Comparison of Cigarette Smoke-Induced Acute Inflammation in Multiple Strains of Mice and the Effect of a Matrix Metalloproteinase Inhibitor on These Responses

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ABSTRACT

The activities of proteases in the lung, specifically matrix metalloproteinases (MMPs), have been implicated in driving the inflammation and lung destruction observed in smokers with chronic obstructive pulmonary disease. Here, our aims were to compare the acute response with cigarette smoke exposure (CSE) in four mouse strains to identify common and distinguishing features and to assess the effect of an MMP inhibitor on this response. To do this, we exposed mice (BALB/C, C57BL/6, A/J, or 129/Sv) to whole-body CSE (1 h/day) for 3 days. CSE induced dose- and time-dependent increases in neutrophils and keratinocyte chemoattractant levels in the airways of all strains; however, the proportion of the neutrophilia differed among strains. In the two most contrasting strains, BALB/C and C57BL/6, we examined MMP gene expression and found only small changes apart from MMP-12, which was highly expressed in both strains. Both strains were then treated with a broad-spectrum MMP inhibitor, PKF242-484 [(2S,3R)-N4-[(S)-2,2-dimethyl-1-methylcarbamoyl-propyl]-N1-hydroxy-2-hydroxymethyl-3-(4-methoxy-phenyl)-succinimide] (0.5–10 mg/kg) either orally or intranasally 1 h before and 5 h after CSE for 3 days. PKF242-484 dose-dependently reduced neutrophilia in BALB/C mice when dosed orally (p < 0.01) or intranasally (p < 0.01) but had no clear effect in C57BL/6 by either route. PKF242-484 reduced BAL macrophages when dosed intranasally (p < 0.05) but had no dose-dependent effect when dosed orally in both strains. These data suggest the inflammation induced by CSE is similar, but not identical, in different mouse strains. In addition, the ability of broad-spectrum MMP inhibitors to inhibit smoke-induced acute neutrophil inflammation is strain-dependent, whereas its ability to limit macrophage infiltration may be route dependent.

Chronic obstructive pulmonary disease (COPD) is a smoking-related syndrome that is currently the fifth leading cause of death worldwide and is projected to become the third leading cause of death by the year 2020. COPD has been defined as “airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious gases and particles” (Pauwels et al., 2001). Cigarette smoke (CS) elicits a repetitive inflammatory insult that is believed to, through the actions of mediators such as proteinases, lead to structural and functional changes in the lung (i.e., emphysema, airway remodeling, and airflow obstruction). Current anti-inflammatory approaches (e.g., steroids) have not been successful in treating this condition, suggesting that there is something unusual about the inflammatory response to CS exposure (CSE) that, at present, is not understood.

Because CS is the principle etiological factor for developing COPD (responsible for >80% of all cases), it has been used to...
develop preclinical in vivo models to investigate prospective disease mechanisms and evaluate the efficacy of candidate compounds. Most investigators have focused on using models of chronic CSE in mice. Some of these studies have demonstrated that there are significant differences in the responses among the different strains of mice (Cavarra et al., 2001; Triantafillopoulos et al., 2004). Understanding these differences may provide insights into potential (genetic) factors that influence COPD susceptibility (only ~25% of smokers are believed to develop COPD); however, understanding the common features of this response may also help us identify central mechanisms underlying the inflammatory response induced by CSE. Furthermore, identifying compounds that inhibit CSE-induced inflammation in multiple strains implies a greater probability for it working in other species, such as man. Two groups have characterized the changes that occur after short-term (<1 week) CSE in multiple strains of mice (Vlahos et al., 2006; Yao et al., 2008). These studies have demonstrated that there are some differences in the degree of the response among the strains; however, the mechanistic consequences of these differences have not been investigated. 

