ORIGINAL RESEARCH

Myeloid-Specific Fos-Related Antigen-1 Regulates Cigarette Smoke–Induced Lung Inflammation, Not Emphysema, in Mice

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Abstract

Heightened lung inflammation is a cardinal feature of chronic obstructive pulmonary disease (COPD). Cigarette smoke (CS)-induced macrophage recruitment and activation, accompanied by abnormal secretion of a number of inflammatory cytokines and matrix metalloproteinases, play a major role in the pathophysiology of COPD. The Fos-related antigen-1 (Fra-1) transcription factor differentially regulates several cellular processes that are implicated in COPD, such as inflammation and immune responses, cell proliferation and death, and extracellular remodeling. Although CS stimulates *Fra-1* expression in the lung, the precise role of this transcription factor in the regulation of CS-induced lung inflammation *in vivo* is poorly understood. Here, we report that myeloid-specific Fra-1 signaling is important for CS-induced lung macrophagic inflammatory response. In response to chronic CS exposure, mice with *Fra-1* specifically deleted in myeloid cells showed reduced levels of CS-induced lung macrophagic inflammation, accompanied by decreased expression levels of proinflammatory cytokines compared with their wild-type counterparts. Consistent with this result, bone marrow–derived Fra-1–null macrophages treated with CS showed decreased levels of proinflammatory mediators and matrix metalloproteinases. Interestingly, deletion of *Fra-1* in myeloid cells did not affect the severity of emphysema. We propose that Fra-1 plays a key role in promoting chronic CS-induced lung macrophagic inflammation *in vivo*, and that targeting this transcription factor may be useful in dampening persistent lung inflammation in patients with COPD.

Keywords: emphysema; inflammation; activator protein-1; lung; macrophages

Clinical Relevance

Persistent macrophagic inflammation in the smoker's lung has been implicated in the development and perpetuation of chronic obstructive pulmonary disease (COPD). Here, we demonstrate, using a tissue-specific knockout model, that Fos-related antigen-1 (Fra-1) transcription factor signaling in myeloid cells plays a key role in promoting chronic cigarette smoke–induced lung macrophagic inflammation *in vivo*. Thus, either depletion or inhibition of Fra-1 activity may be useful in dampening lung inflammation in patients with COPD.

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of chronic morbidity and mortality in the United States, and is projected to rank fifth in 2020 in burden of disease worldwide. The disease is characterized by chronic inflammation of peripheral and central airways, increased thickness of airway walls, and destruction of alveolar septa, ultimately leading to the destruction of the alveolar walls (1). Although cigarette smoking is a major risk factor for COPD, the exact mechanism(s) underlying cigarette smoke (CS)-induced lung inflammation and emphysema is not yet fully understood and

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remains an area of intensive investigation. Macrophage recruitment and activation play a significant role in the pathogenesis of CS-induced COPD (2). They accumulate in the alveoli and bronchiolar and small airways and mediate inflammatory responses to CS. Accumulation of macrophages is frequently observed in the bronchiolar region of the lungs in smokers and patients with COPD (3). CS has been postulated to induce the expression of chemotactic factors in alveolar epithelial cells, which then recruit inflammatory cells, including macrophages, neutrophils, and lymphocytes (4). Macrophage recruitment and activation in the lung after CS exposure can lead to the secretion of various proinflammatory cytokines that are known to contribute to the chronic inflammation observed in patients with COPD (5). In addition, macrophages also express and secrete matrix metalloproteinases (MMPs), which, in turn, remodel the extracellular matrix (ECM), leading to the development of emphysema (6). Although an important role for various transcription factors, such as NF-κB (7) and Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (8), in the regulation of COPD pathogenesis has been established using genetic models, the exact role of the activator protein-1 (AP-1) family of proteins in the regulation of CS-induced lung inflammation and emphysema remain poorly understood *in vivo*.

The dimeric AP-1 complex, composed of Jun (c-Jun, Jun-B, and Jun-D), Fos (c-Fos, Fos-B, Fos-related antigen-1 [Fra-1], and Fra-2), and activating transcription factor (ATF) family members distinctly regulates gene expression in a context-dependent manner (9, 10). AP-1 family member activation is modulated by CS and is associated with CS-triggered chronic inflammation in rodents (11–14). However, their contribution to promoting or attenuating the production of inflammatory mediators in response to chronic CS is unclear. We have recently shown that alveolar epithelium–specific c-Jun/AP-1 plays a protective role in CS-induced emphysema (15). Fra-1 (also referred as Fos-like antigen 1 [Fosl1]) is known to modulate the transcriptional response initiated by Jun/AP-1 (16). Previously, we have reported the induction of Fra-1 by CS in bronchial epithelial cells, mainly at the transcription level (13). Elevated levels of Fra-1 expression have been reported in the lungs of mice that had developed emphysema after exposure to CS (17), but the exact role of Fra-1 in mediating CS-induced lung inflammatory responses and other changes associated with the development of COPD are not known *in vivo*. Given the important role played by macrophage recruitment and activation in mediating CS-induced responses in COPD, we sought to determine the role of Fra-1/AP-1 signaling in mediating macrophage-specific lung inflammatory responses induced by CS. Using a mouse model with a deletion of *Fra-1* in myeloid cells, we now provide evidence that Fra-1/AP-1–mediated signaling in macrophages plays an obligatory role in promoting CS-induced lung inflammatory responses, but not emphysema, *in vivo*.

Materials and Methods

See the online supplement for additional details on methods.

Mice

Mice bearing "floxed" alleles of *Fra-1* (*Fra-1*$^{F/F}$) on a C57BL6J/129 background (18) were obtained from Erwin Wagner (Spanish National Cancer Research Centre, Madrid, Spain) and crossed with LysM2-Cre mice on a C57BL/6J background (Jackson Laboratory, Bar Harbor, ME). *Fra-1*$^{F/F}$ and Meox-Cre (Jackson Laboratory) mice were cross-bred to generate Fra-1–null (*Fra-1*$^{\Delta/\Delta}$) mice, as detailed elsewhere (18).

CS Exposure

Mice (8–10 wk old) were exposed to room air (RA) or CS for 5 h/d, 5 d/wk for 6 months, as described previously (9) under the animal protocol approved by the Animal Care and Use Committee at the Johns Hopkins University (Baltimore, MD). Cell cultures were treated with CS condensate (CSC) at a final concentration of 100 μg/ml.

Isolation of Bone Marrow–Derived Macrophages and Lung Alveolar Macrophages

Bone marrow–derived macrophages (BMDMs) and alveolar macrophages (AMϕs) were isolated and cultured using standard methods.

Lung Morphological Measurements

Lungs were inflated with 0.8% low-melting agarose at 25–30 cm water pressure, and fixed in 1.5% paraformaldehyde. The left lung was cut into three sections (2 to 3 mm thick) and embedded in paraffin. Sections (5 μm thick) were cut and stained with hematoxylin and eosin. The slides were numbered and coded blind, and representative images (15 per lung section) were acquired with a DXM1200 digital camera (Nikon, Tokyo, Japan) at 10× magnification. The mean linear intercept (Lm) and alveolar surface was measured using computer-assisted morphometry with NIS-Elements imaging software (Nikon, Melville, NY) (19).

Assessment of Lung Inflammation

Bronchoalveolar lavage (BAL) fluid was collected from the right lung by instilling twice with 0.75 ml of sterile PBS. The left lung was used for RNA extraction or fixed in formalin for histologic examination. Cells from the BAL fluid were centrifuged and total inflammatory cells were counted using a hemocytometer. Differential cell counts were determined by staining with the Diff-Quick Stain Set (Dade-Behring, Newark, DE).

Cytokine Analysis

Cytokine levels in cell-free BAL fluid samples or cell culture supernatants were measured using a milliplex mouse cytokine assay system (Millipore, Billerica, MA), and the values were expressed as picograms per milliliter of the BAL fluid or per milliliter of cell culture supernatant.

Quantification of MMP-12

MMP-12 levels in cell-free BAL fluid samples from the mice were quantitated using a mouse MMP-12 PicoKine ELISA kit (Boster-Immunoleader, Pleasanton, CA), and expressed as picograms per milliliter of BAL fluid.

