ORIGINAL ARTICLE

A Disintegrin and Metalloproteinase Domain-8: A Novel Protective Proteinase in Chronic Obstructive Pulmonary Disease

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Abstract

Rationale: ADAM8 (a disintegrin and metalloproteinase domain-8) is expressed by leukocytes and epithelial cells in health, but its contribution to the pathogenesis of chronic obstructive pulmonary disease (COPD) is unknown.

Objectives: To determine whether the expression of ADAM8 is increased in the lungs of patients with COPD and cigarette smoke (CS)-exposed mice, and whether ADAM8 promotes the development of COPD.

Methods: ADAM8 levels were measured in lung, sputum, plasma, and/or BAL fluid samples from patients with COPD, smokers, and nonsmokers, and wild-type (WT) mice exposed to CS versus air. COPD-like lung pathologies were compared in CS-exposed WT versus *Adam8*$^{-/-}$ mice.

Measurements and Main Results: ADAM8 immunostaining was reduced in macrophages, and alveolar and bronchial epithelial cells in the lungs of patients with COPD versus control subjects, and CS- versus air-exposed WT mice. ADAM8 levels were similar in plasma, sputum, and BAL fluid samples from patients with COPD and control subjects. CS-exposed *Adam8*$^{-/-}$ mice had greater airspace enlargement and airway mucus cell metaplasia than WT mice, but similar small airway fibrosis. CS-exposed *Adam8*$^{-/-}$ mice had higher lung macrophage counts, oxidative stress levels, and alveolar septal cell death rates, but lower alveolar septal cell proliferation rates and soluble epidermal growth factor receptor BAL fluid levels than WT mice. *Adam8* deficiency increased lung inflammation by reducing CS-induced activation of the intrinsic apoptosis pathway in macrophages. Human ADAM8 proteolytically shed the epidermal growth factor receptor from bronchial epithelial cells to reduce mucin expression *in vitro*. *Adam8* bone marrow chimera studies revealed that *Adam8* deficiency in leukocytes and lung parenchymal cells contributed to the exaggerated COPD-like disease in *Adam8*$^{-/-}$ mice.

Conclusions: *Adam8* deficiency increases CS-induced lung inflammation, emphysema, and airway mucus cell metaplasia. Strategies that increase or prolong ADAM8's expression in the lung may have therapeutic efficacy in COPD.

Keywords: lung inflammation; airway mucus cell metaplasia; EGFR shedding; macrophage; intrinsic apoptosis pathway

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At a Glance Commentary

Scientific Knowledge on the Subject: Proteinases have been thought to promote the development of chronic obstructive pulmonary disease (COPD) (the proteinase–antiproteinase hypothesis). However, the contributions of ADAM8 (a disintegrin and metalloproteinase domain-8) to the pathogenesis of COPD have not been explored.

What This Study Adds to the Field: ADAM8 staining in lung epithelial cells and macrophages was lower in patients with COPD than control subjects and lower in the same cells in wild-type mice exposed to cigarette smoke (CS) versus air. Soluble ADAM8 protein levels were similar in plasma, sputum, and BAL fluid samples from patients with COPD and control subjects. CS-exposed *Adam8*$^{-/-}$ mice had greater lung inflammation, airspace enlargement, and airway mucus cell metaplasia (but not small airway fibrosis) than CS-exposed wild-type mice. *Adam8* deficiency increased CS-induced emphysema development by reducing the rate at which lung macrophages undergo apoptosis, increasing alveolar septal cell apoptosis, and reducing alveolar epithelial repair. Adam8 likely protects the lung from CS-induced mucus metaplasia, in part, by shedding the epidermal growth factor receptor from airway epithelial cells. Thus, our study puts a new twist on the old proteinase-antiproteinase hypothesis by identifying ADAM8 as the first proteinase to protect the lung from CS-induced emphysema and mucus cell metaplasia. Strategies that increase or prolong the expression of ADAM8 in the CS-exposed lung may have therapeutic efficacy in COPD.

Chronic obstructive pulmonary disease (COPD) is characterized by lung inflammation, emphysema development, small airway fibrosis, and mucus hypersecretion, and is projected to be the third leading cause of death worldwide by 2030 (1). Cigarette smoke (CS) is the main risk factor for COPD in developed countries (2). CS contributes to COPD development, in part, by stimulating the recruitment of leukocytes into the lung, which release mediators of inflammation, proteinases, and reactive oxygen species that promote lung inflammation and injury (2). Macrophages are major culprits in the pathogenesis of COPD (3). CS-activated macrophages produce matrix metalloproteinases and cysteine proteinases that promote lung inflammation, degrade extracellular matrix proteins, and injure alveolar septal cells to cause airspace enlargement (2). Little is known about the contributions of other metalloproteinase subfamilies to COPD.

ADAM proteinases are a subfamily of multidomain metalloproteinases that are type-I transmembrane proteinases with a disintegrin and a metalloproteinase domain (4). The metalloproteinase domain of some ADAMs proteolytically sheds proteins and receptors from cell surfaces (4, 5), and the disintegrin domain of some ADAMs binds to integrins to regulate cell adhesion and migration (6). The cysteine-rich and EGF (epidermal growth factor)-like domains of some ADAMs regulate cell adhesion. The cytoplasmic tail of some ADAMs regulates intracellular signaling (7, 8).

ADAM8 (also known as CD156a and MS2) is most highly expressed by activated macrophages (9) but is also expressed by all other leukocytes except for T lymphocytes (10). ADAM8 is expressed by airway epithelium in healthy mice and human subjects (11, 12). ADAM8 has an active MP domain that cleaves CD23, CD40 ligand, and several adhesion molecules (8, 13, 14), but little is known about the function of ADAM8's other domains.

The contributions of ADAMs to COPD pathogenesis are not known. Until now, proteinases have been shown to have exclusively deleterious activities during CS-induced emphysema development in mice (2). Thus, we initially hypothesized that the expression of ADAM8 is increased in the lungs of patients with COPD and CS-exposed mice, and that ADAM8 promotes the development of COPD. To test these hypotheses, we compared ADAM8 staining in the lungs of patients with COPD versus control subjects and wild-type (WT) mice exposed to air or CS, and soluble ADAM8 (sADAM8) levels in plasma and lung samples from patients with COPD versus control subjects. COPD-like lung pathologies were compared in air-exposed versus CS-exposed WT, *Adam8*$^{-/-}$, and *Adam8* bone marrow (BM) chimeric mice.

These results have previously been reported in the form of an abstract (15).

Methods

Further details are provided in the online supplement.

Human Studies

Table 1, the METHODS section in the online supplement, and Tables E1–E3 in the online supplement provide details on the lung immunostaining, plasma, sputum, and BAL cohorts.

Immunofluorescence staining for ADAM8. Lung sections from patients with COPD and control subjects (Table 1) were double immunostained for ADAM8 and markers of alveolar macrophages (AMs; CD68), epithelial cells (pancytokeratin [Pan-CK]), or type-II alveolar epithelial cells (SP-C [surfactant protein-C]).

sADAM8 levels. These were measured in plasma, sputum, and BAL fluid (BALF) using an ELISA.

Animal Studies

Murine studies were approved by the local Institutional Animal Care and Use Committee. C57BL/6 strain *Adam8*$^{-/-}$ and WT mice were studied. BM from unchallenged WT and *Adam8*$^{-/-}$ mice was injected intravenously into lethally irradiated recipient mice to generate four groups of *Adam8* BM chimeras (16).

CS exposures. Female and male adult WT, *Adam8*$^{-/-}$, and *Adam8* BM chimeric mice were exposed to air or CS 6 d/wk for 1–6 months (17).

Adam8 levels in WT murine lungs. Lung sections were double immunostained for Adam8 protein and markers of AMs (CD68), lung epithelial cells (Pan-CK), or type II alveolar epithelial cells (SP-C). Adam8 levels were measured in lungs using an ELISA.

Respiratory mechanics. The online supplement provides information on respiratory mechanics (17).

COPD-like lung pathologies. Emphysema and small airway remodeling were measured on lung sections using morphometry methods (17). Lung sections were immunostained for Muc5ac.

Table 1. Demographic and Clinical Characteristics of the Lung Immunostaining Cohort

Characteristics	Nonsmokers* ($n = 8$)	Smokers* ($n = 8$)	COPD GOLD Stages I–II ($n = 8$)	COPD GOLD Stages III–IV ($n = 9$)	P Value†
Males, %	4 (50)	4 (50)	6 (65)	6 (67)	NS
Age, yr	66 ± 4	62 ± 3	62 ± 3	56 ± 2	NS†
Pack-years of smoking	0 (0)	34 ± 5	41 ± 8	39 ± 5	<0.001‡
Current smokers, %	0	25	25	0	NS
FEV_1, % of predicted	95 ± 8	94 ± 12	78 ± 6	27 ± 4	0.001§
FEV_1/FVC, % of predicted‖	83 ± 5	82 ± 5	61 ± 2	32 ± 3	<0.001¶

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; NS = not significant.

Demographic and clinical characteristics of the patients with COPD, smokers without COPD, and nonsmokers in the Boston lung immunostaining cohort who underwent a lung surgery or a lung biopsy (*see* online supplement for details).

*Nonsmokers were all never-smokers. Smokers were defined as subjects who had a ⩾10 pack-year smoking history. Current smokers were defined as active smokers at the time of the surgery or lung biopsy, or former smokers who had stopped smoking ⩽1 year before the surgery.

†Categorical variables were analyzed with chi-square tests. Continuous variables (age, FEV_1% predicted, FEV_1/FCV ratio, and pack/years of smoking) are presented as mean ± SEM and analyzed using one-way ANOVA followed by pairwise comparisons using two-sided Student's t tests.