Therefore, we had two aims for the studies reported here. First, we intended to characterize the kinetics of CSE-induced acute inflammation in multiple strains of mice (which had not previously been done) to identify common and distinct features of the response among the different strains. The strains chosen for these studies are commonly used in other models of lung inflammation and were a mix of those studied previously (Vlahos et al., 2006; Yao et al., 2008). The second aim was to determine whether strains that differed in their response to cigarette smoke responded differently to pharmacological intervention. We specifically focused on one mechanism that has been shown to be important to both the acute and chronic response to CSE: the activity of matrix metalloproteinases (MMPs). MMPs are believed to drive the inflammation and pathological changes associated with CSE through their ability to activate latent cytokines and degrade components of the lung matrix (Churg et al., 2002, 2003; Houghton et al., 2006). MMP-9 and -12, in particular, have been implicated in COPD because the expression of these proteinases is elevated in the lungs of COPD patients and appear to localize to areas of the lung that are diseased (Segura-Valdez et al., 2000; Grunelli et al., 2004). In mice, MMP-12 has been shown to be important for mediating CSE-induced acute inflammation by activating latent, membrane-bound tumor necrosis factor-α (Churg et al., 2003) and generating chemotactic matrix fragments (Churg et al., 2002; Houghton et al., 2006). In addition, mice deficient for MMP-12 have less inflammation and appear to be completely protected from developing emphysema after chronic CSE (Hautamaki et al., 1997). These data suggest that MMP-12 is central to the response to CSE in mice; however, most of these studies were conducted in a single strain. Thus, we assessed the anti-inflammatory efficacy of a broad-spectrum MMP inhibitor, PKP242-484 (Trifili et al., 2002), in a mouse model of short-term CSE-induced acute inflammation using the two strains whose responses differed most in this system.

In the data reported here, we demonstrate that the principle acute response to CSE shared by each strain is an increase in airway neutrophilia. There are, however, some disparities in the degree of this response among the strains, and we also show that PKP242-484 has different anti-inflammatory effects in the two strains in which it was tested.

Materials and Methods

Materials. BALB/C and C57BL/6 mice were obtained from Charles River (Margate, Kent, UK). A/J and 129/Sv mice were obtained from Harlan UK Limited (Bicester, Oxon, UK). PKP242-484 (Trifili et al., 2002) was made in-house (Novartis Institutes for BioMedical Research, Basel, Switzerland). University of Kentucky Research Cigarettes (brand 1R3F) were obtained from the University of Kentucky (Louisville, KY). All enzyme-link immunosorbent assays (ELISAs) were purchased from R&D Systems Europe Ltd (Abingdon, Oxfordshire, UK).

Animal Maintenance Conditions. Female (BALB/C, C57BL/6, 129/Sv, A/J) mice (16–20 g) were housed in rooms maintained at constant temperature (21 ± 2°C) and humidity (55 ± 15%) with a 12-h light cycle and 15 to 20 air changes/h. Ten animals were housed per cage containing two nest packs filled with grade 6 sawdust (Datessand Group, Manchester, UK), nesting material (Enviro-Dri, Lillico, UK), maxi fun tunnels, and Aspen chew blocks (Lillico, UK) to provide environmental enrichment. Animals were allowed food, RM1 Pellets (SDS, Witham, Essex, UK), and water ad libitum.

Statement on Animal Welfare. Studies described herein were performed under a Project License issued by the United Kingdom Home Office, and protocols were approved by the Local Ethical Review Process at Novartis Institutes for BioMedical Research (Hornasham, UK).

Cigarette Smoke Exposure Methodology. CSE was performed as described by Stevenson et al. (2005). In brief, CS was delivered in 40-ml puffs of smoke every 60 s with fresh air being pumped in for the remaining time. The smoke was generated using 1R3F Research Cigarettes (University of Kentucky) and was drawn into the chambers via a peristaltic pump. This exposure regimen generated 481 mg/m² total suspended particulate mass in the exposure chambers. This was measured by dividing the difference of the mass of exposed and unexposed Whatman glass microfiber filters by the volume of air sampled from the chambers through the filter beginning at the 12th min of the exposure period for a total of 3 min. Sham and age- and sex-matched control animals were exposed to air only in the same manner for 50 min, which was the same duration of exposure for animals exposed to five cigarettes (i.e., approximately 10 min/cigarette used during the exposure period). This regimen was repeated for 3 consecutive days.

CSE Dose-Response Studies. Female mice from four strains (BALB/C, C57BL6, 129/Sv, and A/J) were exposed to two, three, four, or five cigarettes per exposure period and sham controls were exposed to room air only as described above. Animals were sacrificed with an overdose of terminal anesthetic (200 mg i.p. of sodium pentobarbitone) followed by exsanguination 24 h after the last exposure.