Real-Time RT-PCR Analyses

Total RNA was isolated and real-time RT-PCR was performed using TaqMan assays (Applied Biosystems, Carlsbad, CA) and SYBR green gene expression assays using gene-specific primers.

Statistical Analysis

Data were expressed as means ± SD. Student's two-tailed *t* test was used to determine the significant differences between various experimental groups. *P* values of 0.05 or less were considered statistically significant.

Results

Generation of Mice with Specific Deletion of *Fra-1* in Myeloid Cells

To determine the role of macrophage-specific Fra-1/AP-1 signaling in CS-induced lung inflammation and emphysema, we generated mice with a specific deletion of *Fra-1* in the myeloid cell lineage, which includes monocytes, mature macrophages, and granulocytes. Mice bearing "floxed" alleles of *Fra-1* (*Fra-1*$^{F/F}$; C57BL6/129 background) were crossed with mice bearing LysM2-Cre to generate mice heterozygous for *Fra-1* floxed alelle and LysM2-Cre (*Fra-1*$^{F/wt}$LysM2-Cre mice). These mice were then back-crossed to the parental *Fra-1*$^{F/F}$ mice to obtain mice homozygous for *Fra-1* "floxed" alleles and bearing LysM2-Cre (*Fra-1*$^{\Delta/\Delta LysM}$ mice; Figure 1A). *Fra-1*$^{F/F}$ mice not expressing LysM2-Cre (*Fra-1*$^{F/F}$ mice) were used as controls. Mice were genotyped using tail DNA for the presence of *Fra-1* "floxed" and *Cre* alleles (Figure 1B). Deletion of *Fra-1* in macrophages was confirmed by genotyping DNA isolated from BMDMs for the presence of *Fra-1* and *Cre* alleles (Figure 1C). BMDMs were also treated with CSC for 90 minutes and *Fra-1* expression was analyzed by quantitative RT-PCR (Figure 1D). CSC stimulated *Fra-1* messenger RNA (mRNA) expression in wild-type (*Fra-1*$^{F/F}$) BMDMs. As expected, *Fra-1* expression was markedly lower in *Fra-1*$^{\Delta/\Delta LysM}$ (henceforth macrophages isolated from *Fra-1*$^{\Delta/\Delta LysM}$ mice and treated *in vitro* are referred as *Fra-1*$^{\Delta/\Delta}$) BMDMs, and furthermore, it was not induced by CSC. c-Fos and c-Jun expression modestly increased and decreased, respectively, in both cell types in response to CSC (Figure 1D).

Deletion of *Fra-1* Reduces CS-Induced Inflammatory Mediator Expression in Macrophages *In Vitro*

We next analyzed proinflammatory cytokine expression in BMDMs isolated from *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice and treated with CSC for 6 hours. There was a significant increase in macrophage inflammatory protein (MIP)-1α, MIP-2α, IL-6, and TNF-α mRNA expression in *Fra-1*$^{F/F}$ BMDMs treated with CSC as compared with vehicle-treated controls (Figure 2A). In contrast, the induction of these inflammatory cytokines was not observed in *Fra-1*$^{\Delta/\Delta}$ BMDMs treated with CSC. IL-1β and keratinocyte-derived chemokine (KC) mRNA expression was not induced by CSC in either cell type at the time point tested (*see* Figure E1 in the online supplement). Culture supernatants from these CSC-treated BMDMs were also collected and cytokine levels were measured using a multiplex ELISA assay (Figure 2B). Levels of macrophage colony–stimulating factor (MCSF) and IL-1β were significantly increased in *Fra-1*$^{F/F}$ BMDMs, but not in *Fra-1*$^{\Delta/\Delta}$ cells. Likewise, we found an increased secretion of monocyte chemoattractant protein (MCP)-1, KC, IFN-γ, and TNF-α in *Fra-1*$^{F/F}$ but not *Fra-1*$^{\Delta/\Delta}$ BMDMs after CSC treatment. CSC modestly increased IL-10 levels in *Fra-1*$^{F/F}$ BMDMs, but it was undetectable in their *Fra-1*$^{\Delta/\Delta}$ counterparts. These results suggest an important role for Fra-1 in the regulation of CS-stimulated inflammatory responses by macrophages.

Fra-1 Regulates CS-Induced ECM Gene Expression in Macrophages

CS stimulates the expression of MMPs, which, in turn, degrade the components of the ECM, such as collagen and elastin, and contribute to the development and progression of emphysema (6, 20). As macrophages produce MMPs and tissue inhibitors of metalloproteinases (TIMPs) (21), we evaluated whether Fra-1 modulates their expression by CSC in BMDMs. CSC significantly increased MMP-1α, MMP-1β, and MMP-9 expression in *Fra-1*–sufficient (*Fra-1*$^{F/F}$) BMDMs, but this induction was not observed in *Fra-1*–deficient (*Fra-1*$^{\Delta/\Delta}$) BMDMs (Figure 3). CS markedly stimulated MMP-12 expression in both *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta}$ BMDMs (Figure 3); however, the magnitude of induction was significantly lower in *Fra-1*$^{\Delta/\Delta}$ BMDMs than in their *Fra-1*$^{F/F}$ counterparts. CSC did not increase the expression levels of MMP-2, MMP-3, and TIMP-1 in both *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta}$ BMDMs (Figure E2). TIMP-2 expression was significantly decreased in both groups of cells after treatment with CSC. These results suggest that Fra-1 regulates CS-induced MMP-1, MMP-9, and MMP-12 expression in macrophages.

Disruption of *Fra-1* in Myeloid Cells Reduces Chronic CS-Induced Lung Inflammation and MMP Levels *In Vivo*

Given the importance of macrophages in mediating CS-induced inflammatory responses in the lung, we were interested in

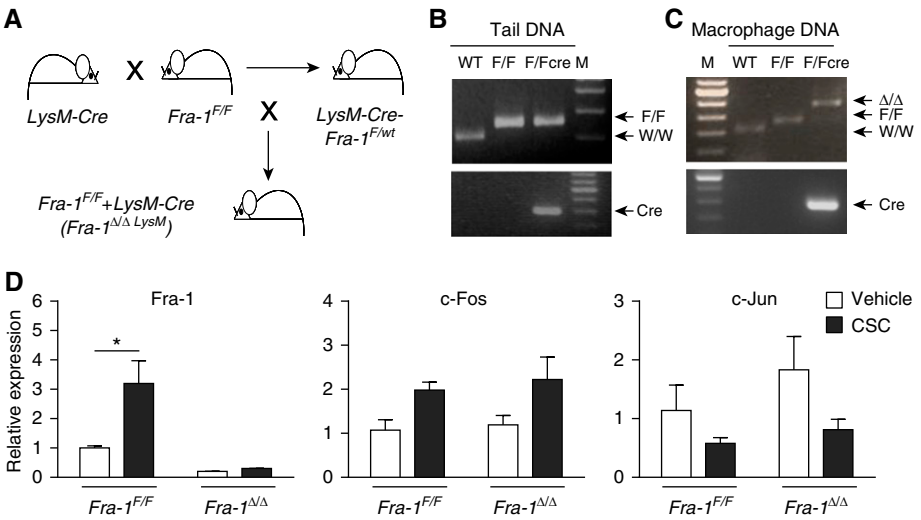

Figure 1. Generation of mice lacking Fos-related antigen-1 (Fra-1) in myeloid cells. (*A*) Schematic representation showing generation of mice with a deletion of Fra-1 in cells of the myeloid lineage (Fra-1$^{\Delta/\Delta LysM}$ mice). (*B* and *C*) PCR amplification of genomic DNA isolated from tails (*B*) and bone marrow–derived macrophages (BMDMs) (*C*) of wild-type (WT), Fra-1$^{F/F}$, and Fra-1$^{\Delta/\Delta LysM}$ mice. *Upper panels* show amplification of Fra-1 wild-type (Fra-1 wt), Fra-1$^{F/F}$, and deletion bands of Fra-1 (Fra-1$^{\Delta/\Delta}$), and *lower panels* show that of Cre. (*D*) Quantitative RT-PCR (qRT-PCR) analysis of Fra-1, c-Fos, and c-Jun expression in BMDMs isolated from Fra-1$^{F/F}$ and Fra-1$^{\Delta/\Delta LysM}$ mice after treatment with vehicle or cigarette smoke condensate (CSC) for 6 hours. Data are expressed as mean ± SD. *$P < 0.05$ vehicle versus CSC.

