

ORIGINAL PAPER

Cysteinyl leukotrienes enhance tumour necrosis factor- α -induced matrix metalloproteinase-9 in human monocytes/macrophages

T. Ichiyama, M. Kajimoto, M. Hasegawa, K. Hashimoto, T. Matsubara and S. Furukawa

Department of Pediatrics, Yamaguchi University School of Medicine, Yamaguchi, Japan

Clinical and Experimental Allergy

Summary

Background Matrix metalloproteinase-9 (MMP-9) is an important enzyme responsible for airway remodelling. Monocytes/macrophages have a cysteinyl leukotriene 1 (cysLT1) receptor, but its function is poorly understood.

Objective To elucidate the function of the cysLT1 receptor of human monocytes/macrophages in MMP-9 production.

Methods We examined the effect of cysLTs (LTC₄, -D₄ and -E₄) on TNF- α -induced MMP-9 production in THP-1 cells, a human monocytic leukaemia cell line and peripheral blood CD14⁺ monocytes/macrophages. In addition, we examined the effect of pranlukast, a cysLT1 receptor antagonist, on the enhancement of TNF- α -induced MMP-9 production by cysLTs.

Results ELISA revealed that LTC₄ and -D₄, but not -E₄, enhanced TNF- α -induced MMP-9 production in THP-1 cells and peripheral blood CD14⁺ monocytes/macrophages. Real-time polymerase chain reaction demonstrated that LTC₄ and -D₄, but not -E₄, increased MMP-9 mRNA expression induced by TNF- α in THP-1 cells. Moreover, we demonstrated that pranlukast completely inhibited the enhancement of TNF- α -induced MMP-9 production by LTC₄ and -D₄ in THP-1 cells and peripheral blood CD14⁺ monocytes/macrophages.

Conclusion LTC₄ and -D₄ enhanced the TNF- α -induced MMP-9 production via binding the cysLT1 receptor in human monocytes/macrophages. Pranlukast inhibited the enhancements by LTC₄ and D₄.

Keywords cysteinyl leukotrienes, matrix metalloproteinase-9, monocytes/macrophages, pranlukast, remodelling

Submitted 4 August 2006; revised 14 January 2007; accepted 8 February 2007

Correspondence:

Takashi Ichiyama, Department of Pediatrics, Yamaguchi University School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan.
E-mail: ichiyama@yamaguchi-u.ac.jp

Introduction

Matrix metalloproteinases (MMPs) constitute a family of enzymes that mediate the degradation of extracellular matrix proteins [1]. MMPs play important roles in normal and pathological processes, including embryogenesis, wound healing, inflammation, cardiovascular diseases, pulmonary diseases and cancer [2]. MMP-9 is a member of this family, and is capable of degrading collagen IV, a major component of the basement membrane of airways [3]. The activity of MMPs is further controlled by specific tissue inhibitors of metalloproteinases (TIMPs) [4]. TIMP-1 has a high avidity for MMP-9 [5]. The MMP-9, TIMP-1 and MMP-9/TIMP-1 balance is understood to be related to airway remodelling [6, 7]. A recent study demonstrated that allergen-challenged MMP-9-deficient mice had less

peribronchial fibrosis and total lung collagen compared with allergen-challenged wild-type mice [8].

Cysteinyl leukotrienes (cysLTs), such as leukotriene C₄ (LTC₄), -D₄ and -E₄, induce contraction of the tracheal muscle and have potent effects on leucocyte trafficking, airway mucus secretion and collagen synthesis [9–12]. Normal peripheral blood leucocytes, such as basophils, eosinophils, B lymphocytes and monocytes/macrophages, have a cysLT1 receptor [13]. However, the function of these cells, especially monocytes/macrophages, is poorly understood.

Alveolar macrophages (AM) are the most abundant cells, not only in the alveoli and distal air spaces but also in the conducting airways [14]. Activated AM in asthma may participate in the inflammatory events associated with allergic disease of the lower airways, including the

release of cytokines, chemokines, arachidonic acid metabolites, products of activated O₂ and by direct interaction with T lymphocytes [15–19].

We examined the effect of cysLTs on the productions of MMP-9 and TIMP-1, and on MMP-9 release by previously reported inducers of MMP-9, including IL-13, IL-17, TGF- β 1, TNF- α and lipopolysaccharide (LPS), in human monocytes/macrophages [20–24].

