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Original Article

Eotaxin-induced expression of membrane type 1 matrix metalloproteinase mRNA in human eosinophils

Izumi Kurosaki,[1] Taku Satoh,[1] Koji Murakami,[1] Takeshi Tastumi,[1,2] Noriyasu Mitani[1] and Ikuo Saiki[1]

[1]Department of Pathogenic Biochemistry, Institute of Natural Medicine and [2]Department of Japanese Oriental (Kampo) Medicine, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan

Abstract

Eosinophil penetration across the basement membrane (BM) is thought to be dependent on the degradation of membrane components. In this process, matrix metalloproteinases (MMP) appear to be primarily responsible for degradation of the BM. Matrix metalloproteinases -2 and MMP-9 degrade type IV collagen, which is a major component of the BM. In the present study, the effects of eotaxin, a selective chemoattractant for eosinophils, on the expression of MMP mRNA were examined. Incubation with chemotactically active concentrations of eotaxin for 24 h enhanced the expression of mRNA for membrane-type 1 MMP (MT1-MMP), but not of mRNA for MMP-2 and MMP-9. An increase in the protein level of MT1-MMP was also detected in the cell lysate of eotaxin-treated eosinophils. These results suggest that up-regulation of MT1-MMP expression may be involved in the eotaxin-induced penetration of eosinophils.

Key words: basement membrane, degradation, eosinophil, eotaxin, matrix metalloproteinase-2, matrix metalloproteinase-9, membrane-type matrix metalloproteinase.

Correspondence: Ikuo Saiki, Professor, Department of Pathogenic Biochemistry, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan.
Email: byosei@ms.toyama-mpu.ac.jp

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Introduction

The accumulation of eosinophils into the sites of inflammation is an important event in several allergic diseases, including allergic rhinitis, atopic dermatitis and asthma. In response to the stimulus of an allergic reaction, eosinophils adhere to the endothelial cell surface of postcapillary venules, cross the endothelium through interendothelial cell junctions and probably penetrate the basement membrane (BM), leaving the vascular system to accumulate at the inflammatory site. It is therefore considered that enzymatic degradation of the BM by eosinophils is one of the important steps in infiltration into inflammatory sites, similar to tumor cell invasion. However, little is known about the mechanism of eosinophil penetration through the BM, although some of the mechanisms by which adhesion molecules and cytokines promote eosinophil binding to and migration through the endothelium in allergic inflammation have been elucidated.[1] Degradation of the BM by eosinophils is an important step for the inflammatory cell infiltration, similar to tumor invasion. Several classes of proteinases, including serine proteinases, cysteine proteinases and matrix metalloproteinases (MMP), have been implicated in the degradation of the extracellular matrix (ECM).[2] Among these proteinases, MMP appear to be primarily responsible for much of the ECM degradation during the invasive process. A previous study showed that MMP-2 and MMP-9 are involved in the migration of lymphocytes through reconstituted BM *in vitro*.[3] Elastase and MMP-9 have also been reported to be responsible for the migration of neutrophils through the BM.[4] Okada *et al*. have reported that MMP-9 plays a major role in eosinophil migration to the site of allergic inflammation.[5] However,

anti-MMP-9 antibody shows only a 50% inhibition of the eosinophil migration induced by interleukin (IL)-5 and platelet-activating factor (PAF). These results indicate that some other molecules that promote the migration of eosinophils are probably involved in this process.

In general, MMP are secreted in a latent form (pro-MMP) and are consequently activated by serine proteinases or other activated MMP to an active form. A recent study has demonstrated that proteolytic maturation of pro-MMP-2 is involved in a specific mechanism not involving the other tested MMP.[6,7] Pro-MMP-2 activation is mediated by membrane type 1 MMP (MT1-MMP/MMP-14) on the cell surface, a novel MMP possessing a hydrophobic C-terminal transmembrane domain.[8–11]

In the present study, we focused our attention on the modulation of MMP expression in eosinophils stimulated with eotaxin, which is a potent chemoattractant for eosinophils.