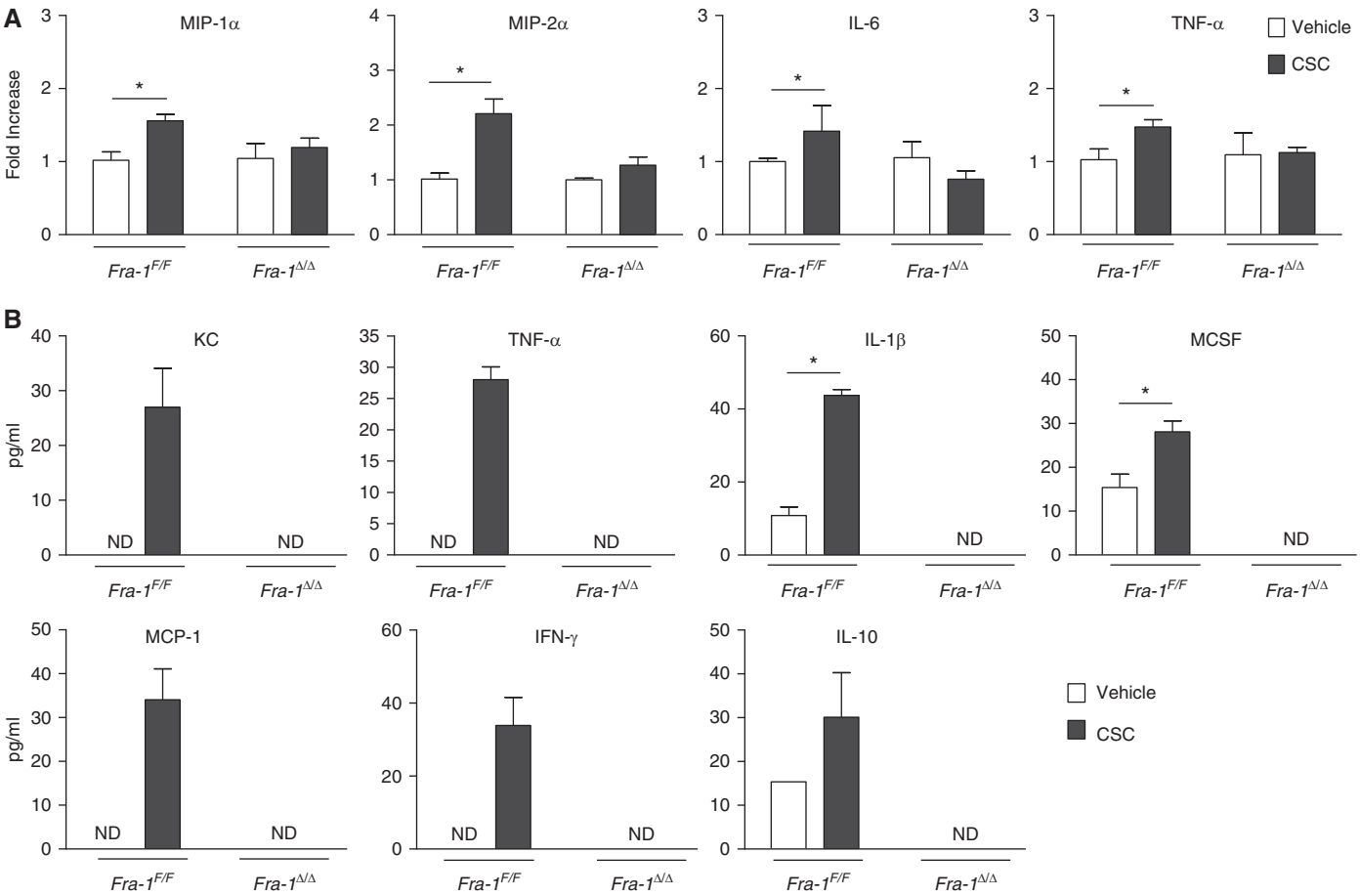

Figure 2. Effect of Fra-1 deletion on CS-induced inflammatory mediator expression in BMDMs. BMDMs isolated from Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta LysM}$ mice ($n = 5$) were treated with vehicle (DMSO) or CSC (100 μg/ml) for 6 hours. (*A*) Inflammatory mediator expression analyzed by qRT-PCR. (*B*) Quantitation of inflammatory mediators in culture supernatants of CSC-treated BMDMs analyzed by multiplex ELISA. Data are expressed as mean ± SD. The cytokine levels found below detection limits were considered as nondetectable (ND), and therefore *P* values were not assigned for CS samples. The values for the vehicle-treated control group were from at least two to three independent biological samples, and CSC experimental groups were from at least three to four independent biological samples. *$P < 0.05$ vehicle versus CSC. KC, keratinocyte-derived chemokine; MCP, monocyte chemoattractant protein; MCSF, macrophage colony–stimulating factor; MIP, macrophage inflammatory protein.

determining the role of macrophage-specific Fra-1 signaling in mediating CS-induced inflammatory responses. *Fra-*$1^{F/F}$ and *Fra-*$1^{\Delta/\Delta LysM}$ mice were exposed to CS or RA for 6 months, after which lungs were fixed or lavaged for BAL fluid collection to measure lung injury and inflammation, respectively. As anticipated, chronic CS elicited lung inflammation accompanied predominantly by macrophagic infiltration in the lungs of *Fra-*$1^{F/F}$ and *Fra-*$1^{\Delta/\Delta LysM}$ mice (Figure 4). However, CS-induced lung macrophagic inflammation was significantly reduced in *Fra-*$1^{\Delta/\Delta LysM}$ mice as compared with their *Fra-*$1^{F/F}$ counterparts. We found no difference in the number of neutrophils between the two genotypes exposed to CS.

To determine if the reduction in macrophage recruitment coincided with a reduction in inflammatory cytokine expression, we quantitated cytokine levels in the BAL (cell-free) fluid. We observed elevated levels of MIP-1α, MIP-1β, IL-12 (p40), and MIP-2α in BAL fluids of both *Fra-*$1^{F/F}$ and *Fra-*$1^{\Delta/\Delta LysM}$ mice as compared with their respective RA controls (Figure 5A). However, their levels in the lungs of *Fra-*$1^{\Delta/\Delta LysM}$ mice were markedly lower than in their wild-type counterparts. In contrast, we did not find significant differences in CS-induced KC, IL-13, and transforming growth factor-β1 levels between *Fra-*$1^{F/F}$ and *Fra-*$1^{\Delta/\Delta LysM}$ mice (Figure E3). Levels of MCSF were also elevated in BAL fluid from smoke-exposed *Fra-*$1^{F/F}$ mice, although it was not detectable in their respective RA controls. On the other hand, MCSF was not detectable in BAL fluid from RA and smoke-exposed *Fra-*$1^{\Delta/\Delta LysM}$ mice. An increase in the levels of IL-10 was observed in *Fra-*$1^{F/F}$ mice exposed to CS, but its levels in the BAL fluid from *Fra-*$1^{\Delta/\Delta LysM}$ mice exposed to RA or CS were below the detection limits of the multiplex ELISA assay (Figure 5B). IFN-γ levels were similarly increased in both genotypes exposed to CS (Figure 5B). The levels of MCP-1, IL-6, TNF-α, IL-1β, and IL-12 (p70) were found to be largely undetectable in the BAL samples of both genotypes (data not shown). These results demonstrate that myeloid-specific *Fra-1* regulates

