

Matrix metalloproteinase inhibitor regulates inflammatory cell migration by reducing ICAM-1 and VCAM-1 expression in a murine model of toluene diisocyanate-induced asthma

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Background: Matrix metalloproteinase-9 (MMP-9) has been reported to play a crucial role in the transmigration of neutrophils, lymphocytes, and eosinophils. Neutrophils, eosinophils, and lymphocytes migrate from the blood to the lungs in response to inflammatory mediators produced in the airways and are subsequently released into the circulation. This traffic is mediated by adhesion molecules. However, little is known about the migration of inflammatory cells through the endothelial and epithelial basement membranes in toluene diisocyanate (TDI)-induced asthma.

Objectives: An aim of this study was to evaluate the effect of MMP inhibitors on the expression of ICAM-1 and VCAM-1 in the migration of inflammatory cells in a murine model of TDI-induced asthma.

Methods: We used a murine model to investigate TDI-induced asthma to examine the possible involvement of ICAM-1 and VCAM-1 in the pathogenesis of that disease and the effect of MMP inhibitors on the expression of ICAM-1 and VCAM-1.

Results: In mice, the following typical pathophysiologic features develop in the lungs: increased numbers of inflammatory cells and increased expression of MMP-9, ICAM-1, and VCAM-1 mRNA and protein. Administration of MMP inhibitors reduced the increased numbers of inflammatory cells and the increased expression of ICAM-1 and VCAM-1 mRNA expression and protein. In addition, MMP inhibitors significantly abrogated the increased expression of IL-1 β , IL-4, and TNF- α mRNA in lung tissues and levels of IL-1 β , IL-4, and TNF- α in bronchoalveolar lavage fluids after TDI inhalation.

Conclusions: These results suggest that MMP inhibitors regulate inflammatory cell migration by reducing ICAM-1 and VCAM-1 expression and possibly also by suppressing IL-1 β ,

IL-4, and TNF- α expression. (J Allergy Clin Immunol 2003;111:1278-84.)

Key words: Toluene diisocyanate, matrix metalloproteinase, asthma, adhesion molecules, cytokines

Toluene diisocyanate (TDI)-induced asthma is characterized by hyperresponsiveness and inflammation of the airways.¹ This inflammation is associated with the infiltration of lymphocytes, eosinophils, and neutrophils into the bronchial lumen.^{1,2} These cellular infiltrates release various chemical mediators, which can cause airway hyperresponsiveness.³⁻⁵ The recruitment of these inflammatory cells from the blood to the site of inflammation is regarded as an essential event in the development and maintenance of airway inflammation. The infiltration of inflammatory cells through endothelial and epithelial structures has been investigated and shows the involvement of adhesion molecules, cytokines, and chemokines.

Matrix metalloproteinases (MMPs) are major proteolytic enzymes that are involved in extracellular matrix (ECM) turnover because of their ability to cleave all proteins constituting ECM. Previous studies have reported that MMP-9 might play a role in chronic airway inflammation and remodeling in asthma.^{6,7} MMP-9 also induces the migration of eosinophils, lymphocytes, and neutrophils across basement membranes.⁸⁻¹⁰ Recently, we demonstrated that the administration of an MMP inhibitor reduces the migration of inflammatory cells through the endothelial and epithelial basement membranes.¹¹ Recruitment of leukocytes from the circulating blood into tissues requires a series of cell adhesion molecule-mediated interactions between the vascular endothelium and leukocyte cell surfaces. Adhesion molecules such as ICAM-1 and VCAM-1 are shown to play important roles in the induction of airway inflammation. However, little is known about the migration of inflammatory cells through the endothelial and epithelial basement membranes in TDI-induced asthma.

In this study, we used a murine model to investigate TDI-induced asthma to examine the possible involvement of ICAM-1 and VCAM-1 in the pathogenesis of that disease. An additional aim of the study was to evaluate the effect of MMP inhibitors on the expression of ICAM-1 and VCAM-1.

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Abbreviations used

BAL: Bronchoalveolar lavage
ECM: Extracellular matrix
MMP: Matrix metalloproteinase
TDI: Toluene diisocyanate

METHODS

Animals and experimental protocol

Female BALB/c mice, 10 to 12 weeks of age, free of murine-specific pathogens, were obtained from the Korean Research Institute of Chemistry Technology (Daejeon, Korea). All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School. Standard guidelines for laboratory animal care were followed.¹² Mice were sensitized twice by 2 courses of intranasal administration of 3% TDI dissolved in ethyl acetate/olive oil (1:4) once per day for 5 consecutive days with a 3-week interval, as previously described.^{11,13} Seven days later, mice were challenged through the airways with 1% TDI dissolved in ethyl acetate/olive oil (1:4) by ultrasonic nebulization (NE-U12, Omron, Tokyo, Japan). As a control, mice were sensitized and challenged with the use of the same protocol but only with the solvent, ethyl acetate/olive oil (1:4).

