

LEVELS OF MATRIX METALLOPROTEINASE-9 AND ITS INHIBITOR IN GUINEA PIG ASTHMA MODEL FOLLOWING OVALBUMIN CHALLENGE

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□ *Our objective was to investigate the role of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and their cellular sources in guinea pig asthma model following ovalbumin (OA) challenge. Twenty guinea pigs were divided into 2 groups: OA-challenged group were sensitized by intraperitoneal injection with OA and exposed to OA while the control group used saline in a similar manner. The positive level index (PLI), defined as the percentage of positive-stained cells $\times$ average optical density, was used to assess the expressing levels of MMP-9 and TIMP-1. The levels of MMP-9 from OA-challenged group were significantly higher than those of controls. However, no significant difference of the TIMP-1 levels between the OA-challenged group and controls were found. MMP-9 was expressed strongly in both inflammatory and structural cells while TIMP-1 was expressed weakly in inflammatory cells and structural cells. Our results implied that MMP-9 may contribute to tissue remodeling in asthma and both structural and inflammatory cells within lung tissue are the main cellular sources of MMP-9. Excessive expression of MMP-9 in the early or acute stage of asthma may be associated with the initiation of bronchial injury and inflammatory cells accumulation.*

Keywords asthma, matrix metalloproteinase-9, remodeling, tissue inhibitor of matrix metalloproteinase-1

INTRODUCTION

Bronchial asthma is characterized by airways infiltration of inflammatory cells, namely eosinophils and T lymphocytes. Moreover, an imbalance between matrix degradation and resynthesis leads to an unregulated pathological process instead of normal tissue repair [1]. Structural changes, including subepithelial fibrosis, epithelial desquamation, goblet cell hyperplasia, and smooth muscle hyperplasia, all potentially contribute to the relatively increased airway narrowing following bronchoconstriction that

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is evident even after intensive anti-inflammatory therapy [2, 3]. These structural changes are poorly responsive to the current therapeutic strategies in asthma. Hence, the current goals in asthma research are, first, to identify key mediators of tissue remodeling, second to correlate physiology with pathology, and finally to provide better means for limiting airflow obstruction in asthma.

Matrix metalloproteinase-9 (MMP-9) is one of the matrix metalloproteinases (MMPs) that may play a central role in regulating matrix degradation. Tissue matrix metalloproteinases inhibitor-1 (TIMP-1) is the natural inhibitor of MMP-9. MMPs and their inhibitors, under normal circumstances, degrade damaged matrix and maintain the normal tissue homeostasis. However, under pathological conditions, MMPs and their inhibitors may be produced in excess and contribute to tissue damage and activation of inappropriate repair mechanisms. MMP-9 and TIMP-1 are of particular relevance to respiratory diseases, including pulmonary emphysema, hyperoxic lung injury, foreign body aspiration, and so on [4–7].

Our study aims to investigate the role of MMP-9 and TIMP-1 in a guinea pig asthma model following ovalbumin (OA) challenge as well as their cellular sources.

MATERIALS AND METHODS

Animal Mode

A total of 20 guinea pigs, including 10 males and 10 females with weight of 300 to 500 g, were purchased from Zhejiang Chinese Traditional Medicine and Drug Academy (No220010017). They were randomly divided into two groups. The OA-challenged group (10 guinea pigs) was sensitized by intraperitoneal injection daily with 1 ml 50% OA (Shanghai Chemistry Reagent Company F20011118) on day 0 to day 14. Starting from day 22, they were exposed to 1% OA (w/v diluted in sterile normal saline) through inhalation in a $30 \times 30 \times 30 \text{ cm}^3$ air-tightened vitreous box for 30 min every day for 1 week. The control group (10 guinea pigs) were injected with saline and exposed to inhaled saline in a similar manner.

Animals were sacrificed 8 hr after the last antigen inhalation. Lung tissue was fixed with 10% buffered neutral formalin. Then 4- μm -thick routine sections were used for immunohistochemistry study.

Immunohistochemistry

HRP-streptavidin complex method was used in immunohistochemical study. Goat ImmunoCruz Staining System (sc-2053) purchased from Santa

Cruz Biotechnology (USA) was used for immunohistochemical study in lung tissue samples according to the manufacturer's protocol. Negative and positive controls were done simultaneously.