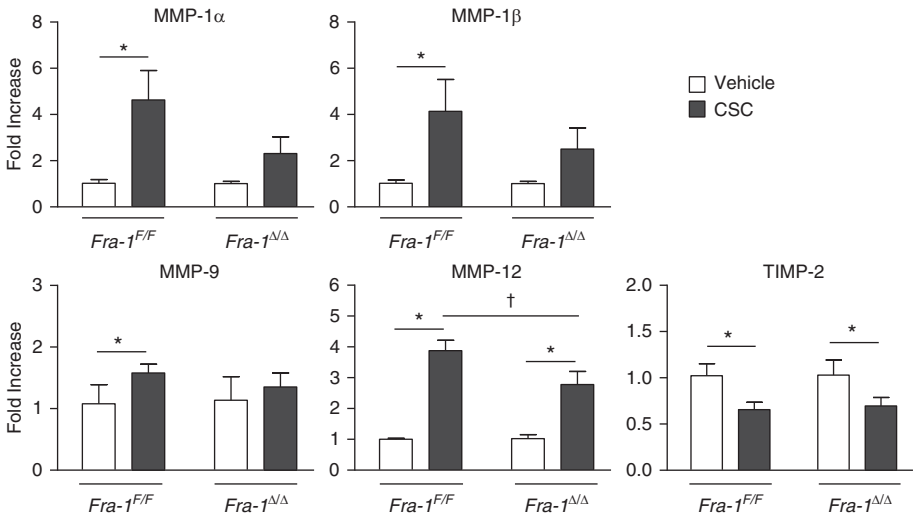

Figure 3. Effect of Fra-1 deletion on CS-induced matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) expression in BMDMs. BMDMs isolated from Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta LysM}$ mice ($n = 5$) were treated with vehicle or CSC (100 μg/ml) for 6 hours, and MMP and TIMP expression was analyzed by qRT-PCR. Data are expressed as mean ± SD. *$P < 0.05$ vehicle versus CSC; †$P < 0.05$ Fra-$1^{F/F}$ versus Fra-$1^{\Delta/\Delta}$ genotype.

CS-induced inflammatory responses in the lung by selectively modulating cytokine gene expression.

One of the major MMPs secreted by macrophages is MMP-12, which has been shown to be required for CS-induced emphysema in mice (22). Therefore, we quantitated MMP-12 levels in the BAL fluid of *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice exposed to RA or CS for 6 months. An increase in MMP-12 levels was observed in the BAL fluids of both *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice exposed to CS as compared with their RA counterparts. However, *Fra-1*$^{\Delta/\Delta LysM}$ mice had significantly reduced levels of MMP-12 compared with *Fra-1*$^{F/F}$ mice (Figure 5C).

Fra-1 Deficiency in Myeloid Cells Does Not Affect CS-Induced Alveolar Space Enlargement

Having observed decreased levels of CS-induced lung inflammation in *Fra-1*$^{\Delta/\Delta LysM}$ mice and a reduction in CS-induced inflammatory cytokine and MMP (MMP-1, MMP-9, and MMP-12) expression in *Fra-1*$^{\Delta/\Delta}$ macrophages *in vitro*, we sought to determine the role of macrophage-specific Fra-1 signaling in the development of emphysema. Alveolar space enlargement was assessed by measuring Lm and alveolar surface area in *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice exposed to RA and chronic CS. Histological evaluation of lung sections indicated emphysematous changes in both *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice exposed to CS compared with corresponding RA control groups (Figure 6A). There was a significant increase in Lm (Figure 6B) and alveolar surface area (Figure 6C) in both *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice. Lm was increased by around 20% and alveolar surface area by 36% (Figures 6B and 6C), both in *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice as compared with their corresponding RA counterparts. However, there were no significant differences either in Lm or alveolar surface area between *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta LysM}$ mice, suggesting that the absence of Fra-1 in myeloid cells alone is not sufficient to attenuate the development of emphysema induced by CS.

Fra-1 Distinctly Modulates CS-Induced Cytokine and ECM Expression in AMϕs

To determine whether Fra-1 deficiency affects CS-induced inflammatory and ECM gene expression in AMϕs, we isolated resident AMϕs from the lungs of *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta}$ mice and treated them immediately with CSC for 6 hours. As shown in Figure 7A, CSC significantly increased *Fra-1* mRNA expression in wild-type AMϕs, whereas its expression was significantly lower in *Fra-1*$^{\Delta/\Delta}$ cells. c-Fos was modestly induced by CSC in wild-type AMϕs, but the basal level expression of c-Fos was significantly higher in *Fra-1*$^{\Delta/\Delta}$ AMϕs, and it was not induced further by CSC. We found no significant differences in CSC-induced c-Jun expression between the wild-type and *Fra-1*$^{\Delta/\Delta}$ AMϕs (Figure 7A). Analysis of inflammatory cytokine and chemokine expression in *Fra-1*$^{F/F}$ and *Fra-1*$^{\Delta/\Delta}$ AMϕs treated with CSC revealed

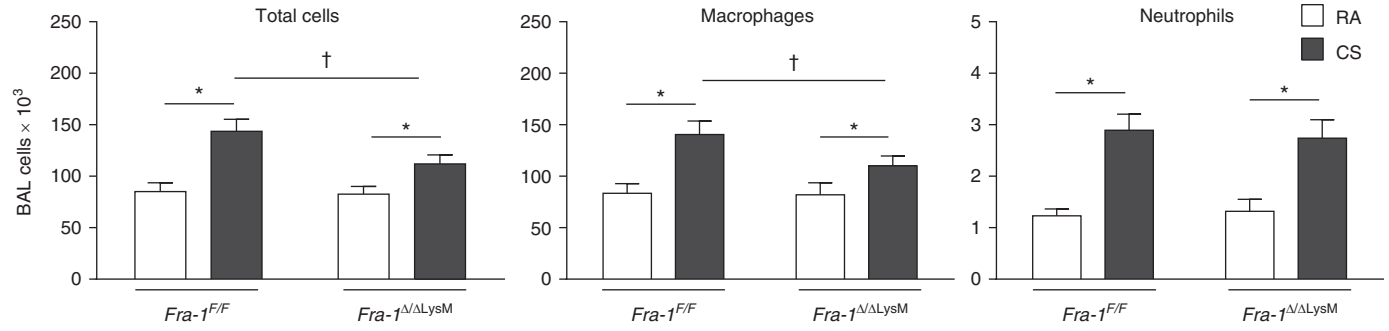

Figure 4. Effect of chronic CS exposure on lung inflammation *in vivo*. Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta LysM}$ mice ($n = 5$ per group) were exposed to filtered room air (RA) or CS for 6 months. Bronchoalveolar lavage (BAL) fluid was collected from the right lung, centrifuged, and stained with the Diff-Quik stain kit. Differential cell counts were performed and expressed as mean ± SD for total cells, neutrophils, and macrophages. *$P < 0.05$, RA versus CS; †$P < 0.05$, Fra-$1^{F/F}$ versus Fra-$1^{\Delta/\Delta LysM}$ genotype.

