Role of Matrix Metalloproteinases in the Inflammatory Response in Human Airway Cell-Based Assays and in Rodent Models of Airway Disease

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ABSTRACT
Since the discovery of the first matrix metalloproteinase (MMP), this ever-growing family of proteinases has been the subject of intense research. Although it was initially believed that MMPs were solely involved in matrix turnover and degradation, there are now data suggesting MMPs are actively involved in the inflammatory process. In previous studies, we have demonstrated an increase in MMP expression in human cell-based assays and in preclinical rat models of airway inflammation. Therefore, the aim of this study was to characterize the role of MMPs in these models by profiling the impact of a broad-spectrum MMP inhibitor. In lipopolysaccharide (LPS)-stimulated THP-1 cells and primary human lung tissue macrophages, the MMP inhibitor had no significant effect on the release of tumor necrosis factor-α, interleukin (IL)-8, IL-1β, growth-regulated oncogene-α, macrophage inflammatory protein-1α, or IL-6 whereas dexamethasone has a significant impact on all cytokines from both cell types. Similarly, in the more biologically complex LPS-driven rat model of airway inflammation, the MMP inhibitor did not have an impact on mediator release and cellular burden. The compound did, however, significantly reduce levels of lung MMP-9. Furthermore, in a “disease” model, the compound did not affect cellular inflammation but did significantly reduce elastase-induced experimental emphysema. In summary, these data demonstrate for the first time that MMPs do not play a role in the increase in inflammatory mediators or cellular burden observed in these preclinical models. However, they do appear to be involved in the elastase-driven breakdown of airway structure, which is not due to a direct effect of the stimulus.

Since the discovery of the first matrix metalloproteinase (MMP), this ever-growing family of proteinases has been the subject of intense research. Although it was initially believed that MMPs were solely involved in the turnover and degradation of the extracellular matrix, there is now data suggesting that MMPs act on cytokines, chemokines, and protein mediators to regulate various aspects of inflammation and immunity (expertly reviewed by Parks et al., 2004). The exact role of most MMPs in inflammation/immunity is not known and, in fact, it is not clear whether they are involved in promoting or reducing these responses. MMPs are believed to have an impact on many of the cytokines/chemokines involved in the inflammatory response, often with apparently opposing outcomes. In vitro, MMPs have been shown to reduce the activity of chemokines such as epithelial cell-derived neutrophil-activating peptide-78 (Van den Steen et al., 2003), while increasing the activity of others such as IL-8 (Van den Steen et al., 2000). They have been shown to release immobilized chemokine complexes, such as syndecan-1/IL-8 (Li et al., 2002) but also convert others (i.e., monocyte chemotactic protein family) into chemokine receptor antagonists (McQuibban et al., 2000). IL-1β is activated by MMP-driven proteolytic processing (Schonbeck et al., 1998) but is then degraded by MMPs (Ito et al., 1996). Although it is known that TNFα is converted to an active form by TNF-converting enzyme (TACE) (Black et al., 1997), studies have shown that MMPs can also be involved (English et al., 2000). In addition, in vitro studies have shown that an MMP inhibitor can
reduce trans-basement membrane neutrophil migration (Deleclaux et al., 1996). In vivo studies have shown that a dual MMP/TACE inhibitor and dual MMP/neutrophil elastase (NE) inhibitors reduced endotoxin-driven airway cellular inflammation (McCann et al., 1999; Trifilieff et al., 2002). In other models of respiratory diseases such as bleomycin-induced fibrosis and antigen-driven allergic inflammation, MMP inhibitors have been shown to inhibit cellular recruitment (Kumagai et al., 1999; Corbel et al., 2001a).

In previous studies, an increase in MMP expression has been described in our fully characterized human cell-based assays and preclinical rodent models of airway inflammation. In cultured THP-1 cells and primary human lung tissue macrophages, we have shown that the expression of a range of MMPs is increased after stimulation (Wong et al., 2005). Furthermore, we have shown a similar increase in MMP expression at the gene and protein level in a rat model of LPS-induced innate airway inflammation and an elastase-driven disease model of airway inflammation/experimental emphysema (McCluskie et al., 2004; Birrell et al., 2005; Wong et al., 2005). Therefore, the aim of this study was to determine the role of these proteases in the models by utilizing a broad-spectrum MMP inhibitor, Bay 17-4003. Through this series of studies we aim to study, for the first time, the role of MMPs in the inflammatory response in human cell-based assay systems. Finally, we will use this inhibitor to dissect out the role of MMPs in the more biologically complex innate response seen in the rodent airway after LPS challenge and in an elastase-driven “disease” model that exhibits inflammation and structural changes.

