Matrix Metalloproteinase-8 Deficiency Promotes Granulocytic Allergen-Induced Airway Inflammation

Maud M. Gueders, Milagros Balbin, Natacha Rocks, Jean-Michel Foidart, Philippe Gosset, Renaud Louis, Steven Shapiro, Carlos Lopez-Otin, Agnes Noël and Didier D. Cataldo

*J Immunol* 2005; 175:2589-2597; doi: 10.4049/jimmunol.175.4.2589

http://www.jimmunol.org/content/175/4/2589

**References** This article cites 41 articles, 10 of which you can access for free at:
http://www.jimmunol.org/content/175/4/2589.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

---

*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2005 by The American Association of Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Matrix Metalloproteinase-8 Deficiency Promotes Granulocytic Allergen-Induced Airway Inflammation

Maud M. Gueders,*† Milagros Balbin,‡ Natacha Rocks,† Jean-Michel Foidart,† Philippe Gosset,§ Renaud Louis,* Steven Shapiro,¶ Carlos Lopez-Otin,‡ Agnes Noël,† and Didier D. Cataldo2*†

Matrix metalloproteinases (MMPs) are involved in inflammatory reaction, including asthma-related airway inflammation. MMP-8, mainly produced by neutrophils, has recently been reported to be increased in the bronchoalveolar lavage fluid (BALF) from asthmatic patients. To evaluate the role of MMP-8 in asthma, we measured MMP-8 expression in lung tissue in an OVA-sensitized mouse model of asthma and addressed the effect of MMP-8 deletion on allergen-induced bronchial inflammation. MMP-8 production was increased in lungs from C57BL/6 mice exposed to allergens. After allergen exposure, MMP-8−/− mice developed an airway inflammation characterized by an increased neutrophilic inflammation in BALF and an increased neutrophilic and eosinophilic infiltration in the airway walls. MMP-8 deficiency was associated with increased levels of IL-4 and anti-OVA IgE and IgG1 in BALF and serum, respectively. Although allergen exposure induced an enhancement of LPS-induced CXC chemokine, KC, and MIP-2 levels in BALF and lung parenchyma, no difference was observed between the two genotypes. Inflammatory cell apoptosis was reduced in the lungs from MMP-8−/− mice. For the first time, our study evidences an important role of MMP-8 in the control of neutrophilic and eosinophilic infiltration during allergen-induced lung inflammation, and demonstrates that the anti-inflammatory effect of MMP-8 is partly due to a regulation of inflammatory cell apoptosis. The Journal of Immunology, 2005, 175: 2589–2597.

Asthma is a complex inflammatory disease characterized by an eosinophilic inflammation of the bronchial walls and airway hyperresponsiveness. Neutrophils also seem to play a significant role in asthma as soon as the disease becomes severe (1, 2). A significant contribution of neutrophils to the bronchial hyperresponsiveness is suggested by in vitro studies (3) and by the increased migratory activity (4) observed in neutrophils from human asthmatics. The airways of human asthmatics display significant morphological changes referred to as bronchial remodeling (5). Among those changes, many are linked to an excessive degradation of the extracellular matrix and mainly of its collagen components. The main feature of remodeling in humans is the deposition of a dense layer made of different collagen and fi-
MMP-8 mRNA has been detected in bronchial biopsies and was correlated with the intensity of the disease (24). MMP-8 could contribute to inflammatory cell trafficking and inflammation in different ways, through the cleavage of fibrillar collagens, or through the degradation or activation of protease inhibitors (α1-antitrypsin, α2-macroglobulin) and/or chemokines such as LPS-induced CXC chemokine, a neutrophil chemoattractant (25–28). However, the functions and mechanisms of action of MMP-8 in inflammatory disorders are not yet well established. Recently, unexpected inhibitory effects of MMP-8 in cancer progression have been evidenced (25, 29). Indeed, MMP-8 gene deletion in mice increased tumor development after carcinogen treatment (25), and genetic manipulation of metastatic cells to overproduce MMP-8 resulted in decreased metastatic dissemination (29). These observations suggest that MMP-8 may have multiple functions regulating protective immune functions and/or tissue infiltration.