Methods

Cell culture and stimulation conditions

THP-1 cells, a human monocytic leukaemia cell line that has a cysLT1 receptor [25], obtained from the American Type Culture Collection, were maintained at 37 °C under humidified 5% CO₂ as stationary cultures. The cells were grown in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 100 U/mL of penicillin and 100 μ g/mL of streptomycin. Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood from 10 healthy medication-free volunteers, with informed consent, by Histopaque 1077 (Sigma Chemical Co., St Louis, MO, USA) gradient centrifugation and washing. Purification of individual cell subpopulations was achieved with a high magnetic gradient Mini MACS purification system (Miltenyi, Sunnyvale, CA, USA). CD14⁺ monocytes/macrophages were isolated by depletion of non-monocytes (negative selection) with a Monocyte Isolation Kit II (Miltenyi). The purity of the isolated cells was determined using the respective fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (Becton-Dickinson Biosciences, San Diego, CA, USA) and flow cytometric analysis (FACScan; Becton-Dickinson Biosciences).

Cells were exposed to 5 ng/mL of IL-13, 5 ng/mL of IL-17, 10 ng/mL of TGF- β 1, 20 ng/mL of TNF- α and 1 ng/mL of LPS (from *Escherichia coli*, 0111:B4) and/or cysLTs (Sigma Chemical Company). The doses were determined in accordance with previous studies that investigated MMP-9-release by each mediator [20–24]. Some samples were pretreated with pranlukast, a cysLT1 receptor antagonist, provided by ONO Pharmaceutical Co. (Osaka, Japan) for 30 min before the addition of cysLTs. The supernatant fluid, both before and after the addition of cysLTs, was harvested for the determination of MMP-9 and TIMP-1 levels, and then stored at –20 °C.

Determination of MMP-9 and TIMP-1 concentrations

The concentrations of MMP-9 and TIMP-1 were determined with sandwich-type ELISA kits (Amersham, Buckinghamshire, UK). Assays were performed following the instructions of the manufacturer. The detection limits were 0.125 ng/mL for MMP-9 and 2.4 ng/mL for TIMP-1.

The assay of MMP-9 recognizes the pro and active forms of MMP-9.

Real-time reverse transcription polymerase chain reaction

Real-time reverse transcription (RT)-polymerase chain reaction (PCR) was performed to determine the mRNA levels of MMP-9 in the THP-1 cells subjected to the indicated treatments. Total cellular RNA was isolated using the TRIzol reagent (Invitrogen, Leek, the Netherlands) according to the manufacturer's instructions. RT was carried out with 2 μ g of total RNA and Random Primers (Invitrogen) in a reaction volume of 20 μ L using the SuperScriptTM III RTS First-strand Strips System (Invitrogen) following the instructions provided. Real-time PCR was carried out using TaqMan Gene Expression Assays and the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The PCRs were recorded in real time and analyzed using the accompanying software, Relative Quantification Program. The mRNA level of the GAPDH housekeeping gene was also determined by real-time RT-PCR in each cDNA sample to normalize the expression of the genes of interest (MMP-9).

Statistical analysis

The values of MMP-9 and TIMP-1 concentrations are expressed as means $\pm$ SD. Statistical analysis was performed with the Wilcoxon matched-paired test, with a *P*-value of < 0.05 considered to be significant.

Results

Figure 1 demonstrates total MMP-9 release by the stimulation of LTC₄, IL-13, IL-17, TGF- β 1, TNF- α and LPS for 24 h in THP-1 cells. Only TNF- α and LPS induced MMP-9 in THP-1 cells. LTC₄ significantly enhanced the MMP-9 production induced by IL-13, IL-17, TGF- β 1 and TNF- α , but not by LPS (Fig. 1). The enhancement of TNF- α -induced MMP-9 release by LTC₄ was significantly greater than that of IL-13, IL-17 and TGF- β 1 (*P* < 0.01 for all). The levels of active MMP-9 were lower than the detection limit in the media of cells treated with any stimulators.

LTC₄ and -D₄ but not -E₄ (10–1000 ng/mL) significantly increased MMP-9 release by TNF- α in THP-1 cells, and the potencies of LTC₄ and -D₄ for TNF- α -induced MMP-9 production were similar (Fig. 2). The potencies of LTC₄ and -D₄ were not dose related.

The time course of MMP-9 production was examined by incubating THP-1 cells with TNF- α and 100 ng/mL LTC₄ during a 48-h period (Fig. 3). MMP-9 production could be observed 8 h after the addition of TNF- α and LTC₄, and gradually increased over 48 h.

