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Altered lymphocyte trafficking and diminished airway reactivity in transgenic mice expressing human MMP-9 in a mouse model of asthma

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Mehra D, Sternberg DI, Jia Y, Canfield S, Lemaitre V, Nkyimbeng T, Wilder J, Sonett J, D'Armiento J. Altered lymphocyte trafficking and diminished airway reactivity in transgenic mice expressing human MMP-9 in a mouse model of asthma. *Am J Physiol Lung Cell Mol Physiol* 298: L189–L196, 2010. First published November 25, 2009; doi:10.1152/ajplung.00042.2009.—Matrix metalloproteinase-9 (MMP-9) is hypothesized to facilitate leukocyte extravasation and extracellular remodeling in asthmatic airways. Careful descriptive studies have shown that MMP-9 levels are higher in the sputum of asthmatics; however, the consequence of increased MMP-9 activity has not been determined in this disease. We induced asthma in transgenic mice that express human MMP-9 in the murine lung tissue macrophage to determine the direct effect of human MMP-9 expression on airway inflammation. Transgenic (TG) and wild-type (WT) mice were immunized and challenged with ovalbumin. Forty-eight hours after the ovalbumin challenge, airway hyperresponsiveness (AHR) was measured, and inflammatory cell infiltration was evaluated in bronchoalveolar lavage fluid (BALF) and lung tissue. Baseline levels of inflammation were similar in the TG and WT groups of mice, and pulmonary eosinophilia was established in both groups by sensitization and challenge with ovalbumin. There was a significant reduction in AHR in sensitized and challenged transgenics compared with WT controls. Although total BALF cell counts were similar in both groups, the lymphocyte number in the lavage of the TG group was significantly diminished compared with the WT group (0.25 ± 0.08 vs. 0.89 ± 0.53 ; $P = 0.0032$). In addition, the draining lymphocytes were found to be larger in the TG animals compared with the WT mice. Equal numbers of macrophages, eosinophils, and neutrophils were seen in both groups. IL-13 levels were found to be lower in the sensitized TG compared with the WT mice. These results demonstrate an inverse relationship between human MMP-9 and AHR and suggest that MMP-9 expression alters leukocyte extravasation by reducing lymphocyte accumulation in the walls of asthmatic airways.

airway remodeling; matrix metalloproteinases; bronchoalveolar lavage; airway hyperresponsiveness

TISSUE REMODELING IS A PROMINENT feature of inflammatory disorders of the airway and lung parenchyma. Histological studies of airway changes in asthma patients demonstrate increased subepithelial fibrosis and collagen deposition (4, 10, 11). Matrix metalloproteinase (MMP) activity has been implicated as key in this process because its physiological function is to cleave structural proteins, such as collagens and elastin.

MMPs are a family of zinc-binding endopeptidases that are secreted in a proform requiring cleavage for enzymatic activa-

tion (37). Individual MMPs contain unique domains that determine substrate specificity and the mechanism through which they are catalytically activated. MMP-9 is one of two family members referred to as a gelatinase because fibronectin II-like repeats within its catalytic domain confer a high binding affinity for gelatin and elastin (1, 40, 50). In the lung, MMP-9 is released into the intercellular compartment by a variety of cell types including macrophages, eosinophils, and airway epithelial cells (40). The multiple potential substrates in this compartment and the diversity of inflammatory cell types that express MMP-9 in the lung suggest broad involvement in the inflammatory response in this organ (40).

Several MMP family members have been studied in asthma and asthma models, but MMP-9 is the predominant MMP found in serum, bronchoalveolar lavage fluid (BALF), sputum, and transbronchial biopsy specimens of individuals with asthma (3, 47). Studies examining MMP-9 levels in patients, however, have yielded apparently conflicting results. The ratio of MMP-9 to TIMP-1 in sputum was reduced in asthmatics compared with controls, and an excess in TIMP-1, an endogenous inhibitor of MMP-9, has been proposed to favor airway remodeling and pathological progression in asthma (47). However, allergen challenge has been reported to increase MMP-9 levels and the MMP-9-to-TIMP-1 ratio (6, 32). Interestingly, MMP-9 knockout (KO) mice exhibit decreased cellular recruitment in the alveoli and in lavage fluid (34). Moreover, studies using MMP inhibitors suggest that inhibition of MMP activity decreases the intensity of inflammatory cell infiltrate and reduces airway hyperresponsiveness (AHR) in the ovalbumin (OVA) and toluene diisocyanate sensitization models of asthma (20, 23).

To better understand the functional contribution of MMP-9 in asthma, we examined the specific consequences of increased MMP-9 in the asthma model. This was done by inducing asthma in the MMP-9 transgenic (TG) mice at 3 mo of age before the development of lung disease (12). Based on previous studies, we hypothesized that MMP-9 would affect the fibrotic response in these mice. However, the expression of MMP-9 led to reduced lymphocyte extravasation in the lung and reduced AHR. These results present an additional role for MMP-9 in the modulation of the immune response.

