is seen in the setting of type I or type II tissue inflammation. If exaggerated levels of IL-13 are noted in tissues from patients with COPD, investigations will also be needed to determine why IL-13 is associated with and potentially causally related to emphysematous responses in some individuals (patients with COPD with asthmatic features) but not in others (patients with "classic" bronchial asthma). These studies will need to determine whether the different outcomes can be attributed, at least in part, to: (a) differences in the location or magnitude of IL-13 or antiproteinase production; (b) individual host susceptibility factors such as polymorphisms of IL-13 or the IL-13 receptor; (c) the possibility that antiproteinase paradigms are induced in asthmatics that negate the proteolytic effects of IL-13; and (d) the possibility that cigarette smoke induces a variety of proteolytic responses, with emphysema being the result of the combined effects of the IL-13 and other proteolytic pathways. Consideration will also need to be directed at the possibility that diagnostic transference is responsible, at least in part, for the belief that asthmatics do not develop emphysema. This issue is raised because physicians tend to diagnose young people with eosinophilia and airways obstruction as asthmatics (regardless of their smoking status) and then change their diagnosis to COPD later on in life when emphysema or irreversible airways obstruction are appreciated.

In summary, these studies demonstrate that the targeted expression of IL-13 in the adult lung causes emphysema, mucus metaplasia, and inflammation that mirror, in many ways, the lesions seen in human COPD. They also demonstrate that this emphysematous response is mediated, via matrix metalloproteinase and cathepsin-dependent pathways. IL-13 may play an important role in the pathogenesis of COPD, particularly in patients with asthma-like features including AHR and eosinophilia.

Acknowledgments

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1. Senior, R.M., and Shapiro, S.D. 1998. Chronic obstructive pulmonary disease: epidemiology, pathophysiology, and pathogenesis. In *Fishman's pulmonary diseases and disorders*. Volume 1. A.P. Fishman et al., editors. McGraw-Hill Inc. New York, New York, USA. 659–681.
2. O'Byrne, P.M., and Postma, D.S. 1999. The many faces of airway inflammation: asthma and chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **159**:S41–S66.
3. Rijcken, B., et al. 1993. Long term variability of bronchial responsiveness to histamine in a random sample of adults. *Am. Rev. Respir. Dis.* **148**:944–949.
4. Rijcken, B., Schouten, J.P., Xu, X., Rosner, B., and Weiss, S.T. 1995. Airway hyperresponsiveness to histamine associated with accelerated decline in FEV_1. *Am. J. Respir. Crit. Care Med.* **151**:1377–1382.
5. O'Connor, G.T., Sparrow, D., and Weiss, S.T. 1995. A prospective longitudinal study of methacholine airway responsiveness as a predictor of pulmonary-function decline: the Normative Aging Study. *Am. J. Respir. Crit. Care Med.* **152**:87–92.
6. Lebowitz, M.D., Postma, D.S., and Burrows, B. 1995. Adverse effects of eosinophilia and smoking on the natural history of newly diagnosed chronic bronchitis. *Chest.* **108**:55–61.
7. Tashkin, D.P., et al. 1996. Methacholine reactivity predicts changes in lung function over time in smokers with early chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **153**:1802–1811.
8. Parker, D.R., O'Connor, G.T., Spararow, D., Segal, M.R., and Weiss, S.T. 1990. The relationship of nonspecific airway responsiveness and atopy to the rate of decline of lung function. The Normative Aging Study. *Am. Rev. Respir. Dis.* **141**:589–594.
9. Xu, X., Rijcken, B., Schouten, J.P., and Weiss, S.T. 1997. Airway responsiveness and development and remission of chronic respiratory symptoms in

adults. *Lancet.* **350**:1431–1434.
10. Vestbo, J., and Prescott, E. 1997. Update on the "Dutch hypothesis" for chronic respiratory disease. *Lancet*. **350**:1431–1434.
11. Sluiter, H.J., et al. 1991. The Dutch hypothesis (chronic non-specific lung disease) revisited. *Eur. Respir. J.* **4**:479–489.
12. Burrows, B., Knudson, R.J., Cline, M.G., and Lebowitz, M.D. 1988. A reexamination of risk factors for ventilatory impairment. *Am. Rev. Respir. Dis.* **138**:829–836.