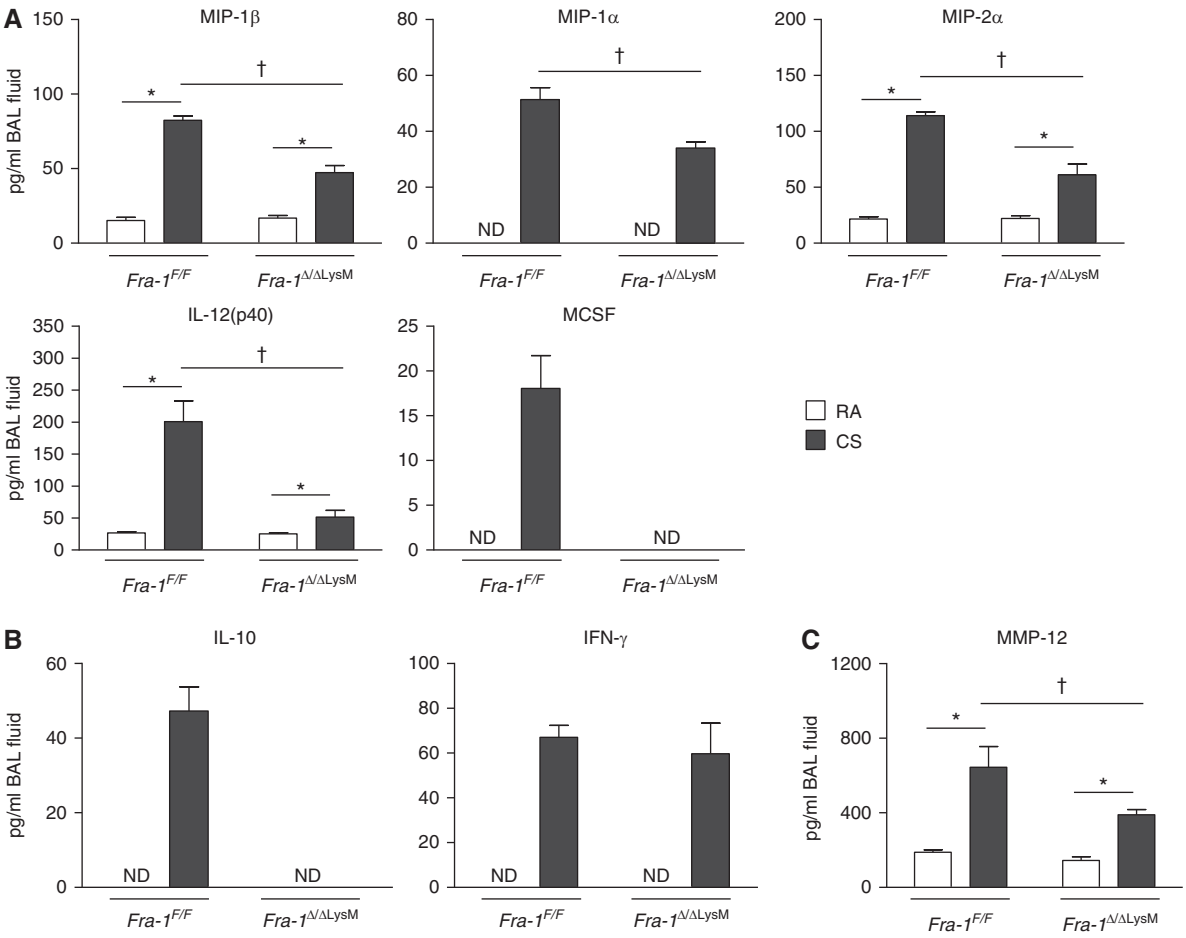

Figure 5. Effect of chronic CS exposure on lung-inflammatory cytokine and MMP expression *in vivo*. Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta LysM}$ mice ($n = 5$ per group) were exposed to RA or CS for 6 months, and (*A*) levels of inflammatory cytokines and (*B*) markers of the M1- and M2-like macrophage phenotype were analyzed in cell-free BAL fluid by multiplex ELISA. (*C*) Levels of MMP-12 in cell-free BAL fluid were analyzed by ELISA. Data are expressed as mean ± SD. The cytokine levels found below detection limits were considered as nondetectable (ND), and therefore *P* values were not assigned for CS samples. The values for the RA control group were from at least two to three mice, and CS experimental groups were from at least three to four mice. $^{*}P < 0.05$, RA versus CS; $^{\dagger}P < 0.05$, Fra-$1^{F/F}$ versus Fra-$1^{\Delta/\Delta LysM}$ genotype.

that *MIP-1α*, *MIP-2α*, KC, and IL-6 mRNA levels were increased significantly in response to CSC, but no significant difference was observed between the two genotypes (Figure E4). CSC-induced *IL-1β* expression in *Fra-1*$^{\Delta/\Delta}$ cells was significantly lower than in their *Fra-1*$^{F/F}$ counterparts (Figure 7B). *TNF-α* expression was not induced by CSC in AMϕs of either genotype (Figure E4). Analysis of *MMP* expression revealed a significant increase in *MMP-2* and *MMP-12* levels in AMϕs of both genotypes treated with CSC, but their induction did not differ significantly between the two genotypes (Figure E4). MMP-9 was not induced by CSC in either genotype, and MMP-3 was modestly induced in *Fra-1*$^{\Delta/\Delta}$ cells (Figure E4). CSC-induced *TIMP-2* and *TIMP-3* expression was similar between *Fra-1*$^{\Delta/\Delta}$ and *Fra-1*$^{F/F}$ cells. We also evaluated *INF-γ* and *IL-10* expression as markers of M1- and M2-like macrophage phenotypes, respectively. *INF-γ* expression was not altered significantly by CSC in either cell type (Figure 7C). CSC-induced *IL-10* expression in *Fra-1*$^{\Delta/\Delta}$ cells was significantly lower than in their *Fra-1*$^{F/F}$ counterparts (Figure 7C).

Discussion

Given the increased global incidence and health care costs associated with the morbidity of COPD, identifying the mechanisms underlying chronic lung inflammation in COPD is imperative to develop novel treatment strategies for mitigating this disease. Because the AP-1 transcription factor is a critical regulator of tissue injury and inflammatory responses (23), in the present study, we investigated the exact role of Fra-1/AP-1 in mediating CS-induced lung experimental emphysema. Our studies provide genetic evidence that Fra-1 is required for CS-induced lung inflammatory responses, as the disruption of *Fra-1* specifically in myeloid cells attenuated lung inflammation induced by chronic CS (Figures 4 and 5). This decrease in lung inflammation is mainly attributable to the reduced levels of macrophages. Interestingly, our results suggest that, although Fra-1 is required for CS-induced lung macrophagic inflammation, this transcription factor–mediated signaling in

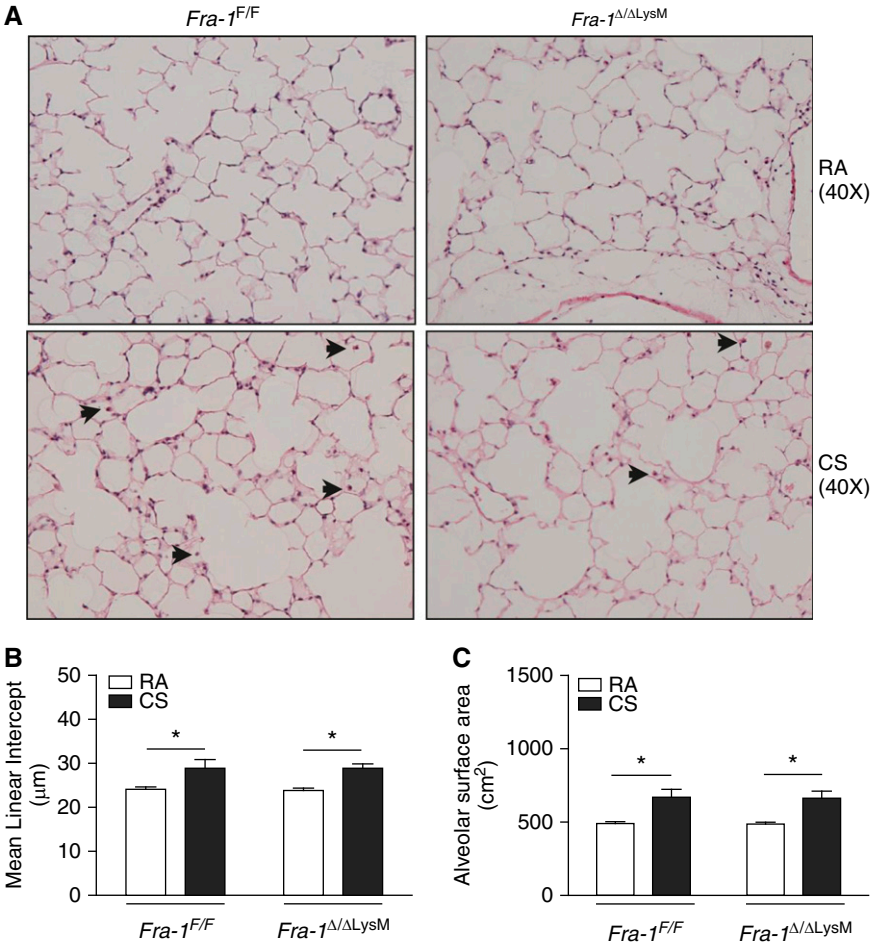

Figure 6. Effect of chronic CS exposure on lung morphology. (*A*) Representative hematoxylin and eosin–stained lung sections from Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta LysM}$ mice exposed to RA or CS for 6 months. Increased alveolar space enlargement was seen in CS-exposed mice as compared with their respective RA controls. *Arrowheads* indicate the presence of macrophages. (*B* and *C*) Morphometric analysis of lung sections from Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta LysM}$ mice ($n = 6$ per group) to measure mean linear intercept (Lm) (*B*) and alveolar surface area (*C*). Data are expressed as mean ± SD. Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta LysM}$ mice exposed to CS show significant increases in Lm and alveolar surface area as compared with their respective RA-exposed controls. *$P < 0.05$, RA versus CS.

myeloid cells alone is not sufficient to drive CS-induced airway space enlargement *in vivo*.