In the present work, the key role played by MMP-8 in the processes leading to airway inflammation after allergen exposure is demonstrated, for the first time, in an experimental mouse model of asthma. Our data have therapeutic implications warning against the use of broad spectrum inhibitors in asthma therapy.

Materials and Methods

Sensitization and allergen exposure protocol

Wild-type (MMP-8+/+) and MMP-8 knockout (MMP-8−/−) mice were generated in a C57BL/6/J29 genetic background, as previously described (25). In additional experiments, we used C57BL/6 mice from Denmark (Taconic Farms). Males of 6–8 wk old were sensitized by i.p. injection of 10 μg of OVA (Sigma-Aldrich) emulsified in aluminum hydroxide (AlumInject; Perbio) on days 1 and 8. Mice were subsequently exposed to allergens by daily inhalation of an aerosol of 1% OVA, for 30 min, generated by ultrasonic nebulizer (Devilbiss 2000), from day 21 to 27. Mice were challenged mice 24 h after the last allergen inhalation. Immunohistochemical staining of MMP-8 was performed on lung sections of sham (PBS-) or allergen-challenged (OVA) C57BL/6 mice. An increase in the number of MMP-8-positive cells was assessed by counting the cells on 10 areas for each mouse, and of inflammatory cells; and a value of 3 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 are expressed as a mean value per mouse and can be compared between groups. After Congo red staining, the eosinophilic infiltration in the airway walls was quantified by manual count and reported to the perimeter of epithelial basement membrane defining an eosinophilic inflammatory score. Immunohistochemistry was performed on paraffin sections to detect MMP-8 and neutrophils. Slides were heated in autoclave in citrate buffer (Dako Target Retrival Solution; DakoCytomation) and incubated with primary Ab (rabbit anti-mouse MMP-8, Abcam; rat anti-mouse neutrophils, Serotec) and secondary Ab (swine anti-rabbit HRP and rabbit anti-rat HRP; DakoCytomation). Slides were then incubated with 3-amino-9-ethylcarbazole (DakoCytomation). Five different areas were analyzed for each mouse, and the percentage of neutrophils undergoing apoptosis was calculated for each mouse. Apoptosis was also studied by measuring the percentage of granulocytes displaying a caspase 3 activation. Caspase 3 was detected in situ using a primary Ab (rabbit anti-human/mouse caspase 3 active; R&D Systems) and secondary Abs coupled with biotin (biotinylated goat anti-rabbit; DakoCytomation) and streptavidin (streptavidin/HRP; DakoCytomation). Slides were then incubated with 3-amino-9-ethylcarbazole (DakoCytomation). Five different areas were analyzed for each mouse, and

![FIGURE 1. MMP-8 production in the lungs of 7-day allergen-challenged mice 24 h after the last allergen inhalation. Immunohistochemical staining of MMP-8 was performed on lung sections of sham (PBS-) or allergen-challenged (OVA) C57BL/6 mice. An increase in the number of MMP-8-positive cells was assessed by counting the cells on 10 areas for each mouse (n = 10) (p < 0.0001).](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org)
Reverse transcription was performed at 70°C for 15 min, followed by 95°C for 2 min at 72°C. Amplification cycle was repeated 18 times for detection of MMP and TIMP.

MMPs, and chemokine mRNAs were measured by RT-PCR using the GeneAmp Thermostable rTth reverse-transcriptase RNA PCR kit (Roche Molecular Systems). Oligonucleotides used for RT-PCR are shown in Table I. Molecular Systems). Oligonucleotides used for RT-PCR are shown in Table I. Reverse transcription was performed at 70°C for 15 min, followed by 95°C for 2 min at 72°C. Amplification cycle was repeated 18 times for detection of 28S rRNA and 38S rRNA (ribosomal RNA), the 28S rRNA (ribosomal RNA), and MMP-9, as an internal standard. Gelatinolytic activity of the murine HT1080 cells, known to produce spontaneously high amounts of MMP-2 and MMP-9 was determined by the lysis band in the 72- and the 95-kDa area, respectively.