Bronchoalveolar lavage (BAL) was performed at 0, 1, 6, 24, 48, and 72 hours after the challenge. After euthanasia with sodium pentobarbital (100 mg/kg body wt), prewarmed 0.9% NaCl solution was slowly infused into the lungs and withdrawn. Total cell numbers were counted with a hemocytometer. Smears of BAL cells prepared with a Cytospin II (Shandon, Runcorn, UK) were stained with Diff-Quik solution (Dade Diagnostics of P.R. Inc, Aguada, PR) for differential cell counting. Two independent, blinded investigators counted the cells, using a microscope. Approximately 400 cells were counted in each of four different random locations.

Measurements of IL-1 β , IL-4, TNF- α , and MMP-9 in BAL fluids

Levels of IL-1 β , IL-4, and TNF- α (Endogen, Inc, Woburn, Mass) and the proform of MMP-9 (pro-MMP-9) (Fuji Chemical Industries, Toyama, Japan) were quantified by enzyme immunoassays according to the manufacturer's protocols. The hydrolytic activity of MMP-9 was measured by gelatin zymography as described previously.^{6,11}

Administration of MMP inhibitors

Inhibitors of MMPs, MMP inhibitor I (MMPI-I; IC₅₀ for MMP-9 = 150 μ mol/L; 4-Abz-Gly-Pro-D-Leu-D-Ala-NHOH; Calbiochem, San Diego, Calif), and MMP-2/MMP-9 inhibitor II (MMPI-II; IC₅₀ for MMP-9 = 30 nmol/L; (2R)-2-[(4-biphenylsulfonfyl)amino]-N-hydroxy-3-phenylpropionamide; Calbiochem) were used to inhibit MMP-9 activity. MMPI-I (20 mg/kg body wt per day IP) dissolved in distilled water or MMPI-II (2 mg/kg body wt per day IP) dissolved in DMSO was administered 3 times at 24-hour intervals, beginning 30 minutes before the TDI challenge.

Western blot analysis

Lung tissues were homogenized, washed with PBS, and incubated in lysis buffer plus a protease inhibitor cocktail (Sigma, St Louis, Mo) to obtain extracts of lung proteins. Western blot analysis was performed as described previously.¹³ Samples were loaded on 8 SDS-PAGE gels and were separated at 120 V for 90 minutes. The blots were incubated with an anti-MMP-9 antibody, diluted 1:800, anti-ICAM-1 antibody, diluted 1:800, or anti-VCAM-1 antibody,

diluted 1:1000 (all from Santa Cruz Biotechnology, Santa Cruz, Calif) overnight at 4°C. The membranes were stripped and reblotted with antiactin antibody (Sigma) to verify equal loading of protein in each lane.

RNA Isolation and RT-PCR

Total RNA from lung tissues was isolated with the use of a rapid extraction method (TRI-Reagent), as previously described.¹⁴ Total RNA was reverse-transcribed to cDNA in a buffer. The first-strand cDNAs were used for the PCR amplification of MMP-9, ICAM-1, VCAM-1, IL-1 β , IL-4, TNF- α , or GAPDH. PCR amplification was performed by mixing 3 μ L of RT reaction with 47 μ L of buffer containing 2.5 U of Taq DNA polymerase (Promega, Madison, Wis) and 30 pmol/L of specific primer pairs for mouse cDNAs of MMP-9, ICAM-1, VCAM-1, IL-1 β , IL-4, TNF- α , or GAPDH, designed from published mouse gene sequences.

Densitometric analyses and statistics

All signals were visualized and analyzed by densitometric scanning (LAS-5000; Fuji Film, Tokyo, Japan). Data are expressed as mean $\pm$ SD. Statistical comparisons were performed by 1-way analysis of variance, followed by the Fisher test. Significant differences between groups were determined by the unpaired Student *t* test. Correlations were calculated by means of the Spearman rank test. Statistical significance was set at *P* < .05.

RESULTS

ICAM-1, VCAM-1, or MMP-9 expression in the lung tissues and BAL fluids of mice sensitized and challenged with TDI