Several studies support an important role for macrophages in mediating CS-induced lung-inflammatory responses in COPD (21). CS-induced infiltrated macrophages secrete a number of inflammatory mediators, such as TNF-α, MIP-1α, MIP-2α, IL-6, and IL-1β, which regulate inflammatory responses and contribute to the pathogenesis of COPD (5, 24, 25). We found a reduced number of macrophages in the BAL fluid of *Fra-*$1^{\Delta/\Delta LysM}$ mice exposed to CS (Figure 4), and this was accompanied by decreased levels of inflammatory cytokines, such as MIP-1α, MIP-1β, MIP-2α, and IL-12 (p40) (Figure 5A). Consistent with this result, we found reduced levels of CS-stimulated inflammatory cytokine expression in BMDMs, further corroborating the *in vivo* results of reduced cytokine expression in mice lacking *Fra-1* in myeloid cells (Figure 2). MCP-1 is a potent chemoattractant of monocytes, and is involved in the recruitment of macrophages in COPD (26). Moreover, increased levels of MCP-1 have been observed in the sputum samples of patients with COPD (27). MCSF has also been shown to aggravate emphysema and cause an accumulation of AMϕs in mice (28). Consistent with these observations, we found reduced levels of MCP-1 and MCSF secreted by *Fra-*$1^{\Delta/\Delta}$ BMDMs (Figure 2B), and no detectable levels of MCSF in the BAL fluid from smoke-exposed *Fra-*$1^{\Delta/\Delta LysM}$ mice (Figure 5A). This could account for a decreased recruitment of macrophages in the BAL fluid of *Fra-*$1^{\Delta/\Delta LysM}$ mice exposed to chronic CS. Elevated levels of *Fra-1* expression, along with IL-8 and TNF-α, has been reported in CSC-treated macrophages obtained from the blood of patients with COPD (29). Our earlier studies have shown that Fra-1 mediates endotoxin-induced lung-inflammatory responses (30), suggesting that this transcription factor plays a larger role in regulating lung inflammation induced by various toxic stimuli.

In addition to inflammatory cytokines and chemokines, MMPs secreted by infiltrated macrophages also contribute to the development of COPD/emphysema. Through their proteolytic activity, MMPs play an important role in ECM remodeling and in the destruction of alveolar walls, and also in inducing inflammatory responses in the lung (6, 31). Among various MMPs, MMP-1, MMP-9, and MMP-12 have been shown to play an important role in COPD pathogenesis, particularly emphysema (22, 32, 33). Overexpression of human MMP-1 causes progressive emphysema in mice (32). Elevated levels of MMP-9 were found in lung macrophages of patients with COPD (33, 34). Transgenic overexpression of human MMP-9 in macrophages leads to alveolar space enlargement in mice (35), but deletion of *MMP-9* in mice did not affect the severity of CS-induced emphysema (34). *MMP-12*–null mice show a diminished inflammatory response to CS and are resistant to the development of emphysema, suggesting that MMP-12 plays a critical role in CS-induced lung inflammation and emphysema (22). Although our results show that Fra-1 deficiency in myeloid cells significantly attenuated CS-induced *MMP-12* expression in BMDMs (Figure 3) and in the lung (Figure 5C), deleting it had no significant effect on the severity of emphysema (Figure 6). Because deletion of *Fra-1* did not completely block CS-induced MMP-12 expression (Figures 3 and 5C), it is likely that even the reduced MMP-12 present in the BAL fluid of *Fra-*$1^{\Delta/\Delta LysM}$ mice is sufficient to optimally promote airway space enlargement in our experimental settings. In addition, although inflammation can contribute to and

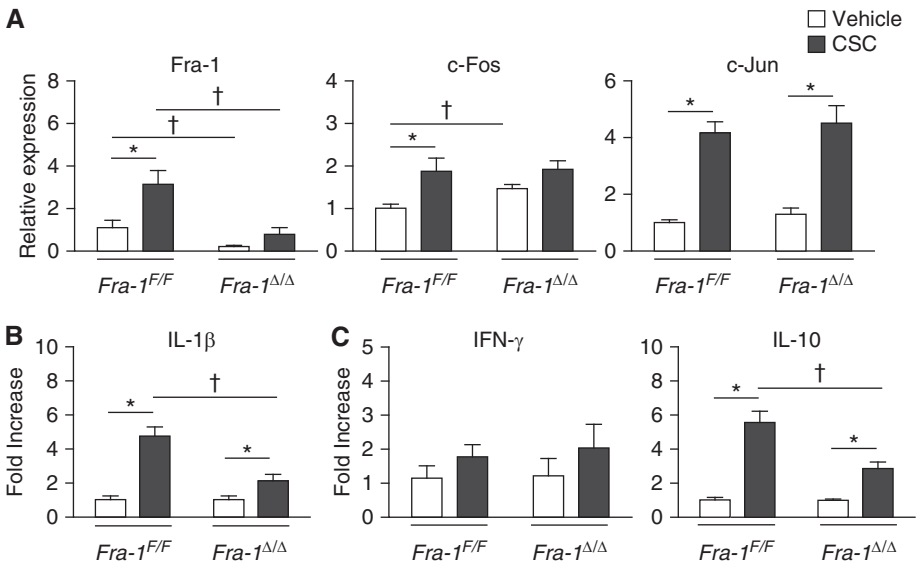

Figure 7. Effect of Fra-1 deletion on CS-induced inflammatory mediator expression in alveolar macrophages (AMϕs) *in vitro*. AMϕs isolated from Fra-$1^{F/F}$ and Fra-$1^{\Delta/\Delta}$ mice were treated with vehicle (DMSO) or CSC (100 μg/ml) for 6 hours. RNA was extracted, cDNA prepared, and expression of (*A*) Fra-1, c-Fos, and c-Jun, (*B*) IL-1β, and (*C*) M1- and M2-like macrophage phenotype markers were analyzed by qRT-PCR. Values in *A* show relative expression with respect to vehicle-treated Fra-$1^{F/F}$ cells, whereas values in *B* and *C* are expressed as fold increase with respect to their own vehicle controls. $^{*}P < 0.05$, DMSO versus CSC; †Fra-$1^{F/F}$ versus Fra-$1^{\Delta/\Delta}$ after CSC treatment. Data shown are means ± SD from at least three to four independent biological samples.

exacerbate COPD pathogenesis, both epithelial and endothelial cell death is required for the development of emphysema (36, 37). Lung epithelial cells also contribute to the secretion of proteases, such as MMPs and elastase (37–39). Therefore, whether a lack of difference in CS-induced lung alveolar space enlargement between the *Fra-*$1^{F/F}$ and *Fra-*$1^{\Delta/\Delta LysM}$ mice is due to the presence of a functional Fra-1 in other cell types of the lung is unclear. Further studies using lung–epithelial and endothelial-specific *Fra-1* knockout mouse models are warranted to ascertain this notion.