Measurements of cytokines by ELISA and Western blots

IL-4, IL-5, IFN-γ, IL-17, and KC levels were assessed using commercial ELISAs (R&D Systems), while ELISAs for MIP-2 and LIX proteins were developed using Abs from R&D Systems and the R&D Duoset ELISA kit (R&D Systems). Western blots were performed to quantify the percentage of neutrophils undergoing apoptosis was calculated. Detection of cells bearing on their surface the orphan receptor T1/ST2 was performed by immunohistochemistry using a rat mAb to mouse T1/ST2 purchased from Morwell Diagnostics. T1/ST2-positive lymphocyte-shaped cells were counted in the peribronchial area of six bronchi per mouse. The left lung of each animal was exposed using a Mikro-Dismembrator (Braun). For protein extraction, the crushed lung tissue was incubated overnight at 4°C in a solution containing 2 M urea, 1 M NaCl, and 50 mM Tris (pH 7.5), and subsequently centrifuged 15 min at 16,000 g. The supernatant was stored at −80°C for zymography. Total RNA was extracted with Rneasy Mini kit (Qiagen). RNA levels and purity were assessed using a NanoDrop spectrophotometer (NanoDrop). Oligonucleotides used for RT-PCR are shown in Table I. Reverse transcription was performed at 70°C for 15 min, followed by 95°C for 2 min at 72°C. Amplification cycle was repeated 18 times for detection of 28S rRNA and 38S rRNA (ribosomal RNA), the 28S rRNA (ribosomal RNA), and MMP-9, as an internal standard. Gelatinolytic activity of the murine HT1080 cells, known to produce spontaneously high amounts of MMP-2 and MMP-9 was determined by the lysis band in the 72- and the 95-kDa area, respectively.

Measurements of cytokines by ELISA and Western blots

IL-4, IL-5, IFN-γ, IL-17, and KC levels were assessed using commercial ELISAs (R&D Systems), while ELISAs for MIP-2 and LIX proteins were developed using Abs from R&D Systems and the R&D Duoset ELISA kit (R&D Systems). Western blots were performed to quantify the percentage of neutrophils undergoing apoptosis was calculated. Detection of cells bearing on their surface the orphan receptor T1/ST2 was performed by immunohistochemistry using a rat mAb to mouse T1/ST2 purchased from Morwell Diagnostics. T1/ST2-positive lymphocyte-shaped cells were counted in the peribronchial area of six bronchi per mouse. The left lung of each animal was crushed using a Mikro-Dismembrator (Braun). For protein extraction, the crushed lung tissue was incubated overnight at 4°C in a solution containing 2 M urea, 1 M NaCl, and 50 mM Tris (pH 7.5), and subsequently centrifuged 15 min at 16,000 g. The supernatant was stored at −80°C for zymography. Total RNA was extracted with Rneasy Mini kit (Qiagen). RNA levels and purity were assessed using a NanoDrop spectrophotometer (NanoDrop). Oligonucleotides used for RT-PCR are shown in Table I. Reverse transcription was performed at 70°C for 15 min, followed by 95°C for 2 min at 72°C. Amplification cycle was repeated 18 times for detection of 28S rRNA and 38S rRNA (ribosomal RNA), the 28S rRNA (ribosomal RNA), and MMP-9, as an internal standard. Gelatinolytic activity of the murine HT1080 cells, known to produce spontaneously high amounts of MMP-2 and MMP-9 was determined by the lysis band in the 72- and the 95-kDa area, respectively.