MATERIALS AND METHODS

Generation of human MMP-9 TG mouse. We used the macrophage as a sensitization-dependent delivery system for MMP-9 in the lung. Our laboratory (26) has previously successfully used this system to examine the role of MMP-9 in atherosclerosis and fibrosis. The

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macrophage delivery system provides specific directed expression of the transgene (26, 27).

The 2.4-kb cDNA of human MMP-9 (gelatinase B) was placed under the control of the human scavenger receptor-enhancer/promoter (SREP; a gift from Dr. Chris Glass, University of California, San Diego). The human gelatinase B construct is shown in Fig. 1A. This promoter yields specific expression of the transgene in tissue macrophages and not in monocytes (17). The construct was microinjected into fertilized mouse eggs [F1 (C57BL/6 × CBA/J) × F1 (C57BL/6 × CBA/J)]. Lines that expressed the transgene were established through Southern blotting and PCR analysis. For the experiments described below, we used the line of mice that exhibited MMP-9 expression within their macrophages comparable with the level seen in human macrophages. The generation of these mice has been previously described (12). This was determined by direct comparison of human and TG mouse macrophages with Western blot and zymography. All studies and procedures were approved by the Columbia University Institutional Animal Care and Use Committee.

Induction of asthma with OVA. Three-month-old mice were used for the study. The MMP-9 mice do not develop air space enlargement until 1 yr of age. Lungs from 3-mo-old mice are phenotypically normal (12). Groups of MMP-9 TG mice and wild-type (WT) control mice were sensitized by intraperitoneal injection of 20 µg of OVA (Sigma-Aldrich, St. Louis, MO) in 0.2 ml of aluminum (Pierce Chemical, Rockford, IL) on days 0 and 14 as previously described (14). Control mice received the same volume of PBS in aluminum. All mice were challenged daily with 1% OVA (aerosolized for 20 min) via the airways between days 28 and 30. Mice were then used to study AHR or anesthetized to measure the pulmonary resistance (R_L) at 24 h after final OVA challenge. The mice were euthanized, and the following parameters were analyzed: airway histology, BAL cell counts and cytokines, and serum IgE levels. Another group of mice was sensitized and challenged but was killed 1 wk after the final OVA challenge to examine the kinetics by which the inflammation resolved.

Determination of airway responsiveness. Airway responsiveness was measured with barometric plethysmography (Buxco, Troy, NY) after methacholine (MCh) challenge as previously described (14). Pressure differences (box pressure signal) were measured between the main chamber of the plethysmograph, containing conscious, spontaneously breathing animals and the reference chamber. Mice were

challenged with aerosolized saline (for the baseline measurements) or MCh (1.56–25 mg/ml) for 3 min, and readings were taken and averaged for 3 min after each nebulization. Data were expressed using the dimensionless parameter enhanced pause (Penh) as previously described (42).

R_L was measured in anesthetized, tracheostomized, and ventilated mice. A four-way connector was attached to the tracheostomy tube with two ports connected to the inspiratory and expiratory sides of the ventilator. Ventilation was achieved with a rate of 150 breaths/min and tidal volume of 1 ml/100 g body wt during MCh challenge. MCh (30 mg/ml) was administered as an aerosol for the period of 10 breaths via the tracheostomy. Changes in pressure, flow, and volume were recorded, and R_L was calculated from peak values after each challenge.

BAL and measurement of BALF cytokines. After assessment of AHR, lungs were lavaged via the tracheal tube with two aliquots of 1-ml warmed saline and combined. The volume of collected BALF was measured in each sample, and the number of BAL cells were counted. Cytospin (Thermo Fisher Scientific, Waltham, MA) slides were stained and differentiated in a blinded fashion by counting at least 300 cells under light microscopy. Cytokine concentrations in the BALF supernatants were measured by ELISA (R&D Systems, Minneapolis, MN). The colorimetric measurements were performed according to the manufacturer's instructions. The limits of detection were 4 pg/ml for IL-4, IL-5, and IL-13.

Measurement of serum OVA-specific IgE antibody. IgE antibody levels were measured by ELISA, 48 h after the last airway challenge as previously described (44). The antibody titers of samples were related to pooled standards that were generated in the laboratory and expressed as ELISA units per milliliter.

Gelatin zymography. At the time of specimen collection, the lung was excised, homogenized in cold PBS, and electrophoretically separated on precast zymogram gelatin and casein gels (Bio-Rad, Hercules, CA). Following electrophoresis, gels were renatured in Novex zymogram renaturing buffer (Invitrogen, Carlsbad, CA) for 30 min at room temperature and then incubated overnight at 37°C in Novex zymogram developing buffer (Invitrogen) to allow degradation of the substrate (gelatin or casein) in the gel matrix. The gels were then washed and stained with 0.4% (wt/vol) Coomassie brilliant blue R-250 (Thermo Fisher Scientific, Fairlawn, NJ) for 1 h and successively destained until distinct bands appeared against a blue background, indicating degradation of the substrate in the gel matrix. Gelatin zymograms are used to detect gelatinase activity, whereas detectable activity on casein zymograms is generally taken to reflect elastase activity.