Several studies have shown differential responses of lung-resident AMΦs and infiltrated (exudated) macrophages (BMDMs) to infectious and noninfectious stimuli (40–42). Our data revealed that AMϕs and BMDMs respond differently to CS. In contrast to BMDMs, *MMP-9* and *TNF-α* expression was not induced by CS in AMΦs. *MMP-2* and *MMP-3* expression was not induced in either CS-stimulated *Fra-*$1^{\Delta/\Delta}$ or *Fra-*$1^{F/F}$ BMDMs (Figure E2), but their expression was stimulated to a similar extent both in *Fra-*$1^{\Delta/\Delta}$ and *Fra-*$1^{F/F}$ AMΦs (Figure E4). We noted a decreased expression of TIMP-2 in BMDMs treated with CS, but its expression was induced by CS in AMΦs. We also found that Fra-1 differentially regulates the expression of inflammatory mediators in AMϕs and BMDMs in response to CS. CS-induced MIP-1α and MIP-2α mRNA expression was significantly lower in *Fra-*$1^{\Delta/\Delta}$ than in *Fra-*$1^{F/F}$ BMDMs (Figure 2), but these differences were not noted between *Fra-*$1^{\Delta/\Delta}$ and *Fra-*$1^{F/F}$ AMΦs (Figure E2). However, IL-1β levels were lower in both CSC-treated *Fra-*$1^{\Delta/\Delta}$ AMΦs and supernatants from smoke-exposed *Fra-*$1^{F/F}$ BMDMs (Figures 2B and 7B). These results suggest that AMϕs and BMDMs respond differently to CS, and that Fra-1 distinctly regulates inflammatory and ECM gene expression in a cell-dependent manner. It has been recently shown that mouse tissue macrophages exhibit both shared and distinct gene expression profiles compared with infiltrated macrophages (43). In a different study, exudated (infiltrated) macrophages, but not resident AMΦs, were shown to produce TNF-α and nitric oxide synthase-2 and promote influenza infection–induced pathology (44). In response to noninfectious (bleomycin-induced) lung injury, exudated macrophages, but not AMΦs, produced CXCL10 (41). It is noteworthy that different culture conditions could affect macrophage cellular responses (45). In our studies, BMDMs are cultured for 7 days with L929-conditioned medium before treatment with CS, whereas AMΦs are isolated from the lung and treated with CSC immediately. This difference in culture conditions could, in part, explain the differences in Fra-1–mediated gene expression in response to CSC. Nonetheless, our *in vivo* results show significantly reduced levels of macrophage accumulation and inflammatory mediator expression in the lungs of *Fra-*$1^{\Delta/\Delta LysM}$ mice exposed to chronic CS (Figures 4 and 5A), suggesting that Fra-1 plays a key role in modulating lung-inflammatory responses during emphysema. It should be pointed out that the present study, although using *Fra-*$1^{F/F}$ mice as controls, lacks an experimental group with the wild-type mice expressing Cre, which could potentially interact with locus of X-over P1 (loxP)-like DNA sequences in the genome.

Macrophages are shown to polarize into either an M1-like (classically activated) or M2-like (alternatively activated) phenotype upon cigarette smoking (46). Fra-1 is known to alter tumor-associated macrophage polarization (47, 48). Thus, we studied whether Fra-1 affects macrophage polarization after CS exposure. As shown in Figure 2B, INF-γ levels were increased in supernatants from *Fra-*$1^{F/F}$ BMDMs after CSC treatment, whereas it was not detectable in their *Fra-*$1^{\Delta/\Delta}$ counterparts. IL-10 levels were modestly higher in the supernatant of *Fra-*$1^{F/F}$ BMDMS, but were undetectable in their *Fra-*$1^{\Delta/\Delta}$ counterparts. In contrast, CSC significantly induced IL-10, but not INF-γ mRNA expression, in AMΦs of both genotypes (Figure 7C). In mice exposed to chronic CS, INF-γ levels increased in the BAL fluids of both genotypes. However, IL-10 levels were modestly higher in the BAL fluid of *Fra-*$1^{F/F}$ mice, but were undetectable in *Fra-*$1^{\Delta/\Delta LysM}$ mice (Figure 5B). Macrophage polarization is a complex process consisting of multiple subtypes and requires very thorough analysis using appropriate markers, FACS analysis, identical culture conditions, and multiple time point analysis (45). Further detailed studies are needed to define the exact role of Fra-1 in macrophage polarization

caused by acute and chronic CS exposure conditions during the development of COPD.

We have previously shown that CS stimulates *Fra-1* expression at the transcription level via MMP-dependent epidermal growth factor receptor–mediated extracellular signal–regulated kinase 1/2 signaling (13, 49). In addition, we have shown that extracellular signal–regulated kinase 1/2 signaling is critical for cytokine (TNF-α) -induced *Fra-1* expression in lung epithelial cells (50). Fra-1 requires a dimerization partner, such as Jun (c-Jun, Jun-B, Jun-D) and ATF (ATF-4) family members to mediate its effects on gene transcription (10). Dimerization of Fra-1 with Jun proteins is thought to play a key role in suppressing chronic AP-1 activity mediated by the Jun/Fos dimeric complex, as Fra-1, unlike c-Fos, lacks a strong transactivation domain (10). We also found that not all cytokine expression induced by CS was modulated by Fra-1, suggesting that this transcription factor regulates cytokine gene expression in a contextual manner. Further studies using global RNA sequencing and chromatin immunoprecipitation sequencing are warranted to define whether Fra-1 selectively regulates CS-induced cytokine and ECM gene expression, and whether this occurs via a c-Jun–dependent and/or –independent manner using Fra-1– and/or c-Jun–deficient macrophages and mouse models.

To our knowledge, this study is the first to demonstrate that the Fra-1/AP-1 transcription factor plays a significant role in the regulation of CS-induced lung macrophagic inflammation during experimental emphysema. We propose that either depletion of Fra-1 or dampening its activation may be useful to mitigate chronic CS-induced inflammation *in vivo*. ■

