CD8⁺ T Cells Are Required for Inflammation and Destruction in Cigarette Smoke-Induced Emphysema in Mice¹

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Increased numbers of T lymphocytes are observed in the lungs of patients with chronic obstructive pulmonary disease, but their role in the disease process is not known. We investigated the role of CD8⁺ T cells in inflammatory cell recruitment and lung destruction in a cigarette smoke-induced murine model of emphysema. In contrast to wild-type (C57BL/6J) mice that displayed macrophage, lymphocyte, and neutrophil recruitment to the lung followed by emphysema in response to cigarette smoke, CD8⁺ T cell-deficient (CD8⁻/⁻) mice had a blunted inflammatory response and did not develop emphysema when exposed to long-term cigarette smoke. Further studies supported a pathogenetic pathway whereby the CD8⁺ T cell product, IFN-γ-inducible protein-10, induces production of macrophage elastase (matrix metalloproteinase 12) that degrades elastin, both causing lung destruction directly and generating elastin fragments that serve as monocyte chemokines augmenting macrophage-mediated lung destruction. These studies demonstrate a requirement for CD8⁺ T cells for the development of cigarette smoke-induced emphysema and they provide a unifying pathway whereby CD8⁺ T cells are a central regulator of the inflammatory network in chronic obstructive pulmonary disease. The Journal of Immunology, 2007, 178: 8090–8096.

Pulmonary emphysema is a major component of chronic obstructive pulmonary disease (COPD),¹ a disease that currently affects over 18 million Americans and is the fourth leading cause of death in the United States. The burden of COPD promises to increase throughout the world as smoking rates have accelerated in many countries (1). Emphysema also serves as a useful model for the manifestations of chronic inflammation on tissue structure and function. Cigarette smoke exposure leads to inflammation, release of destructive proteinases, and ultimately to the destruction and enlargement of alveolar walls that define emphysema (2, 3).

These concepts arose from the elastase:antielastase hypothesis proposed over 40 years ago to explain the development of emphysema. Initially, attention was focused exclusively on the neutrophil and neutrophil elastase. Although neutrophils are recruited acutely following smoke exposure and neutrophil elastase is a powerful enzyme, it later became clear that the macrophage, a prominent cell in chronic inflammation following cigarette smoke exposure, regulates inflammation and also produces destructive elastases as well (4–6). We have previously shown that mice lacking matrix metalloproteinase 12 (MMP-12) (MMP-12⁻/⁻) are protected from the accumulation of lung macrophages and airspace enlargement in response to cigarette smoke exposure (7). More recently, we have demonstrated that the reduced lung macrophage content observed in MMP-12⁻/⁻ smoke-exposed mice is the result of the reduced production of elastin fragments (EFs), which are the major monocyte chemotactic factor generated in the murine model of cigarette smoke-induced emphysema (8–11).

T lymphocytes, particularly CD8⁺ T cells, are increased in lungs of patients with COPD (12), and it was recently shown that human CD8⁺ T cells have the capacity to produce cytokines that may contribute to emphysema (13, 14). Overexpression of IFN-γ in transgenic mice results in apoptosis, inflammation, and emphysema (15). Moreover, the IFN-γ and IFN-γ-inducible protein-10 (IP-10) have been shown to be increased in CD8⁺ T cells from patients with emphysema; IP-10 was also shown to induce the production of MMP-12 in human alveolar macrophages (16). In addition to this potentially indirect influence of CD8⁺ T cells on emphysema, they may also directly cause cytotoxicity contributing to emphysema, as supported by increased perforin expression and cytotoxic activity of sputum CD8⁺ T cells in patients with COPD (17).

To determine the role of CD8⁺ T cells in the pathogenesis of emphysema, we subjected wild-type (WT) (C57BL/6J), CD8⁺ T cell-deficient (CD8⁻/⁻) mice and CD4⁺ T cell-deficient (CD4⁻/⁻) mice to a model of cigarette smoke-induced emphysema.