Histological and immunohistochemistry studies. After obtaining the BALF, right lungs were inflated through the tracheal tube with 2 ml of 10% formalin and then fixed in 10% formalin. Peribronchial sections were then stained with hematoxylin-eosin and periodic acid-Schiff (PAS) and examined. The extent of peribronchial infiltrates was estimated by an inflammation score. Slides were coded, and the peribronchial inflammation was graded in a blinded manner using a reproducible scoring system (7). A value of 0 indicates no detectable inflammation. A value of 1 indicates occasional cuffing with inflammatory cells, 2 indicates that most bronchi were surrounded by a thin layer (1–5 cells) of inflammatory cells, and 3 indicates when most bronchi were surrounded by a thick layer (>5 cells) of inflammatory cells. Because 5–7 randomly selected tissue sections per mouse were scored, inflammation scores could be expressed as a mean value per animal and could be compared between groups. For lymphocyte quantization, station 10 nodes were carefully dissected and mechanically dissociated. Lymphocytes were then separated, analyzed, and quantitated using Cytospin (Thermo Fisher Scientific, Waltham, MA). Draining lymph nodes were isolated, and total number of CDC11⁺ cells were measured by FACS analysis.

Statistical analysis. All results were expressed as the means ± SE. ANOVA was used to determine the levels of difference among all

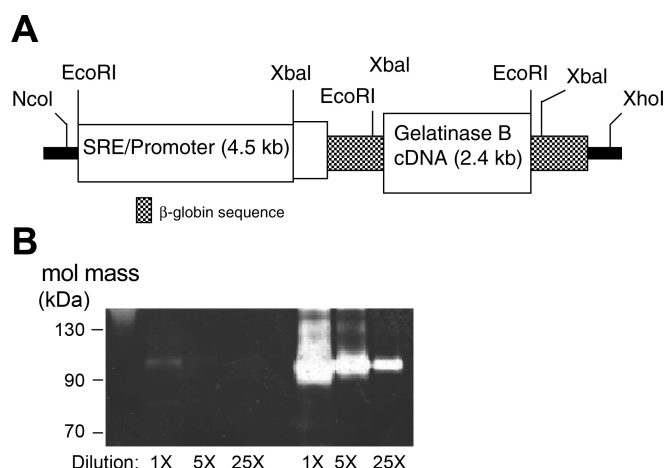


Fig. 1. A: scavenger receptor-enhancer (SREP)/promoter (SREP)-gelatinase B construct. The gelatinase B cDNA was cloned into a vector containing the polyadenylation sequence of the β -globin gene and placed under the control of the human SREP. The final construct (8.3 kb) was removed from the cloning vector by *NcoI/XhoI* digestions. B: gelatin zymography of culture media from a wild-type (WT) and transgenic peritoneal macrophages. After a 48-h incubation, dilutions (1, 5, and 25 $\times$) of the respective media were loaded in a SDS-PAGE 10% copolymerized with gelatin.

groups. Pairs of groups were compared with unpaired, two-tailed Student's *t*-test. The significance was set at $P < 0.05$.

RESULTS

Human MMP-9 TG mouse. The expression of human gelatinase B in the macrophages of TG mice was confirmed by Western blotting and gelatin zymography (24, 38) of the media of peritoneal macrophages after petri dish culture for 48 h (37°C, 5% CO₂, DMEM media with 10% FBS; Fig. 1B). The culture media was collected and concentrated 10-fold and loaded on a SDS-PAGE 10% gel copolymerized with gelatin for zymography.

Serum IgE antibody level is not affected by MMP-9 expression. After OVA sensitization and challenge, serum IgE (OVA-specific) antibody levels were increased in both WT and TG mice compared with unsensitized mice (Fig. 2). There was no statistical difference between the IgE levels in the TG and WT group, suggesting that the animals were similarly sensitized.

MMP-9 inhibits lymphocyte accumulation in BALF. Challenging the airways of sensitized mice with allergen recruits inflammatory leukocytes into the lung. Cell recruitment into the airway was analyzed by studying BALF. In unsensitized groups, cells in the BALF were primarily macrophages (>90%). Antigen sensitization and challenge induced a dramatic increase in the number and variety of cells in this model (Fig. 3A). Cells from the airways of these animals included eosinophils, lymphocytes, and macrophages. As predicted by the model, there were significantly more cells in the airway of sensitized mice compared with the unsensitized mice. Examination of differential BALF cell counts revealed that whereas eosinophil and macrophage recruitment did not differ significantly between WT and TG mice, sensitized TG mice exhibited diminished recruitment of lymphocytes vs. WT controls (Fig. 3B).