The positive level index (PLI), defined as the percentage of positive-stained cells (P/A) $\times$ average optical density (AOD) in 10 random microscopic fields (magnification $\times$ 400), was used to assess expressing levels of MMP-9 and TIMP-1 [8, 9].

Statistical Analysis

Statistical analyses were conducted using SPSS software (10.0). Quantitative data were expressed as mean $\pm$ sd and Student's *t*-test was used to estimate the statistical significance. A 2-tailed $p < 0.05$ was considered significant.

RESULTS

All 10 OA-challenged guinea pigs showed cough, wheeziness, and deep breathe after OA inhalation for 1 week, which mimic the asthmatic reaction in human.

As shown in Table 1, P/A, AOD, and PLI of MMP-9 in lung tissue of OA-challenged group were significantly increased compared with the controls ($p < 0.001$, respectively). The PLI of MMP-9 in lung tissue of OA-challenged group increased over 25-fold relative to the controls. However, P/A, AOD, and PLI of TIMP-1 in lung tissue of OA-challenged group and controls were not significantly different ($p > 0.05$, respectively). The ratio of PLI between MMP-9 and TIMP-1 (MMP-9/TIMP-1 ratio) in OA-challenged group was significantly higher than that of controls ($p = 0.034$).

With regard to the source of MMP-9 and TIMP-1, we found that MMP-9 was expressed strongly in inflammatory cells (e.g., lymphocytes and

TABLE 1 MMP-9 and TIMP-1 Levels of OA-Challenged Group and Control Group

		Control group	OA-challenged group	<i>t</i> value	<i>p</i> value
MMP-9	P/A	4.40 $\pm$ 2.41	61.60 $\pm$ 14.77	12.089	<0.001
	AOD	0.47 $\pm$ 0.14	0.87 $\pm$ 0.08	7.538	<0.001
	PLI	2.01 $\pm$ 1.13	53.27 $\pm$ 13.08	12.342	<0.001
TIMP-1	P/A	4.20 $\pm$ 1.18	4.40 $\pm$ 2.95	0.183	0.857
	AOD	0.35 $\pm$ 0.07	0.40 $\pm$ 0.12	0.981	0.340
	PLI	1.50 $\pm$ 0.80	1.69 $\pm$ 1.03	0.442	0.664
MMP-9/TIMP-1	(PLI)	1.87 $\pm$ 0.02	64.76 $\pm$ 79.65	2.497	0.034

PA = positive-stained cells, AOD = average optical density, and PLI = positive index level.

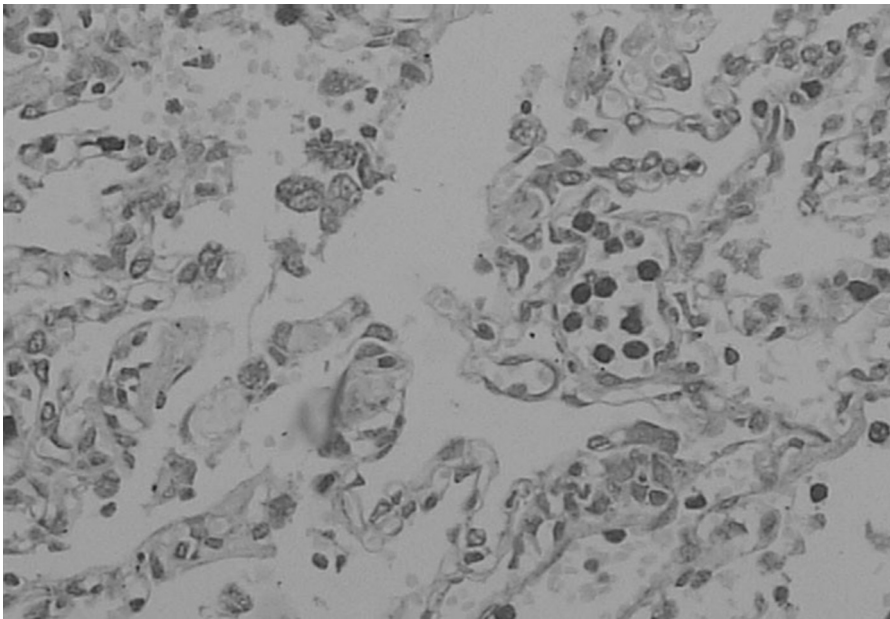


FIGURE 1 MMP-9 immunoreactivity in lung section from guinea pigs. Most macrophages and lymphocytes in lung and lymphocytes in microvasculature were positive ($\times 400$).