Materials and Methods

Reagents

Cigarettes were purchased from the University of Kentucky (7, 18). Mac-3 Ab, CD4 Ab, and CD8 Ab were purchased from BD Biosciences. Hema3 was purchased from Biochemical Sciences. Vectastain Elite ABC kit was purchased from Vector Laboratories. Histopaque-1077 was purchased from Sigma-Aldrich. Human CD14 microbeads were purchased from Miltenyi Biotec. Western blot chemiluminescence reagents were purchased from Pierce. Prestained protein ladder was purchased from Invitrogen Life Technologies. Anti-mouse HRP-conjugated Ab was purchased from Amersham Biosciences. IP-10 Ab (1/200) was purchased from R&D Systems.
MMP-12 polyclonal Ab (1/500) was purchased from GenWay Biotech. BA4 is a mouse anti-bovine IgG1 subclass mAb (1/2000) that was derived from an immunization with the α-elastin fraction of insoluble elastin (provided by R. P. Mecham, Washington University School of Medicine, St. Louis, MO). It has subsequently been shown to recognize the VGVAPG hexapeptide found in human elastin (19).

**Mice**

CD8\(^+\) T cell-deficient mice (CD8\(^{-/-}\)) were generated by Fung-Leung et al. (20) and purchased from The Jackson Laboratory. CD4\(^+\) T cell-deficient mice (CD4\(^{-/-}\)) were generated by Rahemtulla et al. (21) and purchased from The Jackson Laboratory. These mice are on a pure C57BL/6j background (>10 backcrosses). Age- and sex-matched WT C57BL/6j mice were used as controls. All animals were housed within a pathogen-free sterile barrier facility at the Harvard School of Public Health. The Harvard Standing Committee for Animal Research at Harvard University School of Public Health approved all animal experiments.

**Cigarette smoke exposure**

Groups of CD8\(^{-/-}\), CD4\(^{-/-}\), and WT female mice, all 10–12 wk of age, were subjected to the smoke of two unfiltered cigarettes per day (University of Kentucky), 6 days a week for 6 mo, using a smoking apparatus as previously described (7). Mice tolerated cigarette smoke exposure without evidence of toxicity (carboxyhemoglobin levels ~10% and no weight loss).

**Tissue processing**

On completion of the smoking protocol, the right ventricle was perfused with saline and the mice were sacrificed by CO\(_2\) inhalation. In some mice, the left lungs were ligated and tissue was removed for Western blot analysis and chemotaxis assay. Lung tissue homogenates were prepared by homogenizing the lungs in 1 ml of 1× PBS. In other mice, the left lung was inflated by instilling OCT solution diluted with PBS (1/1) for the immunohistochemistry. The right lung was inflated by instilling 10% formalin at a constant pressure of 25 cm H\(_2\)O (for 10 min) and then ligated and removed. Inflated lungs were fixed for 24 h before embedding in paraffin. Serial midsagittal sections were obtained for morphological and histological analyses.

**Morphometry**

After fixation, midsagittal sections were stained with H&E. The mean linear intercept (Lm), that is the average distance between alveolar walls and proportional to the amount of pulmonary emphysema, was determined by light microscopy as previously described (7, 22, 23). All measurements were performed by a single blinded investigator (S. D. Shaprio).

**Bronchoalveolar lavage (BAL) fluid analysis**

At the appropriate time point, a subset of mice designated for BAL analysis was sacrificed and BAL was performed via a 22-g i.v. catheter inserted into the trachea. The lungs were lavaged with 0.75 ml of PBS × 4 to obtain the BAL fluid. The BAL fluid was centrifuged at 3000 rpm × 3 min, the RBC were lysed, and the BAL fluid was centrifuged at 3000 rpm × 3 min. Cell pellets were resuspended in 1.0 ml of PBS and used to determine the total cell counts and differential cell counts using a hemocytometer and cyto-spins stained with Hema3 (Biological Sciences).