CSE-Induced Inflammation Time Course Studies. Female mice from four strains (BALB/C, C57BL6, 129/Sv, and A/J) were exposed to five cigarettes per exposure period, and sham controls were exposed to room air only as described above. Animals were sacrificed with an overdose of terminal anesthetic (200 mg of sodium pentobarbitone i.p.) followed by exsanguination 3, 6, 24, 48, and 72 h after the last exposure. There were sham time-matched controls for each time point.

Evaluating the Effect of PKP242-484 on CSE-Induced Inflammation. Animals (female, BALB/C, or C57BL/6) were dosed with either vehicle or compound, PKP242-484 (0.5–10 mg/kg), 1 h before and 5 h after each sham or smoke exposure (five cigarettes for BALB/C and four cigarettes for C57BL/6 per exposure period). After the preliminary dose-response and time course studies, a small number of C57BL/6 mice began to show some signs of distress during the exposure period in subsequent studies; therefore, we chose to reduce the duration of the exposures (to 40 min) for both smoke- and...
sham-exposed C57/BL6 mice during the compound studies to ensure their exposure level was not too high. Two routes of administration were evaluated, intranasally and orally. For intranasal studies, animals were treated with 0.025 ml of compound or vehicle (phosphate-buffered saline) under isoflurane/oxygen/nitrous oxide anesthesia. For oral studies, animals were administered 0.2 ml of either compound or vehicle (polyethylene glycol 300). There was one exception, the study of which C57/BL6 mice were dosed with 0.5, 1, and 5 mg/kg PKP242-494 where the dosing volume for compound and vehicle was 0.1 ml.

Preparation of Bronchoalveolar Lavage Fluid and Lung Tissue Leukocytes. After exsanguination, the lungs were lavaged using a cannula inserted into the trachea and instilling the lungs with 4 × 0.3-ml aliquots of sterile phosphate-buffered saline. All aliquots were combined for individual mice. Cytospins were prepared for differential staining. The cells were then spun down (300g) and resuspended in 0.5 ml of methyl violet fixative to perform total cell counts using a hemocytometer. Differential cell counts were performed using standard morphological criteria on Hema-Gurr-stained cytospins (300 cells/sample) (Merck (Darmstadt, Germany). Leukocyte numbers were determined by multiplying the percentage of each cytospin (300 cells/sample) (Merck (Darmstadt, Germany). Leukocyte subpopulation with the total number of cells for each sample and expressed as cells per milliliter for bronchoalveolar lavage (BAL) cells.

Preparation of Bronchoalveolar Lavage Fluid and Lung Tissue Cytokine Analysis. After collection, aliquots of cell-free BAL fluid and lung tissue were frozen in liquid N2 and stored at −80°C. Tissues were further processed before cytokine analysis by homogenizing approximately 250 mg of tissue in 2 ml of ice-cold homogenization buffer (saline with 1 mg/ml protease inhibitor cocktail; Roche Diagnostics, Mannheim, Germany). Homogenates were spun at 800g, and the supernatants along with cell-free BAL fluid were analyzed for cytokine levels by ELISA. KC (mouse GRO-α homolog) was measured using ELISA Duo-Sets from R&D Systems Europe Ltd. Tissue homogenate supernatant protein levels were measured using the Bio-Rad Protein assay (Bio-Rad, Hemel Hempstead, UK), and cytokine values were normalized against protein levels for individual homogenate samples. Bronchoalveolar lavage fluid protein levels were below the level of detection of the assay.