Materials and Methods

Effect of the MMP Inhibitor on MMP Activity: Fluorogenic Assay

This assay used a fluorogenic MMP substrate (Bachem UK, Merseyside, UK) (10 μM) for MMP-2 and -9, at a concentration slightly above the Km values for MMP-2 and MMP-9, and a concentration of MMP active enzyme (Merck Biosciences Ltd., Nottingham, UK) low enough to ensure that the substrate was in excess (30 μM for MMP-2 and 10 μM for MMP-9). The inhibitor (structure as in Fig. 1) was preincubated with MMP-2 or -9 for 20 min before addition of the substrate, which was then kept in the dark at room temperature for 2 h, and the fluorescence was measured using a fluorometer set at 330 ± 12 nm excitation and 405 ± 10 nm emission.

The Bayer MMP inhibitor

![Fig. 1. The structure of the Bayer broad-spectrum MMP inhibitor. The potency (K_i) of the compound on isolated enzymes is reported to be 16, 3.1, 3.7, 0.2, and 0.5 nM for MMP-1, MMP-2, MMP-3, MMP-9, and MMP-12, respectively (G. Benz, Bayer, personal communication).](image)

Effect of the Compound on LPS-Induced Cytokine Release from THP-1 Cells

The human monocytic cell line THP-1 was purchased from the European Collection of Cell Cultures (Health Protection Agency, Salisbury, Wiltshire, UK). The cells were grown in RPMI 1640 with Glutamax I (Invitrogen, Paisley, UK) supplemented with 10% FCS and 1% antibiotic and antimycotic solution (penicillin/streptomycin; Sigma-Aldrich, Poole, UK) at 37°C in a humidified atmosphere [95% air-5% (v/v) CO2].

On experiment days the cells were centrifuged at 1000 rpm for 5 min, the supernatant was discarded, and the pellet of cells were washed in serum-free RPMI 1640 with Glutamax I (Invitrogen) supplemented with 1% antibiotic and antimycotic solution (penicillin/streptomycin; Sigma-Aldrich). After a second centrifugation, the cells were resuspended in RPMI 1640 with Glutamax I, supplemented with 3% FCS and 1% antibiotic and antimycotic solution, and transferred to 24-well plates (400,000/well). Vehicle (DMSO, 0.1% v/v, final concentration), Bayer compound (0.001–1 μM; a gift from Bayer Plc, Newbury, UK), or dexamethasone (1 μM) was added to the wells for 60 min at 37°C in a humidified atmosphere [95% air, 5% (v/v) CO2], after which vehicle (RPMI 1640 with Glutamax I, supplemented with 3% FCS and 1% antibiotic and antimycotic solution) or LPS (0.1 μg/ml) was added, and the plate was then incubated at 37°C in a humidified atmosphere [95% air-5% (v/v) CO2] for 24 h. The supernatants were retained for cytokine protein expression by ELISA (TNFα, IL-8, IL-1β, GROα, MIP-1α, and IL-6, DuoSet; R&D Systems, Minneapolis, MN) and cell viability was assessed by trypan blue exclusion. TNFα convertase (TACE) inhibitor (Calbiochem, San Diego, CA) and IL-1β-converting enzyme (ICE) inhibitor (ICE inhibitor II; Calbiochem) were included as positive controls and tested as above at 1 μM. MMP-9 levels in the cell supernatants were determined using zymography in accordance with the manufacturer’s instructions (Invitrogen) and detailed in McCluskie et al. (2004).