**Immunohistochemistry**

Alveolar and interstitial macrophages were quantified using mac-3 (rat anti-mouse Ab at 1/250 dilution; BD Biosciences) immunostaining, using the Vectastain Elite ABC kit (Vector Laboratories) in which 3,3’-diaminobenzidine was the chromogenic substrate. Alveolar and interstitial T lymphocytes were quantified using frozen sections. T lymphocytes were quantified using murine CD8α (rat anti-mouse Ab at 1/250 dilution; BD Biosciences) and CD4 (rat anti-mouse Ab at 1/250 dilution; BD Biosciences), using the avidin-biotin HRP technique in which 3,3’-diaminobenzidine was the chromogenic substrate. IP-10 (goat anti-mouse at 1/200 dilution; from R&D Systems) immunostaining was performed in the same way. Results are represented as the average count from 10 different high-powered fields (×100) per slide that have been corrected for millimeter alveolar wall using the morphometric data (as above).

**Real-time PCR**

Following cigarette smoke exposure as described above, mouse lungs (n = 6) were homogenized in 1 ml of cold TRIzol before performing RNA isolation as per the manufacturer’s instructions. The samples were reverse-transcribed using RT-PCR beads with poly(dT) oligos as per manufacturer’s instructions (Amersham Biosciences). Real-time PCR analysis was performed using GeneAmp 5700 Sequence Detection System (Applied Biosystems). The comparative cycle threshold method was used using GAPDH as an endogenous reference housekeeping gene. SYBR green buffer was used as the fluorophore. All experiments were performed in triplicate. The primer sequences for MCP-1, MCP-2, MIP-1α, MIP-1β, and RANTES have been described elsewhere (8).

**ELISA**

BAL fluid was obtained as described above. Each sample of BAL fluid was normalized for total protein content using the BCA Protein Assay kit (Pierce). The levels of MCP-1 and MIP-1α were determined using the commercially available Quantakine kits (R&D Systems) as per the manufacturer’s instructions. Each sample was performed in triplicate (n = 6 samples).

**Monocyte chemotaxis**

Assays of monocyte chemotaxis were performed on human peripheral blood monocytes using lung homogenates from CD8\(^{-/-}\) and WT smoke-exposed and nonsmoking control mice as the chemoattractants. Human peripheral blood monocytes were obtained from healthy donors (using an approved protocol by Brigham & Women’s Hospital Institutional Review Board) by phlebotomy and purified using Histopaque-1077 and CD14 microbeads. The purity of monocytes was 99%. Monocytes were placed in the top wells of a microchemotaxis chamber at a concentration of 1.0 × 10\(^6\) cells/well in DMEM. The lower compartments contained the chemoattractant from CD8\(^{-/-}\) and WT smoke-exposed and nonsmoking control mice.

Three experiments were performed with the same result. The result of one representative experiment performed in triplicate is shown.

**Western blot analysis**

Lungs were removed en bloc, perfused with saline until free of blood, then frozen and homogenized in 1 ml of 1× PBS. Samples were normalized for total lung protein content for further analysis. SDS-PAGE was performed with 10% gels according to the standard procedure after boiling with sample buffer and protein in the gel were transferred electrophoretically to a nitrocellulose membrane in Tris-glycine buffer containing 20% methanol at 180 mA for 1.5 h. Western blots were visualized using Western Blot Chemiluminescence Reagent (Pierce). The blots depicted are representative images of a total (n = 6).

**Statistics**

Data are expressed as the mean value ± SEM unless otherwise indicated. The results of the experiments were analyzed by two-way ANOVA to evaluate the effects of two factors (mice and smoking) on Lm. The Tukey honestly significant difference test was used for post hoc comparison, when the ANOVA resulted significant, to detect particular statistically significant differences between subgroups.