ICAM-1 mRNA expression in the lung tissues was significantly increased at 24, 48, and 72 hours after TDI inhalation compared with levels before inhalation or in the control group. In contrast, no significant change in ICAM-1 mRNA expression was observed after inhalation of the vehicle control (Fig 1, A). Western blot analysis also showed that ICAM-1 protein levels in the lung tissues were significantly increased at 24, 48, and 72 hours after TDI inhalation compared with levels before inhalation or in the control group. No significant changes in ICAM-1 protein levels were observed after inhalation of the vehicle control (Fig 1, B). VCAM-1 and MMP-9 mRNA expression were significantly increased at 6, 24, 48, and 72 hours after TDI inhalation compared with levels before inhalation or in the control group. In contrast, no significant changes in VCAM-1 and MMP-9 mRNA expression were observed after inhalation of the vehicle control (Fig 1, C and E). Western blot analysis revealed that VCAM-1 and MMP-9 protein levels were significantly increased at 6, 24, 48, and 72 hours after TDI inhalation compared with levels before inhalation or in the control group. In contrast, no significant changes in VCAM-1 and MMP-9 protein levels were observed after inhalation of the vehicle control (Fig 1, D and F). Gelatin zymography revealed that pro-MMP-9 levels in the BAL fluids were significantly increased at 6, 24, 48, and 72 hours after TDI inhalation compared with levels before inhalation or in the control group. No significant change in the pro-MMP-9 level was observed after inhalation of the vehicle control (Fig 1, G).