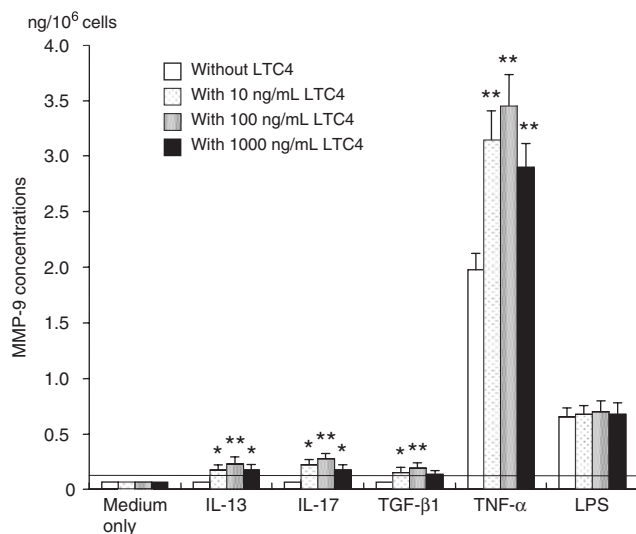


Fig. 1. Matrix metalloproteinase-9 (MMP-9) production, as measured by ELISA, in THP-1 cells stimulated by IL-13, IL-17, TGF- β 1, TNF- α or lipopolysaccharide (LPS) with/without LTC4, for 24 h. Data ($n=8$) are presented as means $\pm$ SD. A line indicates the detection limit. ** $P < 0.01$ and * $P < 0.05$ compared with cells treated without LTC4.

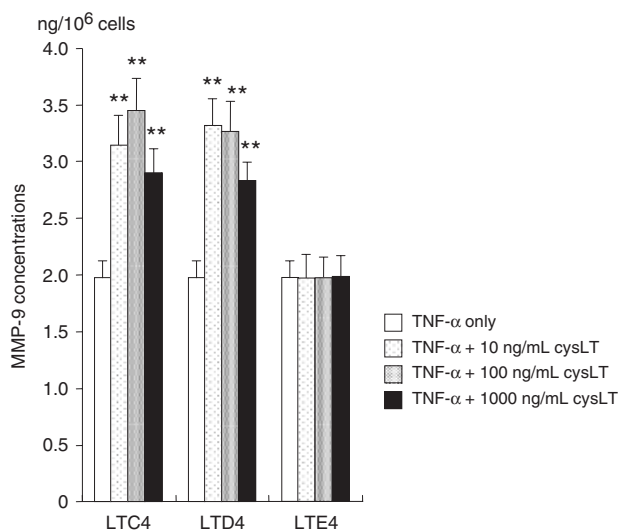


Fig. 2. Matrix metalloproteinase-9 (MMP-9) production, as measured by ELISA, in THP-1 cells stimulated with TNF- α and cysteinyl leukotriene 1 (cysLTs) (LTC4, -D4 or -E4) for 24 h. Data ($n=8$) are presented as means $\pm$ SD. ** $P < 0.01$ compared with cells treated with TNF- α only.

ELISA demonstrated that LTC4, IL-13, IL-17, TGF- β 1, TNF- α or LPS did not induce TIMP-1 in THP-1 cells (Fig. 4).

The time courses of the inhibitory effects of pretreatments with 10^{-7} M pranlukast on MMP-9 production induced by TNF- α and 100 ng/mL LTC4 were examined in THP-1 cells over 48 h (Fig. 3). Pranlukast blocked MMP-9 production during the experiment. Pranlukast similarly blocked MMP-9 production induced by TNF- α and 100 ng/mL LTD4 during the experiment (data not shown).