**Results**

**In the absence of CD8\(^+\) T cells there is reduced inflammation in response to long-term cigarette smoke exposure in mice**

To evaluate the inflammatory cell accumulation in response to cigarette smoke exposure, we analyzed the numbers of inflammatory cells using BAL fluid as well as quantification in lung sections of WT and CD8\(^{-/-}\) smoke-exposed and nonsmoke-exposed control mice. In WT mice, there was significant accumulation of T lymphocytes (p < 0.001), macrophages (p = 0.0002), and neutrophils (p = 0.0005) in BAL fluid in response to long-term cigarette smoke exposure (Fig. 1). In contrast, CD8\(^{-/-}\) mice did not display elevated total cell numbers, nor increase in any subset of inflammatory/immune cells obtained from BAL fluid (Fig. 1).

To assess for T lymphocytes and macrophages in lung tissue, we performed immunohistochemistry for CD8, CD4, and mac-3 in midsagittal sections. CD8 staining showed a significant increase in CD8\(^+\) T cells in lung tissue of WT smoke-exposed mice as compared with nonsmoke-exposed controls (p = 0.0003) (Fig. 2, A and B). As previously shown (20), there was a small amount of CD8\(^+\) expression in CD8\(^{-/-}\) mice, however, there was no significant increase in CD8\(^+\) T cells in response to cigarette smoke.
FIGURE 1. Inflammatory cell content in BAL fluid in response to long-term cigarette smoke exposure. BAL was performed in CD8<sup>−/−</sup> and WT smoke-exposed and nonsmoking control mice. BAL total cell (A), BAL macrophage (B), BAL lymphocyte (C), and BAL neutrophil (D) counts were tabulated using a hemocytometer and Hema-3-stained cytospins; n = 8 mice/group. NS, nonsmoking mice; Sm, smoke-exposed mice. Bars, SEM. *Significantly different from WT nonsmoking mice (p < 0.01); **Significantly different from WT smoke-exposed mice (p < 0.01). Note, there is significant increase in macrophages, neutrophils, and lymphocytes in response to cigarette smoke in WT, but not CD8<sup>−/−</sup> mice.

FIGURE 2. T lymphocyte content within lung tissue in response to long-term cigarette smoke exposure. Lungs were inflated with OCT/PBS (1:1) and frozen section was prepared for CD8 and CD4 staining. Lung T lymphocyte counts are expressed as CD8<sup>+</sup> cells/hpf (A) and CD4<sup>+</sup> cells/hpf (B) adjusted for tissue density on the stained sections; n = 4 mice/group. Bars, SEM. *Significantly different from WT nonsmoking mice (p < 0.01). Representative CD8-stained sections are shown for WT nonsmoking mice (C), WT smoke-exposed mice (D), CD8<sup>−/−</sup> nonsmoking mice (E), and CD8<sup>−/−</sup> smoke-exposed mice (F). NS, Nonsmoking mice; Sm, smoke-exposed mice. Note, CD8<sup>+</sup> T cells are increased in WT, but not CD8<sup>−/−</sup>, mice in response to cigarette smoking. CD4<sup>+</sup> T cell content is not changed in response to cigarette smoke.

CD8<sup>+</sup> T cells are required for airspace enlargement in response to long-term cigarette smoke exposure

Following 6 mo of smoke exposure, the mice were killed and lungs were inflated at a constant pressure of 25 cm H<sub>2</sub>O, fixed, and midsagittal sections were obtained for morphometry as described in Materials and Methods (Fig. 4). To estimate the morphological change in response to long-term cigarette smoke exposure, we measured the Lm, which measures the average distance between alveolar walls and is proportional to the amount of emphysema. In WT mice, Lm was significantly increased by 21% in response to long-term cigarette smoke exposure (33.04 ± 0.46 to 39.29 ± 0.76, p < 0.0004) (Table I). In contrast, Lm was not changed in response to cigarette smoke exposure in CD8<sup>−/−</sup> mice (34.51 ± 0.70 to 34.66 ± 1.03, 0.4%, p = 0.697) (Table I).