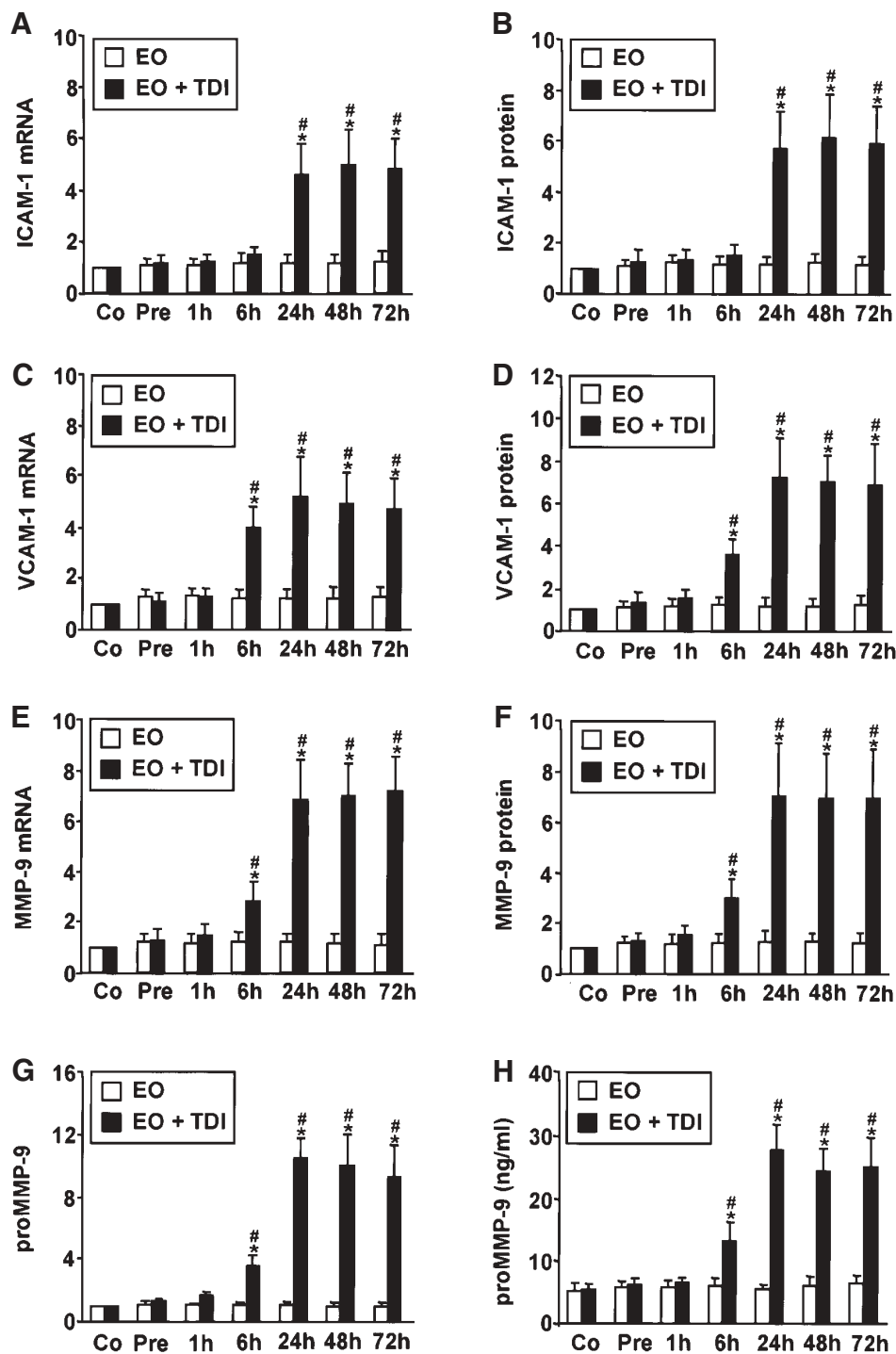


FIG 1. Effect of TDI challenge on ICAM-1, VCAM-1, and MMP-9 expression in lung tissues and in BAL fluids. **A, C, and E,** RT-PCR of ICAM-1, VCAM-1, and MMP-9. Densitometric analyses are presented as relative ratio of each molecule mRNA to GAPDH mRNA. Relative ratio of each molecule mRNA in lung tissues of control mice (Co) is arbitrarily presented as 1. **B, D, and F,** Western blotting of ICAM-1, VCAM-1, and MMP-9. Densitometric analyses are presented as relative ratio of each protein to actin. Relative ratio of each protein in lung tissues of control mice is arbitrarily presented as 1. **G,** Gelatin zymography. Densitometric analyses are presented as relative ratio of induction of pro-MMP-9 before and after TDI challenge. Pro-MMP-9 in BAL fluids of control mice is arbitrarily presented as 1. **H,** Enzyme immunoassay of pro-MMP-9. Data represent mean $\pm$ SD from 6 independent experiments. EO, Vehicle control; Co, no treatment; Pre, before challenge; 1, 6, 24, 48 and 72 hours, after challenge. * $P < .05$ vs Pre; # $P < .05$ vs EO.

Consistent with the results obtained from the gelatin zymography, enzyme immunoassays revealed that the level of pro-MMP-9 was increased after challenge with TDI (Fig 1, *H*). Total cells, lymphocytes, neutrophils, and eosinophils were counted in the BAL fluids at the same time points, and there were significant correlations between the level of pro-MMP-9 and the numbers of total cells ($r = 0.729$, $P = .000$), lymphocytes ($r = 0.457$, $P = .005$), neutrophils ($r = 0.776$, $P = .000$), or eosinophils ($r = 0.699$, $P = .000$) (data not shown).

MMP inhibitors decrease MMP-9 mRNA expression and protein levels in BAL fluids and lung tissues of TDI-sensitized and TDI-challenged mice

The administration of an MMP inhibitor (MMPI-I or MMPI-II) significantly reduced the increase in expression of MMP-9 mRNA and protein in lung tissues 72 hours after TDI inhalation (data not shown). In addition, the administration of either MMP inhibitor dramatically reduced the level of pro-MMP-9 in BAL fluids 72 hours after TDI inhalation. Consistent with the results obtained from the gelatin zymography, enzyme immunoassays revealed that MMP inhibitors decreased the levels of pro-MMP-9 in the BAL fluids 72 hours after the TDI inhalation (data not shown).

MMP inhibitors decrease the increased numbers of inflammatory cells in BAL fluids of TDI-sensitized and TDI-challenged mice

Total cell numbers in BAL fluids were significantly greater from 6 to 72 hours after TDI inhalation compared with cell numbers before the inhalation or those in the control group. At 6 hours after TDI inhalation, neutrophils were increased in the BAL fluid, and the increased number of neutrophils continued up to 72 hours after TDI inhalation. The numbers of lymphocytes and eosinophils in BAL fluids were also increased significantly from 6 to 72 hours after TDI inhalation. In contrast, no significant changes in cellular components were observed after inhalation of the control agent, ethyl acetate/olive oil. The administration of MMPI-I or MMPI-II significantly reduced the increase in total cells, in neutrophils, in lymphocytes, and in eosinophils, elicited in the airway lumen 3 days after TDI inhalation compared with control animals (Fig 2).

MMP inhibitors reduce expression of ICAM-1 and VCAM-1 mRNA and protein in lung tissues and decrease IL-1 β , IL-4, and TNF- α mRNA expression in lung tissues and IL-1 β , IL-4, and TNF- α in BAL fluids of TDI-sensitized and TDI-challenged mice

The administration of an MMP inhibitor (MMPI-I or MMPI-II) significantly reduced the increase in expression of ICAM-1 and VCAM-1 mRNA and protein in the

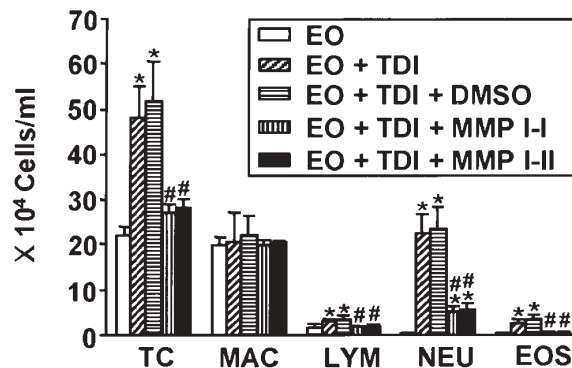


FIG 2. Effect of MMPI-I or MMPI-II on total and differential cellular components of BAL fluids in TDI-sensitized and TDI-challenged mice. Sampling was performed at 72 hours after treatment with vehicle only (EO), EO plus TDI, EO plus TDI plus DMSO, EO plus TDI plus MMPI-I, or EO plus TDI plus MMPI-II. Numbers of each cellular component were counted. Bars represent mean $\pm$ SD from 6 independent experiments. TC, Total cell; MAC, macrophage; LYM, lymphocyte; NEU, neutrophil; EOS, eosinophil. * $P < .05$ vs EO; # $P < .05$ vs EO+TDI.