MMP-9 reduces lymphocyte infiltration into the lung. Airway inflammation after allergen challenge is characterized by peribronchiolar and perivascular inflammatory cell infiltrates (16, 18, 31). Antigen challenge of sensitized mice, either WT or TG, induced a characteristic widespread peribronchiolar and perivascular inflammation that was not seen in unsensitized mice. The inflammation index was determined by the mean value of the inflammation score (Fig. 4). There was a trend toward lower inflammation scores in sensitized TG mice com-

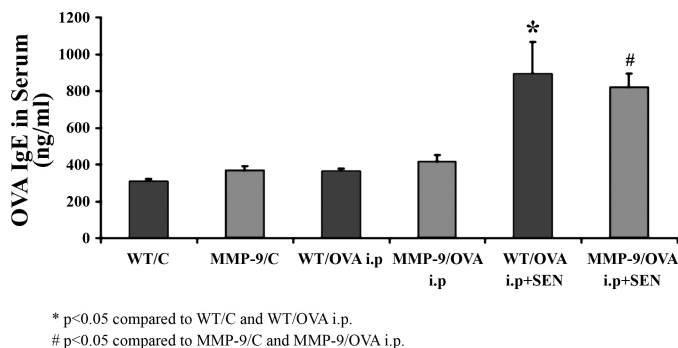
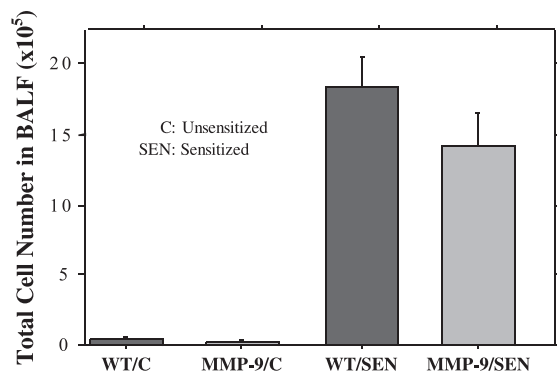


Fig. 2. Ovalbumin (OVA)-specific IgE levels measured in serum of transgenic and WT mice. IgE levels are higher in sensitized mice (SEN) compared with unsensitized. Matrix metalloproteinase-9 (MMP-9) expression does not affect serum OVA-specific IgE levels. Data are expressed as means $\pm$ SE, $n = 6-8$ each group from 2 separate experiments. C, control.



$p = 0.0772$ when MMP-9 compared to WT in sensitized mice, $n = 8$

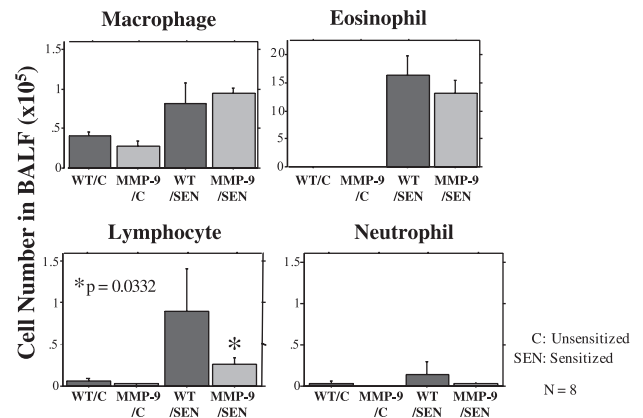


Fig. 3. Effect of increased expression of MMP-9 on airway cell recruitment. Bronchoalveolar lavage fluid (BALF) was recovered 24 h after the final allergen challenge, and the total cell number and differential cell counts were tallied. Values are expressed as means $\pm$ SE, $n = 8-12$ each group from 3 separate experiments.

pared with sensitized WT mice, $P = 0.070$. Peribronchial sections were stained with PAS to see changes in mucus metaplasia. PAS staining in sensitized mice was higher than control. Although the PAS staining is not quantitative, we do see a qualitative decrease in the PAS staining in the MMP-9 TG mice compared with controls (Fig. 4). CD3 immunostaining for T lymphocytes was used to quantitate lymphocyte infiltration. Peribronchial T cell infiltration is markedly increased in the lungs of sensitized WT but not TG mice (Fig. 5). This is consistent with the reduced number of lymphocytes found in TG airways.