We replicated this study in a separate group of mice with identical results. That is, in contrast to WT mice, we did not observe macrophage accumulation or airspace enlargement in the absence of CD8<sup>+</sup> T cells (data not shown).

CD4<sup>+</sup> T cells are not required for the development of airspace enlargement in response to long-term cigarette smoke exposure in mice

In contrast to CD8<sup>+</sup> T cells, we did not observe a significant increase in total CD4<sup>+</sup> T cell numbers (Fig. 2), nor did we observe protection from emphysema in the absence of CD4<sup>+</sup> T cells. Exposure of WT and CD4<sup>−/−</sup> mice to cigarette smoke (2 cigarettes/day for 6 mo) resulted in an 18.9% increase in Lm in WT mice (33.04 ± 0.46 to 39.29 ± 0.76, p = 0.0004), and an 18.5% increase in CD4<sup>−/−</sup> mice (33.76 ± 1.37 to 39.99 ± 1.78, p = 0.012) (Table II). Although there was no effect of CD4 deficiency on the development of emphysema, ~20% of CD4<sup>−/−</sup> mice had baseline inflammation and slightly elevated Lm compared with other nonsmoke-exposed mice, possibly related to infection and inflamma
tion from their immunocompromised state, despite being housed within a sterile barrier facility. Due to the complex nature of the phenotype and the multiple defects that could generate such results in these mice, they were eliminated from analysis; however, the results were the same with inclusion. That is, there was no protection from airspace enlargement in response to smoking in the absence of CD4<sup>+</sup> T cells.
CD8+ T cells are required for MMP-12 production and elastin turnover in lungs of mice exposed to long-term cigarette smoke

It has been previously shown that activated CD8+ T cells, via IFN-γ, leads to IP-10 production, which in turn induces expression of macrophage elastase (MMP-12) in human cells in culture (16). Moreover, we have previously shown that MMP-12 is required for airspace enlargement in experimental emphysema (7). We (8) and others (9–11) have also shown that EFs are chemotactic for macrophages hence amplifying inflammation and lung destruction.

As a unifying mechanism, immunohistochemistry for IP-10 demonstrated induction in bronchial epithelial cells, T lymphocytes, and macrophages in response to cigarette smoke in WT mice (Fig. 5, A–D). In CD8−/− mice, there were few IP-10+ cells (Fig. 5, E and F) and no increased IP-10 production in smoke-exposed CD8−/− mice (Fig. 5, G and H). We attempted to measure IFN-γ using both ELISA and real-time PCR, but found that it was below the detections limits for both modalities. In WT mice, MMP-12 was induced upon exposure to cigarette smoke, as shown by Western blots on lung homogenates from WT nonsmoke exposed and smoke-exposed mice (Fig. 6A). However, CD8−/− mice had minimal baseline expression without induction upon exposure to cigarette smoke (Fig. 6A).

Upon exposure to long-term cigarette smoke exposure, WT mice also demonstrated expected degradation of elastin and generation of the chemotactic 45-kDa EFs as shown in lung homogenates (Fig. 6B). In the absence of CD8+ T cells (and inflammatory cell elastases), no EFs were observed in response to long-term cigarette smoke exposure (Fig. 6B).

Lungs of CD8−/− mice exposed to cigarette smoke have reduced monocyte chemotactic activity

Given the importance of EFs in low-level macrophage accumulation in response to cigarette smoke (8), we would predict that the

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<th>Table I. Morphological change in response to 6-mo cigarette smoke exposure in C57BL/6J and CD8−/− mice</th>
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*aLm is mean linear intercept, which is proportional to emphysema. Data are expressed as the mean value ± SEM in each experimental group, n = 8/group.
absence of elastin turnover in CD8−/− mice would lead to diminished chemotactic activity for monocytes. To clarify the mechanism whereby CD8+ T cells regulate lung macrophage content, we measured monocyte chemotactic activity in these mice.