METHODS

Cell preparation

Eosinophils were purified as previously described.[12] Briefly, peripheral blood polymorphonuclear leukocytes (PMNL; containing neutrophils and eosinophils) from whole blood of volunteer donors who had an anamnesis for allergy were isolated by 4.5% dextran-sedimentation followed by density-gradient centrifugation with Lymphocyte Separation Medium (LSM®) (ICN Biomedicals Inc., Aurora, OH, USA). Then, eosinophils were negatively collected by incubation with mouse antihuman CD16 monoclonal antibody (Nichirei Co., Tokyo, Japan) followed by treatment with beads coupled to sheep antimouse Ig antibody (Dynabeads, Dynal AS, Oslo, Norway). The purity of the eosinophil population was > 96%, as assessed by Diff-Quick staining (Baxter, Dudigen, Switzerland; Fig. 1). The positively collected neutrophil population was harvested by trypsinization. Peripheral blood mononuclear cells (PBMNC) were collected from mononuclear cell fraction of LSM centrifugation.

Treatment of leukocytes with eotaxin

Eosinophils (5×10^6), PBMNC (5×10^6) and neutrophils (1×10^7), suspended in RPMI-1640 + 10% fetal bovine serum, were cultured in 12-well plates with or without recombinant human eotaxin (100 ng/mL; Bachem Fine Chem. AG, Bubendorf, Switzerland) for 24 h at 37°C in a 5% CO_2 incubator.

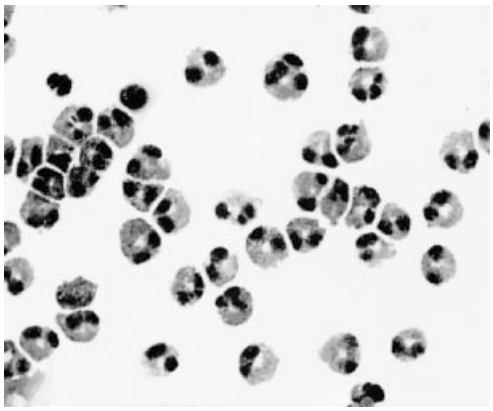

Fig. 1 Micoscopic observation of highly purified eosinophils. Eosinophils were purified by the procedure described in Methods. The purity of the eosinophil preparations used in this study was greater than 96.0%.

Preparation of total RNA and reverse transcriptase–polymerase chain reaction

Total RNA was isolated from human eosinophils using ISOGEN (Wako Chemicals, Osaka, Japan), and reverse transcription (RT) was performed using a First-strand cDNA Synthesis kit (Takara Biochemicals, Siga, Japan) at 42°C for 45 min to maximize cDNA synthesis and was terminated by heating at 99°C for 5 min. Polymerase chain reaction (PCR) was performed using cDNA templates (100 ng) and specific oligonucleotide primers with denaturation at 94°C for 30 s, annealing for 60 s (appropriate temperatures for each gene), and extension at 72°C for 90 s, using TAKARA Ex Taq (Takara Biochemicals) according to the manufacturer's instructions. The sequences of the primers for each gene were as follows: 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ (glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sense),[13] 5′-CATGTGGGCCATGAGGTCCACCAC-3′ (GAPDH antisense),[13] 5′-CCACGTGACAAGCCCATGGGGCCCC-3′ (MMP-2 sense),[14] 5′-GGAGCCTAGCCAGTCGGATTTGATG-3′ (MMP-2 antisense),[14] 5′-AACGCTATGGTTACACTCGG-3′ (MMP-9 sense; Genbank, J05070), 5′-AACTGGATGACGATGTCTGC-3′ (MMP-9 antisense; GenBank, J05070), 5′-CCCTATGCCTACATCCGTGA-3′ (MT1-MMP sense)[15], 5′-TCCATCCATCACTTGGTTAT-3′ (MT1-MMP antisense).[15]

The predicted PCR products were 983 bp, 480 bp, 361 bp and 550 bp for GAPDH, MMP-2, MMP-9 and MT1-MMP, respectively. The PCR products were detected using electrophoresis on 1.5% agarose gels (Takara Biochemicals, Siga, Japan) and staining with ethidium

bromide. The relative abundance of MMP was indicated as the density relative to that of GAPDH, determined by using a Master Scan Gel Analysis System (Scanalytics, Billeica, MA, USA).