MMP-9 inhibits AHR. AHR is a characteristic feature of the pulmonary allergic response and has been associated with recruitment of inflammatory cells in the lung. AHR was measured with whole body plethysmography 24 h after the final serial OVA challenge and was assessed by MCh-induced airflow obstruction in conscious mice. Measurement of MCh responsiveness was obtained by exposing mice to nebulized PBS followed by incremental doses of nebulized MCh and monitoring Penh (a calculated value indicating airway constriction). There was no difference in the response to MCh in unsensitized WT mice and TG mice. Both sensitized WT and TG mice showed increases in Penh following allergen challenge, however, with increasing doses of MCh, sensitized TG mice showed significantly reduced AHR relative to sensitized

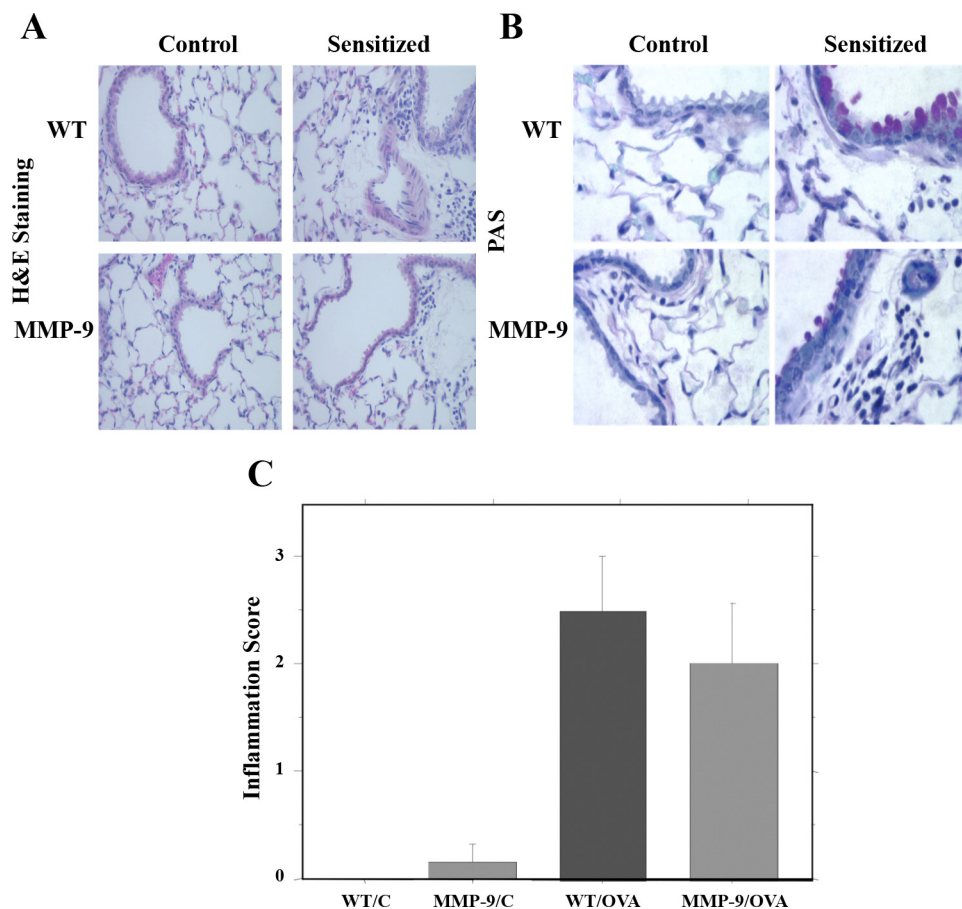


Fig. 4. Histopathological analysis of lungs 24 h after the last allergen exposure. Representative photomicrographs of hematoxylin-eosin (H&E) (A) and periodic acid-Schiff (PAS) (B) stained paraffin-embedded lung are shown. C: quantification of histological inflammation. Mean peribronchial inflammation scores were determined in the 4 groups as described in the text.

WT controls, $P = 0.0308$ (data not shown). To confirm the airway responsiveness, R_L was also measured in anesthetized, tracheostomized, and ventilated mice. Unsensitized mice showed a slight increase in R_L in response to MCh (30 mg/ml). Sensitized WT mice exhibited a statistically significant greater increase in R_L compared with sensitized TG mice, $P < 0.01$ (Fig. 6).

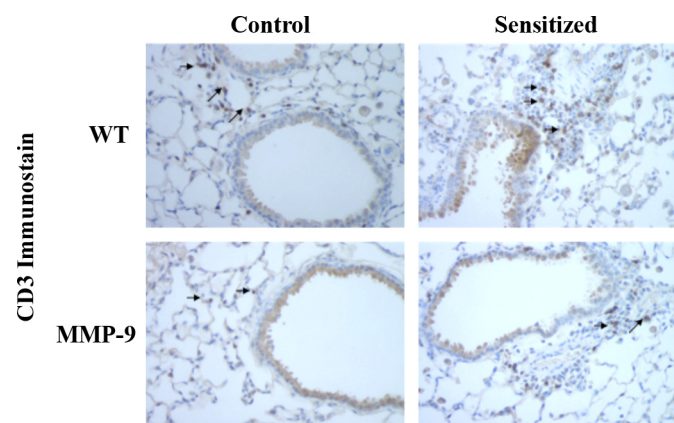
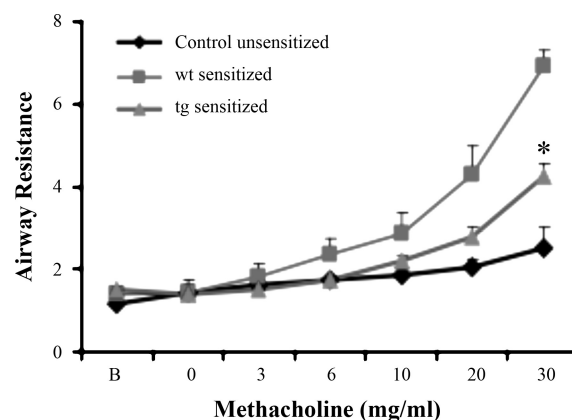