Effect of the MMP Inhibitor on LPS-Induced Cytokine Release from Human Lung Tissue Macrophages

Human lung tissue macrophages were obtained from nondiseased, donor tissue that was not suitable for transplant as outlined below. Ethical approval for the study was obtained along with consent from relatives. Lung tissue was cut into small pieces and flushed with phosphate-buffered saline using a needle and syringe (without calcium and magnesium) (Sigma-Aldrich). The pooled cell suspension (consistently >99%) were incubated overnight, for treatment the vehicle was added (as above) at 0.001, 0.01, 0.1, or 1 μM. MMP-9 levels in the cell supernatants were determined using zymography in accordance with the manufacturer’s instructions (Invitrogen) and detailed in McCluskie et al. (2004).
Effect of the MMP Inhibitor on Porcine Pancreatic Elastase-Induced Airway Inflammation in the Rat

Animals. Male Wistar rats (150–180 g) were purchased from Harlan-Olac (Bicester, UK) and kept for at least 5 days before experiments were initiated. Food and water were supplied ad libitum. UK Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) Act 1986 were strictly observed. Experiments were conducted with $n = 8$ animals.

Rats were orally dosed with vehicle (0.5% methylcellulose and 0.2% Tween 80 in distilled water, 2 ml/kg) or Bayer compound (0.1, 1, or 10 mg/kg) 1 h before an aerosol challenge of endotoxin-free saline for 30 min (Fresenius Kabi; Warrington, UK) or LPS (0.3 mg/ml, *Escherichia coli* serotype 0111:B4; Sigma-Aldrich) in a Perspex box (600 × 240 × 350 mm). A positive standard, dexamethasone (1 mg/kg), was included in the experimental design.

Quantification of Airway Inflammation. Six hours after saline or LPS challenge, animals were euthanized with sodium pentobarbitone (200 mg/kg i.p.), and the trachea was cannulated. Bronchoalveolar lavage (BAL) cells were recovered from the airway lumen by flushing the airways with 10 ml/kg RPMI 1640 (Invitrogen) delivered through the tracheal cannula and removed after a 30-s interval. This procedure was repeated, and samples were then pooled for each animal.

The inflammatory cells were extracted from the lung tissue by collagenase digest as described by Birrell et al. (2005). Total white cell numbers in the BAL and lung tissue samples were determined on the F820 hematology analyzer (Sysmex, Linford Wood, Milton Keynes, UK). Cytospins of these samples were prepared by centrifugation of 100-μl aliquots in a cytospin (Shandon, Runcorn, UK) at 700 rpm for 5 min, low acceleration at room temperature. Slides were fixed and stained on a Hema-Tek 2000 (Ames, Elkhart, IN) with modified Wright-Giemsa stain. Three-part differential counts on 200 cells/slide were performed following standard morphological criteria, and the percentage of eosinophils, lymphomononuclear (LMN) cells, and neutrophils were determined.

Inflammatory Biomarker Level Determination in the Airways after LPS Challenge. Levels of MMP-9 were determined usingzymography as outlined above. The amounts of TNF-α, IL-6, and IL-1β in the BAL fluid (BALF) and lung tissue were determined using DuoSet ELISAs from R&D Systems, according to the manufacturer’s instructions. The detection limit of these assays was determined to be in the range of 1 to 5 pg/ml. The cytokine levels in the lung tissue were corrected for total protein content, which was measured using the Bradford assay. Myeloperoxidase activity was measured in lung tissue homogenate supernatants using o-dianisidine hydrochloride as the substrate/hydrogen donor as described in Mccluskie et al. (2004).

Effect of the MMP Inhibitor on Porcine Pancreatic Elastase-Induced Airway Inflammation

Before determining the impact of the MMP inhibitor on PPE-driven airway inflammation, we confirmed that the MMP inhibitor was indeed selective and did not have any direct effect on PPE. Bay 17-4003 or the positive control (elastase inhibitor III, Calbiochem) was prepared in DMSO (0.01, 0.1, and 10 μM, final concentration) and added to black plates (catalog no. 77600-04; BMG LABTECH GmbH (Offenburg, Germany) along with buffer (0.05 M Trizma base, 0.5 M NaCl, and 0.01 M CaCl₂, pH 7.5) containing 1 U/ml (final concentration) of PPE (Calbiochem) and left in the dark for 30 min. Then the fluorogenic substrate (N-methoxyxycinn-4-Val-Val-7-amido-4-hydrochloride as the substrate/hydrogen donor as described in Mccluskie et al. (2004).