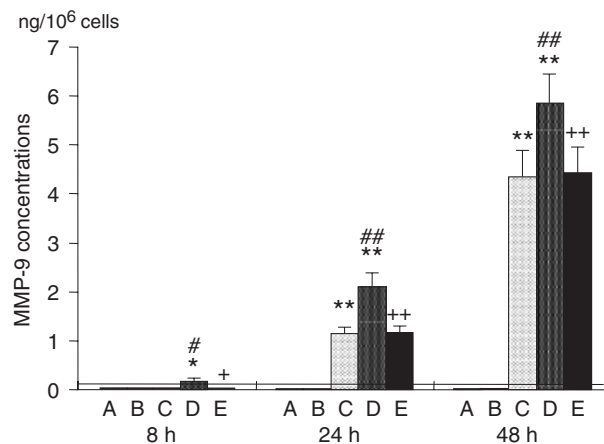


Fig. 3. Kinetics of matrix metalloproteinase-9 (MMP-9) production and the inhibitory effect of pranlukast on MMP-9 release, as measured by ELISA, in THP-1 cells stimulated with LTC4 (B), TNF- α (C) and TNF- α +LTC4 (D) for 8, 24 and 48 h, and cells pretreated with pranlukast for 30 min before TNF- α +LTC4 treatment (E). (A) Cells with no treatment. Data ($n=8$) are presented as means $\pm$ SD. A line indicates the detection limit. ** $P < 0.01$ and * $P < 0.05$ compared with cells with no treatment. ## $P < 0.01$ and # $P < 0.05$, compared with cells treated with TNF- α only. ++ $P < 0.01$, and + $P < 0.05$ compared with cells treated with TNF- α and LTC4.

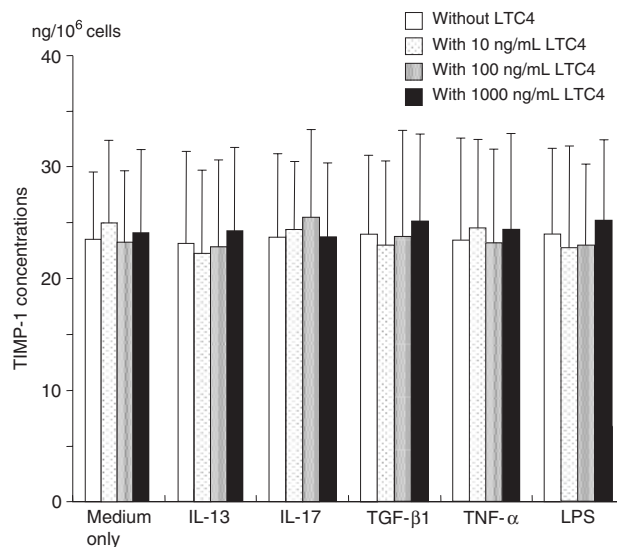


Fig. 4. Tissue inhibitors of metalloproteinases (TIMP)-1 production, as measured by ELISA, in THP-1 cells stimulated by IL-13, IL-17, TGF- β 1, TNF- α or lipopolysaccharide (LPS) with/without LTC4, for 24 h. Data ($n=8$) are presented as means $\pm$ SD.

The concentrations of MMP-9 in the culture fluid of THP-1 cells exposed to TNF- α and 100 ng/mL LTC4, -D4 or -E4, in the presence or absence of pranlukast, for 24 h are shown in Fig. 5. The production of MMP-9 induced by TNF- α and cysLTs was significantly inhibited by 10^{-6} – 10^{-8} M pranlukast. The effect of pranlukast was dose related.

Real-time PCR demonstrated that MMP-9 mRNA expression was significantly increased 24 h after the

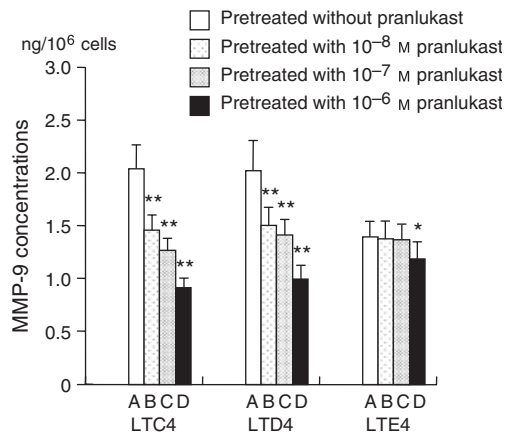


Fig. 5. Inhibitory effect of pranlukast on matrix metalloproteinase-9 (MMP-9) release, as measured by ELISA, in THP-1 cells stimulated with TNF- α and LTC4, -D4 or -E4 for 24 h. The cells were pretreated with pranlukast for 30 min before TNF- α and LTC4, -D4 or -E4 treatments. Cells pretreated without pranlukast (A) Cells pretreated with 10⁻⁸ (B), 10⁻⁷ (C) or 10⁻⁶ M (D) pranlukast. Data ($n=8$) are presented as means $\pm$ SD. ** $P < 0.01$ and * $P < 0.05$ compared with cells pretreated without pranlukast.