Measurements of cytokines by ELISA and Western blots

IL-4, IL-5, IFN-γ, IL-17, and KC levels were assessed using commercial ELISAs (R&D Systems), while ELISAs for MIP-2 and LIX proteins were developed using Abs from R&D Systems and the R&D Duoset ELISA kit (R&D Systems). Western blots were performed to quantify the percentage of neutrophils undergoing apoptosis was calculated. Detection of cells bearing on their surface the orphan receptor T1/ST2 was performed by immunohistochemistry using a rat mAb to mouse T1/ST2 purchased from Morwell Diagnostics. T1/ST2-positive lymphocyte-shaped cells were counted in the peribronchial area of six bronchi per mouse. The left lung of each animal was crushed using a Mikro-Dismembrator (Braun). For protein extraction, the crushed lung tissue was incubated overnight at 4°C in a solution containing 2 M urea, 1 M NaCl, and 50 mM Tris (pH 7.5), and subsequently centrifuged 15 min at 16,000 g. The supernatant was stored at −80°C for zymography. Total RNA was extracted with Rneasy Mini kit (Qiagen). RNA levels and purity were assessed using a NanoDrop spectrophotometer (NanoDrop). Oligonucleotides used for RT-PCR are shown in Table I. Reverse transcription was performed at 70°C for 15 min, followed by 95°C for 2 min at 72°C. Amplification cycle was repeated 18 times for detection of 28S rRNA and 38S rRNA (ribosomal RNA), the 28S rRNA (ribosomal RNA), and MMP-9, as an internal standard. Gelatinolytic activity of the murine HT1080 cells, known to produce spontaneously high amounts of MMP-2 and MMP-9 was determined by the lysis band in the 72- and the 95-kDa area, respectively.

Measurements of cytokines by ELISA and Western blots

IL-4, IL-5, IFN-γ, IL-17, and KC levels were assessed using commercial ELISAs (R&D Systems), while ELISAs for MIP-2 and LIX proteins were developed using Abs from R&D Systems and the R&D Duoset ELISA kit (R&D Systems). Western blots were performed to quantify the percentage of neutrophils undergoing apoptosis was calculated. Detection of cells bearing on their surface the orphan receptor T1/ST2 was performed by immunohistochemistry using a rat mAb to mouse T1/ST2 purchased from Morwell Diagnostics. T1/ST2-positive lymphocyte-shaped cells were counted in the peribronchial area of six bronchi per mouse. The left lung of each animal was crushed using a Mikro-Dismembrator (Braun). For protein extraction, the crushed lung tissue was incubated overnight at 4°C in a solution containing 2 M urea, 1 M NaCl, and 50 mM Tris (pH 7.5), and subsequently centrifuged 15 min at 16,000 g. The supernatant was stored at −80°C for zymography. Total RNA was extracted with Rneasy Mini kit (Qiagen). RNA levels and purity were assessed using a NanoDrop spectrophotometer (NanoDrop). Oligonucleotides used for RT-PCR are shown in Table I. Reverse transcription was performed at 70°C for 15 min, followed by 95°C for 2 min at 72°C. Amplification cycle was repeated 18 times for detection of 28S rRNA and 38S rRNA (ribosomal RNA), the 28S rRNA (ribosomal RNA), and MMP-9, as an internal standard. Gelatinolytic activity of the murine HT1080 cells, known to produce spontaneously high amounts of MMP-2 and MMP-9 was determined by the lysis band in the 72- and the 95-kDa area, respectively.
**Results**

**MMP-8 expression in the lungs of allergen-challenged mice**

To determine whether MMP-8 production is modulated in the OVA mice model of asthma, we performed an immunohistochemical analysis on lung sections from C57BL/6 mice exposed either to OVA or PBS. Mice exposure to allergen led to a large increase of the number of cells positive for MMP-8 in lung tissue ($p < 0.0001$) (Fig. 1).

**BALF**

Exposure of mice to aerosolized OVA induced a significant increase in total cell counts in MMP-8/−/− mice ($p < 0.05$). Eosinophil counts were significantly increased after allergen exposure in both MMP-8+/+ ($n = 43$) and MMP-8/−/− ($n = 48$) genotypes, as compared with their nonexposed counterparts ($p < 0.0001$). Interestingly, BALF of MMP-8/−/− mice were characterized by a 10-fold increase in neutrophil counts after allergen exposure when compared with their nonexposed counterpart (Table II). In sharp contrast, only a 2-fold enhancement of neutrophil number was induced by allergen treatment in MMP-8+/+ mice. Such an effect of MMP-8 deficiency on cell infiltration in BALF was specific to neutrophils, because other cell counts (epithelial cells, lymphocytes, or macrophages) were not affected.