Examining CSE-Induced MMP Gene Expression in Lung Tissue. Total RNA was isolated from ~25 mg of lung tissue using the RNasy mini RNA isolation kit (QIAGEN, Dorking, Surrey, UK) according to the manufacturer’s instructions. First strand cDNA was prepared from total RNA using the reagents and protocol provided in the first strand cDNA synthesis kit (Roche Diagnostics). For quantitative PCR (TaqMan Analysis), messenger RNA levels in total RNA samples were measured by TaqMan reverse transcriptase-PCR. Inventoried TaqMan gene expression assays for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH), MMP-9, MMP-12, MMP-8, MMP-2, tissue inhibitor of metalloproteinase (TIMP) 1, and TIMP2 were purchased from Applied Biosystems (Foster City, CA). Quantitative reverse transcriptase-PCR reactions were performed in triplicate in 20-μl final volumes and contained final concentrations of 1× TaqMan Universal PCR master mix with 10 ng of the target cDNA preparation in each reaction. Experiments were performed using an ABI PRISM 7900 sequence detector (Applied Biosystems) and analyzed using ABI PRISM 7900 Sequence Detection System software. Amplification conditions were as follows: 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The data were quantitated by extrapolation from the standard curve, normalized to GAPDH, and the mean ± S.E.M. was plotted.

Statistical Analysis. All data are presented as mean ± S.E.M. For CSE dose-response studies, a Kruskal-Wallis test with a post hoc Dunn’s multiple comparison test was used. For time course studies, a Student’s t test was used comparing all smoke-exposed animals with their corresponding time-matched sham-exposed controls. For the compound dose-response studies, a one-way analysis of variance with Dunnett correction for multiple comparisons was used. A p value of less than 0.05 was considered significant.

Results

CSE-Induced Changes in BAL Inflammatory Cell Numbers. CSE-induced a predominantly neutrophilic inflammation in the lungs of all four strains of mice in a dose-dependent fashion (Fig. 1, A–D). The greatest numbers of infiltrating neutrophils were recovered in the BAL fluid of

![Fig. 1. Dose response of CSE-induced BAL neutrophil infiltration. CSE induces a dose-dependent increase in the number of BAL neutrophils in BALB/C (A), C57BL/6 (B), A/J (C), and 129/Sv (D) mice. Data plotted as the mean ± S.E.M., with n = 10 for each group. Significance (*, p < 0.05; **, p < 0.01; *** p < 0.001) was determined versus the sham control group.](image-url)
CSE also increased KC levels in the BAL of A/J mice (Fig. 1C). The fewest numbers of neutrophils were recovered in the BAL of 129/Sv (Fig. 1D) and C57BL/6 (Fig. 1B) mice. There were no changes in macrophages and lymphocytes recovered in the lavage fluid in any strain (data not shown). Proportionally, the BALB/C mice had the greatest percentage of neutrophils in the BAL fluid (60 ± 3% after five cigarettes), and C57BL/6 had the lowest (27 ± 3%).

CSE-Induced Changes in BAL Neutrophil Chemokine Levels. CSE also increased the neutrophil chemokine, KC (murine GRO-α homolog), in a dose-dependent fashion in the BAL fluid of all four strains (Fig. 2, A–D). The greatest levels achieved were in 129/Sv mice in the group exposed to four cigarettes (Fig. 2D). However, this was the only dose in 129/Sv mice to have increased levels of KC in the BAL. BALB/C and A/J mice (Fig. 2, A and C, respectively) had increases in BAL KC levels in the groups exposed to three, four, and five cigarettes. Again, the overall lowest levels measured were in C57BL/6 mice (Fig. 2B).

CSE-Induced Changes in Lung Tissue Neutrophil Chemokine Levels. CSE also increased KC levels in the lung tissue in a dose-dependent fashion in all four strains of mice (Fig. 3, A–D). Similar levels were measured in BALB/C and 129/Sv mice in the groups exposed to four cigarettes (p < 0.001 for BALB/C and p < 0.001 for 129/Sv) (Fig. 3, A and D, respectively). The levels in BALB/C were only marginally less than the A/J and 129/Sv groups exposed to five cigarettes. Again, the lowest levels measured were in C57BL/6 mice (Fig. 3C).

Time-Dependent Changes in Lung KC Levels after 3 Days of CSE. Because five cigarettes elicited the greatest and most consistent response across all four strains, it was the dose of CS chosen for studies investigating the kinetics of the inflammatory response. The levels of KC in the lung tissue were increased at maximal levels at the earliest time point examined in BALB/C and A/J mice (Fig. 4, A and C, respectively). In 129/Sv mice, the levels were increased at 3 h and peaked 24 h after the final exposure (Fig. 4D). The maximal concentrations of KC measured were similar in BALB/C, A/J, and 129/Sv mice, and levels began to subside after 24 h, returning to control levels by 72 h. The levels in C57BL/6 mice followed a similar pattern as the 129/Sv mice but failed to reach significance at any time point examined (Fig. 4B).