We performed in vitro assays of monocyte chemotaxis using a modified Boyden chamber. Assays of monocyte chemotaxis were performed on human peripheral blood monocytes using lung homogenates from CD8−/− and WT smoke-exposed and nonsmoking control mice as the chemoattractants. The lung homogenates from WT smoke-exposed mice demonstrated potent chemotactic activity as opposed to WT nonsmoking control mice (p < 0.0001) (Fig. 6C). The lung homogenates from CD8−/− smoke-exposed mice did not have increased chemotactic activity as compared with CD8−/− nonsmoking control mice (p = 0.218) (Fig. 6C).

Although differences in EF production can fully explain differences in monocyte chemotaxis, we also evaluated CC chemokine production in these mice. We performed real-time PCR to evaluate the expression of MCP-1, MCP-2, MIP-1α, MIP-1β, and RANTES. There was no significant expression of these genes at baseline or in response to long-term cigarette smoke in WT or CD8−/− mice (data not shown). We also performed ELISA of MCP-1 and MIP-1α but failed to detect expression at the protein level as well (data not shown).

**Discussion**

In this study, we have shown that CD8+ T cells are required for the development of cigarette smoke-induced emphysema in mice.
effect is likely due to CD8+ T cell regulation of monocyte recruitment and macrophage activation and not a direct effect of CD8+ T cells on lung destruction. Combining previous concepts developed by others (24) and ourselves (7–8, 16), we hypothesized that cigarette smoke-induced activation of CD8+ T cells leads production of IFN-γ-inducible proteins such as IP-10, that has been shown to induce macrophage production of MMP-12 (16). MMP-12 degrades elastin, disrupting lung architecture leading to airspace enlargement (7). In addition, we have recently shown that EFs, byproducts of elastin destruction, are the major chemotactic factors responsible for macrophage accumulation and the subsequent development of experimental emphysema (8). We provide data that demonstrate the requirement for CD8+ T cells in smoke-induced emphysema and provide a unifying pathway placing CD8+ T cells as a central regulator of inflammation and lung destruction in COPD. Our findings are in contrast of those by d’Hulst et al. (25) demonstrating that Scid mice are not protected from cigarette smoke-induced emphysema, however, the complete lack of an immune system and ability to generate an inflammatory cell response make this a less than ideal model.

Over the past decade, we have come to realize that emphysema is not caused by a single cell type or proteinase but that multiple inflammatory and immune cells are involved and we are now trying to determine how they interact in a complex network to contribute to lung destruction in COPD. Macrophages are prominent in chronic inflammatory conditions of the lung including emphysema (4–6). Initially, their capacity to degrade elastin and hence contribute to emphysema was questioned. However, investigators demonstrated that macrophages have the capacity to produce both cysteine (5) and matrix metalloproteinases (6, 26) capable of elastolysis.

We developed a murine model of cigarette-smoke induced emphysema to directly determine the role of individual proteinases in the development of emphysema. We, and others, have found that exposure of two to four cigarettes daily results in similar carboxyhemoglobin levels as humans following acute exposure (10–14%). The airspace enlargement and inflammatory cell infiltrate found within the alveolar compartment in cigarette smoke-induced emphysema in the mouse recapitulates the human disease very well. The same is not necessarily true for the airways component of the disease, given the sparse six to eight generations of branching airways present in mice as compared with 20+ generations in humans. Therefore, the contribution of the airways and any unique inflammatory cell populations within would be underrepresented in the mouse and represents a limitation of the model. We have previously used this model to show that macrophage elastase (MMP-12) was required for lung destruction as well as macrophage accumulation (7). Recently, we have concluded that the basis for MMP-12-mediated macrophage accumulation was not due to the requirement of MMP-12 to allow monocytes to penetrate basement membranes and egress from the vasculature (in fact, monocytes have little or no MMP-12 before differentiation to, and activation of, macrophages). Instead, the production of EFs by MMP-12 and other elastases, known to be monocyte chemokines in vitro (9, 11), is a major chemokine in this low-level macrophage accumulation observed in response to cigarette smoke exposure in vivo (8).