Fig. 2. Effect of the Bayer compound on MMP-2 and MMP-9 activity. Vehicle (DMSO) or compound (0.01, 0.1, or 1 μM) was preincubated with active MMP-2 (A) or MMP-9 (B) for 20 min before addition of the fluorogenic substrate. Fluorescence was measured 2 h later using a fluorometer set at 330 ± 12 nm excitation and 405 ± 10 nm emission. The assay was performed in duplicate.

Values are expressed as means ± S.E.M. of $n$ independent observations. Statistical analysis was assessed by one-way ANOVA using the appropriate post-test analysis. $p < 0.05$ was considered to be statistically significant.

Results

Effect of the Bayer Compound on MMP Activity. To demonstrate the activity of the broad-spectrum MMP inhibitor, it was profiled in fluorogenic assays. Active MMP-2 or MMP-9 increased the amount of fluorescence in the assay and addition of the compound reduced the amount of fluorescence to the basal level (Fig. 2). The $IC_{50}$ of the compound on both...
MMP-2 and MMP-9 activity was approximately 10 nM, which is similar to that reported by Bayer Plc (see legend to Fig. 1).

**Effect of the Compound on LPS-Induced Cytokine Release from THP-1 Cells and Human Lung Tissue Macrophages.** LPS (0.1 μg/ml) caused a submaximal release of TNFα, IL-8, IL-1β, GROα, MIP-1α, IL-6, and MMP-9 from THP-1 cells and 0.01 μg/ml LPS caused a submaximal release of TNFα, IL-8, IL-1β, GROα, MIP-1α, and IL-6 from human lung tissue macrophages (Figs. 3, 4, and 5). Pretreatment with the compound had no significant impact on cytokine release from THP-1 cells, whereas the intra-assay positive control, dexamethasone, inhibited the release of all cytokines (Figs. 3 and 4). In comparison, the TACE inhibitor caused a significant 51% inhibition of TNFα release whereas the ICE inhibitor caused a significant 47% inhibition of IL-1β release. Neither inhibitor had a significant impact on the other cytokines measured (data not shown).

Unlike the intra-assay positive control, dexamethasone, the MMP inhibitor did not have a significant impact on MMP-9 levels in the supernatant (Fig. 4). There was no significant impact on cell viability by either the MMP inhibitor or dexamethasone (Fig. 4).

![Fig. 3. Effect of the compound on cytokine release from cultured THP-1 cells. Vehicle (DMSO, 0.1% v/v final concentration), compound (0.001, 0.01, 0.1, or 1 μM) or dexamethasone (1 μM) was added to the cells 1 h before stimulation with LPS (0.1 μg/ml). Twenty-four hours later, the supernatants were collected and assayed for cytokine levels by ELISA (A, TNFα; B, IL-8; C, IL-1β; D, GROα; E, MIP-1α; and F, IL-6). TACE inhibitor (1 μM) caused 51% inhibition of TNFα levels and ICE inhibitor (1 μM) caused 47% inhibition of IL-1β levels (both p < 0.05; neither had a significant impact on the other cytokines measured). Results represent means ± S.E.M. (n = 6). *, significantly different from vehicle stimulation control, +, significantly different from LPS stimulation control.](image-url)
The LPS challenge caused a significant increase in BALF TNFα and IL-β, which, unlike the positive control, dexamethasone, was not significantly affected by the MMP inhibitor (Fig. 6). To confirm that the lack of effect of the MMP inhibitor was not restricted to cytokines in the airway lumen, levels of cytokines were determined in the lung tissue. Similar to the result in the BALF, unlike the glucocorticoid, the MMP inhibitor did not have an impact on the LPS-induced increase in IL-1β and IL-6 levels in the lung tissue (Fig. 6). No significant increase in TNFα was observed in the lung tissue at this time point after LPS challenge (data not shown).

Aerosolized LPS caused a significant increase in BALF neutrophilia and eosinophilia and lung tissue neutrophilia (Fig. 7). Treatment with the MMP inhibitor failed to have any significant impact on LPS-induced lung cellular inflammation, whereas the positive control, dexamethasone, significantly inhibited neutrophilia and eosinophilia (Fig. 7). To determine whether the MMP inhibitor affected the activation status of the neutrophils myeloperoxidase levels were determined. Figure 6F clearly shows that the activation status of the neutrophils in the lung tissue was not affected by the MMP inhibitor. There was no significant change in LMN cell number in either the BALF or lung tissue by any treatment (data not shown).