Time-Dependent Changes in BAL Fluid KC Levels after 3 Days of CSE. Levels of KC appeared to peak slightly later in the BAL fluid than they did in the lung tissue. In BALB/C mice, levels of KC in the BAL fluid were increased at 3 h (p < 0.01) and reached maximal levels at 6 h (p < 0.001) after the final exposure (Fig. 5A). In the three other strains, the levels were increased at 3 h and peaked 24 h after the final exposure (Fig. 5, B–D). The levels began to subside after 24 h, returning to control levels by 72 h in all four strains. The maximal concentration of KC measured was greatest in BALB/C and lowest in C57BL/6.

**Fig. 2.** Dose-response of CSE-induced BAL KC levels. CSE induces a dose-dependent increase in the KC in the BAL fluid of BALB/C (A), C57BL/6 (B), A/J (C), and 129/Sv (D) mice. Data plotted as the mean ± S.E.M., with n = 10 for each group. Significance (*, p < 0.05; **, p < 0.01; ***, p < 0.001) was determined versus the sham control group.
neutrophils in the lavage fluid (63 ± 3%), and C57BL/6 mice had the lowest (13 ± 3%) 24 h after CSE.

**Time-Dependent Changes in BAL Macrophage Numbers after 3 Days of CSE.** In BALB/C (p < 0.001) and A/J (p < 0.05) mice, the number of macrophages recovered in the BAL fluid was only increased 72 h after the final exposure (Fig. 7, A and C, respectively). It is interesting that the number of macrophages recovered in the BAL fluid of C57BL/6 mice was increased at 3 and 6 h after the final exposure (p < 0.05) (Fig. 7B). In addition, C57BL/6 mice had the highest proportion of macrophages in the BAL fluid 3 and 6 h after the third exposure to CS (84 ± 2 and 83 ± 3%, respectively), whereas BALB/C mice had the lowest (36 ± 4 and 38 ± 3%, respectively).

**Time-Dependent Changes in BAL Lymphocyte Numbers after 3 Days of CSE.** The number of lymphocytes recovered in the BAL fluid of all four strains was much lower than the numbers of neutrophils and macrophages recovered (Figs. 6–8). In BALB/C, C57BL/6, and 129/Sv mice, the number of lymphocytes increased marginally at the earliest time...
point examined and was only significant in BALB/C mice ($p < 0.05$) (Fig. 8, A, B, and D, respectively). In BALB/C and C57BL/6, the number of lymphocytes recovered in the BAL fluid were increased 24 h and remained elevated up to 72 h after the final exposure (Fig. 8, A and B, respectively). Lymphocyte numbers were also elevated 48 and 72 h after exposure in A/J mice (Fig. 8C). The number of lymphocytes recovered in the BAL of 129/Sv mouse was variable and was not significant at any time point (Fig. 8D).

**Time-Dependent Changes in Matrix Metalloproteinase Expression in Lung Tissue after 3 Days of CSE.** Investigations into the expression of matrix metalloproteinases after an acute (3 days) exposure to cigarette smoke were conducted in BALB/C and C57BL/6 mice. BALB/C mice were chosen because the inflammatory changes consistently appeared to be the greatest in this strain. The reason for using C57BL/6 was that the inflammation induced by smoke in these mice appeared to be different from the other three

![](image1)

**Fig. 5.** Time course of KC levels in BAL fluid after 3 days of CSE. CSE induced a time-dependent increase in KC levels in the BAL fluid of BALB/C (A), C57BL/6 (B), A/J (C), and 129/Sv (D) mice. Data from smoke-exposed mice are represented by black squares connected by solid black lines. Data from sham controls were represented by gray triangles connected by gray hashed lines. Data plotted as the mean ± S.E.M., with $n = 5$ for each group. Significance ($*$, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) was determined versus the sham control group.