lung tissues at 72 hours after TDI inhalation (Fig 3). The administration of an MMP inhibitor (MMPI-I or MMPI-II) also reduced significantly the increase in IL-1 β , IL-4, and TNF- α mRNA expression in the lung tissues (Fig 4, *A* through *F*). In addition, the administration of either MMP inhibitor dramatically reduced the levels of IL-1 β , IL-4, and TNF- α in BAL fluids 72 hours after the TDI inhalation (Fig 4, *G*).

DISCUSSION

In this murine model of TDI-induced asthma, expression of MMP-9, ICAM-1, and VCAM-1 mRNA and levels of proteins were increased in the lung after TDI challenge. These effects were MMP dependent in that the administration of an MMP inhibitor reduced both the increased numbers of inflammatory cells and the increase in expression of ICAM-1 and VCAM-1 mRNA and protein. In addition, MMP inhibitors abrogated the increase in IL-1 β , IL-4, and TNF- α mRNA expression in lung tissues and in IL-1 β , IL-4, and TNF- α levels in BAL fluids after TDI inhalation.

MMPs are a family of zinc- and calcium-dependent endopeptidases capable of proteolytically degrading many components of the ECM. Of the MMPs, MMP-9 is of particular relevance to asthma. In a murine model, inhibition of MMP-9 by administration of an MMP inhibitor decreased antigen-induced airway inflammation and hyperresponsiveness.¹⁵ MMP-9 has been reported to play a crucial role in the transmigration of eosinophils, lymphocytes, and neutrophils in vitro.⁸⁻¹⁰ We recently reported that the release of MMP-9 was upregulated in association with the accumulation of inflammatory cells in the airways in a murine model of TDI-induced asthma.¹¹ These findings have suggested that the inhibition of MMP-9 might be a good therapeutic

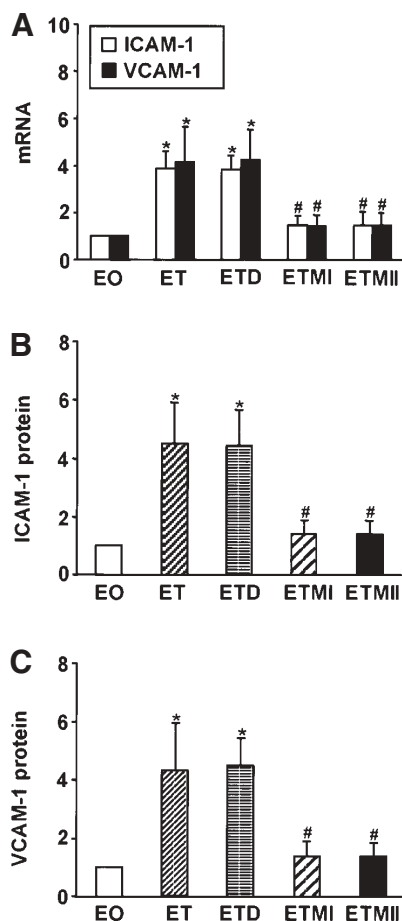


FIG 3. Effect of MMPI-I or MMPI-II on ICAM-1 and VCAM-1 expression in lung tissues of TDI-sensitized and TDI-challenged mice. Sampling was performed at 72 hours after treatment with vehicle only (EO), EO plus TDI (ET), EO plus TDI plus DMSO (ETD), EO plus TDI plus MMPI-I (ETMI), or EO plus TDI plus MMPI-II (ETMII). **A**, RT-PCR of ICAM-1 and VCAM-1. Densitometric analyses are presented as relative ratio of ICAM-1 or VCAM-1 mRNA to GAPDH mRNA. Relative ratio of ICAM-1 or VCAM-1 mRNA in lung tissues of EO mice is arbitrarily presented as 1. **B** and **C**, Western blotting of ICAM-1 and VCAM-1. Densitometric analyses are presented as relative ratio of ICAM-1 or VCAM-1 to actin. Relative ratio of ICAM-1 or VCAM-1 in lung tissues of EO mice is arbitrarily presented as 1. Data represent mean $\pm$ SD from 6 independent experiments. * $P < .05$ vs EO; # $P < .05$ vs ET.