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References

1. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: Molecular and cellular mechanisms. *Eur Respir J* 2003;22: 672–688.
2. Tetley TD. Macrophages and the pathogenesis of copd. *Chest* 2002;121 (5 suppl):156S–159S.
3. Finkelstein R, Fraser RS, Ghezzo H, Cosio MG. Alveolar inflammation and its relation to emphysema in smokers. *Am J Respir Crit Care Med* 1995;152:1666–1672.
4. Shapiro SD. COPD unwound. *N Engl J Med* 2005;352:2016–2019.
5. Barnes PJ. The cytokine network in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2009;41:631–638.
6. Churg A, Zhou S, Wright JL. Series "matrix metalloproteinases in lung health and disease": matrix metalloproteinases in COPD. *Eur Respir J* 2012;39:197–209.
7. Rajendrasozhan S, Chung S, Sundar IK, Yao H, Rahman I. Targeted disruption of NF-{kappa}B1 (p50) augments cigarette smoke–induced lung inflammation and emphysema in mice: a critical role of p50 in chromatin remodeling. *Am J Physiol Lung Cell Mol Physiol* 2010;298: L197–L209.
8. Rangasamy T, Cho CY, Thimmulappa RK, Zhen L, Srisuma SS, Kensler TW, Yamamoto M, Petrache I, Tuder RM, Biswal S. Genetic ablation of Nrf2 enhances susceptibility to cigarette smoke–induced emphysema in mice. *J Clin Invest* 2004;114:1248–1259.
9. Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. *Nat Rev Cancer* 2003;3:859–868.
10. Reddy SP, Mossman BT. Role and regulation of activator protein-1 in toxicant-induced responses of the lung. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1161–L1178.
11. Müller T. Expression of c-Fos in quiescent Swiss 3T3 cells exposed to aqueous cigarette smoke fractions. *Cancer Res* 1995;55: 1927–1932.
12. Chang WC, Lee YC, Liu CL, Hsu JD, Wang HC, Chen CC, Wang CJ. Increased expression of iNOS and c-Fos via regulation of protein tyrosine phosphorylation and MEK1/ERK2 proteins in terminal bronchiole lesions in the lungs of rats exposed to cigarette smoke. *Arch Toxicol* 2001;75:28–35.
13. Zhang Q, Adiseshaiah P, Reddy SP. Matrix metalloproteinase/ epidermal growth factor receptor/mitogen-activated protein kinase signaling regulate Fra-1 induction by cigarette smoke in lung epithelial cells. *Am J Respir Cell Mol Biol* 2005;32:72–81.
14. Li YT, He B, Wang YZ. Exposure to cigarette smoke upregulates AP-1 activity and induces TNF-alpha overexpression in mouse lungs. *Inhal Toxicol* 2009;21:641–647.
15. Reddy NM, Vegiraju S, Irving A, Paun BC, Luzina IG, Atamas SP, Biswal S, Ana NA, Mitzner W, Reddy SP. Targeted deletion of Jun/AP-1 in alveolar epithelial cells causes progressive emphysema and worsens cigarette smoke–induced lung inflammation. *Am J Pathol* 2012;180:562–574.
16. Cohen DR, Curran T. Fra-1: a serum-inducible, cellular immediate-early gene that encodes a Fos-related antigen. *Mol Cell Biol* 1988;8: 2063–2069.
17. Hirama N, Shibata Y, Otake K, Machiya J, Wada T, Inoue S, Abe S, Takabatake N, Sata M, Kubota I. Increased surfactant protein-D and foamy macrophages in smoking-induced mouse emphysema. *Respirology* 2007;12:191–201.
18. Eferl R, Hoebertz A, Schilling AF, Rath M, Karreth F, Kenner L, Amling M, Wagner EF. The Fos-related antigen Fra-1 is an activator of bone matrix formation. *EMBO J* 2004;23:2789–2799.
19. Knudsen L, Weibel ER, Gundersen HJ, Weinstein FV, Ochs M. Assessment of air space size characteristics by intercept (chord) measurement: an accurate and efficient stereological approach. *J Appl Physiol* 2010;108:412–421.
20. Shapiro SD. The macrophage in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:S29–S32.
21. Barnes PJ. Alveolar macrophages in chronic obstructive pulmonary disease (COPD). *Cell Mol Biol (Noisy-le-grand)* 2004;50 Online Pub: OL627–637.
22. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD. Requirement for macrophage elastase for cigarette smoke–induced emphysema in mice. *Science* 1997;277:2002–2004.
23. Schonthaler HB, Guinea-Viniegra J, Wagner EF. Targeting inflammation by modulating the Jun/AP-1 pathway. *Ann Rheum Dis* 2011;70: i109–i112.
24. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;153:530–534.
25. Chung KF. Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* 2001;34:50s–59s.
26. de Boer WI, Sont JK, van Schadewijk A, Stolk J, van Krieken JH, Hiemstra PS. Monocyte chemoattractant protein 1, interleukin 8, and chronic airways inflammation in copd. *J Pathol* 2000;190: 619–626.
27. Traves SL, Culpitt SV, Russell RE, Barnes PJ, Donnelly LE. Increased levels of the chemokines GROalpha and MCP-1 in sputum samples from patients with COPD. *Thorax* 2002;57:590–595.
28. Ishikawa T, Aoshiba K, Yokohori N, Nagai A. Macrophage colony–stimulating factor aggravates rather than regenerates emphysematous lungs in mice. *Respiration* 2006;73:538–545.

29. Kent L, Smyth L, Clayton C, Scott L, Cook T, Stephens R, Fox S, Hext P, Farrow S, Singh D. Cigarette smoke extract induced cytokine and chemokine gene expression changes in COPD macrophages. *Cytokine* 2008;42:205–216.
30. Vaz M, Reddy NM, Rajasekaran S, Reddy SP. Genetic disruption of Fra-1 decreases susceptibility to endotoxin-induced acute lung injury and mortality in mice. *Am J Respir Cell Mol Biol* 2012;46: 55–62.
31. Demedts IK, Morel-Montero A, Lebecque S, Pacheco Y, Cataldo D, Joos GF, Pauwels RA, Brusselle GG. Elevated MMP-12 protein levels in induced sputum from patients with COPD. *Thorax* 2006;61: 196–201.
32. Foronjy RF, Okada Y, Cole R, D'Armiento J. Progressive adult-onset emphysema in transgenic mice expressing human MMP-1 in the lung. *Am J Physiol Lung Cell Mol Physiol* 2003;284:L727–L737.
33. Russell RE, Culpitt SV, DeMatos C, Donnelly L, Smith M, Wiggins J, Barnes PJ. Release and activity of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2002;26:602–609.
34. Atkinson JJ, Lutey BA, Suzuki Y, Toennies HM, Kelley DG, Kobayashi DK, Ijem WG, Deslee G, Moore CH, Jacobs ME, *et al*. The role of matrix metalloproteinase-9 in cigarette smoke–induced emphysema. *Am J Respir Crit Care Med* 2011;183:876–884.
35. Foronjy R, Nkyimbeng T, Wallace A, Thankachen J, Okada Y, Lemaitre V, D'Armiento J. Transgenic expression of matrix metalloproteinase-9 causes adult-onset emphysema in mice associated with the loss of alveolar elastin. *Am J Physiol Lung Cell Mol Physiol* 2008;294: L1149–L1157.
36. Giordano RJ, Lahdenranta J, Zhen L, Chukwueke U, Petrache I, Langley RR, Fidler IJ, Pasqualini R, Tuder RM, Arap W. Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse. *J Biol Chem* 2008;283:29447–29460.
37. Mercer BA, Lemaitre V, Powell CA, D'Armiento J. The epithelial cell in lung health and emphysema pathogenesis. *Curr Respir Med Rev* 2006;2:101–142.
38. Qu P, Du H, Wang X, Yan C. Matrix metalloproteinase 12 overexpression in lung epithelial cells plays a key role in emphysema to lung bronchioalveolar adenocarcinoma transition. *Cancer Res* 2009;69:7252–7261.
39. Thorley AJ, Tetley TD. Pulmonary epithelium, cigarette smoke, and chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2007;2:409–428.
40. Herold S, Mayer K, Lohmeyer J. Acute lung injury: how macrophages orchestrate resolution of inflammation and tissue repair. *Front Immunol* 2011;2:65.
41. Tighe RM, Liang J, Liu N, Jung Y, Jiang D, Gunn MD, Noble PW. Recruited exudative macrophages selectively produce CXCL10 after noninfectious lung injury. *Am J Respir Cell Mol Biol* 2011;45:781–788.
42. Redente EF, Higgins DM, Dwyer-Nield LD, Orme IM, Gonzalez-Juarrero M, Malkinson AM. Differential polarization of alveolar macrophages and bone marrow–derived monocytes following chemically and pathogen-induced chronic lung inflammation. *J Leukoc Biol* 2010;88: 159–168.
43. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, Helft J, Chow A, Elpek KG, Gordonov S, *et al*. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* 2012;13: 1118–1128.
44. Lin KL, Suzuki Y, Nakano H, Ramsburg E, Gunn MD. $CCR2^{+}$ monocyte-derived dendritic cells and exudate macrophages produce influenza-induced pulmonary immune pathology and mortality. *J Immunol* 2008;180:2562–2572.
45. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, *et al*. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 2014;41:14–20.
46. Yuan F, Fu X, Shi H, Chen G, Dong P, Zhang W. Induction of murine macrophage M2 polarization by cigarette smoke extract via the Jak2/Stat3 pathway. *PLoS One* 2014;9:e107063.
47. Luo YP, Zhou H, Krueger J, Kaplan C, Liao D, Markowitz D, Liu C, Chen T, Chuang TH, Xiang R, *et al*. The role of proto-oncogene Fra-1 in remodeling the tumor microenvironment in support of breast tumor cell invasion and progression. *Oncogene* 2010;29: 662–673.
48. Wang Q, Ni H, Lan L, Wei X, Xiang R, Wang Y. Fra-1 protooncogene regulates IL-6 expression in macrophages and promotes the generation of M2D macrophages. *Cell Res* 2010;20:701–712.
49. Zhang Q, Adiseshaiah P, Kalvakolanu DV, Reddy SP. A phosphatidylinositol 3-kinase–regulated Akt-independent signaling promotes cigarette smoke–induced Fra-1 expression. *J Biol Chem* 2006;281:10174–10181.
50. Adiseshaiah P, Kalvakolanu DV, Reddy SP. A JNK-independent signaling pathway regulates TNF alpha–stimulated, c-Jun-driven Fra-1 protooncogene transcription in pulmonary epithelial cells. *J Immunol* 2006;177:7193–7202.