Fig. 5. Immunohistochemical staining for CD3. CD3 is a marker for T lymphocytes. Representative photomicrographs of lung sections from unsensitized and sensitized WT mice and unsensitized and sensitized MMP-9 transgenic (TG) mice are shown (magnification, $\times 20$). Note nuclear and cytoplasmic staining in cells in the interstitial compartment (arrows).

Pulmonary Th2 cytokine secretion is increased in the draining lymph nodes of MMP-9 TG mice. Production of cytokines such as IL-4, IL-5, and IL-13 is characteristic of a robust T helper type 2 (Th2) response and is a feature of allergic pulmonary reactions. Levels of Th2-type cytokines and IFN- γ



* $p < 0.05$ compared to wt sensitized

Fig. 6. Effect of increased expression of MMP-9 on airway hyperresponsiveness (AHR) measured by enhanced pause (Penh). AHR was measured 24 h after the final allergen challenge using a system in which mice were exposed to increasing concentrations of methacholine (MCh; 3–30 mg/ml). Values are expressed as means $\pm$ SE, $n = 8$ –12 each group in 3 separate experiments, * $P < 0.05$ compared with WT-sensitized mice. B, baseline measurements.

were measured in the BALF and in the culture media of cells from hilar lymph nodes. Levels of IL-4 in BALF tended to increase in sensitized mice compared with unsensitized mice, but there was no significant difference between TG and control mice (Fig. 7A). The level of IL-13 in serum was higher in sensitized mice compared with unsensitized mice, and the mean TG group IL-13 level was significantly higher than similarly sensitized WT mice (Fig. 7B). There were no differences in the levels of IL-5 or IFN- γ in any group (data not shown). Since the station 10 (hilar) nodes were generally larger in sensitized mice, we measured the total cell numbers from these nodes. Consistent with these results, cell counts from station 10 nodes were higher in sensitized than unsensitized mice and were significantly higher in nodes from sensitized TG mice than from sensitized WT controls (Fig. 8). FACS analysis showed that CD11c⁺ cells were higher in sensitized mice compared with unsensitized mice. The levels in the TG mice were slightly higher than WT, but the difference was not statistically significant (data not shown).

DISCUSSION

The present study demonstrates that mice with expression of human MMP-9 in their macrophages develop reduced IL-13 levels, decreased lymphocytes in the BAL, and decreased airway responsiveness in an OVA-induced asthma model. The findings suggest that human MMP-9 expression reduces airway constriction likely secondary to the decrease in lymphocyte

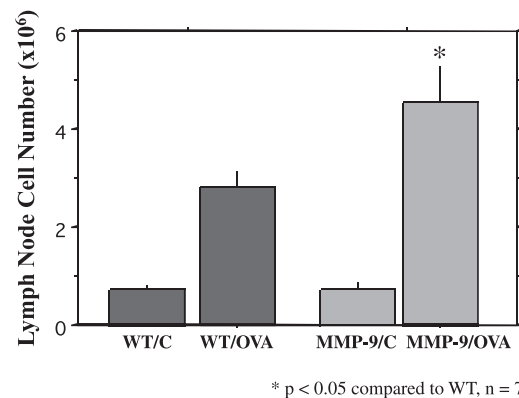


Fig. 8. Cell number from draining lymph nodes, $n = 4-10$ each group from 3 separate experiments, * $P < 0.05$ compared with WT-sensitized mice.

accumulation in the asthmatic airway of antigen-challenged mice. Lymphocytes are thought to directly contribute to airway function and attenuate airway constriction (9, 10, 39). Prior reports have suggested a role for lymphocytes in the cellular inflammation in bronchi and in the pathophysiology of human AHR (2, 5). A similar link between AHR and lymphocyte migration was also observed in rats where CD4⁺ T cells could increase AHR after they were transferred from one animal to another and from humans to SCID mice (13, 15, 45).