**Histopathology of the lungs**

No developmental abnormalities were observed in the lungs of MMP-8/−/− mice (data not shown). The airways from MMP-8+/+ and MMP-8/−/− mice exposed to sham challenge (PBS) showed normal histology (Fig. 2, A and B). After sensitization and subsequent allergen exposure, both MMP-8/−/− and MMP-8+/+ displayed a significant peribronchial and periangular inflammation (Fig. 2, C and D). Such an enhanced peribronchial inflammation in both genotypes was confirmed by the higher inflammation scores ($p < 0.0001$) (Fig. 3A).

Eosinophilic and neutrophilic tissue infiltration was studied on Congo red-stained slides (Fig. 2, E and F) and by immunohistochemistry (Fig. 2, G and H), respectively. Quantitative results were expressed as a number of cells/mm epithelial basement membrane (Fig. 3, B and C). Although the number of eosinophils was increased in both groups after allergen exposure ($p < 0.0001$), the enhancement was even higher in MMP-8/−/− mice than in MMP-8+/+ ($p < 0.05$) (Fig. 3B).

The neutrophilic infiltration was increased after allergen exposure in both genotypes. Again, the enhancement of neutrophil counts in the bronchial wall was 2 times higher in MMP-8/−/− when compared with MMP-8+/+ (Fig. 3C).

**Blood cell counts in MMP-8+/+ and MMP-8/−/− mice**

To address some potential differences regarding blood inflammatory cells between MMP-8+/+ and MMP-8/−/−, differential cell counts were performed, and no significant difference was found when comparing the counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils (Fig. 4A).

**Assessment of sensitization to allergen**

Serum levels of anti-OVA-specific IgE were detected by ELISA. Specific IgE levels were increased in the sera of allergen-exposed mice from both genotypes ($p < 0.0001$). However, an enhancement at higher extent was detected in the sera of MMP-8/−/− mice.
as compared with MMP-8−/− (p < 0.05) (Fig. 4B). To investigate a potential Th1/Th2 imbalance in MMP-8−/− mice, we measured the levels of anti-OVA-specific IgG1 and IgG2a. IgG1 levels were significantly increased after allergen exposure in both genotypes (p < 0.05) and even more elevated in MMP-8−/− mice as compared with MMP-8+/+ (p < 0.05) (Fig. 4C). Measurements of OVA-specific IgG2a did not show any difference between allergen-challenged and unchallenged mice, but levels were higher in MMP-8−/− after allergens as compared with MMP-8+/+ (p < 0.01) (Fig. 4D). Total IgE levels were also studied by ELISA, and their levels were found to be increased significantly in both knockout and wild-type mice after allergen exposure without difference between the genotypes (data not shown).

Cytokines in BALF and lung protein extracts

To further investigate the mechanisms leading to an increased inflammation and increased levels of OVA-specific IgE in MMP-8−/− mice, we measured by ELISA, Western blots, and RT-PCR a panel of relevant ILs (IL-4, IL-5, and IFN-γ) and chemokines (KC, IL-17, MIP-2, LIX, eotaxin) that may be involved in inflammatory cell recruitment.

Although IL-4 levels assessed by ELISA were increased in BALF and lung protein extracts after allergen exposure in both genotypes (p < 0.001), statistical differences were only reached in MMP-8−/− mice (p < 0.0001) (Fig. 5, A and B). Allergen exposure induced a greater enhancement of IL-4 levels in BALF of MMP-8−/− mice than in wild-type mice. IL-5 and IL-17 levels in BALF or lung protein extracts were not significantly different between the groups, and IFN-γ levels were only rarely detectable in BALF (Fig. 5, C and H, respectively). Eotaxin, an eosinophil chemoattractant, was measured in BALF and lung protein extracts. Its levels were significantly increased after allergen exposure in both genotypes without any difference between MMP-8+/+ and MMP-8−/− (Fig. 5D).

We also focused on neutrophil chemoattractants: KC, MIP-2, and LIX. Levels of KC measured by ELISA in lung protein extracts were similarly increased after allergen exposure in both genotypes (p = 0.0001) (Fig. 5E) and were rarely detectable in BALF. ELISA analysis of LIX levels in BALF revealed a tendency to increase after allergen exposure in both genotypes, but these differences did not reach statistical significance (data not shown). LIX production was also studied by Western blot analysis in BALF. Although significant increase of LIX was observed in mice exposed to allergens (p < 0.05), again no difference was detected between MMP-8−/− and MMP-8+/+ mice (Fig. 5, F and G). Because MIP-2 levels were rarely detectable by ELISA, RT-PCR analysis was performed in lung tissue extracts. MIP-2 mRNA levels were increased after allergen challenge in both groups without any differences between MMP-8−/− and MMP-8+/+ mice (Fig. 6).