‡The pack/year smoking histories of the GOLD stages III–IV and GOLD stages I–II COPD and the smoker groups were higher than from those of the nonsmoker group ($P < 0.001$ for all comparisons). The pack-year smoking histories of the GOLD stages III–IV and GOLD stages I–II COPD groups were not significantly different from those of the smoker group ($P = 0.51$ and $P = 0.48$, respectively).

§The FEV_1% predicted of the GOLD stages III–IV COPD group was significantly lower than that of the GOLD stages I–II COPD, smoker, and nonsmoker groups ($P = 0.001$ for all three comparisons). The FEV_1% predicted of the GOLD stages I–II COPD group was not significantly different from that of the smoker or nonsmoker groups ($P = 0.24$ and $P = 0.11$, respectively).

‖All patients with COPD had FEV_1/FVC ratio <0.7, whereas smokers without COPD and nonsmoker control subjects had a FEV_1/FCV ratio ⩾0.7.

¶The FEV_1/FVC ratios of the GOLD stages III–IV and GOLD stages I–II COPD groups were significantly lower than those of the smoker and nonsmoker groups by design ($P \leqslant 0.001$ for all comparisons).

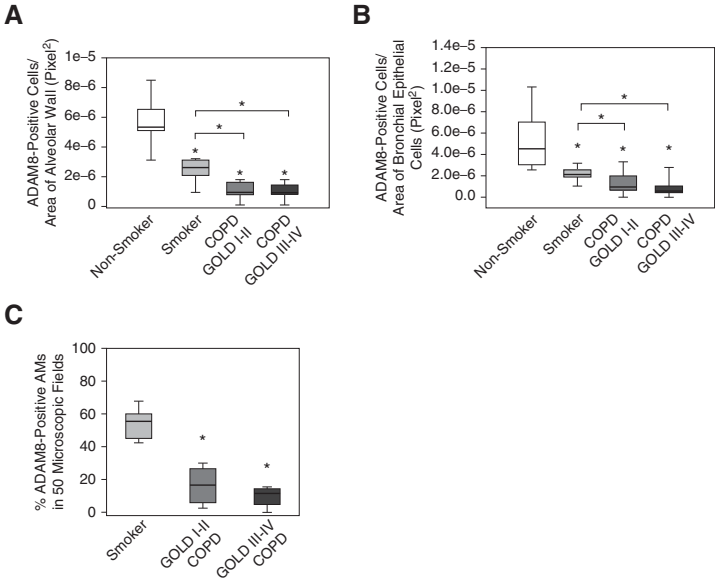

Figure 1. ADAM8 staining is reduced in epithelial cells and alveolar macrophages in smoker and chronic obstructive pulmonary disease lungs. (*A* and *B*) ADAM8-positive cells were quantified in alveolar (*A*) and bronchial (*B*) epithelial cells per unit of alveolar wall or bronchial epithelial cells, respectively, using MetaMorph software. (*C*) The percentage of ADAM8 positively stained macrophages was calculated in 50 randomly acquired images of lung sections from smokers and patients with chronic obstructive pulmonary disease. The boxes in the box plots show the medians and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles for eight to nine subjects/group. The Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney U test. *$P < 0.05$ versus nonsmoker or the group indicated. ADAM8 = a disintegrin and metalloproteinase domain-8; AM = alveolar macrophage; COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease.

Lung inflammation. Leukocytes were counted in BAL samples (16). AM counts in lung sections were normalized to alveolar wall area. Mediators of inflammation and desmosine levels were measured in lung samples using ELISAs.

Lung oxidative stress. Lung thiobarbituric acid reactive substances were measured using a kit.

ADAM8-mediated cleavage of mediators and receptors. The online supplement provides further information.

Alveolar septal cells phenotypes. Lung sections were stained with a TUNEL kit (a cell death marker) or for Ki67 (a proliferation marker). Readouts of Wnt-β-catenin prorepair signaling were measured including lung levels of phospho-β-catenin and total β-catenin using Western blotting and *Wisp2* and *Tcf2* expression using qRT-PCR (18).

Apoptosis of WT versus Adam8$^{-/-}$ cells. Macrophages, polymorphonuclear neutrophils (PMNs), or murine tracheal epithelial cells from unchallenged mice were incubated with or without 2.5–20% CS extract (CSE). Intracellular levels of active caspase-3, -8, or -9 and mitochondrial membrane potential were measured.

PMN adhesion and migration, macrophage gene expression studies. The online supplement provides further information (19, 20).

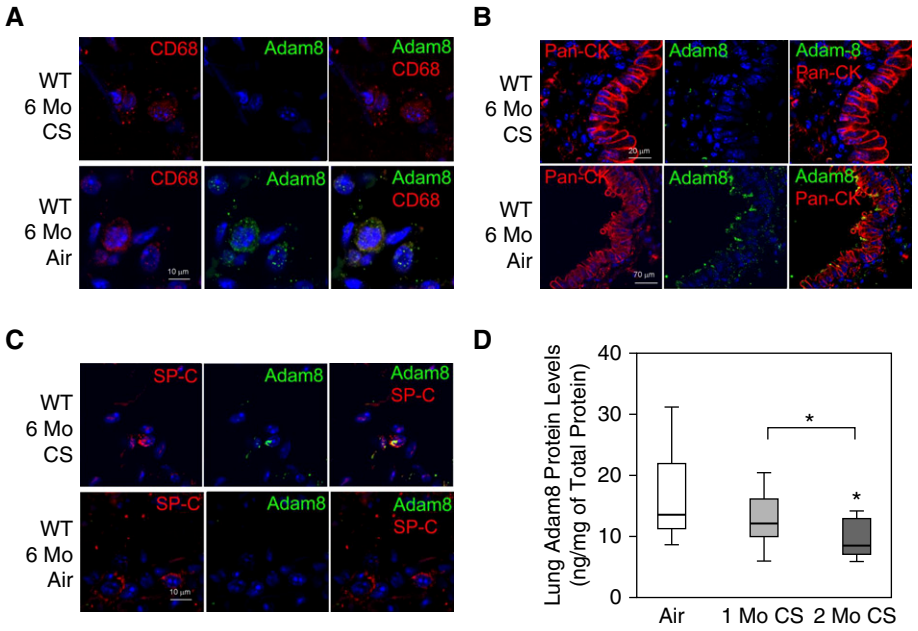

Figure 2. Adam8 staining is reduced in macrophages and epithelial cells in the lungs of cigarette smoke (CS)-exposed wild-type mice. (*A–C*) C57BL/6 wild-type mice were exposed to air or CS for 6 months, and lungs were inflated and removed. Lung sections were double immunostained with a red fluorophore (left) for markers of macrophages (CD68, *A*), bronchial epithelial cells (Pan-CK, *B*), or type II alveolar epithelial cells (SP-C, *C*) and a green fluorophore for Adam8 (middle). The nuclei were counterstained blue with 4′-6-diamidino-2-phenylindole. Lung sections were analyzed using a confocal microscope and merged images are shown on the right. The images shown are representative of three to four mice per group. Lung sections stained with nonimmune primary antibodies showed no or minimal staining (data not shown). (*D*) Adam8 protein levels were measured in homogenates of lungs from mice exposed to air or CS for up to 2 months using an ELISA ($n = 8–12$ mice/group). The boxes in the box plots show the medians and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles for eight to nine subjects/group. The Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. $*P < 0.05$ versus air-exposed mice or the group indicated. Scale bars: (*A*) 10 μm; (*B*) 70 μm; (*C*) 10 μm. Adam8 = a disintegrin and metalloproteinase domain-8; Pan-CK = pancytokeratin; SP-C = surfactant protein-C; WT = wild type.

EGF receptor cleavage by rhADAM8. Human bronchial epithelial cells were incubated with or without active rhADAM8 for 4 hours. Soluble EGFR (EGF receptor) levels in culture supernatants, residual surface EGFR, and mucin gene expression were quantified using an ELISA, immunostaining, and qRT-PCR, respectively.

Statistics

Data are presented as mean ± SEM (if normally distributed), or as box plots showing medians and 25th and 75th percentiles, and whiskers showing 10th and 90th percentiles (if not normally distributed). One-way ANOVA followed by two-sided Student's *t* test or Mann-Whitney *U* test were performed. *P* less than or equal to 0.05 was considered significant.

Results

Human Studies

Nonsmokers had intense staining for ADAM8 in alveolar epithelial cells, especially in type II cells (Figure 1A; *see* Figure E1A), and bronchial epithelial cells (Figure 1B and Table 1; *see* Figure E1B). ADAM8 staining was lower in type II alveolar epithelial and bronchial epithelial cells in smokers and patients with COPD than nonsmokers, and lower in these cells in both Global Initiative for Chronic Obstructive Lung Disease stage I–II and III–IV patients with COPD than smokers. Nonsmokers had few AMs, but AMs that were present in lung sections had intense staining for ADAM8 (*see* Figure E2). The percentage of ADAM8-positive AMs was higher in smokers than both Global Initiative for Chronic Obstructive Lung Disease stage I–II and III–IV stage patients with COPD (Figure 1C).

Plasma sADAM8 levels were lower in patients with COPD than nonsmokers but not smokers (*see* Figure E3A). Sputum sADAM8 levels were similar in patients with COPD and smokers, but lower in smokers than nonsmokers (*see* Figure E3B). BALF sADAM8 were higher in patients with COPD than smokers (*see* Figure E3C). However, after adjusting the *P* values for differences in sex, age, pack-years, and/or current smoking, there were no significant differences in plasma, sputum, or BALF sADAM8 levels between patients with COPD and control subjects (*see* Tables E1–E3). Plasma, sputum, and BALF sADAM8 levels were not related to Global Initiative for Chronic Obstructive Lung Disease stage (not shown).