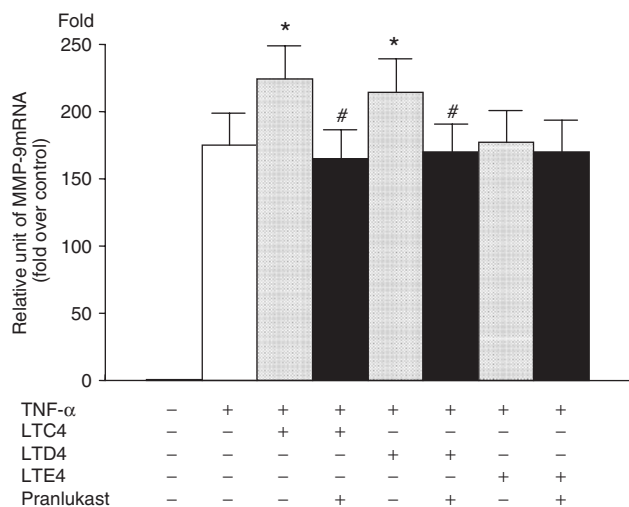


Fig. 6. Matrix metalloproteinase-9 (MMP-9) mRNA expression and the inhibitory effect of pranlukast on MMP-9 mRNA expression, as measured by real-time PCR, in THP-1 cells stimulated with TNF- α and 10⁻⁶ M LTC4, -D4 or -E4 for 24 h, and cells pretreated with 10⁻⁷ M pranlukast for 30 min before TNF- α and LTC4, -D4 or -E4 treatments. Data ($n=8$) are presented as means $\pm$ SD. * $P < 0.05$ compared with cells treated with TNF- α only. # $P < 0.05$ compared with cells treated with TNF- α and LTC4 or -D4.

addition of TNF- α in THP-1 cells ($P < 0.01$) (Fig. 6). Moreover, 100 ng/mL LTC4 and -D4, but not -E4, significantly enhanced the MMP-9 mRNA expression induced by TNF- α (Fig. 6). Pretreatment with 10⁻⁷ M pranlukast significantly decreased the MMP-9 mRNA expression caused by the stimulation of TNF- α and LTC4, or -D4 in THP-1 cells (Fig. 6).

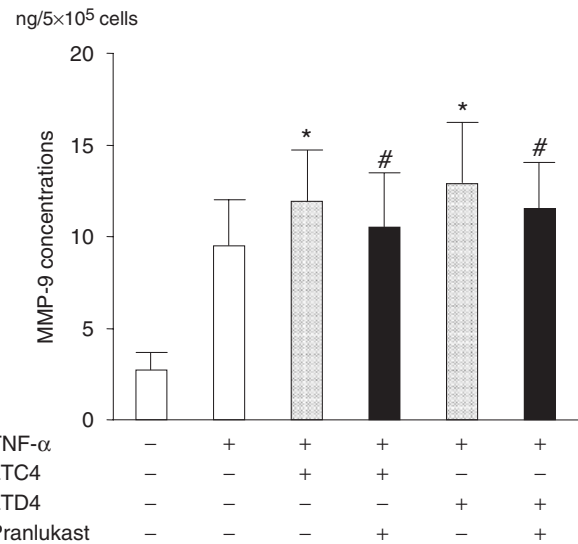


Fig. 7. Inhibitory effect of pranlukast on matrix metalloproteinase-9 (MMP-9) release, as measured by ELISA, in peripheral blood CD14⁺ monocytes/macrophages stimulated with TNF- α and LTC4 or -D4 for 24 h. The cells were pretreated with pranlukast for 30 min before TNF- α and LTC4 or -D4 treatments. Data ($n=10$) are presented as means $\pm$ SD. * $P < 0.05$ compared with cells treated with TNF- α only. # $P < 0.05$ compared with cells treated with TNF- α and LTC4 or -D4.

The purity of the CD14⁺ cells obtained after negative selection with the MACS system was 85.8 $\pm$ 6.1%. The MMP-9 production and the inhibitory effect of pranlukast on MMP-9 production were examined by incubating peripheral blood CD14⁺ monocytes/macrophages with TNF- α and LTC4 or -D4 for 24 h, with some samples being pretreated with 10⁻⁷ M pranlukast (Fig. 7). LTC4 and -D4 significantly enhanced the MMP-9 production induced by TNF- α in peripheral blood CD14⁺ monocytes/macrophages ($P < 0.05$). The production of MMP-9 induced by TNF- α and LTC4 or -D4 was significantly inhibited by 10⁻⁷ M pranlukast ($P < 0.05$).