Western blot analysis

Cell lysates of eosinophils were isolated using RIPA buffer set (Boehringer Mannheim GmbH, Mannheim, Germany) and stored at – 80°C until use. Aliquots of 10 μg protein were electrophoresed on 10–20% gradient-polyacrylamide gels and were blotted onto nitrocellulose filters (Bio-Rad Laboratories, CA, USA). The filters were developed with rabbit polyclonal antibodies specific for MT1-MMP (Chemicon International Inc., Temecula, CA, USA), horseradish peroxidase-conjugated goat antirabbit antibody (Amersham Pharmacia Biotech UK Ltd, Buckinghamshire, England) and superSignal (Pierce Inc., Billeica, IL, USA) detection. The density of MT1-MMP was determined by using the Master Scan Gel Analysis System (Scanalytics).

Statistics

Two-sided Welch's *t*-test was performed for the expression of mRNA for MMP in eosinophils.

RESULTS

Increase in expression of MMP mRNA in eotaxin-treated eosinophils

We investigated the expression of mRNA for MT1-MMP, MMP-2 and MMP-9 in human eosinophils by RT-PCR. As shown in Fig. 2a, expression of mRNA for MT1-MMP and MMP-9 was detected in human eosinophils from three donors. The expression of mRNA for MT1-MMP was markedly enhanced by the treatment with eotaxin, whereas that for MMP-9 was not increased. The signal of mRNA for MMP-2 was weak both in untreated or eotaxin-treated eosinophils. The density ratio of MT1-MMP relative to GAPDH was increased in eotaxin-treated eosinophils, whereas those of MMP-2 or MMP-9 were not significantly changed (Fig. 3). To further confirm the specificity of eotaxin-induced expression of MT1-MMP in the eosinophils, we also examined the effect of eotaxin on the expression of MMP mRNA in PBMNC and neutrophils. As shown in Fig. 2b,c and Fig. 3, the expression of mRNA for MMP in either PBMNC or neutrophils was not significantly affected by the treatment with eotaxin.