tic strategy for TDI-induced asthma. In the current study, we have found that the expression of MMP-9 mRNA and protein in the lung tissues was significantly increased at 6, 24, 48, and 72 hours after TDI inhalation compared with the levels before inhalation or in the control group. Furthermore, pro-MMP-9 levels in the BAL fluids were significantly increased at 6, 24, 48, and 72 hours after TDI inhalation compared with the levels before inhalation or in the control group. These findings are consistent with previous reports that concentrations of MMP-9 were increased in the BAL fluids, sputum, and serum of asthmatic patients.^{6,16-18} In this study, the results showed that there were significant correlations between the level of

MMP-9 and the numbers of lymphocytes, neutrophils, or eosinophils. In addition, inhibitors of MMP-9 decreased the increased numbers of inflammatory cells in BAL fluids of TDI-sensitized and TDI-challenged mice. Therefore, we conclude that MMP-9 may play a crucial role in facilitating the migration of these cells to the airways in TDI-induced asthma.

TDI-induced asthma has been recognized as a disease resulting from chronic airway inflammation characteristically associated with the infiltration of lymphocytes, eosinophils, and neutrophils into the bronchial lumen.^{1,2} There is increasing evidence that cytokine-inducible leukocyte-endothelial adhesion molecules are important in the recruitment and migration of leukocytes to the sites of inflammation.^{19,20} Vascular endothelial cell adhesion molecules are now regarded as important contributors to the migration of leukocytes into inflamed tissues. Representative cell adhesion molecules include ICAM-1 and VCAM-1. ICAM-1 is constitutively expressed on endothelial and epithelial cells in subjects with or without asthma.²¹ ICAM-1 is upregulated on the bronchial vascular endothelium after bronchial allergen challenge in the allergen-sensitized patients with asthma.²² VCAM-1 is markedly upregulated on the human bronchial vascular endothelium of subjects with asthma who have airflow limitation.²³ In this study, we found that the expression of ICAM-1 and VCAM-1 mRNA and protein in lung tissues was significantly increased after TDI inhalation compared with the levels before inhalation or in the control group. Therefore, our data suggest that ICAM-1 and VCAM-1 may play an important role in inducing and maintaining TDI-induced asthma.

We administered MMPI-I or MMPI-II to evaluate the effect of MMP inhibitors on the expression of ICAM-1 and VCAM-1. MMP inhibitors reduced the expression of ICAM-1 and VCAM-1 mRNA and protein in lung tissues of TDI-sensitized and TDI-challenged mice. One likely mechanism for the inhibitory effect of MMPI-I or MMPI-II on the expression of ICAM-1 and VCAM-1 is that MMP-9 alters ECM to allow the migration of inflammatory cells, including neutrophils, lymphocytes, and eosinophils, to the airways. In this study, the administration of MMP inhibitors not only reduces MMP-9, ICAM-1, and VCAM-1 levels in the BAL fluids and/or lung tissues but inhibits the migration of inflammatory cells through the endothelial and epithelial basement membranes. It has long been appreciated that the expression of ICAM-1 or VCAM-1 is modulated by cytokines such as IL-1 β , IL-4, and TNF.²⁴⁻²⁶ Our data demonstrate that MMP inhibitors reduce the increased numbers of inflammatory cells in the airways, which are the source of IL-1 β , IL-4, and TNF, and then decrease the increased expression of IL-1 β , IL-4, and TNF- α mRNA in lung tissues and the levels of IL-1 β , IL-4, and TNF- α in BAL fluids of TDI-sensitized and TDI-challenged mice. Decrease in the expression of ICAM-1 and VCAM-1 mRNA and protein may be due to the reduced IL-1 β , IL-4, and TNF- α mRNA and protein in the lungs. Thus, the MMP inhibitors abolish the migration of inflammatory