Discussion

Airway remodelling is an important factor in the pathogenesis of bronchial asthma. MMP-9 is thought to be an important mediator in the airway remodelling of asthma [6, 7, 26–29]. The expressions of MMP-9 in the sputum [26, 30], bronchoalveolar lavage fluid [31] and airway epithelial cells [29, 32] were increased in asthma. In addition, ovalbumin (OVA)-challenged MMP-9-deficient mice have reduced levels of peribronchial fibrosis compared with OVA-challenged wild-type mice [28]. Moreover, MMP-9 induces T cells, neutrophils and eosinophils through the basement membrane [33–35]. It is likely that MMP-9 promotes airway remodelling and inflammation in asthma.

Several recent papers have demonstrated that TNF- α plays a critical role in the initiation and amplification of airway inflammation in asthmatic patients [36–39]. TNF- α induces the adhesion, migration and activation of inflammatory cells, secretion of mucin, apoptosis and the modulation of repair of airway epithelial cells, increased responsiveness of smooth-muscle cells to contractile agents, proliferation and activation of myofibroblasts and fibroblasts and increased synthesis of extracellular matrix glycoproteins [36]. The expression of TNF- α in the airway was related to the severity of asthma [38]. The soluble TNF- α receptor etanercept was beneficial in patients with refractory asthma [39].

Our present study demonstrated that only cysLTs could not increase the expression of MMP-9 in THP-1 cells, but LTC₄ and -D₄ could enhance TNF- α -induced MMP-9 expression. It is likely that LTC₄ and -D₄ exert an enhancement of MMP-9 expression induced by TNF- α via the cysLT₁ receptor because pranlukast completely blocked the enhancement of TNF- α -induced MMP-9 expression by LTC₄ and -D₄. The potency of LTC₄ and -D₄ for TNF- α -induced MMP-9 expression was not dose related. The whole MMP-9 detected in our experiment was the pro form because the levels of active MMP-9 were lower than the detection limit in the media of cells treated with TNF- α in the presence or absence of cysLTs. Ménard and Bissonnette reported that macrophage inflammatory protein-1 α (MIP-1 α) production induced by 10^{-11} M LTD₄ in AM was more than that induced by 10^{-6} , 10^{-8} , 10^{-10} , 10^{-12} or 10^{-14} M LTD₄ [14]. We previously reported that monocyte chemoattractant protein-1 (MCP-1) production by cysLTs in human monocytes/macrophages was not dose related [40]. Therefore, it is unlikely that the potency of cysLTs is dose related. Another hypothesis is that higher dosages of LTC₄- and -D₄ decreased the secretion of MMP-9 because of the potential cell toxicity of LTC₄ and -D₄ for THP-1 cells. LTD₄ would be expected to be about 10 times more potent for calcium flux at the cysLT₁ receptors than LTC₄ (and 100 times more than LTE₄) [41, 42]. The mechanism of enhancement of TNF- α -induced MMP-9 production via cysLT₁ receptors of monocytes/macrophages stimulated by cysLTs is different from that of the contraction via cysLT₁ receptors of airway smooth muscle stimulated by cysLTs. The potencies of LTC₄ and -D₄ for the enhancement of TNF- α -induced MMP-9 production in monocytes/macrophages may be different from those for the contraction of airway smooth muscle via the calcium flux.

Pranlukast has some actions in asthma other than as an antagonist of the cysLT₁ receptor, including immunomodulation [43]. We have previously demonstrated that pranlukast inhibits the TNF- α -induced nuclear factor- κ B (NF- κ B) activation [44], and cysLTs-induced MCP-1 production in monocytes/macrophages [40]. In adults administered a single oral dose of 225 mg, C_{max} was

642.3 ± 151.0 ng/mL (1.31 ± 0.31 μ M) [45], but this value included pranlukast bound to plasma protein. The concentration of free pranlukast was probably less than a tenth of this [46]. Therefore, we think that 10^{-7} and 10^{-8} M pranlukast are clinical doses, and 10^{-6} M is a pharmacological dose. In the animal model, cysLTs promoted airway remodelling in asthma, and the cysLT receptor antagonists inhibited it [10]; however, the mechanism has remained unclear. We revealed one of the mechanisms of airway remodelling associated with cysLTs, and the inhibitory effect of pranlukast. However, the contribution of the anti-remodelling activity of pranlukast at oral therapeutic doses in asthmatic patients remains unclear.