Murine Studies

Adam8 staining was reduced in lung macrophages, bronchial epithelial cells, and type II alveolar epithelial cells in WT mice exposed to CS versus air for 6 months (Figure 2). Exposing WT mice to CS for up to 2 months reduced lung Adam8 protein levels (Figure 2D).

Phenotype studies. Unchallenged 8-week-old WT and $Adam8^{-/-}$ mice had similar distal airspace size indicating that $Adam8^{-/-}$ mice have normal lung development (Figures 3A and 3B). After 6 months of exposure to air, $Adam8^{-/-}$ mice developed modestly greater distal airspace size than WT mice. However, 6 months of CS exposure induced even greater emphysema development in $Adam8^{-/-}$ versus WT mice (Figures 3A and 3B). CS-exposed $Adam8^{-/-}$ mice had greater lung elastin degradation than WT mice (assessed as by BALF desmosine levels; Figure 3C) and a left shift in their pressure-volume flow curves (*see* Figures E4A and E4B), higher quasistatic lung compliance, and lower tissue and peripheral airway elastance values than WT mice (indicative of greater loss of elastic recoil in their lungs) (Table 2). CS-exposed $Adam8^{-/-}$ mice had

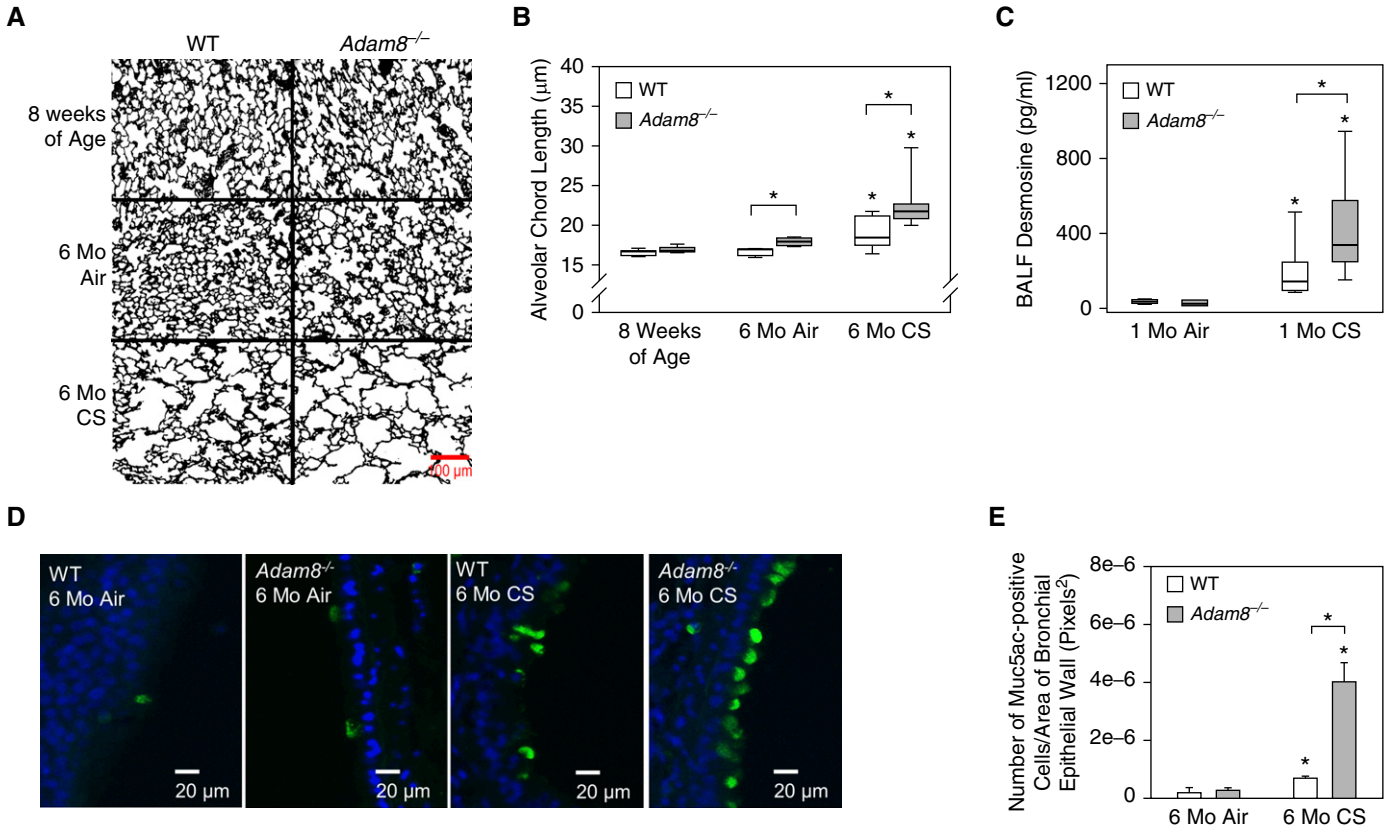

Figure 3. *Adam8*$^{-/-}$ mice have increased cigarette smoke (CS)-induced airspace enlargement and mucus cell metaplasia. (*A* and *B*) Wild-type (WT) and *Adam8*$^{-/-}$ mice were exposed to air for 8 weeks, or to air or CS for 6 months. (*A*) Images of Gill-stained inflated lung sections from mice (scale bar, 100 μm) that are representatives of five to eight mice/group. (*B*) Box plots of alveolar chord lengths as a measure of airspace enlargement. The boxes in the box plots show the medians and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles for mice exposed to air for 8 weeks (six mice/group), mice exposed to air for 6 months (five to six mice/group), and mice exposed to CS for 6 months (seven to eight mice/group). (*C*) WT and *Adam8*$^{-/-}$ mice were exposed to air (three to five mice/group) or CS (nine mice/group) for 1 month, BAL was performed, and desmosine levels (as a readout of lung elastin degradation) were measured in BAL fluid samples using an ELISA. Data are presented as box plots with the boxes showing the median values and 25th and 75th percentiles, and the whiskers showing the 10th and 90th percentiles. (*D*) Inflated lung sections were immunostained with a green fluorophore for Muc5ac to identify airway epithelial cells with mucus metaplasia. Images of the immunostained lung sections from air- and CS-exposed WT and *Adam8*$^{-/-}$ mice that are representative of five to six mice/group are shown. (*E*) Muc5ac immunostaining in bronchial epithelial cells was quantified as described in the online supplement. The boxes in the box plots show the medians and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles. In *B*, *C*, and *E*, the Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. *$P < 0.05$ versus mice belonging to the same genotype exposed to air, or the group indicated. Adam8 = a disintegrin and metalloproteinase domain-8; BALF = BAL fluid.

greater mucus cell metaplasia than WT mice (Figures 3D and 3E). CS exposure induced similar small airway fibrosis in WT and *Adam8*$^{-/-}$ (*see* Figures E4C and E4D).

CS-induced lung inflammation, oxidative stress, alveolar septal cell death, and impaired alveolar septal cell repair contribute to emphysema development (2, 18, 21, 22) and were measured in WT and *Adam8*$^{-/-}$ mice.

Lung inflammation. *Adam8*$^{-/-}$ mice had modestly higher BAL total leukocyte counts than WT mice after 1 month of exposure to air (Figure 4A) mostly because of higher BAL lymphocyte counts (Figures 4C and 4D), and also after 6 months of exposure to air because of higher BAL macrophage and PMN counts (Figures 4B and 4C). CS-exposed *Adam8*$^{-/-}$ mice had higher BAL total leukocyte counts than WT mice mainly because of increased BAL macrophage counts, but BAL PMN and lymphocyte counts were also higher in CS-exposed *Adam8*$^{-/-}$ mice (Figures 4A–4D). *Adam8*$^{-/-}$ mice had modestly lower blood total leukocyte counts after 3 months of CS exposure than WT mice (*see* Table E4).

To determine whether the higher lung leukocyte counts in CS-exposed *Adam8*$^{-/-}$ lungs reflected higher lung levels of chemokines and cytokines, lung levels of mediators that regulate leukocyte recruitment and survival were measured. Levels of chemokines (Ccl-2, Ccl-3, and Cxcl-1), proinflammatory mediators (Il-1β, Il-6, and Tnf-α), myeloid leukocyte prosurvival factors (G-csf [granulocyte colony–stimulating factor] and Gm-csf [granulocyte–macrophage

Table 2. Respiratory Mechanics in WT and *Adam8*$^{-/-}$ Mice Exposed to Air or CS

Parameter	6 Months Air		6 Months CS	
	WT (*n* = 24)	*Adam8*$^{-/-}$ (*n* = 30)	WT (*n* = 31)	*Adam8*$^{-/-}$ (*n* = 19)
Tissue elastance, cm H_2O/ml	20.0 (2.8)	19.1 (3.0)	21.2 (4.2)	18.7 (1.5)*
Peripheral airway elastance, cm H_2O/ml	22.4 (20.4–24.0)	20.3 (18.8–22.2)†	24.6 (22.0–27.4)	19.8 (19.0–21.2)*
Quasistatic compliance, ml/cm $H_2O \times 10^2$	8.5 (0.2)	9.1 (0.1)	8.0 (0.1)	9.2 (0.2)*