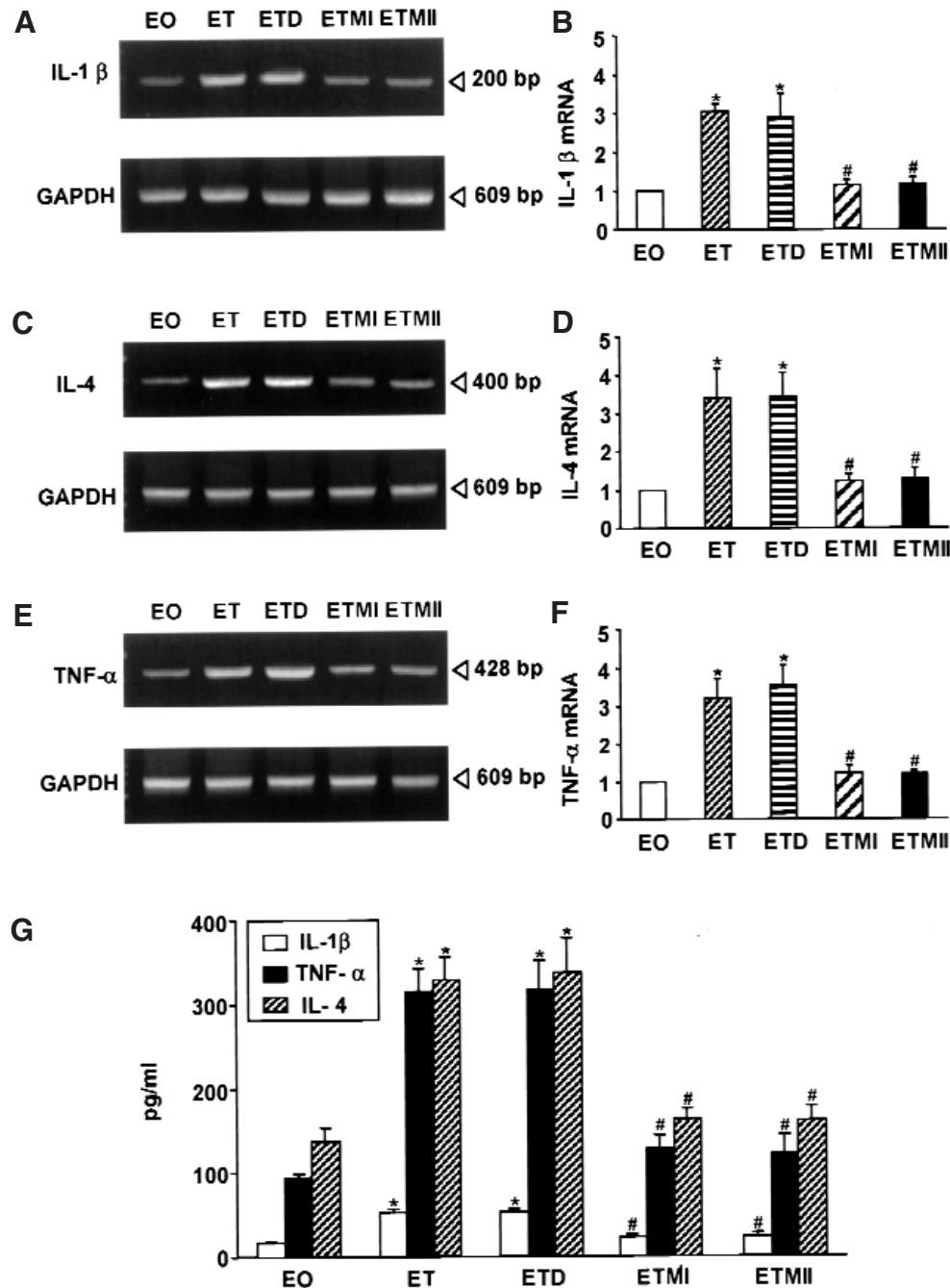


FIG 4. Effect of MMPI-I or MMPI-II on IL-1 β , IL-4, and TNF- α mRNA expression in lung tissues and IL-1 β , IL-4, and TNF- α levels in BAL fluids of TDI-sensitized and TDI-challenged mice. Sampling was performed at 72 hours after treatment with vehicle only (EO), EO plus TDI (ET), EO plus TDI plus DMSO (ETD), EO plus TDI plus MMPI-I (ETMI), or EO plus TDI plus MMPI-II (ETMII). **A**, **C**, and **E**, RT-PCR of IL-1 β , IL-4, and TNF- α . **B**, **D**, and **F**, Densitometric analyses are presented as relative ratio of each molecule mRNA to GAPDH mRNA. Relative ratio of each molecule mRNA in lung tissues of EO mice is arbitrarily presented as 1. **G**, Enzyme immunoassay of IL-1 β , IL-4, and TNF- α . Data represent mean $\pm$ SD from 6 independent experiments. * P < .05 vs EO; # P < .05 vs ET.

cells. Hence, these results strongly indicate that MMP inhibitors regulate inflammatory cell migration by reducing ICAM-1 and VCAM-1 expression and possibly also by suppressing IL-1 β , IL-4, and TNF- α expression.

REFERENCES

1. Mapp CE, Boschetto P, Zocca E, Milani GF, Pivrotto F, Tegazzin V, et al. Pathogenesis of late asthmatic reactions induced by exposure to isocyanates. *Bull Eur Physiopathol Respir* 1987;23:583-6.