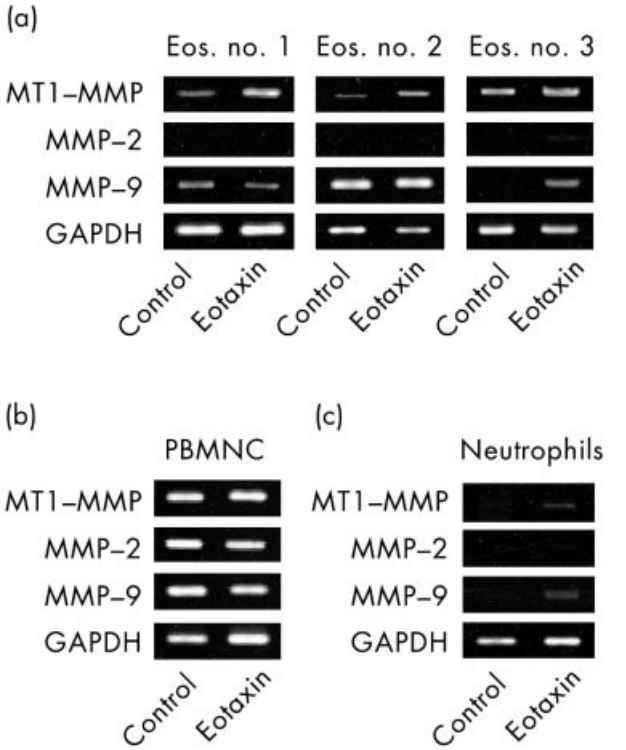

Fig. 2 Expression of mRNA for matrix metalloproteinases (MMP) in human eosinophils, peripheral blood mononuclear cells (PBMNC) and neutrophils. Highly purified eosinophils (Eos.) were collected from three volunteers (a). In addition, PBMNC (b) and neutrophils (c) were collected from volunteer no. 1. These cells were incubated with or without eotaxin (100 ng/mL) for 24 h. Total RNA was isolated and reverse transcriptase polymerase chain reaction was performed. The numbers of amplification cycles for MT1-MMP, MMP-2, MMP-9 and reduced glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were 36, 39, 36 and 30, respectively. The PCR products were electrophoresed on 1.5% agarose gels.

Detection of MT1-MMP by western blotting

Increase in the protein level of MT1-MMP in eotaxin-treated eosinophils was examined by the western blotting method. A marked enhancement of expression of MT1-MMP protein was detected in eotaxin-treated eosinophils in comparison with untreated eosinophils (Fig. 4).

DISCUSSION

Eosinophil infiltration into the site of inflammation is a crucial step in allergic diseases. The selective accumulation of eosinophils into the inflammatory site suggests that there may be a local release of chemoattractants

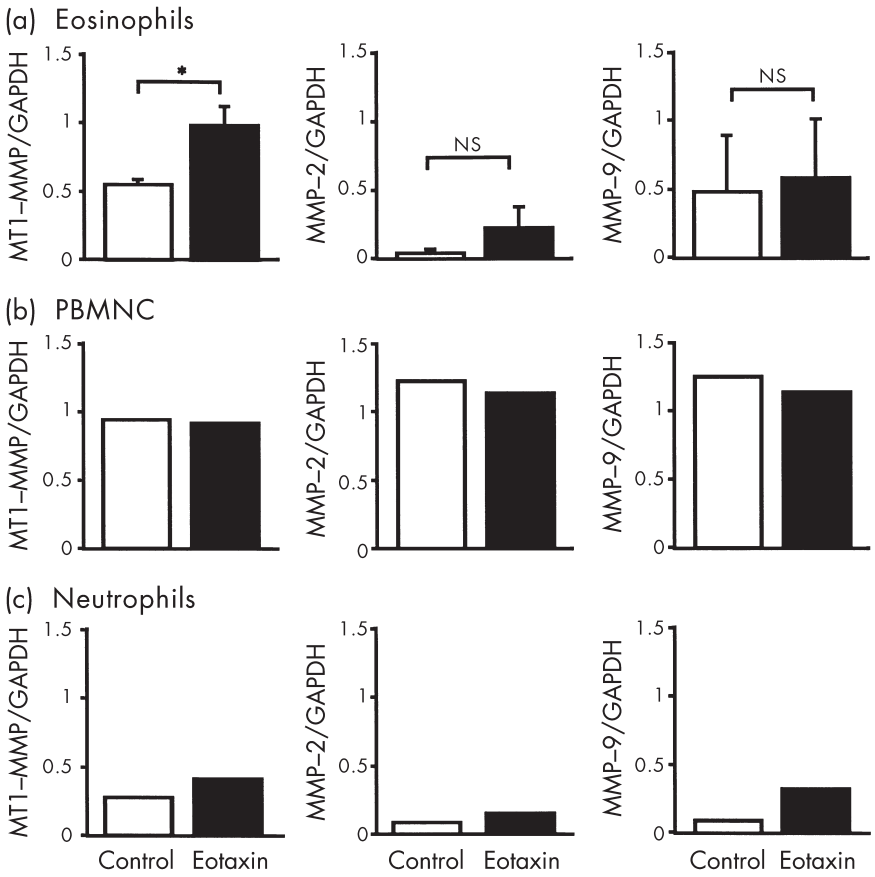

Fig. 3 Densitometric analysis for the expression of matrix metalloproteinases (MMP) in eosinophils, peripheral blood mononuclear cells (PBMNC) and neutrophils. The intensity of MT1-MMP, MMP-2 and MMP-9 expression in untreated (□) and eotaxin-treated (■) cells is shown as a density ratio relative to reduced glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The results of eosinophils are the mean ± SD of triplicate samples. $^*P < 0.05$ (two-sided Welch's *t*-test).