Definition of abbreviations: CS = cigarette smoke; IQR = interquartile range; WT = wild type.
WT and *Adam8*$^{-/-}$ mice were exposed to air or CS for 6 months, and respiratory mechanics were measured using a FlexiVent device. Data are mean (SEM) for data that are normally distributed or medians (IQR) for data that are not normally distributed. Data were analyzed with ANOVA followed by pairwise comparisons using two-sided Student's *t* tests (for data that are normally distributed) or the Mann-Whitney *U* tests (for data that are not normally distributed).
*The result for CS-exposed *Adam8*$^{-/-}$ mice is significantly different from that of the CS-exposed WT mice ($P < 0.05$).
†The result for air-exposed *Adam8*$^{-/-}$ mice is significantly different from that of the air-exposed WT mice ($P < 0.05$).

colony–stimulating factor]), and antiinflammatory mediators (Il-10 and active Tgf-β) were higher in CS-exposed *Adam8*$^{-/-}$ versus WT lungs (*see* Figure E5). However, active human ADAM8 did not degrade CCL-2, CCL-3, IL-8 (the human ortholog of Cxcl1), IL-6, GM-CSF, or TGF-β (transforming growth factor-β), and only weakly cleaved tumor necrosis factor-α and G-CSF *in vitro* (*see* Figure E6 or not shown). Thus, the higher lung levels of proinflammatory mediators in CS-exposed *Adam8*$^{-/-}$ lungs were unlikely to be caused by the lack of Adam8-mediated degradation of these mediators. It is also unlikely that *Adam8* deficiency increases leukocyte transendothelial migration into the lung because WT and *Adam8*$^{-/-}$ PMNs did not differ in their capacity to adhere to an extracellular matrix protein or migrate toward chemoattractants (*see* Figures E7A–E7C), and WT and *Adam8*$^{-/-}$ monocytes had similar adhesion and chemotactic responses *in vitro* (23).

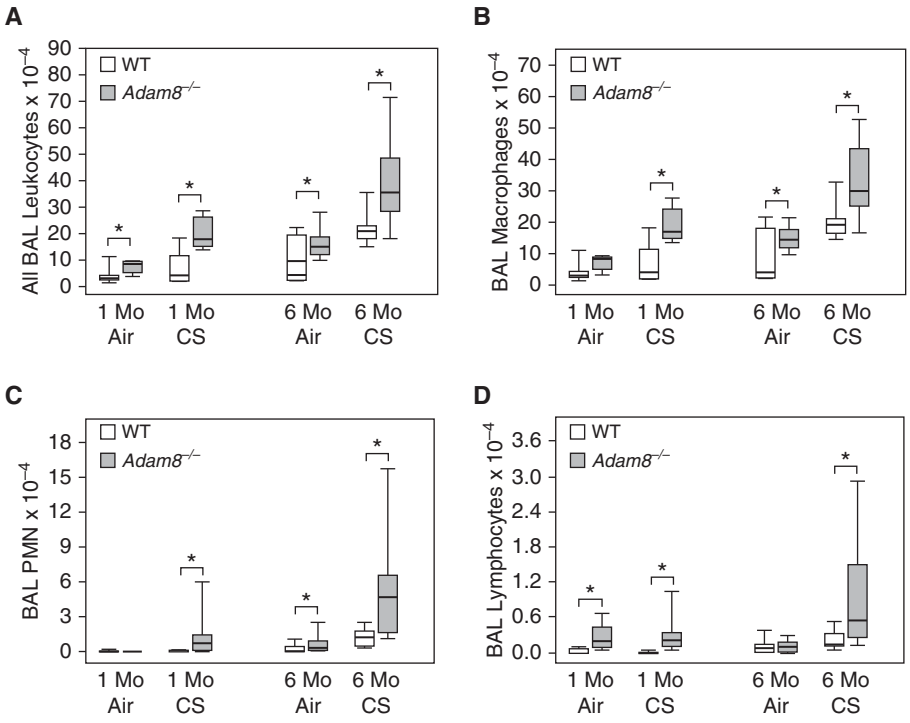

Figure 4. *Adam8*$^{-/-}$ mice have increased cigarette smoke–induced lung inflammation. Total leukocytes (*A*), macrophages (*B*), polymorphonuclear neutrophils (*C*), and lymphocytes (*D*) were counted in BAL samples from wild-type and *Adam8*$^{-/-}$ mice exposed to air or cigarette smoke for up to 6 months ($n = 7$–19 mice/group). The boxes in the box plots show the median values and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles. The Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. *$P < 0.05$ versus air-exposed mice belonging to the same genotype, or the group indicated. Adam8 = a disintegrin and metalloproteinase domain-8; CS = cigarette smoke; NS = not significant; PMN = polymorphonuclear neutrophils; WT = wild type.

Macrophage polarization influences pulmonary inflammatory responses. However, AMs isolated from air- and CS-exposed WT and *Adam8*$^{-/-}$ mice had similar gene expression levels of M1 and M2 markers (not shown).

Leukocyte apoptosis regulates leukocyte accumulation in tissues (24). Thus, CSE-induced activation of the intrinsic and extrinsic apoptosis pathways was compared in WT and *Adam8*$^{-/-}$ leukocytes. CSE-induced apoptosis was similar in WT and *Adam8*$^{-/-}$ PMNs (*see* Figure E7D). However, CSE induced lower rates of activation of the intrinsic apoptosis pathway in *Adam8*$^{-/-}$ versus WT macrophages *in vitro* as assessed by lower intracellular active caspase-3 and -9 levels (Figures 5A–5C), and reduced loss of mitochondrial membrane potential in *Adam8*$^{-/-}$ cells (Figure 5D). CSE-treated WT and *Adam8*$^{-/-}$ macrophages did not differ in their rates of activation of the extrinsic apoptosis pathway (measured

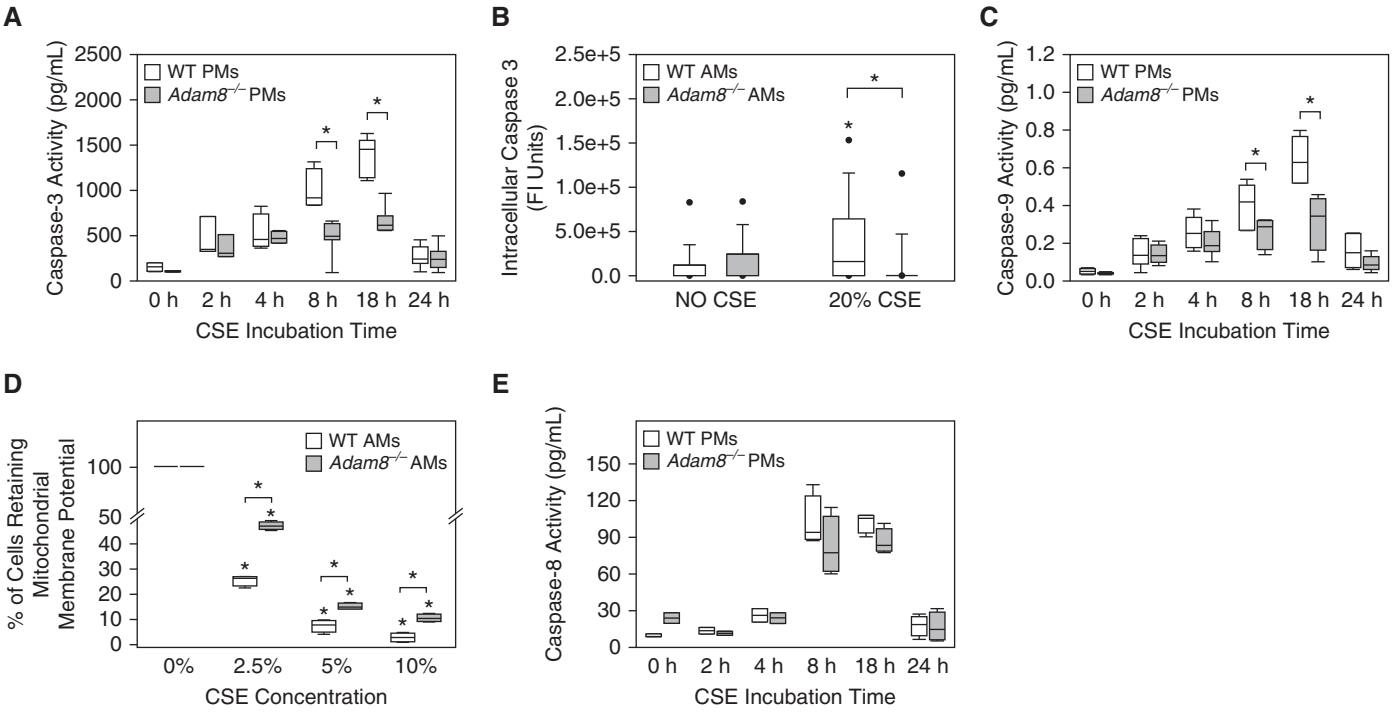

Figure 5. Adam8 deficiency in macrophages reduces cigarette smoke extract (CSE)-induced activation of the intrinsic apoptosis pathway in macrophages *in vitro*. Quiescent peritoneal macrophages (PMs, *A*, *C*, and *E*) or alveolar macrophages (AMs, *B* and *D*) from unchallenged wild-type and *Adam8*$^{-/-}$ mice were incubated for varying times with 2.5–20% CSE to induce apoptosis ($n = 3$–6 separate experiments). At the indicated time points, apoptosis of macrophages was measured as intracellular levels of active caspase-3 using a fluorogenic substrate that is specific for caspase-3 in PMs in *A*, or immunostaining for active caspase-3 in AMs in *B*. Readouts of activation of the intrinsic apoptosis pathway (intracellular levels of active caspase-9 in PMs in *C*, or loss of mitochondrial membrane potential in AMs in *D*) were measured as described in the online supplement. Activation of the extrinsic apoptosis pathway was also measured as intracellular levels of active caspase-8 in PMs in *E*. In *A*–*E*, the boxes in the box plots show the median values and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles. The Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. *$P < 0.05$ versus cells from mice belonging to the same genotype that were incubated without CSE, or the group indicated. Adam8 = a disintegrin and metalloproteinase domain-8; WT = wild type.

as intracellular active caspase-8 levels) (Figure 5E). AMs isolated from CS-exposed *Adam8*$^{-/-}$ mice had lower intracellular levels of active caspase-3 and reduced loss of mitochondrial membrane potential compared with AMs from CS-exposed WT mice (Figure 6). Signaling of GM-CSF and G-CSF *via* their receptors inhibits activation of the intrinsic apoptosis pathway in myeloid leukocytes (25, 26). However, active human ADAM8 did not cleave G-CSF or GM-CSF receptors from human monocyte surfaces *in vitro* (*see* Figure E8).