![](image2)

**Fig. 6.** Time course of neutrophil infiltration after 3 days of CSE. CSE induced a time-dependent increase in neutrophil numbers recovered in the BAL fluid of BALB/C (A), C57BL/6 (B), A/J (C), and 129/Sv (D) mice. Data from smoke-exposed mice are represented by black squares connected by solid black lines. Data from sham controls were represented by gray triangles connected by gray hashed lines. Data plotted as the mean ± S.E.M., with $n = 10$ for each group. Significance ($*$, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) was determined versus the sham control group.
strains; i.e., a greater component of the inflammation coming was from macrophages in this strain versus neutrophils in the three other strains.

There were no consistent changes in the expression of MMP-9 in the lung tissue after 3 days of CSE, although there were small decreases in C57BL/6 mice at some time points (Fig. 9, A and B). In general, the expression of MMP-9 was higher in C57BL/6 compared with BALB/c mice. MMP-12 was increased in both strains (p < 0.001) in a time-dependent fashion and to similar levels relative to GAPDH (Fig. 9, C and D).

In general, the expression of MMP-2 was reduced in both strains after smoke (p < 0.01) (Fig. 10, A and B), with the reduction appearing to be greater in BALB/C mice. In addition, there appeared to be greater expression of MMP-2 in BALB/C versus C57BL/6 mice. MMP-8 expression was increased at 3, 48, and 72 h after exposure in C57BL/6 mice (p < 0.05) (Fig. 10C). In BALB/C mice, MMP-8 levels were only increased 3 h after exposure (p < 0.01) (Fig. 10D) and subsequently returned to control levels.

CSE increased TIMP-1 expression in both C57BL/6 and BALB/C mice (Fig. 11, A and B, respectively). TIMP-1 ex-
pression levels were greater and consistently up-regulated in the lungs of BALB/C mice between 3 (\(p < 0.01\)) and 48 (\(p < 0.01\)) h after CSE. In general, the expression of TIMP-2 was reduced in both strains after CSE (Fig. 11, C and D). The decrease in TIMP-2 expression was more consistent in BALB/C mice, with levels reduced 3, 6, and 48 h after CSE.

Effect of Twice-Daily, Prophylactic, Oral Administration of PKF242-484 on CSE-Induced Inflammation.

In BALB/C mice, 3 days of CSE induced an increase in BAL neutrophil numbers (\(p < 0.01\)) (Fig. 12A). PKF242-484 dose-dependently reduced the number of neutrophils recovered in the lavage fluid. The greatest level of inhibition was achieved at the 10 mg/kg dose, where there was a 46% reduction in BAL neutrophils (\(p < 0.01\)). There were no significant changes in macrophages, except for an increase in the number recovered from the group dosed with 10 mg/kg PKF242-484 relative to sham vehicle controls (Fig. 12B).

In C57BL/6 mice, 3 days of CSE also increased BAL neu-
trophil numbers ($p < 0.01$) (Fig. 12C). PKF242-484 dosed in the same manner, but at slightly different doses, had no effect on neutrophil numbers in C57BL/6 mice. CSE did increase BAL macrophage numbers in C57BL/6 mice ($p < 0.01$), and PKF242-484 only reduced the numbers of macrophages recovered in the group receiving the lowest dose (0.5 mg/kg; $p < 0.05$) and trended toward increasing at higher doses (Fig. 12D).

**Effect of Twice-Daily, Prophylactic, Intranasal Administration of PKF242-484 on CSE-Induced Inflammation.** In BALB/C mice, 3 days of CSE induced an increase in BAL neutrophil numbers ($p < 0.01$) (Fig. 13A). PKF242-484 dose-dependently reduced the number of neutrophils recovered in the lavage fluid. The greatest level of inhibition was achieved at the 10 mg/kg dose, where there was a 62% reduction in BAL neutrophils ($p < 0.01$).
increasing BAL macrophage numbers but did not achieve significance \( (p = 0.07) \). PKF242-484 at 10 mg/kg did reduce macrophage numbers compared with the CSE-vehicle control group \( (p < 0.05) \) (Fig. 13B).