2. Boschetto P, Fabbri LM, Zocca E, Milani G, Pivrotto F, Dal Vecchio A, et al. Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. *J Allergy Clin Immunol* 1988;80:261-7.
3. Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
4. Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991;87:893-910.
5. Busse WW, William FC, Sedgwick JD. Mechanism of airway inflammation in asthma. *Am Rev Respir Dis* 1993;147:S20-4.
6. Vignola AM, Riccobono L, Mirabella A, Profita M, Chanez P, Bellia V, et al. Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airway obstruction in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1998;158:1945-50.
7. Hoshino M, Takahashi M, Takai Y, Sim JJ. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol* 1999;104:356-63.
8. Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane component in vitro: role of matrix metalloproteinase-9. *Am J Respir Cell Mol Biol* 1997;17:519-28.
9. Leppert D, Waubant E, Galaray N, Bunnett W, Hauser SL. T cell gelatinases mediate basement membrane transmigration in vitro. *J Immunol* 1995;154:4379-89.
10. Delclaux C, Delacourt C, D'Ortho MP, Boyer V, Lafuma C, Harf A. Role of gelatinase B and elastase in human polymorphonuclear neutrophil migration across basement membrane. *Am J Respir Cell Mol Biol* 1996;14:288-95.
11. Lee YC, Song CH, Lee HB, Oh JR, Rhee YK, Park HS, et al. A murine model of toluene diisocyanate-induced asthma can be treated with matrix metalloproteinase inhibitor. *J Allergy Clin Immunol* 2001;108:1021-6.
12. Institute of Laboratory Animal Resources Commission of Life Sciences NRC. Guide for the care and use of laboratory animals. Washington, DC: National Academy Press; 1996.
13. Lee YC, Kwak YG, Song CH. Contribution of vascular endothelial growth factor to airway hyper-responsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. *J Immunol* 2002;168:3595-600.
14. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid-guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
15. Kumagai K, Ohno I, Okada S, Ohkawara Y, Suzuki K, Shinya T, et al. Inhibition of matrix metalloproteinase prevents allergen-induced airway inflammation in a murine model of asthma. *J Immunol* 1999;162:4212-9.
16. Mautino G, Oliver N, Chanez P, Bousquet J, Capony F. Increased release of matrix metalloproteinase-9 in bronchoalveolar lavage fluid and by alveolar macrophages of asthmatics. *Am J Respir Cell Mol Biol* 1997;17:583-91.
17. Lemjabbar H, Gosset P, Lamblin C, Tillie I, Hartmann D, Wallaert B, et al. Contribution of 92 kDa gelatinase type IV in bronchial inflammation during status asthmaticus. *Am J Respir Crit Care Med* 1999;159:1298-307.
18. Bosse M, Chakir J, Rouabhi M, Boulet LP, Audette M, Laviolette M. Serum matrix metalloproteinase-9: tissue inhibitor of metalloproteinases-1 ratio correlates with steroid responsiveness in moderate to severe asthma. *Am J Respir Crit Care Med* 1999;159:596-602.
19. Montefort S, Holgate ST. The role of adhesion molecules in inflammation. *Respir Med* 1991;85:91-9.
20. Wegner CD, Gundel RH, Reilly P, Haynes N, Gordon Letts L, Rothlein R. ICAM-1 in the pathogenesis of asthma. *Science* 1990;247:456-9.
21. Montefort S, Roche WR, Howarth PH, Djukanovic R, Gratziau C, Carroll M, et al. Intercellular adhesion molecule-1 (ICAM-1) and endothelial leukocyte adhesion molecule-1 (ELAM-1) expression in the bronchial mucosa of normal and asthmatic subjects. *Eur Respir J* 1992;5:815-23.
22. Montefort S, Gratziau C, Goulding D, Polosa R, Haskard DO, Howarth PH, et al. Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules: 6 hours after local allergen challenge of sensitized asthmatic airways. *J Clin Invest* 1994;93:1411-21.
23. Ohkawara Y, Yamauchi K, Maruyama N, Hoshi H, Ohno I, Honma M, et al. In situ expression of the cell adhesion molecules in bronchial tissues from asthmatics with airflow limitation: in vivo evidence of VCAM-1/VLA-4 interaction in selective eosinophil infiltration. *Am J Cell Mol Biol* 1995;12:4-12.
24. Dustin ML, Rothlein R, Bhhan AK, Dinarello CA, Springer TA. Induction by IL-1 and IFN- γ : tissue distribution, biochemistry and function of a natural adherence molecule (ICAM-1). *J Immunol* 1986;137:245-54.
25. Osborn L, Heisson C, Tizard R, Vassallo C, Luhowskyi R, Chi-Rosso C, et al. Direct expression cloning of Vascular Cell Adhesion Molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 1989;59:1203-11.
26. Hirata N, Kohrogi H, Iwagoe H, Goto E, Hamamoto J, Fujii K, et al. Allergen exposure induces the expression of endothelial adhesion molecules in passively sensitized human bronchus: time course and the role of cytokines. *Am J Respir Cell Mol Biol* 1998;18:12-20.