macrophages) both in lung tissue and vessel, as shown in Figure 1. Moreover, MMP-9 was positive in structural cells as well (e.g., epithelial cells, smooth muscle, and fibroblasts), as illustrated in Figure 2. TIMP-1 also was expressed in inflammatory and structural cells, but it was much weaker both in OA-challenged guinea pig (Figure 3) and controls.

DISCUSSION

Recent studies demonstrated that basement membranes of lung were damaged during asthma with the deposition of a provisional extracellular matrix (ECM) [10, 11]. The integrity of ECM is controlled by a dynamic equilibrium between synthesis and local degradation of its different components. The MMPs are believed to be the main physiological mediators of ECM degradation [5–7].

In this study, we developed an antigen-challenged guinea pig asthma model that may mimic the asthmatic reaction in humans. In this model, an increased expression of MMP-9 in lung tissue from OA-challenged guinea pig was noted when compared with controls. These results are in agreement with the study that showed an association among MMP-9, inflammatory cell accumulation, and cytokine release in sensitized and challenged mice [12].

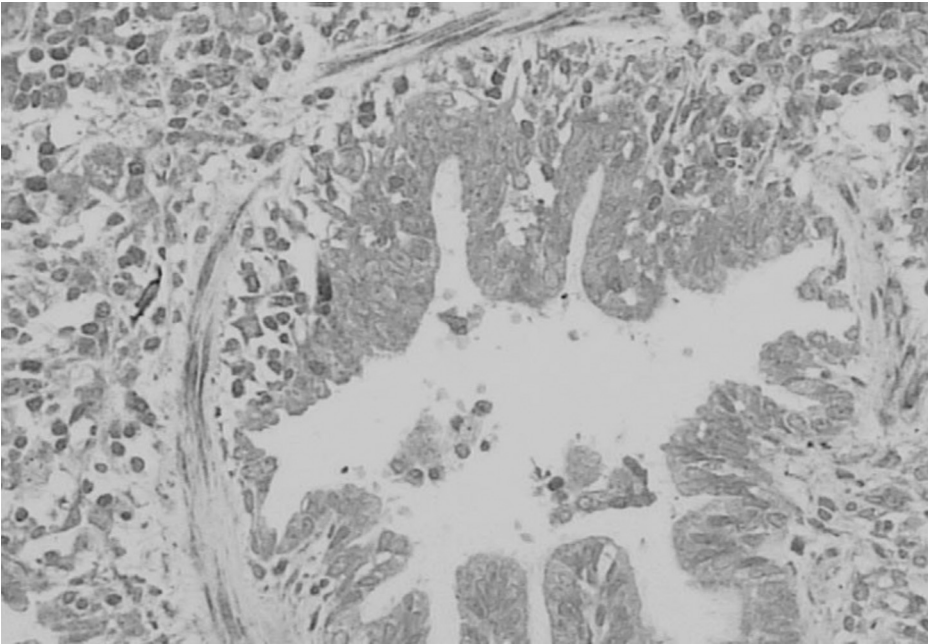


FIGURE 2 MMP-9 immunoreactivity in lung section of guinea pigs. Some epithelia and, smooth muscle and interstitial cells were positive ($\times 400$).