Oxidative stress and alveolar septal cell phenotypes. Air-exposed *Adam8*$^{-/-}$ mice had greater lung oxidative stress levels (measured as thiobarbituric acid reactive substances) than air-exposed WT mice, and this difference was greater after CS exposure (Figure 7A). Alveolar septal cell death rates were higher in CS-exposed *Adam8*$^{-/-}$ versus WT mice (Figure 7B), but CSE-induced apoptosis was similar in WT and *Adam8*$^{-/-}$ murine tracheal epithelial cells *in vitro* (*see* Figure E7E).

CS induced lower alveolar septal cell proliferation rates (assessed as lower Ki67 staining) in *Adam8*$^{-/-}$ versus WT lungs (Figure 7C). Wnt-β-catenin signaling contributes to alveolar septal cell proliferation and repair (18). CS activated the β-catenin pathway in WT (but not *Adam8*$^{-/-}$) lungs as assessed by a reduction in phosphorylated/total β-catenin protein ratios (Figure 7D), because phosphorylation of β-catenin prevents it from translocating to the nucleus by targeting β-catenin for proteasomal degradation. CS also increased the expression of two genes activated by nuclear translocation of β-catenin (*Tcf7* and *Wisp2*) in WT but not *Adam8*$^{-/-}$ lungs (Figures 7E and 7F).

Airway mucus metaplasia and EGFR shedding. EGFR activation in airway epithelial cells promotes mucus cell metaplasia (27). Proteolytic shedding of the EGFR inhibits this pathway. CS-exposed *Adam8*$^{-/-}$ had lower BALF sEgfr levels than WT mice (Figure 8A). Incubating human bronchial epithelial cells with active human ADAM8 decreased surface EGFR protein levels (Figure 8B), increased supernatant soluble EGFR levels (Figure 8C), and reduced mucin gene expression in human bronchial epithelial cells (Figures 8D–8F). Thus, ADAM8 directly sheds the EGFR from airway epithelial cells to reduce mucin gene expression.

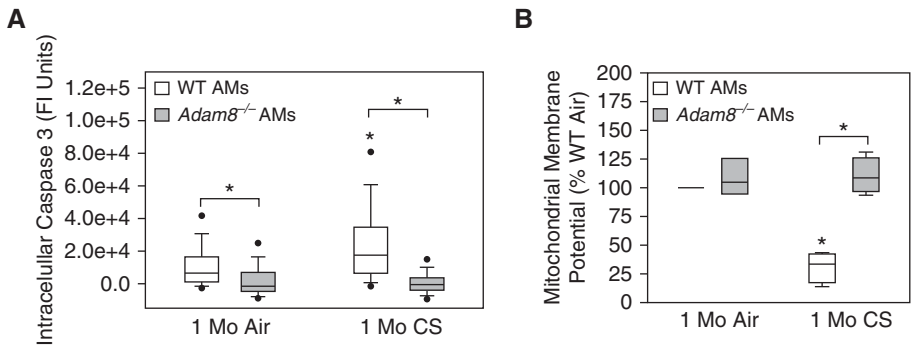

Figure 6. Cigarette smoke–exposed *Adam8*$^{-/-}$ mice have reduced apoptosis of their alveolar macrophages (AMs) *ex vivo*. AMs were isolated from the lungs of wild-type and *Adam8*$^{-/-}$ mice that had been exposed to air or cigarette smoke for 1 month using BAL, and apoptosis was measured immediately afterward. (*A*) Alveolar macrophages were fixed and immunostained for intracellular active caspase-3, and staining was quantified, as described in the online supplement ($n = 4$ separate experiments). (*B*) Loss of mitochondrial membrane potential was measured by staining the cells with JC-1 dye and quantifying the staining using image analysis software ($n = 4$ separate experiments). The boxes in the box plots show the median values and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles. The Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. *$P < 0.05$ versus air-exposed mice belonging to the same genotype or the group indicated. Adam8 = a disintegrin and metalloproteinase domain-8; CS = cigarette smoke; WT = wild type.

BM chimera studies. *Adam8* BM chimera studies were performed to identify the cells in which *Adam8* deficiency contributes to exaggerated COPD-like lung disease. Immunostaining experiments confirmed that Adam8 staining was detected in lung parenchymal cells (but not lung leukocytes) in air-exposed WT recipients that received *Adam8*$^{-/-}$ BM, and in lung leukocytes (mainly macrophages) but not lung parenchymal cells in air-exposed *Adam8*$^{-/-}$ recipients that received WT BM (*see* Figure E9). Loss of both leukocyte-derived and lung parenchymal cell–derived *Adam8* increased emphysema development in CS-exposed *Adam8*$^{-/-}$ mice because WT recipients with *Adam8*$^{-/-}$ BM had greater CS-induced emphysema than WT recipients with WT BM; and *Adam8*$^{-/-}$ recipients with WT BM or *Adam8*$^{-/-}$ BM had similar CS-induced emphysema (Figure 9A). Loss of both leukocyte- and lung parenchymal cell–derived *Adam8* contributed to the increased AM counts and reduced alveolar septal cell proliferation in CS-exposed *Adam8*$^{-/-}$ mice because *Adam8*$^{-/-}$ recipients with WT BM had lower AM counts and higher alveolar septal cell Ki67 staining than *Adam8*$^{-/-}$ recipients with *Adam8*$^{-/-}$ BM; and WT recipients with *Adam8*$^{-/-}$ or WT BM had similar AM counts and alveolar septal cell Ki67 staining (Figures 9B and 9C). *Adam8* deficiency in both leukocyte and lung parenchymal cells contributed to the increased alveolar septal cell death in CS-exposed *Adam8*$^{-/-}$ mice because WT recipients transplanted with *Adam8*$^{-/-}$ BM had greater CS-induced caspase-3 activation in alveolar septal cells than WT recipients with WT BM; and *Adam8*$^{-/-}$ recipients with WT BM or *Adam8*$^{-/-}$ BM had similar CS-induced caspase-3 activation in alveolar septal cells (Figure 9D). *Adam8* deficiency in leukocytes was mainly responsible for the greater mucus cell metaplasia in CS-exposed *Adam8*$^{-/-}$ airways because WT recipients with *Adam8*$^{-/-}$ BM had greater CS-induced mucus cell metaplasia than WT recipients with WT BM; and *Adam8*$^{-/-}$ recipients with WT BM had less mucus cell metaplasia than *Adam8*$^{-/-}$ recipients with *Adam8*$^{-/-}$ BM (Figure 9E).

Discussion

Patients with COPD, human smokers, and CS-exposed mice have lower ADAM8 staining in lung macrophages and epithelial cells than control subjects. ADAM8 staining is strikingly reduced in these cells in the lung even in mild COPD cases. *Adam8*$^{-/-}$ mice have exaggerated CS-induced lung inflammation, lung elastin degradation, emphysema development, and airway mucus cell metaplasia (but not small airway fibrosis). *Adam8* deficiency increases CS-induced emphysema by: *1*) reducing CS-induced activation of the intrinsic apoptosis pathway in lung macrophages leading to increased survival and increased numbers of these cellular culprits in the lung; *2*) increasing lung levels of oxidative stress and some proinflammatory mediators; and *3*) increasing apoptosis rates and reducing proliferation rates in alveolar septal cells (Figure 10). Adam8 protects murine airways from CS-induced mucus cell metaplasia in mice, in part, by shedding the EGFR from airway epithelial cells (Figure 10). Until now, studies of gene-targeted mice implicating proteinases in emphysema development have shown that these proteinases have exclusively deleterious activities in CS-exposed mice (2, 28–30). We identify ADAM8 as the first proteinase that protects mice from CS-induced COPD-like lung disease.