Accumulating evidence also suggests that MMP activity is important in asthma pathogenesis (19, 22, 33). MMPs are postulated to degrade the ECM and basement membrane, facilitating recruitment of cells across endothelial basement membranes, and to catalytically activate proinflammatory chemokines, such as IL-8 (46, 48). In addition to its earliest described role digesting components of the ECM, MMP-9 modulates the activity of other proteases and cytokines that are important in lung disease (50). MMP-9 degrades α_1 -antitrypsin, protects neutrophil elastase activity, and when bound to CD44 can release latent transforming growth factor- β_1 (TGF- β_1) (1, 30). A TG mouse that overexpresses IL-13 exhibits an increase in MMP-9 transcripts in the whole lung and develops airway inflammation and mucus metaplasia similar to the airway pathology of severe asthma (51). When crossed with the MMP-9-null mouse, a decrease in bioactive TGF- β and a decrease in airway and alveolar pathology are seen in the IL-13 TG mouse lung (51). These studies have led to the recognition that MMP-9, the predominant protease in the lung, may participate in an inflammatory cascade that results in collagen deposition and fibrosis in asthma (1).

More recently, it has been shown that the expression of MMP-9 in macrophages is capable of altering the ECM and generating progressive air space enlargement in aging mice. The experiments were done using mice that specifically express human MMP-9 in their macrophages; the development of air space enlargement and matrix destruction was confirmed using morphometric, biochemical, and histological analyses. Similarly, these same TG mice were used to study effects of increased MMP-9 expression on bleomycin-induced pulmonary fibrosis. In the bleomycin study, the MMP-9 TG mice developed reduced lung fibrosis compared with WT mice. These studies have demonstrated the usefulness of the MMP-9 macrophage TG mouse in the investigation of lung diseases and confirmed its role in matrix degradation.

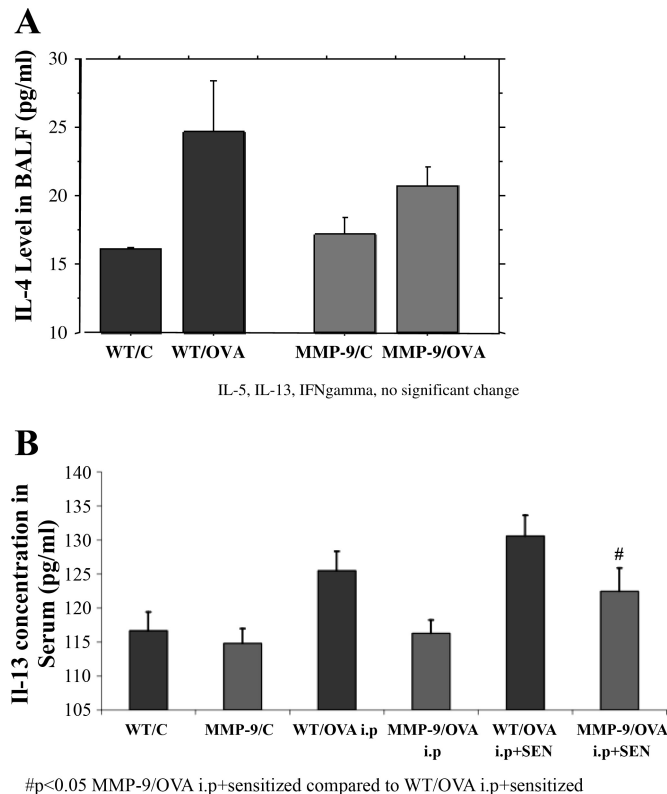


Fig. 7. Effect of increased expression of MMP-9 on IL-4 and IL-13 production. IL-4 levels were measured in BALF of cells from lung draining lymph nodes by ELISA (A). IL-13 levels were measured in serum by ELISA (B). Data are expressed as means $\pm$ SE, $n = 4-10$ each group from 3 separate experiments, $P < 0.05$ compared with WT-sensitized mice.

A study by McMillan et al. (35) showed that the AHR was increased in MMP-9 KO mice. They also found that pulmonary inflammation, Th2 cytokine production, and eotaxin/macrophage-derived chemokine generation were all markedly increased in the absence of MMP-9 (34). Additionally, resolution of inflammation was slower in the absence of MMP-9. However, Cataldo et al. (7) demonstrated that monocytic cell recruitment into airways was decreased in MMP-9 KO mice compared with WT mice, although eosinophil recruitment was unaffected by the absence of MMP-9. The inflammation induced by the protocol in the study by Cataldo et al. (7) was relatively mild in WT C57BL/6J mice, showing minimal peribronchial or perivascular infiltrates and few eosinophils in the lavage ($6.4 \times 10^4/\text{ml}$). In contrast, both McMillan et al. (35) and our protocol induce a florid eosinophilic inflammation, with large numbers of eosinophils in the tissue and lavage ($40\text{--}60 \times 10^5$ cells/ml). Furthermore, in the study by Cataldo et al. (7), AHR was significantly decreased in MMP-9 KO mice compared with WT controls after allergen challenge. However, the AHR was significantly higher in MMP-9 KO mice in the absence of allergen challenge, making the results difficult to interpret. The differences between these studies are likely to be due to subtle differences in the induction protocol as well as perhaps the genetic backgrounds of the mice.