Determination of Th2 recruitment in lung parenchyma

An anti-T1/ST2 (orphan receptor expressed by Th2 cells) immunohistochemistry was performed on slides from lungs of MMP-8+/+ (n = 10) and MMP-8−/− (n = 12) mice exposed to OVA. Numbers of lymphocyte-shaped cells positive for this staining were significantly increased in MMP-8−/− mice as compared with MMP-8+/+ (p < 0.05), indicating that Th2 recruitment is increased in the lungs from MMP-8−/− (Fig. 7).

Assessment of cell apoptosis in lung sections

TUNEL analysis and caspase 3 immunohistochemistry performed on paraffin-embedded lung sections revealed that the number of
apoptotic neutrophils was significantly increased after allergen exposure both in MMP-8−/− and MMP-8−/−. Interestingly, the extent of neutrophil apoptosis was significantly higher in MMP-8−/− when compared with MMP-8−/− mice when compared with MMP-8−/− in BALF. IL-5 levels measured in BALF by ELISA were not significantly different between the groups (C). Eotaxin levels measured in the lung protein extracts were increased after allergen exposure in both genotypes without difference between the groups (D). Levels of KC measured in lung protein extracts (E) were increased after OVA challenge in both groups (p = 0.0001). LIX expression was studied in BALF by Western blots (F) showing a significant increase in mice exposed to allergens (p < 0.05) without any obvious difference between MMP-8−/− and MMP-8−/− (G). IL-17 levels were measured in lung proteins extracts by ELISA, but no differences were found between the groups (H). Results are expressed as means ± SEM.

**FIGURE 5.** Measurements of chemokines and cytokines in the BAL and lung protein extracts 24 h after a 7-day allergen exposure. Levels of IL-4 measured in BALF (A) and lung protein extracts (B). Significant differences were observed between MMP-8−/− mice exposed to OVA aerosol and mice exposed to PBS. IL-4 levels were higher after OVA exposure in BALF of MMP-8−/− mice when compared with MMP-8−/− in BALF. IL-5 levels measured in BALF by ELISA were not significantly different between the groups (C). Eotaxin levels measured in the lung protein extracts were increased after allergen exposure in both genotypes without difference between the groups (D). Levels of KC measured in lung protein extracts (E) were increased after OVA challenge in both groups (p = 0.0001). LIX expression was studied in BALF by Western blots (F) showing a significant increase in mice exposed to allergens (p < 0.05) without any obvious difference between MMP-8−/− and MMP-8−/− (G). IL-17 levels were measured in lung proteins extracts by ELISA, but no differences were found between the groups (H). Results are expressed as means ± SEM.
mice, but the extent of this increase was significantly higher in MMP-8−/− than in MMP-8+/+ mice (Fig. 9C). MMP-8 mRNAs were rarely detectable in the samples, and levels of TIMP-1 did not display significant differences between the groups (Fig. 9D).