In conclusion, cysLTs enhance TNF- α -induced MMP-9 expression via binding cysLT₁ receptor in human monocytes/macrophages *in vitro*, and pranlukast inhibits the enhancement of TNF- α -induced MMP-9 production by cysLTs in the cells.

Acknowledgements

This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (C-17591092), Japan.

References

- Chandler S, Miller KM, Clements JM *et al*. Matrix metalloproteinases, tumor necrosis factor and multiple sclerosis: an overview. *J Neuroimmunol* 1997; **72**:155–61.
- Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem* 2003; **253**:269–85.
- Nagase H. Activation mechanisms of matrix metalloproteinases. *Biol Chem* 1997; **378**:151–60.
- Murphy G, Knäuper V. Relating matrix metalloproteinase structure to function: why the 'hemopexin' domain? *Matrix Biol* 1997; **15**:511–8.
- Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *J Clin Invest* 1995; **96**:2304–10.
- Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. *Am J Respir Cell Mol Biol* 2003; **28**:12–24.
- Kelly EA, Jarjour NN. Role of matrix metalloproteinases in asthma. *Curr Opin Pulm Med* 2003; **9**:28–33.
- Lim DH, Cho JY, Miller M, McElwain K, McElwain S, Broide DH. Reduced peribronchial fibrosis in allergen-challenged MMP-9-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 2006; **291**:L265–71.
- Nakai H, Konno M, Kosuge S *et al*. New potent antagonists of leukotrienes C₄ and D₄. Synthesis and structure-activity relationships. *J Med Chem* 1988; **31**:84–91.
- Henderson WR Jr, Tang LO, Chu SJ *et al*. A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 2002; **165**:108–16.