13. Burrows, B., Bloom, J.W., Traver, G.A., and Cline, M.G. 1987. The course and prognosis of different forms of chronic airways obstruction in a sample from the general population. *N. Engl. J. Med.* **317**:1309–1314.
14. Lams, B.E., Sousa, A.R., Rees, P.J., and Lee, T.H. 1998. Immunopathology of the small-airway submucosa in smokers with and without chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **158**:1518–1523.
15. Cosio, M.G., and Guerassimov, A. 1999. Chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **160**:S21–S25.
16. Saetta, M. 1999. Airway inflammation in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **160**:517–520.
17. Saetta, M., et al. 1993. Activated T-lymphocytes and macrophages in bronchial mucosa of subjects with chronic bronchitis. *Am. Rev. Respir. Dis.* **147**:301–306.
18. Saetta, M., et al. 1994. Airway eosinophilia in chronic bronchitis during exacerbations. *Am. J. Respir. Crit. Care Med.* **150**:1646–1652.
19. O'Shaughnessy, T.C., Ansari, T.W., Barnes, N.C., and Jeffrey, P.K. 1997. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8+ T lymphocytes with FEV1. *Am. J. Resp. Crit. Care Med.* **155**:852–857.
20. Zhu, Z., et al. 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities and eotaxin production. *J. Clin. Invest.* **103**:779–788.
21. Cohn, L., Tepper, J.S., and Bottomly, K. 1998. IL-4-independent induction of airway hyperresponsiveness by Th2, but not Th1, cells. *J. Immunol.* **161**:3813–3816.
22. Bradding, P., Redington, A.E., and Holgate, S.T. 1997. Airway wall remodelling in the pathogenesis of asthma: cytokine expression in the airways. In *Airway wall remodelling in asthma*. A.G. Stewart, editor. CRC Press Inc. Boca Raton, Florida, USA. 29–63.
23. Wills-Karp, M., et al. 1998. Interleukin-13: central mediator of allergic asthma. *Science*. **282**:2258–2260.
24. Ray, P., et al. 1997. Regulated overexpression of interleukin-11 in the lung: use to dissociate development-dependent and -independent phenotypes. *J. Clin. Invest.* **100**:2501–2511.
25. Cohn, L., Homer, R.J., Marinov, A., Rankin, J., and Bottomly, K. 1997. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. *J. Exp. Med.* **186**:1737–1747.
26. Yoon, H.J., Zhu, Z., Gwaltney, J.M., Jr., and Elias, J.A. 1999. Rhinovirus regulation of IL-1 receptor antagonist *in vivo* and *in vitro*: a potential mechanism of disease resolution. *J. Immunol.* **162**:7461–7469.
27. Elias, J.A., et al. 1994. Interleukin-1 and transforming growth factor β regulation of fibroblast-derived interleukin-11. *J. Immunol.* **152**:2421–2429.
28. Hautamaki, R.D., Kobayashi, D.K., Senior, R.M., and Shapiro, S.D. 1997. Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science.* **277**:2002–2004.
29. Senior, R.M., et al. 1991. Human 92- and 72-kilodalton type IV collagenases are elastases. *J. Biol. Chem.* **266**:7870–7875.
30. Shi, G.-P., Munger, J.S., Meara, J.P., Rich, D.H., and Chapman, H.A. 1992. Molecular cloning and expression of human alveolar macrophage cathepsin S, an elastinolytic cysteine protease. *J. Biol. Chem.* **267**:7258–7262.
31. Rasband, W. NIH Image. http://rsb.info.nih.gov/nih-image.
32. Galardy, R.E., et al. 1994. Low molecular weight inhibitors in corneal ulceration. *Ann. NY Acad. Sci.* **732**:315–323.
33. Graesser, D., et al. 1998. The interrelationship of alpha4 integrin and matrix metalloproteinase-2 in the pathogenesis of experimental autoimmune encephalomyelitis. *Lab. Invest.* **78**:1445–1458.
34. Salminen, A. 1984. Effects of the protease inhibitor leupeptin on proteolytic activities and regeneration of mouse skeletal muscles after exercise injuries. *Am. J. Pathol.* **117**:64–70.