Although the present study did not identify changes in smooth muscle cell migration or proliferation, this may be due to the insensitivity of the acute asthma model to detect such processes, and subtle effects of MMP-9 on smooth muscle cell function could account for the alteration in AHR seen in this model. A recent study by Malavia et al. (31) demonstrated that MMP-9 secretion in airway epithelium led to proliferation of smooth muscle cells. In addition, our laboratory (24) documented a fibrotic role for MMP-9 in aneurysm formation when the macrophage MMP-9 TG mice were crossed into a model of atherosclerosis. Future studies will need to examine the effect of MMP-9 in more chronic models of asthma to elucidate the role of this enzyme on the smooth muscle cell in the allergic airway.

A decrease in the number of lymphocytes in the BALF of the MMP-9 TG mice was observed, and IL-13 levels were decreased in the serum of the TG mice. In addition, there were fewer T cells in the lung tissue in TG animals with an increase in the number of lymphocytes in the draining lymph nodes. Therefore, the cleavage of IL-13 by MMP-9 could be a possible mechanism for the phenotype seen in the TG mice. Previous studies have shown that MMP-9 cleaves IL-2R α , thus downregulating the proliferative capacity of T cells (41). It is possible that lymphocyte clearance or egression from the lung to the draining nodes might be promoted in MMP-9 TG mice. This hypothesis is supported by our observation that the lymphocyte number in the draining lymph node was markedly increased in TG mice. It is unlikely that the lymphocyte response to the allergen was blunted in the TG group or that lymphocyte emigration was affected. The normal IgE response and the increase in lymphocytes in draining lymph nodes suggest otherwise.

The methodology of this paper builds on work performed in earlier studies of MMP function in asthma. Mice treated with the MMP inhibitor, R-94138, demonstrate reduced airway inflammation and hyperreactivity after sensitization and allergen challenge (20). However, the R-94138 inhibitor has a

broad spectrum of action, having reported effects on MMP-2, MMP-3, MMP-7, MMP-9, and MMP-13 (43). Since MMPs operate in a tightly regulated interdependent network, the use of such inhibitors cannot provide specific biological information about individual MMPs. Our study, in contrast, uses TG mice expressing MMP-9 specifically and thus yields insight into the role of this important MMP in the asthmatic phenotype. The most striking finding in our study was the decreased number of lymphocytes in the airway of TG mice following allergen challenge. This finding is consistent with MMP-2 and MMP-9 double KO studies conducted by Corry et al. (8). They reported that MMP-9-deficient mice accumulated leukocytes in the pulmonary parenchyma but that lavage fluid yielded diminished lymphocyte counts. Corry et al. (8) observed that MMP-9 deficiency causes extraluminal pulmonary lymphocytosis. We observed that this increase in MMP-9 activity similarly yielded a decreased lymphocytic infiltrate into the airways with an apparent relocation to the regional lymph node basins. Additionally, Corry et al. (8) demonstrated that transepithelial gradients of three chemokines, CCL7, CCL11, and CCL17, were disrupted by MMP-9 deficiency, suggesting that MMP modulation of chemokine gradients may govern leukocyte emigration into airways. However, we were able to identify changes in IL-13 levels in the serum of MMP-9 mice that could be modifying the trafficking and clearance of recruited lymphocytes by altering transepithelial cytokine gradients. This hypothesis is also supported by data from a murine model of contact dermatitis that does not resolve in MMP-9 KO mice (49). It is conceivable that in vivo MMP-9 activity affects chemokine function directly, as described above, or by interaction with other MMPs as described in other model systems (28).

Inflammatory cytokines, such as IL-13, are known to regulate MMP expression, but the reverse is also true: cytokines and their receptors can be substrates for MMP action (21). MMPs can influence the development of AHR by altering the function of inflammatory regulators. Indeed, increased AHR is partially reversed by the MMP inhibitors BB-94, R-94138, and GM6001, suggesting contribution from an MMP-dependent pathway (20, 29). In this model, the identification of IL-13 levels in the serum with a decrease in mucous metaplasia could account for the changes seen in AHR and suggest that the MMP-9 cleavage of IL-13 modulates this process. Several chemokines are also converted by MMPs to form receptor antagonists with anti-inflammatory properties, such as MCP-2, MCP-3, and MCP-4 (36).

In summary, we have shown that increased expression of MMP-9 in lung tissue macrophages is associated with a decreased number of lymphocytes in the airway and the lung following allergen challenge. Furthermore, we have found that there is an increased number of lymphocytes in the draining lymph node in the MMP-9 TG mice. These data suggest a role for MMP-9 in lymphocyte trafficking in asthmatic airways and suggest that MMP-9 expression is protective against AHR and lymphocytic inflammation of the airways.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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