- 11 Diamant Z, Hiltermann JT, van Rensen EL *et al.* The effect of inhaled leukotriene D4 and methacholine on sputum cell differentials in asthma. *Am J Respir Crit Care Med* 1997; **155**: 1247–53.
- 12 Laitinen LA, Laitinen A, Haahtela T, Vilkkä V, Spur BW, Lee TH. Leukotriene E4 and granulocytic infiltration into asthmatic airways. *Lancet* 1993; **341**:989–90.
- 13 Figueroa DJ, Breyer RM, Defoe SK *et al.* Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. *Am J Respir Crit Care Med* 2001; **163**:226–33.
- 14 Ménard G, Bissonnette EY. Priming of alveolar macrophages by leukotriene D4: potentiation of inflammation. *Am J Respir Cell Mol Biol* 2000; **23**:572–7.
- 15 Oosterhoff Y, Hoogsteden HC, Rutgers B, Kauffman HF, Postma DS. Lymphocyte and macrophage activation in bronchoalveolar lavage fluid in nocturnal asthma. *Am J Respir Crit Care Med* 1995; **151**:75–81.
- 16 Chanez P, Vignola AM, Lacoste P, Michel FB, Godard P, Bousquet J. Increased expression of adhesion molecules (ICAM-1 and LFA-1) on alveolar macrophages from asthmatic patients. *Allergy* 1993; **48**:576–80.
- 17 Lane SJ, Sousa AR, Lee TH. The role of the macrophage in asthma. *Allergy* 1994; **49**:201–9.
- 18 Tonnel AB, Joseph M, Gosset P, Fournier E, Capron A. Stimulation of alveolar macrophages in asthmatic patients after local provocation test. *Lancet* 1983; **1**:1406–8.
- 19 Viksman MY, Bochner BS, Peebles RS, Schleimer RP, Liu MC. Expression of activation markers on alveolar macrophages in allergic asthmatics after endobronchial or whole-lung allergen challenge. *Clin Immunol* 2002; **104**:77–85.
- 20 Zheng T, Zhu Z, Wang Z *et al.* Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 2000; **106**: 1081–93.
- 21 Prause O, Bozinovski S, Anderson GP, Linden A. Increased matrix metalloproteinase-9 concentration and activity after stimulation with interleukin-17 in mouse airways. *Thorax* 2004; **59**:313–7.
- 22 Duivenvoorden WC, Hirte HW, Singh G. Transforming growth factor β 1 acts as an inducer of matrix metalloproteinase expression and activity in human bone-metastasizing cancer cells. *Clin Exp Metastasis* 1999; **17**:27–34.
- 23 Rosenberg GA, Estrada EY, Dencoff JE, Stetler-Stevenson WG. Urokinase-type plasminogen activator-induced gelatinase B causes delayed opening of the blood-brain barrier: an expanded therapeutic window. *Brain Res* 1995; **703**:151–5.
- 24 Shankavaram UT, DeWitt DL, Wahl LM. Lipopolysaccharide induction of monocyte matrix metalloproteinases is regulated by the tyrosine phosphorylation of cytosolic phospholipase A2. *J Leukoc Biol* 1998; **64**:221–7.
- 25 Hoshino M, Izumi T, Shimizu T. Leukotriene D4 activates mitogen-activated protein kinase through a protein kinase C α -Raf-1-dependent pathway in human monocytic leukemia THP-1 cells. *J Biol Chem* 1998; **273**:4878–82.
- 26 Vignola AM, Riccobono L, Mirabella A *et al.* Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airflow obstruction in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1998; **158**:1945–50.
- 27 Hoshino M, Takahashi M, Takai Y, Sim J. 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.
- 28 Simpson JL, Scott RJ, Boyle MJ, Gibson PG. Differential proteolytic enzyme activity in eosinophilic and neutrophilic asthma. *Am J Respir Crit Care Med* 2005; **172**:559–65.
- 29 Cho JY, Miller M, McElwain K *et al.* Remodeling associated expression of matrix metalloproteinase 9 but not tissue inhibitor of metalloproteinase 1 in airway epithelium: modulation by immunostimulatory DNA. *J Allergy Clin Immunol* 2006; **117**: 618–25.
- 30 Tanaka H, Miyazaki N, Oashi K, Tanaka S, Ohmichi M, Abe S. Sputum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio in acute asthma. *J Allergy Clin Immunol* 2000; **105**:900–5.
- 31 Lemjabbar H, Gosset P, Lamblin C *et al.* Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus. *Am J Respir Crit Care Med* 1999; **159**: 1298–307.
- 32 Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol* 1998; **102**:783–8.
- 33 Leppert D, Waubant E, Galaray R, Bunnett NW, Hauser SL. T cell gelatinases mediate basement membrane transmigration in vitro. *J Immunol* 1995; **154**:4379–89.
- 34 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.
- 35 Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. *Am J Respir Cell Mol Biol* 1997; **17**:519–28.
- 36 Erzurum SC. Inhibition of tumor necrosis factor α for refractory asthma. *N Engl J Med* 2006; **357**:754–8.
- 37 Cazzola M, Polosa R. Anti-TNF- α and Th1 cytokine-directed therapies for the treatment of asthma. *Curr Opin Allergy Clin Immunol* 2006; **6**:43–50.
- 38 Howarth PH, Babu KS, Arshad HS *et al.* Tumour necrosis factor (TNF- α) as a novel therapeutic target in symptomatic corticosteroid-dependent asthma. *Thorax* 2005; **60**: 1012–8.
- 39 Berry MA, Hargadon B, Shelley M *et al.* Evidence of a role of tumor necrosis factor α in refractory asthma. *N Engl J Med* 2006; **354**:697–708.
- 40 Ichiyama T, Hasegawa M, Ueno Y, Makata H, Matsubara T, Furukawa S. Cysteinyl leukotrienes induce monocyte chemoattractant protein 1 in human monocytes/macrophages. *Clin Exp Allergy* 2005; **35**:1214–9.
- 41 Evans JF. Cysteinyl leukotriene receptors. *Prostaglandins Other Lipid Mediat* 2002; **68–69**:587–97.
- 42 Capra V. Molecular and functional aspects of human cysteinyl leukotriene receptors. *Pharmacol Res* 2004; **50**:1–11.
- 43 Tohda Y, Nakahara H, Kubo H, Haraguchi R, Fukuoka M, Nakajima S. Effects of ONO-1078 (pranlukast) on

- cytokine production in peripheral blood mononuclear cells of patients with bronchial asthma. *Clin Exp Allergy* 1999; **29**: 1532–6.
- 44 Ichiyama T, Hasegawa S, Umeda M, Terai K, Matsubara T, Furukawa S. Pranlukast inhibits NF- κ B activation in human monocytes/macrophages and T cells. *Clin Exp Allergy* 2003; **33**:802–7.
- 45 Nakashima M, Kanamaru M, Uematsu T, Tsubokura S. A phase I clinical study of leukotriene C₄, D₄, and E₄ receptor antagonist; ONO-1078 in healthy volunteers. *Rinsho Iyaku* 1993; **9** (Suppl 1):3–29. Japanese.
- 46 Lynch KR, O'Neill GP, Liu Q *et al*. Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature* 1999; **399**:789–93.