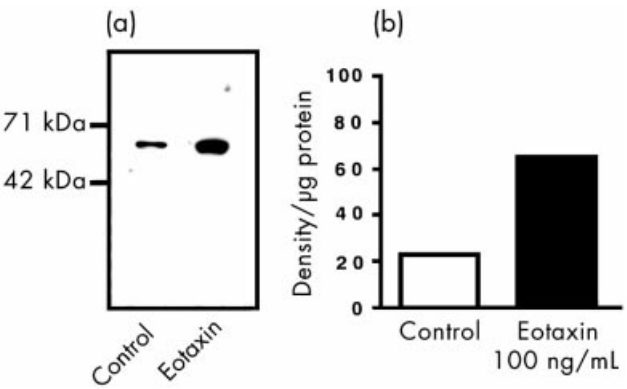

Fig. 4 Western blot analysis of MT1-MMP expression. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed under reducing conditions. After blotting, protein levels of MT1-MMP in eosinophils were detected using monoclonal antibodies for MT1-MMP (a). (b) Densitometric analysis of the expression of MT1-MMP in untreated or eotaxin-treated eosinophils.

specific for these leukocytes. In particular, eotaxin, which belongs to the C-C chemokine family, is considered to play an important role in eosinophil infiltration. Brown *et al*. have observed increased gene transcription and production of eotaxin in the bronchial mucosa and bronchiovascular lavage fluid (BALF) of patients with allergic asthma compared with non-asthmatic subjects.[16] The up-regulation of eotaxin expression is closely correlated with the number of airway eosinophils.[17]

Enzymatic degradation of ECM is an important step in the process of cellular migration and invasion.[18] Gelatinase A (MMP-2) and B (MMP-9) degrade the type IV collagen and mediate the invasion of various types of cells, such as endothelial cells, fibroblasts and macrophages.[19–22] Gelatinase secretion by tumor cells is also correlated with their metastatic potential.[23–26] In addition, MT1-MMP regulates the proteolytic activation of MMP-2 and also degrades type I, II and III collagens, laminin and fibronectin by itself.[27–30] Although MMP-9 has been reported to be expressed in eosinophils,[5] there are few reports on the relationship between the expression of MMP and the eosinophil-specific chemokines, for

example, an eotaxin. Therefore, it is important to clarify the regulatory mechanism of the enzymatic degradation of the ECM by eosinophils. Considering the results of clinical studies,[31–33] we carried out studies to examine the possibility that eotaxin acts, not only as a chemoattractant for eosinophils, but also as a regulatory factor in the infiltration process.

In the present study, we investigated the mRNA expression of MMP in human eosinophils, because MMP are considered to be closely involved in degradation of the BM. Incubation with a chemotactically active concentration of eotaxin (100 ng/mL)[34] for 24 h markedly enhanced the expression of the mRNA for MT1-MMP in human eosinophils, compared with MMP-2 and MMP-9 (Figs 2,3). In contrast, the expression of mRNA for MMP-2, MMP-9 and MT1-MMP in either PBMNC or neutrophils was not significantly affected by the treatment with eotaxin. Thus, MT1-MMP may play a critical role in the regulation of eotaxin-induced eosinophil infiltration. Furthermore, it has previously been reported that the inflammatory response in asthma results in the generation of MMP-2 from the epithelium.[35] Therefore, activation of MMP-2 at the sites of allergic inflammation may be modulated through MT1-MMP on the eosinophil surface.

In conclusion, the present study suggests that MT1-MMP probably plays an important role in eotaxin-induced eosinophil infiltration. In addition to eotaxin, other inflammatory chemokines may also be involved in the modulation of eosinophil invasion in a number of allergic diseases, such as asthma and atopic dermatitis. Further investigation will be needed to examine the mechanism of eotaxin-induced infiltration of eosinophils.

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