Human Studies

There is one prior report on ADAM8 levels in COPD showing that sputum sADAM8 levels were higher in patients with severe COPD than nonsmokers, but smokers were not studied (31). We showed in larger human cohorts that patients with COPD and control subjects had similar sputum, plasma, and BALF sADAM8 levels. Thus, sADAM8 blood and lung levels are unlikely to be useful diagnostic or prognostic biomarkers for COPD. Likely, sADAM8 detected in human blood and lung samples reflected shedding of ADAM8 from alveolar epithelial and leukocyte surfaces by ADAM8 and/or other proteinases (32). The reduced ADAM8 staining in macrophages and epithelial cells in COPD lungs may reflect epigenetic silencing of *ADAM8* expression, as reported for other genes in smoker airways (33). Because *Adam8* deficiency in mice leads to greater COPD-like disease, reduced ADAM8 expression in COPD lungs likely contributes to pulmonary pathologies in

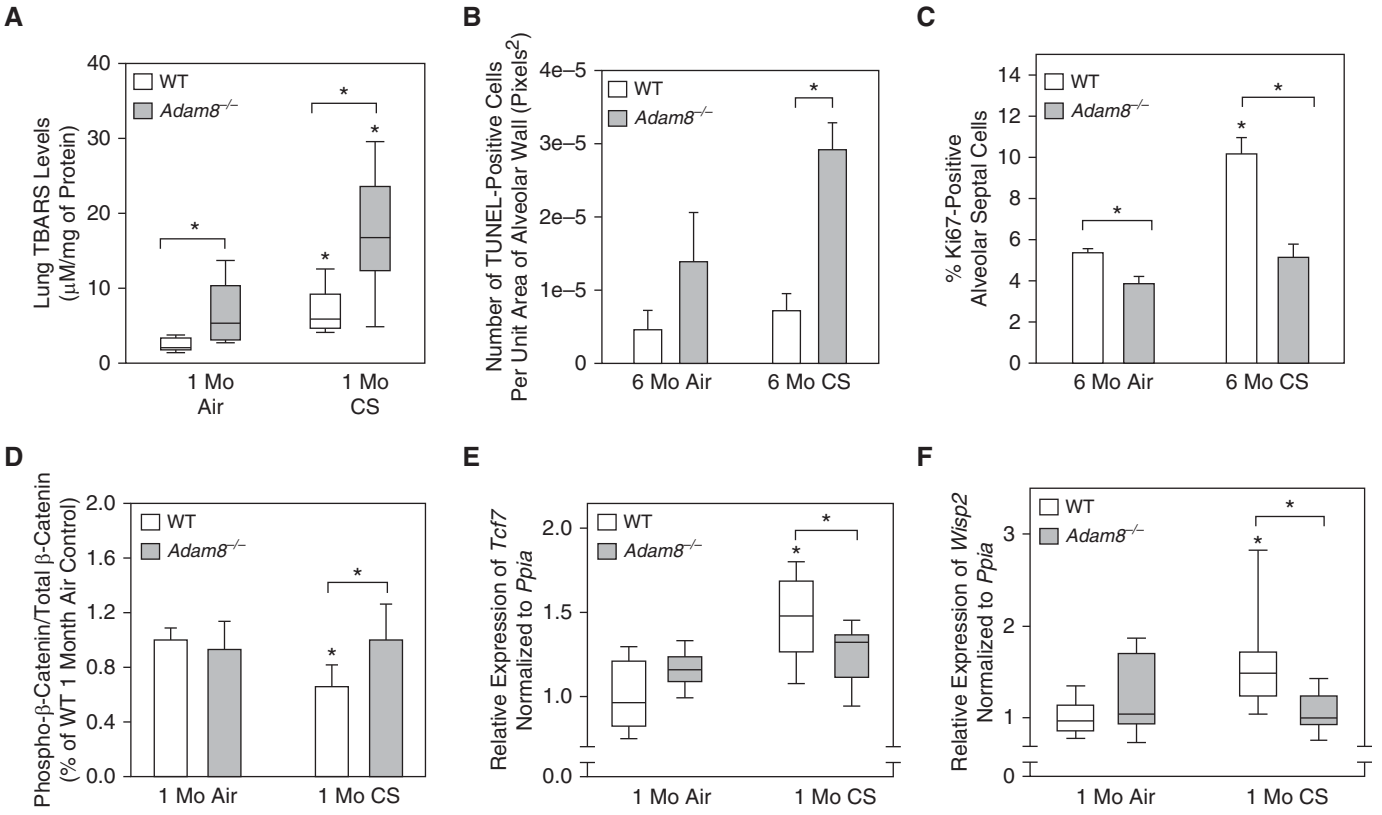

Figure 7. Cigarette smoke (CS)-exposed *Adam8*$^{-/-}$ mice have increased lung oxidative stress levels, increased alveolar septal cell death rates, and reduced alveolar septal cell proliferation rates. (*A*) Lung oxidative stress levels were measured as thiobarbituric acid reactive substances in homogenates of lungs from *Adam8*$^{-/-}$ and wild-type (WT) mice that had been exposed to air or CS for 1 month. Data are presented as box plots with the boxes showing the median values and 25th and 75th percentiles, and the whiskers showing the 10th and 90th percentiles (n = 6–10 mice/group). (*B* and *C*) *Adam8*$^{-/-}$ and WT mice were exposed to air or CS for 6 months and lungs were inflated. In *B*, alveolar septal cell death was measured as positive TUNEL staining in the lung section. In *C*, alveolar septal cell proliferation was measured as positive staining for Ki67 in alveolar septal cells in lung sections. In *B* and *C*, data shown are mean ± SEM values from three to five mice/group. (*D–F*) WT and *Adam8*$^{-/-}$ mice were exposed to air or CS for 1 month. In *D*, lung levels of phosphorylated-β-catenin (which is targeted for proteosomal degradation) and total β-catenin were quantified using Western blotting, and the signals were normalized to the corresponding values for the air-exposed WT mice analyzed on the same blots. The ratio of phosphorylated-β-catenin to total β-catenin was quantified for each animal (decreases in this ratio indicate reduced inactivation of this pathway and vice versa). The bar graphs show the means ± SD; n = 9 mice/group. Tcf7 (*E*) and Wisp2 (*F*) steady-state mRNA levels were measured in the lungs using quantitative real-time RT-PCR and the comparative threshold method. The boxes in the box plots show the median values and 25th and 75th percentiles, and whiskers show the 10th and 90th percentiles (n = 6–8 air-exposed and 9 CS-exposed mice/group). In *A*, *E*, and *F*, Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. In *B–D*, data were analyzed using one-way ANOVA followed by pairwise testing with two-sided Student's *t* test. In *A–F*, *$P < 0.05$ versus mice belonging to the same genotype that were exposed to air, or the group indicated. Adam8 = a disintegrin and metalloproteinase domain-8; TBARS = thiobarbituric acid reactive substances.

patients with COPD and promotes COPD progression.

Murine Studies

The increased lung inflammation, lung elastin degradation, oxidative stress levels, and dysregulated alveolar septal cell apoptosis and repair detected in CS-exposed *Adam8*$^{-/-}$ mice likely contribute to their exaggerated emphysema phenotype, because these processes have been linked to emphysema development in CS-exposed mice (2, 18, 21, 22).

Macrophages are required for CS-induced emphysema development in mice (28, 34). The increased macrophage counts in CS-exposed *Adam8*$^{-/-}$ lungs likely promoted emphysema development by: *1*) increasing lung levels of Mmp-12, which degrades lung elastin and promotes lung inflammation (28, 35); *2*) increasing oxidative stress, which induces lung injury (36, 37); and *3*) releasing proinflammatory mediators that amplify lung inflammation, because macrophages are major sources of these mediators (4), and ADAM8 did not significantly degrade mediators *in vitro*.

The increased lung macrophage and PMN counts in CS-exposed *Adam8*$^{-/-}$ mice are likely caused by reduced activation of the intrinsic apoptosis pathway in macrophages; and increased lung levels of mediators that promote myeloid leukocyte recruitment (Ccl-3 and Cxcl-1), activation (Il-6, and Il-1β), and survival (Gm-csf and G-csf) (Figure 10). Other ADAMs (6, 8, 38, 39)