Investigators have also begun to unravel interactions between macrophages and neutrophils in COPD. MMP-12 is responsible for “shedding” of active TNF-α from the surface of monocyte/macrophages and subsequent recruitment of neutrophils in response to cigarette smoke in mice (27). Human macrophages, via cigarette smoke-related oxidant activity, inactivate histone deacetylase-2-promoting transcription of MMPs and neutrophil chemokines including IL-8 (18, 28). Neutrophil elastase-deficient mice only develop one-third as much cigarette-related emphysema as WT littermates (22). The full protection from emphysema with MMP-12 deficiency and the two-thirds protection in the absence of neutrophil elastase are explained by interactions between macrophages and neutrophils and their elastases. For example, neutrophil elastase degrades tissue inhibitor of metalloproteinase-1 augmenting MMP activity (29, 30) and MMPs degrade α-1 antitrypsin, indirectly augmenting neutrophil elastase activity (31–33). Hence, these cells and proteinases “work together” to cause a greater lung injury than each would generate by itself.

So, how do the T lymphocytes fit into this network? T lymphocytes, particularly CD8+ T cells, are known to be increased in lungs of patients with COPD (12, 14). Moreover, transgenic mice that overexpress IFN-γ develop inflammation, apoptosis, and consequent emphysema (15). Humans with COPD have increased amounts of IP-10 and its receptor CXCR3 (24). Induction of IP-10 in CD8+ T cells and bronchiolar epithelium is thought to signal through CD8+ T cell CXCR3 to recruit T lymphocytes in COPD. Recently, it was shown that alveolar macrophages also express CXCR3 and that IP-10 signals through macrophage CXCR3 to produce MMP-12 (16). In the absence of CD8+ T cells, we do not see an induction of IP-10 in epithelial cells in response to cigarette smoke. In support of the concept that this is a major regulator of MMP-12, we do not see any induction of MMP-12 in the absence of CD8+ T cells either. In general, MMP-12 can only be detected in small amounts when subjecting concentrated BALF to zymography. This low-level expression, which likely requires close contact of macrophages with elastin within the interstitium, is consistent with the chronic nature of the injury observed in COPD. As expected with this network, there is also less neutrophil recruitment in CD8− mice. Hence, with a decrement of macrophage and neutrophil elastases, we see impaired elastin breakdown. This explains both impaired macrophage accumulation and airspace enlargement in lungs of CD8−/− mice.

CD8+ T cells have also been shown to be required for hyperresponsiveness and inflammation in a model of OVA-induced allergic airway hyperresponsiveness (34). This data together with our support the Dutch hypothesis that asthma and COPD have common origins. However, there are many differences between these two lung diseases including the type of inflammation and lack of airspace enlargement in asthma. Our data support an indirect role of CD8+ T cells in lung destruction associated with COPD. CD8+ T cells possess a small complement of matrix degrading proteinases such as MMP-2 and MMP-9. They also contain granzymes that are cytotoxic but have minimal matrix-degrading capacity. Perforin, which forms pores for release of granzymes into target cells, has been shown to be increased in COPD. However, we saw no alteration in smoke-induced emphysema in perforin-deficient mice (data not shown). Thus, we have no evidence for a direct role for CD8+ T cells in inducing emphysema either via proteolysis or by induction of apoptosis. However, direct CD8+ T cell-mediated cytotoxicity might be important in microbial exacerbations in COPD patients.

In conclusion, we have demonstrated that CD8+ T cells play an important role in the pathogenesis of cigarette smoke-induced emphysema in mice by regulating macrophage accumulation and activation. These findings further solidify and define a complex network of inflammatory and immune cell interactions in chronic destructive lung disease and may allow therapeutic targeting to interrupt this pathologic process in humans.

Disclosures
The authors have no financial conflict of interest.
References