Discussion

Based on clinical data depicting increased levels of MMP-8 (neutrophil collagenase) in the sputum from asthmatics (24) and a potentially important role of neutrophils in asthma (31), we studied the MMP-8 expression after allergen exposure in mice and found MMP-8 to be significantly increased after allergens. Therefore, we decided to apply a mouse model of allergen-induced airway inflammation to recently generated MMP-8−/− mice (25). In contrast to initial expectation, we demonstrate in this study that MMP-8 deletion in mice did not impair the development of allergen-induced airway inflammation. On the contrary, when compared with allergen-exposed MMP-8−/− mice, MMP-8+/+ mice displayed: 1) an increased neutrophilic inflammation in the BALF and peribronchial area; 2) an enhanced eosinophilic infiltration in airway walls; and 3) increased levels of IgE and IgG1 in serum and of IL-4 in BALF. Allergen exposure was linked to increased levels of MMP-8 by allergens, but at a lower extent in MMP-8−/− mice (25). Interestingly, IL-4 has been reported to induce a delay of neutrophil recruitment and trafficking. In the present study, in MMP-8+/+ mice exposed to OVA, we report in this work for the first time an increased expression of LIX, KC, and MIP-2 in the BALF or lung parenchyma from allergen-exposed mice. Nevertheless, because their levels were similar in both genotypes, the enhancement of those chemoattractants for neutrophils cannot explain per se our finding of increased neutrophilic inflammation in the BALF of MMP-8−/− mice after allergen exposure. We acknowledge that by measuring mediator levels by ELISA, we cannot exclude the hypothesis that one of these mediators would have been cleaved modulating its chemokine activity, as demonstrated recently (35). Such a mediator processing can only rarely be addressed by Ab-based methods and requires the use of other techniques such as mass spectrometry. Another parameter not addressed by the present study and of potential relevance in the regulation of the MMP-cytokine-chemokine network of reciprocal interactions is the regulation of chemokine receptors on neutrophils. Of interest is our finding that IL-4 levels are increased after allergen exposure in the BALF of MMP-8−/− mice. IL-4 is a very potent inducer of many biological events and is one of the key cytokines in the development of Th2 inflammation. Interestingly, IL-4 has been reported to induce a delay of neutrophil apoptosis (36), suggesting that IL-4 by itself could play a role in the reduction of neutrophilic apoptosis observed in the lung and leading to neutrophil accumulation in the BALF. In addition, IL-4 is a multifunctional cytokine that can regulate the secretion of neutrophil-attracting chemokines such as growth-related onco-gene-α and IL-8 (37), and control the expression of VCAM-1 on the surface of vascular endothelial cells (38), thereby modulating neutrophil recruitment and trafficking. In the present study, increased IL-4 levels are linked with anti-OVA-specific IgE levels that were strongly enhanced in the serum of MMP-8−/− mice. Knowing that neutrophils bear on their plasma membrane a significant number of IgE receptors, these cells could therefore be activated directly by IgE (39). As IL-4 is a cytokine produced by lymphocytes, this suggests that the deletion of MMP-8 in mice could interfere with the function of those cells. In line with the increase of IL-4, specific anti-OVA IgE, and anti-OVA IgG1 levels in MMP-8−/−, suggesting a Th2 profile in serum, we report in this work that the increased neutrophil number in the lungs after allergen in MMP-8−/− mice results at least partly from a reduced apoptosis and a delayed clearance of recruited neutrophils. Accordingly, the protective effect of MMP-8 toward inflammatory responses in a chemically induced cancer mice model could also in part the consequence of a delayed apoptosis of neutrophils in MMP-8−/− mice (25). The exact role of MMP-8 in the cascade of events leading to neutrophil apoptosis remains to be determined.

The complexity of the cytokine/chemokine receptor network puts difficulties in elucidating an eventual contribution of cytokine/chemokine modulation in the observed neutrophil accumulation. We report in this work for the first time an increased expression of LIX, KC, and MIP-2 in the BALF or lung parenchyma from allergen-exposed mice. Nevertheless, because their levels were similar in both genotypes, the enhancement of those chemoattractants for neutrophils cannot explain per se our finding of increased neutrophilic inflammation in the BALF of MMP-8−/− mice after allergen exposure. We acknowledge that by measuring mediator levels by ELISA, we cannot exclude the hypothesis that one of these mediators would have been cleaved modulating its chemokine activity, as demonstrated recently (35). Such a mediator processing can only rarely be addressed by Ab-based methods and requires the use of other techniques such as mass spectrometry. Another parameter not addressed by the present study and of potential relevance in the regulation of the MMP-cytokine-chemokine network of reciprocal interactions is the regulation of chemokine receptors on neutrophils. Of interest is our finding that IL-4 levels are increased after allergen exposure in the BALF of MMP-8−/− mice. IL-4 is a very potent inducer of many biological events and is one of the key cytokines in the development of Th2 inflammation. Interestingly, IL-4 has been reported to induce a delay of neutrophil apoptosis (36), suggesting that IL-4 by itself could play a role in the reduction of neutrophilic apoptosis observed in the lung and leading to neutrophil accumulation in the BALF. In addition, IL-4 is a multifunctional cytokine that can regulate the secretion of neutrophil-attracting chemokines such as growth-related onco-gene-α and IL-8 (37), and control the expression of VCAM-1 on the surface of vascular endothelial cells (38), thereby modulating neutrophil recruitment and trafficking. In the present study, increased IL-4 levels are linked with anti-OVA-specific IgE levels that were strongly enhanced in the serum of MMP-8−/− mice. Knowing that neutrophils bear on their plasma membrane a significant number of IgE receptors, these cells could therefore be activated directly by IgE (39). As IL-4 is a cytokine produced by lymphocytes, this suggests that the deletion of MMP-8 in mice could interfere with the function of those cells. In line with the increase of IL-4, specific anti-OVA IgE, and anti-OVA IgG1 levels in MMP-8−/−, suggesting a Th2 profile in serum, we report in this work that the