In C57BL/6 mice, 3 days of CSE also increased BAL neutrophil numbers \( (p < 0.01) \) (Fig. 13C). PKF242-484 dosed intranasally had no dose-dependent effect on neutrophil numbers in C57BL/6 mice, but there was a significant reduction in the group receiving 3 mg/kg. In a second study, which was a repeat of this same experiment, PKF242-484 had no effect on BAL neutrophil numbers at any dose tested (data not shown). CSE did increase BAL macrophage numbers in C57BL/6 mice \( (p < 0.01) \). PKF242-484 at 10 mg/kg reduced these numbers of BAL macrophages back to control levels \( (p < 0.05) \) (Fig. 13D).

**Discussion**

The predominant change in response to short-term (3 days) CSE was an increase in neutrophil numbers in the BAL fluid and neutrophil chemokines in both BAL fluid and the lung tissue. The kinetics of the inflammation saw neutrophils peak 24 h after the third CSE. Neutrophil infiltration was preceded or paralleled by increases in KC levels, suggesting that this may be a primary mechanism for driving the acute response to CSE. This conclusion is supported by previous reports showing that CXCR2 antagonists attenuate the acute inflammatory response to CSE in both C57BL/6 mice (Thatcher et al., 2005) and Sprague-Dawley rats (Stevenson et al., 2005). After BAL neutrophil numbers peaked, increases in BAL lymphocytes and macrophages followed, the exception being in C57BL/6 mice, where macrophage numbers increased immediately after CSE. In general, this pattern of neutrophil infiltration followed by macrophage and lymphocyte influx agrees with the kinetics of most models of acute neutrophilia in both the airways (Corteling et al., 2002) and other organs, such as the skin (MacKay et al., 1985).

Overall, the types of changes in response to CSE were similar among the strains, although the degree of the changes did vary. In general, BALB/C mice consistently had the greatest response to CSE (i.e., proportion and numbers of neutrophils and levels of KC in the BAL fluid), whereas C57BL/6 mice consistently had the smallest response. These data are consistent with observations reported by Vlahos et al. (Vlahos et al., 2006). In addition, these observations about the different degree of inflammation among these strains are similar to those made from other models of acute lung inflammation, such as the lipopolysaccharide (Corteling et al., 2002) and allergen challenge (Trifili et al., 2000) models. Our data do not correlate entirely with that of Yao et al. (2008), who reported that C57BL6 mice were the most responsive to CSE compared with other strains, including A/J mice and 129/Sv mice, the latter being the least responsive among the strains tested in their study. Our exposure levels were comparable with those used by Yao et al., as was the degree of inflammation in C57BL6 mice; thus, the responses of A/J and 129/Sv mice to CSE are the main discrepancies between the two studies. The reason for this difference is unknown at present. Because responses in BALB/C and C57BL/6 mice differed most in our model system, we studied whether there were mechanistic differences by which CSE induced inflammation in the lungs of these two strains. In addition, because studies investigating the effects of CSE in mice are typically conducted using C57BL6 mice, it is important to identify mechanisms that may be either strain-dependent or -independent.

The activities of MMPs are believed to be central to mediating CSE-induced inflammation and airspace enlargement. To be specific, the activity of MMP-12 was shown to be required for the development of chronic CSE-induced inflam-
Pound's anti-inflammatory effects is that it reduced the inflammatory protein-2) production in response to PKF242/H9252 in cytokine (interleukin-1) or chemokine (KC, macrophage did not observe any clear effects on CSE-mediated increases reduced the neutrophilia in the BALB/C mice is unclear. We explain why these mice were more sensitive to the effects of the greater relative levels of MMP message expression in other model of lung inflammation (Churg et al., 2001). If that is the case, our observations (using a potent, broad-spectrum MMP inhibitor) would be consistent with and clarify their results, i.e., no effect of a broad-spectrum inhibitor (administered orally) on inflammatory cell (neutrophil and macrophage) infiltration in C57BL/6 mice. This does conflict, however, with other reports of reduced neutrophil infiltration in MMP-12-deficient mice (C57/BL6 background) (Churg et al., 2003; Leclerc et al., 2006) and after treatment with a specific MMP-12 inhibitor in C57BL/6 mice after acute CSE (Le Quément et al., 2008). One possible explanation for this discrepancy is that there are different effects using a broad-spectrum MMP inhibitor versus the selective inhibition of MMP-12. In addition, these studies (Leclerc et al., 2006; Le Quément et al., 2008) reported a greater degree of inflammation in the C57/BL6 mice after CSE than we observed (>2-fold greater), which may also account for the differences. The reason for the different level of acute inflammation is not clear, but one possibility is different levels of cigarette smoke exposure.