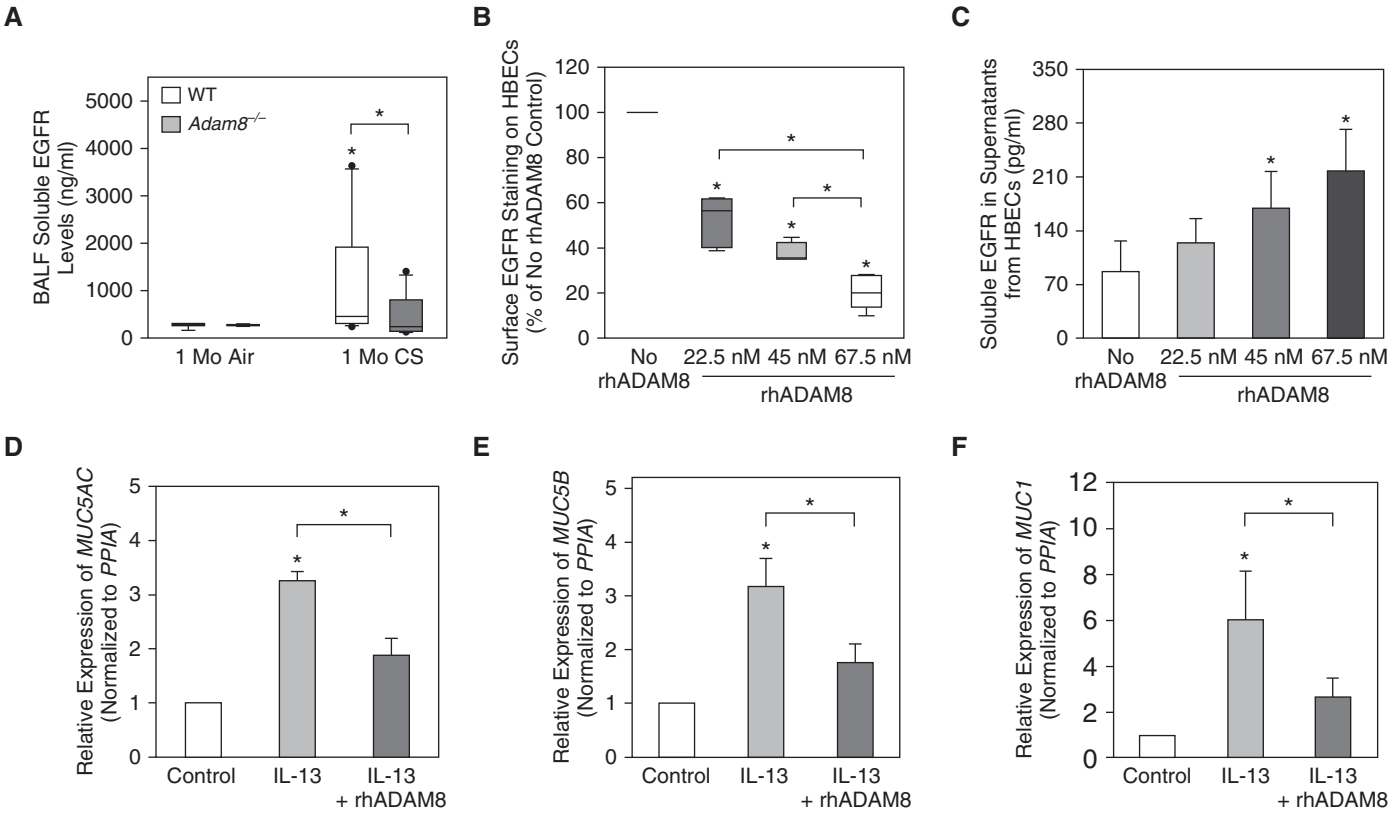

Figure 8. Cigarette smoke–exposed *Adam8*$^{-/-}$ mice have reduced epidermal growth factor receptor (EGFR) shedding in their lungs, and recombinant ADAM8 sheds EGFR from human bronchial epithelial cell (HBEC) surfaces to reduce mucin expression *in vitro*. (*A*) WT and *Adam8*$^{-/-}$ mice were exposed to air or cigarette smoke for 1 month and BAL was performed. Soluble Egfr (sEgfr) levels were measured in BAL fluid (BALF) samples from 7–11 mice/group using an ELISA. The boxes in the box plots show the median values and 25th and 75th percentiles, and the whiskers show the 10th and 90th percentiles. The Shapiro-Wilk test was used to determine whether the data were normally distributed, and Brown-Forsythe test was used to assess equal variance. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. **P* < 0.05 versus air-exposed mice belonging to the same genotype or the group indicated. (*B* and *C*) HBECs were grown to confluence on chamber-slides and incubated with or without 22.5–67.5 nM active recombinant human ADAM8 (rhADAM8) for 4 hours at 37°C. In *B*, HBECs were fixed and immunostained for cell surface–associated EGFR, which was quantified as described in the online supplement. The boxes in the box plot show the median values and 25th and 75th percentiles, and whiskers show the 10th and 90th percentiles ($n = 4$–5 separate experiments). The Shapiro-Wilk test was applied to determine distribution normality and the Brown-Forsythe test as an equal variance test. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. (*C*) Soluble EGFR shed into culture supernatants was quantified using an ELISA ($n = 4$–5 separate experiments). The bar graph shows the mean ± SEM and data were analyzed using a one-way ANOVA followed by pairwise testing with two-sided Student's *t* test. **P* < 0.05 versus the no rhADAM8 control. (*D–F*) HBECs were grown to confluence on cell culture plates, and then incubated with or without 10 ng/ml of IL-13 for 4 days (to induced mucin gene expression in submerged HBEC cultures). HBECs were then incubated with or without 67.5 nM active rhADAM8 for an additional 4 hours. MUC5AC (*D*), MUC5B (*E*), and MUC1 (*F*) steady state mRNA levels were then measured on RNA isolated from the cells using qRT-PCR ($n = 4$ separate experiments). In *D–F*, the bar graphs show the mean ± SEM and data were analyzed using one-way ANOVA followed by pairwise testing with two-sided Student's *t* test. **P* < 0.05 versus the control group or the group indicated. Adam8 = a disintegrin and metalloproteinase domain-8; CS = cigarette smoke; WT = wild type.

have been implicated in regulating leukocyte adhesion and migration. However, there are conflicting reports on whether Adam8 regulates leukocyte function in other models (40, 41). Herein, we found no evidence that myeloid leukocyte-derived Adam8 regulates the adhesion or migration function of these cells.

The mechanism by which Adam8 regulates the intrinsic apoptosis pathway in macrophages is not clear. ADAM8's MP domain did not shed receptors for G-CSF or GM-CSF, which inhibit activation of the intrinsic apoptosis pathway. Signaling via the cytoplasmic tail of ADAM8 may promote activation of the intrinsic apoptosis pathway in macrophages because the cytoplasmic tails of human and murine ADAM8 have Src homology-3 domains (42) having the potential to bind Src family kinases that regulate the intrinsic apoptosis pathway (43, 44).

The increased leukocyte counts in CS-exposed *Adam8*$^{-/-}$ lungs likely contributed to their increased lung oxidative stress levels and increased alveolar septal cell apoptosis, because activated myeloid leukocytes release oxidants, proteinases, and other mediators that injure alveolar septal cells (2). Wnt-β-catenin signaling promotes alveolar

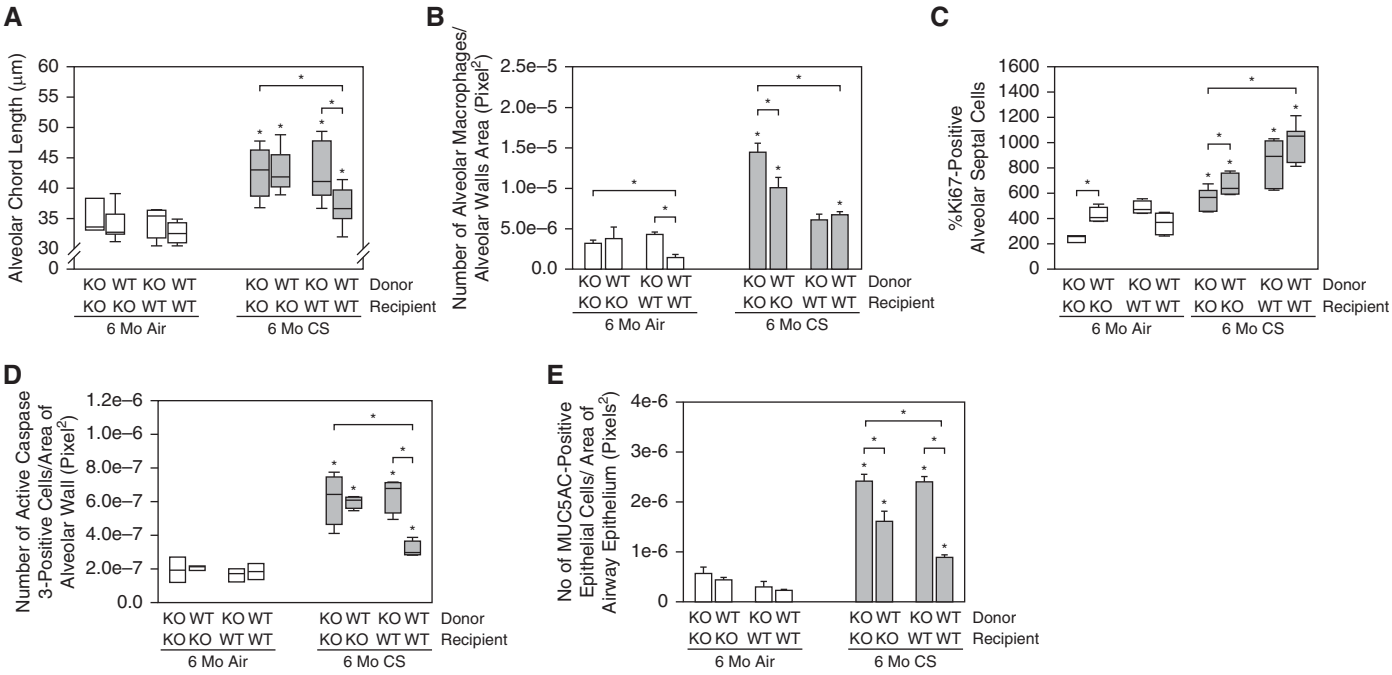

Figure 9. Deficiency of Adam8 in both bone marrow (BM)-derived leukocytes and lung epithelial cells contributes to the exaggerated chronic obstructive pulmonary disease–like lung disease in mice. Four groups of Adam8 BM chimeric mice (wild-type [WT] BM transplanted into WT recipients, WT BM transplanted into *Adam8*$^{-/-}$ recipients, *Adam8*$^{-/-}$ BM transplanted into WT recipients, and *Adam8*$^{-/-}$ BM transplanted into *Adam8*$^{-/-}$ recipients) were generated and exposed to air (white boxes) or cigarette smoke (gray boxes) for 6 months. The following were analyzed: (*A*) alveolar chord lengths as a readout of airspace enlargement; (*B*) the number of alveolar macrophages in lung sections normalized to alveolar wall area; (*C*) the percentage of alveolar septal cells that had positive immunostaining for Ki67 (a readout of cellular proliferation); (*D*) the number of alveolar septal cells that were positively stained for active caspase-3 per unit area of alveolar wall area; and (*E*) the number of bronchial epithelial cells that were positively stained for Muc5ac per unit area of bronchial epithelial cell area. In *A*, *C*, and *D*, the boxes in the box plots show the medians and 25th and 75th percentiles, and whiskers show the 10th and 90th percentiles. The Shapiro-Wilk test was applied to determine distribution normality and the Brown-Forsythe test as an equal variance test. Data were analyzed using one-way ANOVA followed by pairwise testing with Mann-Whitney *U* test. In *B* and *E*, the bar graphs show the mean ± SEM. The data were analyzed using one-way ANOVA followed by pairwise testing with two-sided Student's *t* test. In *A–E*, 3–11 mice/group were studied. *$P < 0.05$ versus mice belonging to the same experimental condition that were exposed to air, or the group indicated. Adam8 = a disintegrin and metalloproteinase domain-8; CS = cigarette smoke; KO = knockout.

epithelial repair in injured lungs (18, 45). *Adam8*$^{-/-}$ mice had impaired CS-triggered β-catenin activation, which likely contributed to their reduced alveolar septal cell proliferation rates. When Wnt binds to Frizzled receptors, this inhibits proteosomal degradation of β-catenin and promotes translocation of β-catenin to the nucleus. This process induces transcription of Wnt-inducible proteins that promote DNA replication and mitosis during cellular proliferation (46). Whether the lack of Adam8's MP domain or cytoplasmic tail reduced Wnt activation in CS-exposed *Adam8*$^{-/-}$ lungs will be investigated in future studies.