![FIGURE 6. MIP-2 expression in lung tissue extracts 24 h after a 7-day allergen exposure. MIP-2 expression was analyzed by RT-PCR in lung tissue extracts, and its expression was increased after allergen in both groups without any differences between MMP-8−/− and MMP-8+/+ mice. Results are expressed as means ± SEM.](image)

![FIGURE 7. Counts of T1/ST2-positive cells in the peribronchial area from MMP-8−/− (n = 10) and MMP-8+/+ (n = 12) mice exposed to OVA (six peribronchial areas/mouse). Results are expressed as mean ± SEM. Measurements were performed 24 h after a 7-day allergen exposure.](image)
The number of cells expressing the orphan receptor T1/ST2 is increased in lung parenchyma of MMP-8−/− exposed to OVA, confirming that Th2 cell recruitment is increased in the lungs from MMP-8−/− in the context of allergenic stimulation. Very interestingly, a recent study by McMillan et al. (40), although using an exposure protocol based on high concentrations of allergens different from ours, demonstrated such an increased Th2 cell recruitment in the BALF of mice knockout for the MMP-9 gene. Taken together, those data clearly suggest that there exists a mutual interplay between the occurrence of Th2 inflammation and MMPs, those latter playing a crucial role in its control.

The increased levels of pro- and activated MMP-9 found in the lung tissue from mice exposed to allergens when compared with sham-challenged mice confirm our previous observations in mice and humans (14, 15, 30, 41). Furthermore, these data suggest that the bronchial morphological changes observed in asthma could be at least in part explained by an increase of the net MMP load in the lung of individuals exposed to allergens. In line with MMP-9, we also describe in this work increased levels of MMP-2 to be present in the lung parenchyma of mice after allergen exposure. MMP-2 could therefore take part in the increased smooth muscle mass described in a model similar to ours (33), because this protease has been reported to be mitogenic for smooth muscle cells in cell culture (34). In accordance with our previous report (30), MMP-12 levels were increased after allergen in both MMP-8+/+ and MMP-8−/−. However, interestingly, MMP-12 levels found in MMP-8−/− were significantly higher than in MMP-8+/+, suggesting an eventual compensatory mechanism of the MMP family following MMP-8 deletion.

In conclusion, our data demonstrate, in the context of allergic asthma, the anti-inflammatory effect of MMP-8 and suggest an implication of MMP-8 in the cascade of events leading to neutrophil apoptosis. As potential therapeutic issues have been proposed with MMP inhibitors, the present study describing an increased neutrophilic inflammation in MMP-8−/− warns against the use of nonspecific MMP inhibition. In this context, other authors have reported that MMP-2−/− mice display increased numbers of various inflammatory cells in the alveola due to a lack of egression of those cells (42). Taken together, these studies suggest that MMP inhibition should be selective and target specific MMPs, such as MMP-9 (30), for the therapy of asthma.

Acknowledgments
We thank Fabienne Perin-Rasquin, Cécile Caulier, and Fabrice Olivier for their excellent and essential technical assistance, and Romain Pauwels (deceased) for fruitful discussions.
Disclosures
The authors have no financial conflict of interest.

References