**Effect of the Compound on PPE-Induced Airway Inflammation in the Rat.** The MMP inhibitor failed to have an impact on LPS-induced inflammation in the human cell-based assays or the more biologically complicated rodent model of lung inflammation. To determine whether the compound would have an effect on a more severe disease model, the MMP inhibitor was profiled in a fully characterized PPE-driven rodent model of lung inflammation (Birrell et al., 2005). Before this experiment was initiated, it was important to demonstrate that the selective MMP inhibitor did not affect the activity of the stimulus used in this preclinical model. Figure 8 clearly demonstrates that, unlike the positive control compound, elastase inhibitor III, Bay 17-4003 had no impact on the activity of PPE up to the highest concentration tested.

Challenge with PPE caused a significant increase in BALF and lung tissue neutrophilia and LMN cell number (Fig. 9). The MMP inhibitor failed to have a significant impact on the PPE-induced increase in lung cellular burden (Fig. 9). There was no glucocorticoid comparator in this study because we have previously shown this model to be steroid-resistant (Birrell et al., 2005).

We have previously shown that after PPE challenge the model exhibits aspects of emphysema, as measured by increases in average airspace area (Birrell et al., 2005). Even at this early stage of experimental emphysema development, i.e., 2 days after challenge rather than 4 weeks, PPE challenge caused a small significant increase in average airspace area (Fig. 10). Although the compound had no impact on inflammation, it appeared to cause a dose-related inhibition of experimental emphysema, which would suggest that the MMPs present are partially responsible for the change in lung structure (Fig. 10). Interestingly, the effect of the MMP inhibitor appeared to be bell-shaped with the highest dose tested having less impact. The high dose/vehicle challenge group had an increase in experimental emphysema compared with the vehicle dosed/vehicle challenge group (Fig. 10). Al-

**Fig. 4.** Effect of the compound on MMP-9 release from cultured THP-1 cells and cell viability. Vehicle (DMSO, 0.1% v/v final concentration), compound (0.001, 0.01, 0.1, and 1 μM), or dexamethasone (1 μM) was added to the cells 1 h before stimulation with LPS (0.1 μg/ml). Twenty-four hours later the supernatants were collected and assayed for MMP-9 levels by zymography (A). Cell viability was determined by trypan blue exclusion (B). Results represent means ± S.E.M. (n = 6). *p*, significantly different from vehicle stimulation control; †, significantly different from LPS stimulation control.

Similar to the result with the THP-1 cells, only the intra-assay positive control had any significant affect on cytokine release from primary human lung tissue macrophages (Fig. 5). In our hands the cultured human lung tissue macrophages do not release any more MMP-9 upon stimulation with LPS (data not shown).

**Effect of the Compound on LPS-Induced Airway Inflammation in the Rat.** To determine whether inhibition of MMP activity would have an impact on the inflammatory response in a more complex biological system, Bay 17-4003 was profiled in a fully characterized LPS-driven model of rodent airway inflammation (McCluskie et al., 2004). The MMP inhibitor caused a significant and dose-related inhibition of MMP-9 activity/release in BALF samples as measured by zymography (Fig. 6). However, Fig. 4 clearly shows that zymography is not appropriate for demonstrating the effect of the MMP inhibitor on MMP activity, which suggests that, in this experiment, the MMP inhibitor has reduced the amount of MMP-9 in the lungs. These data demonstrate that this compound inhibits rat MMP-9, as well as human MMP activity and that the dosing regimen used was appropriate.
though the reason for this is not known, it might explain why the highest dose of MMP inhibitor appeared not to affect the experimental emphysema.

Discussion

MMPs are important for homeostasis and turnover of the extracellular matrix in health and disease. Although it was initially believed that MMPs were solely involved in matrix turnover and degradation, there are now data suggesting that MMPs are actively involved in the inflammatory process. Interestingly, it has been demonstrated that the extracellular matrix is a source for several inflammatory cell chemotactic factors. In fact, fragments of elastin (Senior et al., 1980; Houghton et al., 2006) and collagen (Weathington et al., 2006) have been shown to be chemotactic and could therefore play a role in the inflammatory response.