Neutrophil elastase, ADAM10, and ADAM17 induce mucus metaplasia by shedding EGFR ligands that bind to and activate the EGFR (47, 48). ADAM8 is the first proteinase identified that reduces airway mucus cell metaplasia. Reduced ADAM8 expression in bronchial epithelial cells in smokers and patients with COPD may contribute to mucus hypersecretion and progression of the chronic bronchitis phenotype. *In vitro* and *in vivo* studio supported the notion that ADAM8 reduces airway mucus metaplasia by shedding the EGFR from airway epithelial cells. Studies of CS-exposed *Adam8*$^{-/-}$ X *Egfr*$^{-/-}$ mice to test this hypothesis are not feasible, because *Egfr*$^{-/-}$ mice die shortly after birth (49). EGFR antagonists are an alternative approach (50) that could be tested in studies of *Adam8*$^{-/-}$ mice. Tnf-α and Il-1β also induce mucin synthesis (27), and the increased lung levels of these cytokines in CS-exposed *Adam8*$^{-/-}$ mice may have contributed to their increased mucus metaplasia.

Both leukocyte-derived and lung parenchymal cell–derived *Adam8* contributed to the increased emphysema, lung macrophage counts, and dysregulated alveolar septal cell apoptosis and repair detected CS-exposed *Adam8*$^{-/-}$ mice. Surprisingly, *Adam8* BM chimera studies showed that *Adam8* deficiency in leukocytes (and not airway epithelial cells) was responsible for the increased mucus cell metaplasia in CS-exposed *Adam8*$^{-/-}$ mice. *Adam8* deficiency in airway leukocytes may increase mucus cell metaplasia by reducing leukocyte-derived Adam8-mediated shedding of the EGFR from airway epithelial cells, and/or by increasing the survival of airway macrophages to increase the airway burden of other macrophage products that induce mucus cell metaplasia (47, 48).

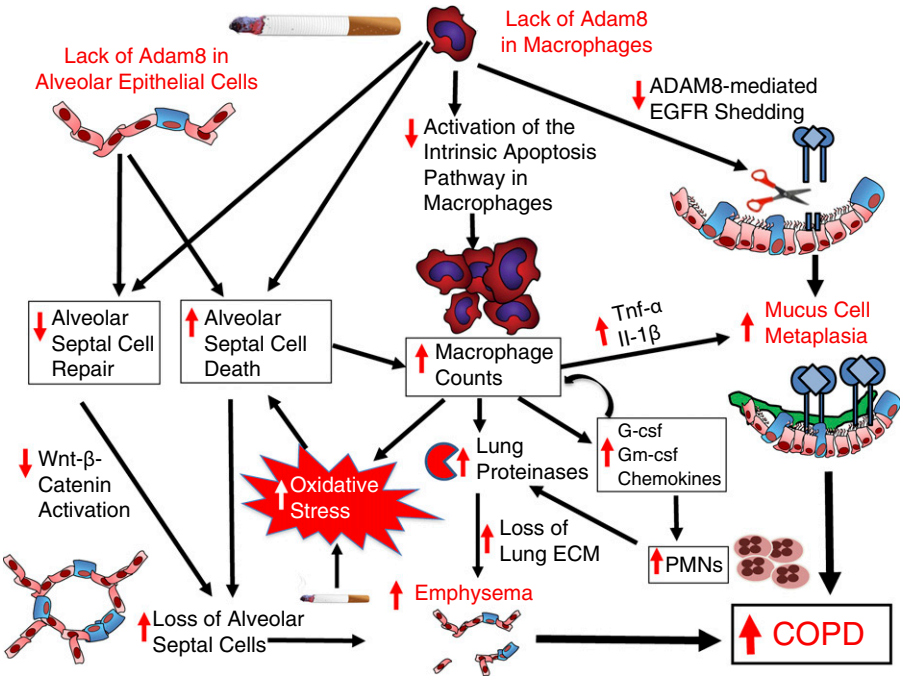

Figure 10. The proposed mechanisms by which Adam8 deficiency in leukocytes and/or lung parenchymal cells leads to exaggerated cigarette smoke (CS)-induced chronic obstructive pulmonary disease in mice. Based on the results of studies of wild-type, *Adam8*$^{-/-}$, and Adam8 bone marrow chimeric mice, deficiency of Adam8 in both leukocytes and epithelial cells increases CS-induced lung pathologies in mice. Deficiency of Adam8 in leukocytes (likely macrophages) increases lung macrophage survival by reducing activation of the intrinsic apoptosis pathway in macrophages to thereby increase the number of lung macrophages and the lung burden of their destructive products (proteinases including matrix metalloproteinases, reactive oxygen/nitrogen species, and proinflammatory mediators). The increased numbers of viable macrophages in CS-exposed *Adam8*$^{-/-}$ lungs secrete increased quantities of chemokines (e.g., Ccl-2, Ccl-3, and Cxcl-1) and survival factors for myeloid cells (e.g., G-csf [granulocyte colony–stimulating factor] and Gm-csf [granulocyte–macrophage colony–stimulating factor]) that increase lung PMN counts and further increase lung macrophage counts. Deficiency of Adam8 in leukocytes (likely macrophages) also increases emphysema development by increasing alveolar septal cell death and decreasing alveolar septal cell repair. Deficiency of Adam8 in leukocytes (likely macrophages) increases airway mucus cell metaplasia as a result of loss of macrophage-derived Adam8 shedding the Egfr from airway epithelial cell surfaces, thereby increasing Egf receptor–ligand–mediated activation of mucin gene expression in airway epithelium. The increased numbers of viable macrophages in CS-exposed *Adam8*$^{-/-}$ lungs secrete increased quantities of Tnf-α (tumor necrosis factor-α) and Il-1β, which may also increase mucus metaplasia in the *Adam8*$^{-/-}$ airways. Deficiency of Adam8 in lung parenchymal cells (likely epithelial cells) increases emphysema development by increasing alveolar septal cell death and reducing alveolar septal cell repair, in part, by inhibiting activation of the Wnt-β-catenin pathway. Deficiency of Adam8 in lung parenchymal cells (likely epithelial cells) also increases lung macrophage counts possibly by increasing alveolar septal cell death to trigger an inflammatory response in the lung. Adam8 = a disintegrin and metalloproteinase domain-8; COPD = chronic obstructive pulmonary disease; ECM = extracellular matrix; EGFR = epidermal growth factor receptor; PMN = polymorphonuclear neutrophil.

Unchallenged *Adam8*$^{-/-}$ mice had higher lung leukocyte counts, lung oxidative stress levels, and airspace enlargement, and lower rates of alveolar septal cell proliferation and AM apoptosis than WT mice. Thus, *Adam8* deficiency is sufficient to perturb lung homeostasis in the unchallenged state, which may be caused (in part) by *Adam8* deficiency increasing lung macrophage survival thereby increasing lung macrophage counts and lung levels of macrophage-derived proinflammatory mediators, proteinases, and oxidants. *Adam8* deficiency in alveolar septal cells may also contribute to the increased lung levels of proinflammatory cytokines and oxidants, and reduced alveolar epithelial repair, and thereby to airspace enlargement in unchallenged *Adam8*$^{-/-}$ mice.

Study limitations. We studied lung samples from small human cohorts. Our results should be confirmed in larger COPD cohorts. We could not relate the reduced ADAM8 staining in lung sections to emphysema severity because computed tomography–defined emphysema measurements were not available on our cohort. The mechanisms by which *Adam8* deficiency protects lung macrophages from the toxic effects of CS and inhibits alveolar repair were not identified. Lung volumes were not measured in the mice to exclude the possibility that the increased emphysema development in CS-exposed *Adam8*$^{-/-}$ mice was caused by increased lung volumes (rather than increased lung destruction). Only a loss-of-function strategy was used to assess the contributions of Adam8 to COPD in mice. Future studies should use gain-of-function approaches to confirm our findings, because inhibitors that are selective for Adam8 do not exist.

Conclusions

According to the proteinase-antiproteinase hypothesis, inhaling CS increases lung proteinase levels causing them to exceed the lung antiproteinase defense, thus promoting lung destruction. However, this study identifies Adam8 as the first proteinase that protects the CS-exposed murine lung from developing COPD-like lung disease. CS exposure and COPD reduce the expression of ADAM8 in lung epithelial cells and macrophages, and this may promote the progression of the chronic bronchitis and emphysema phenotypes in patients with COPD. Strategies that increase or prolong the expression of ADAM8 on cell surfaces may have potential as new therapeutics for patients with COPD. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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