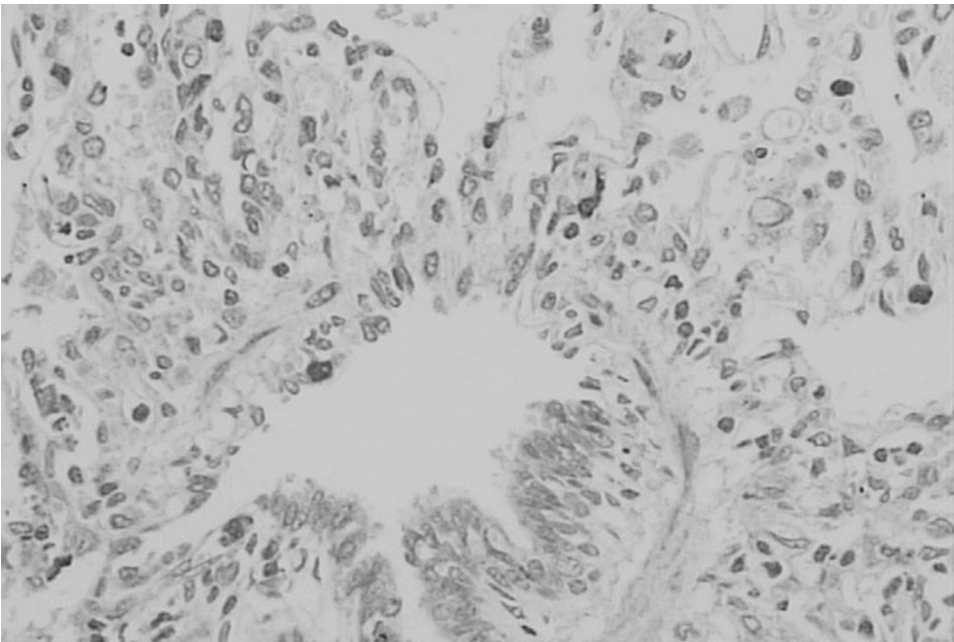


FIGURE 3 TIMP-1 immunoreactivity in lung section of guinea pigs. Few monocytes and lymphocytes were positive ($\times 400$).

There have growing evidences that MMP-9 may be crucial for the migration of airway inflammatory cells. *In vitro* studies have shown that MMP-9 is required for neutrophil, eosinophil and T-lymphocyte migration through synthetic matrix [13–16]. The increased generation of MMP-9 may be associated with the initiation of bronchial injury and inflammatory cell accumulation, which may lead further to inappropriate tissue remodeling in asthma. However, whether MMP-9 acts directly on these substrates or via activation of other proteases is still unclear.

Although MMP-9 shared some substrate specificities, this enzyme is known to be synthesized by different type of cells. Some studies reported that MMP-9 was produced mainly by inflammatory cells, such as neutrophils, lymphocytes, macrophages, and eosinophils [4, 7, 14, 17, 18]. However, based on our data, MMP-9 is produced by inflammatory cells both in lung tissue and microvasculature. In addition, structural cells, such as epithelial cells, smooth muscle, and fibroblasts, strongly expressed MMP-9 as well. These results indicate that both structural and inflammatory cells are important sources of MMP-9 and that the recruitment and activation of inflammatory cells within the microvasculature of the bronchial circulation is most likely to be the source of MMP-9 in the circulation.

Appropriate regulation of MMPs and their inhibitors is important for tissue healing. Endogenous TIMPs presumably exert some protective effects in preventing ECM degradation in tissues. TIMPs also are known to promote fibroblast hyperplasia [19]. Recent studies demonstrated an imbalance in the ratio of MMP-9 to TIMP-1 in serum and sputum from asthmatic subjects [20–22]. In our study, however, the expression of TIMP-1 did not increase and a significantly higher MMP-9/TIMP-1 ratio was found in lung tissue from OA-challenged guinea pig in comparison with those of controls. As this animal model is an acute and early stage model of asthma, the results imply that it is MMP-9, not TIMP-1, that increases in the early or acute stage of asthma. Also, the increased expression of MMP-9 may be associated with initiation of bronchial injury and inflammatory cell accumulation via matrix degradation. It is might possible that increased levels of TIMP-1 following MMP-9 will result in the excessive deposition of ECM component.

SUMMARY

Our findings suggest that MMP-9 produced by structural and inflammatory cells within bronchial tissue contribute to tissue remodeling via matrix degradation in the early or acute stage of asthmatic model. Excessive expression of MMP-9 in the early stage of asthma may be associated with the initiation of bronchial injury and inflammatory cells accumulation.

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