We have previously described an increase in MMP expression in fully characterized human cell-based assays and preclinical rodent models of airway inflammation. More specifically, we have recently demonstrated in cultured THP-1 cells and primary human lung tissue macrophages an increased expression of a range of MMPs after LPS stimulation (Wong et al., 2005). Furthermore, in a rat model of LPS-induced innate airway inflammation and an elastase-driven disease model of airway inflammation and experimental emphysema, we have demonstrated a similar increase in MMP expression both at the gene and protein levels (McCluskie et al., 2004; Birrell et al., 2005; Wong et al., 2005). Therefore, the aim of this study was to determine the role of these proteases in the inflammatory responses in these models by using a broad spectrum MMP inhibitor. To our knowledge this is the first time the role of MMPs has been extensively profiled in a range of preclinical models of airway inflammation and the effect of an inhibitor assessed.

Interestingly, the MMP inhibitor failed to have any significant impact on the cytokine production from LPS-stimulated THP-1 cells, whereas the intra-assay positive control, dexamethasone, inhibited the release of all cytokines. In parallel studies, we confirmed that TNFα release was controlled by TACE and IL-1β release was controlled by ICE in this model system. In addition, the MMP inhibitor did not affect the MMP-9 zymography analysis of the cell supernatant from stimulated THP-1 cells (Fig. 4). As we have shown that the inhibitor blocks the activity of MMP-9 (Fig. 2), it would imply
that the compound is being “removed” from the active site on MMP-9 during the zymography process, possibly during the denaturing steps or through some of the washing phases. Therefore, the zymography result suggests that the MMP inhibitor, unlike the intra-assay positive control, is not having any effect on the amounts of MMP-9 produced. Similar to the THP-1 results, the MMP inhibitor did not have any significant effect on cytokine release from primary human lung tissue macrophages, although there did appear to be a small, nonsignificant reduction in some of the cytokines measured. We were not able to determine the effect on MMP-9 levels in the primary macrophages because, unlike the gene expression of other MMPs, we are unable to measure an increase in the release of this MMP after stimulation with LPS (Wong et al., 2005).

The absence of a significant effect of the MMP inhibitor on cytokine release from our cell-based assays would appear to be at odds with some of the published data discussed in the Introduction (Schonbeck et al., 1998; English et al., 2000). The reason for this is not clear but is likely to be due to the different cells and assay protocols used. Zhang et al. (2004) have recently shown that a dual TACE/MMP inhibitor reduces LPS-induced TNFα secretion in THP-1 cells, human primary monocytes, and human whole blood. Our data sug-

**Fig. 6.** Effect of the compound on LPS-induced inflammatory mediators in the rat lung. Rats were treated with vehicle (0.5% methylcellulose and 0.2% Tween 80 in distilled water, 2 ml/kg), compound (0.1, 1, or 10 mg/kg), or dexamethasone (1 mg/kg) orally 1 h before aerosolized saline or LPS (0.3 mg/ml, 30 min). Six hours after challenge, the animals were culled, and the inflammatory status of the airways was assessed. MMP-9 activity levels in the BALF were determined by zymography (A). The levels of TNFα (B) and IL-1β (C) in the BALF and IL-6 (D) and IL-1β (E) in the lung tissue homogenate were assessed by ELISA (DuoSets from R&D Systems) according to the manufacturer’s instructions. F, levels of myeloperoxidase in the lung tissue. Results represent means ± S.E.M. (n = 8). +, significantly different from vehicle stimulation control; *, significantly different from LPS stimulation control.
suggests that, at least in THP-1 cells, the inhibition observed by Zhang et al. (2004) was due to an effect on TACE activity.

When the impact of the MMP inhibitor on the inflammatory response observed in the more biologically complex in vivo LPS model was assessed, we observed a dose-dependent decrease in the MMP-9 zymography signal. This result suggests that the MMP inhibitor is reducing the amount of MMP-9, presumably through inhibition of the in vivo activity of one or more MMPs. There is evidence for the activity of MMPs to be involved in the production of MMPs; for example, Nenan et al. (2005) have shown that addition of MMP-12 can lead to an increase in gelatinase expression, and Selman et al. (2003) have shown that an MMP inhibitor reduces the amount of MMP-9 in a tobacco smoke-induced model of emphysema. Although in this study it is not clear how the MMP inhibitor is causing the reduction in MMP-9 levels or indeed which MMP is responsible, the study does clearly show that this compound inhibits rat MMP activity (as well as human MMP activity) and validates the use of the dosing regimen employed. This effect on MMP-9 levels was not associated with a reduction in any of the other “inflammatory” indices in this model. The compound appeared not to affect BALF and tissue cytokine levels, the increase in cellular burden in the airway lumen and tissue, or the activation status of the cells. The lack of effect of MMP inhibition on cellular burden in the in vivo LPS model agrees with some published in vivo studies by Corbel et al. (2001b) using an inhibitor and by Betsuyaku et al. (1999) with MMP-9 knockout mice. It ap-