35. Delaisse, J.-M., Eckhout, Y., and Vaes, G. 1984. *In vivo* and *in vitro* evidence for the involvement of cysteine proteinases in bone resorption. *Biochem. Biophys. Res. Commun.* **125**:441–447.
36. Burri, P.H. 1997. Structural aspects of prenatal and postnatal development and growth of the lung. In *Lung growth and development*. Volume 100. J.A. McDonald, editor. Marcel Dekker Inc. New York, New York, USA. 1–35.
37. Snider, G.L., Lucey, E.C., and Stone, P.J. 1994. Pitfalls in antiprotease therapy of emphysema. *Am. J. Respir. Crit. Care Med.* **150**:5131–5137.
38. Snider, G.L., Kleinerman, J., Thurlbeck, W.M., and Bengali, Z.H. 1985. The definition of emphysema: report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases Workshop. *Am. Rev. Resp. Dis.* **132**:182–185.
39. Shapiro, S.D. 2000. Animal models for chronic obstructive pulmonary disease. Age of klotho and marlboro mice. *Am. J. Respir. Cell Mol. Biol.* **22**:4–7.
40. Boushey, H.A. 1999. Glucocorticoid therapy for chronic obstructive pulmonary disease. *N. Engl. J. Med.* **340**:1990–1991.
41. Saetta, M., et al. 1999. CD8+ve cells in the lungs of smokers with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **160**:711–717.
42. Ohnishi, K., Takagi, M., Kurokawa, Y., Satomi, S., and Konttinen, Y.T. 1998. Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema. *Lab. Invest.* **78**:1077–1087.
43. Finlay, G.A., et al. 1997. Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. *Am. J. Respir. Crit. Care Med.* **156**:240–247.
44. Takeyabu, K., et al. 1998. Cysteine proteinases and cystatin C in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Eur. Respir. J.* **12**:1033–1039.
45. Takahashi, H., et al. 1993. Cathepsin L activity is increased in alveolar macrophages and bronchoalveolar lavage fluid of smokers. *Am. Rev. Respir. Dis.* **147**:1562–1568.
46. Reddy, V.Y., Zhang, Q.-Y., and Weiss, S.J. 1995. Pericellular mobilization of the tissue-destructive cysteine proteinases, cathepsins B, L, and S, by human monocyte-derived macrophages. *Proc. Natl. Acad. Sci. USA.* **92**:3849–3853.
47. Chapman, H.A., Riese, R.J., and Shi, G.-P. 1997. Emerging roles for cysteine proteases in human biology. *Annu. Rev. Physiol.* **59**:63–88.
48. Smith, G.N., Jr., Mickler, E.A., Hasty, K.A., and Brandt, K.D. 1999. Specificity of inhibition of matrix metalloproteinase activity by doxycycline: relationship to structure of the enzyme. *Arthritis Rheum.* **42**:1140–1146.
49. Curci, J.A., et al. 2000. Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. *J. Vasc. Surg.* **31**:325–342.
50. Hooper, N.M., Karran, E.H., and Turner, A.J. 1997. Membrane protein secretases. *Biochem. J.* **321**:265–279.
51. Mullberg, J., Althoff, K., Jostock, T., and Rose-John, S. 2000. The importance of shedding of membrane proteins for cytokine biology. *Eur. Cytokine Netw.* **11**:27–38.
52. Diaz, P.T., et al. 2000. Increased susceptibility to pulmonary emphysema among HIV-seropositive smokers. *Ann. Intern. Med.* **132**:369–372.
53. Klein, S.A., et al. 1997. Demonstration of the Th1 to Th2 cytokine shift during the course of HIV-1 infection using cytoplasmic cytokine detection on single cell level by flow cytometry. *AIDS.* **11**:1111–1118.
54. Husain, S.R., et al. 1997. Receptor for interleukin 13 on AIDS-associated Kaposi's sarcoma cells serves as a new target for a potent Pseudomonas exotoxin-based chimeric toxin protein. *Clin. Cancer Res.* **3**:151–156.
55. Minty, A., et al. 1997. The related cytokines interleukin-13 and interleukin-4 are distinguished by differential production and differential effects on T lymphocytes. *Eur. Cytokine Netw.* **8**:203–213.