PKF242-484 administered orally and intranasally reduced CSE-induced neutrophilia in BALB/C mice where the neutrophil response was more robust. The differences in inflammatory cell profiles suggest that there was a different milieu of MMPs in the lungs after acute CSE in BALB/C versus C57BL/6 mice. We found limited evidence of this when looking at the expression of MMPs/TIMP at the message level (MMP-2, MMP-8, and TIMP-1), but the most consistent change in both strains was a time-dependent increase in MMP-12 message levels. Others have characterized MMP activity levels by zymography after short-term CSE (Seagrave et al., 2004) and its likely that the activities of MMP-2, -8, and -9 are up-regulated in this model because of their release from infiltrating inflammatory cells (i.e., not regulated transcriptionally). The fact there were greater numbers of inflammatory cells in the lungs in BALB/C mice may explain why these mice were more sensitive to the effects of an MMP inhibitor. This premise is supported somewhat by the greater relative levels of MMP message expression in BALB/C versus C57BL/6 mice after CSEs. How PKF242-484 reduced the neutrophilia in the BALB/C mice is unclear. We did not observe any clear effects on CSE-mediated increases in cytokine (interleukin-1β) or chemokine (KC, macrophage inflammatory protein-2) production in response to PKF242-484 treatment. Another possible explanation for the compound’s anti-inflammatory effects is that it reduced the production of chemotactic matrix fragments such as Pro-Gly-Pro, which works through the CXCR2 receptor to drive neutrophilia (Weathington et al., 2006).

It is interesting that PKF242-484 was only effective at reducing macrophage infiltration at the top dose (10 mg/kg) in both strains when dosed intranasally but had no effect when dosed orally At present, we cannot explain this anomaly; however, one possibility is systemic administration of PKF242-484 could not achieve high enough levels in the lung to inactivate MMPs involved in mediating macrophage recruitment to the lung. These data would suggest that inhaled delivery may be an attractive approach for the development of an MMP inhibitor for the treatment of COPD. This may not only enhance the anti-inflammatory attributes of MMP inhibition but may also limit the associated systemic (musculoskeletal) toxicities associated with such compounds (Belvisi and Bottomley, 2003).

An advantage to this model is its relative short duration compared with more chronic models that take on average 6 months from beginning to end. Pemberton et al. (2005) tested a broad-spectrum MMP inhibitor, ilomilast, in a chronic (C57BL/6) CSE model and demonstrated that dosed (intranasally) prophylactically and therapeutically the compound effectively attenuated the inflammation and emphysema induced by CSE. These data suggest that the acute model is not only a shorter model but also may be predictive of the effects of a compound in a more chronic inflammatory setting.

In summary, CSE induces dose- and time-dependent inflammatory changes in all strains of mice examined; however, the extent and timing of some of these changes differ among the strains. We evaluated the effects of a broad-spectrum MMP inhibitor in the most contrasting strains, BALB/C and C57BL/6. MMP inhibition attenuated CSE-induced neutrophilia in the strain with the more prominent lung neutrophil inflammation, BALB/C but had no effect on neutrophils in C57BL/6 mice. This disparity may be because of the contrasting levels of inflammatory cells in the lungs, thus providing a greater window to see the effect in BALB/C mice. Another possibility is that the different degree of inflammation could also change the relative contributions of various MMPs to the response. MMP inhibition did not affect macrophage numbers in either strain when dosed systemically but did when dosed topically, suggesting high levels of PKF242-484 were necessary in the lung to reduce BAL macrophage numbers.

These data suggest that this system can be used as a robust model to evaluate the efficacy of prospective candidate compounds being developed for the treatment of COPD. The data also highlight the importance of testing compounds in multiple strains (or species) to understand which strain (or species) will be the most appropriate (mechanistically) for evaluating the in vivo efficacy of test compounds with respect to drug discovery. In addition, understanding these strain-dependent differences may also be important for understanding factors that influence the susceptibility of some smokers for developing COPD, whereas others do not.

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