Fig. 7. Effect of the MMP inhibitor on LPS-induced airway cellular accumulation. Rats were treated with vehicle (0.5% methylcellulose and 0.2% Tween 80 in distilled water, 2 ml/kg), compound (0.1, 1, or 10 mg/kg), or dexamethasone (1 mg/kg) orally 1 h before aerosolized saline or LPS (0.3 mg/ml, 30 min). Six hours after challenge, the animals were culled, and the inflammatory status of the airways was assessed. The number of BALF neutrophils (A) and eosinophils (B) and tissue neutrophils (C) was determined. Results represent means ± S.E.M. (n = 8). +, significantly different from vehicle stimulation control; *, significantly different from LPS stimulation control.

Fig. 8. Effect of the MMP inhibitor on PPE activity in an in vitro assay. The MMP inhibitor was incubated with PPE (1 U/ml) before addition of fluorogenic substrate. PPE activity was then assessed. Elastase inhibitor III was included as a positive control. Results represent means ± S.E.M. (n = 4).
pears, however, to contradict data published by Trifilieff et al. (2002) with a dual TACE/MMP inhibitor and by McCann et al. (1999) with dual MMP/NE inhibitors. The use of dual inhibitors in these studies makes determining the role of the MMPs in isolation on the cellular inflammation extremely difficult. In addition, it may also imply that it is TACE/NE activity or a combination of these activities in addition to the MMP activity that is involved in the innate inflammatory response to LPS in the lung. In summary, even though we have demonstrated an increase in MMP activity that was reduced by an inhibitor, it appears that MMPs play no role in the recruitment of response effector cells in the LPS-driven inflammation seen in this in vivo model.

It would appear from the literature that MMPs may play more of a role in cellular recruitment in “airway disease” models inasmuch as reports demonstrate that a reduction in MMP activity can protect against lung injury (Hautamaki et al., 1997; Kumagai et al., 1999; Corbel et al., 2001b; Li et al., 2002). For example, mice lacking MMP-12 are protected against the development of cigarette smoke-induced emphysema and from the accumulation of lung macrophages normally induced by chronic smoke exposure, an effect thought to be due to the generation of elastin fragments (Houghton et al., 2006). We therefore tested the same compound in a model of steroid-resistant airway inflammation and experimental emphysema (Birrell et al., 2005). Even though we have pre-
viously demonstrated an increase in MMP expression in this elastase-driven model, MMPs do not appear to play a role in cellular trafficking in the airways (Birrell et al., 2005; Wong et al., 2005). Interestingly, although the MMP inhibitor did not block cellular inflammation or have any direct effect on the stimulus used, it did cause a significant reduction in the early stage of experimental emphysema. This is a fascinating observation and suggests that the increased MMP activity caused by the inflammation may be driving the airway damage. It would be interesting to determine whether inhibition of MMP activity would have a significant effect on the fully developed emphysema lesion observed in this model 4 weeks after the insult. However, unfortunately insufficient compound was available to perform these studies. One issue with these data that should be discussed is the bell-shaped response curve exhibited by the MMP inhibitor on the elastase-driven model, MMPs do not appear to play a role in elastase-induced increase in average airspace area such that at the top dose tested the inhibition was lost. The reason for this loss is not known, but as we observed an increase in experimental emphysema in the high-dose vehicle-challenged group, it is possible that the compound has “off-target,” adverse side effects on lung structure at this dose.

In summary, the data presented here demonstrate that MMPs do not play a substantial role in inflammatory mediator production and cellular burden in a comprehensive array of preclinical models of airway inflammation. This study, however, does show for the first time that MMPs are integral in the elastase-induced, inflammation-driven experimental emphysema lesion.

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