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Production of the Chemokine Eotaxin-1 in Osteoarthritis and its Role in Cartilage Degradation

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Abstract The expression of the chemokine, eotaxin-1, and its receptors in normal and osteoarthritic human chondrocytes was examined, and its role in cartilage degradation was elucidated in this study. Results indicated that plasma concentrations of eotaxin-1 as well as the chemokines, RANTES, and MCP-1α, were higher in patients with osteoarthritis (OA) than those in normal humans. Stimulation of chondrocytes with IL-1β or TNF-α significantly induced eotaxin-1 expression. The production of eotaxin-1 induced expression of its own receptor of CCR3 and CCR5 on the cell surface of chondrosarcomas, suggesting that an autocrine/paracrine pathway is involved in eotaxin-1's action. In addition, eotaxin-1 markedly increased the expressions of MMP-3 and MMP-13 mRNA, but had no effect on TIMP-1 expression in chondrocytes. However, pretreatment of anti-eotaxin-1 antibody significantly decreased the MMP-3 expression induced by IL-1β. These results first demonstrate that human chondrocytes express the chemokine, eotaxin-1, and that its expression is induced by treatment with IL-1β and TNF-α. The cytokine-triggered induction of eotaxin-1 further results in enhanced expressions of its own receptor of CCR3, CCR5, and MMPs, suggesting that eotaxin-1 plays an important role in cartilage degradation in OA. J. Cell. Biochem. 93: 929–939, 2004.

Key words: chemokine; eotaxin-1; osteoarthritis; matrix metalloproteinase; chondrocyte

Osteoarthritis (OA), characterized by degradation of articular cartilage and inflammation of the synovium [Radin et al., 1991; Muir, 1995; Pelletier et al., 2001], is a chronic degenerative joint disease caused by biomechanical alterations and the aging process [Yuan et al., 2003]. However, recent studies have revealed a role of an inflammatory process in the pathogenesis of OA, with evidence of an inflammatory process reflected in several clinical signs and symptoms of OA, including swelling of affected joints, synovial effusion, and joint stiffness [Peyron, 1980; Pelletier et al., 2001]. Cartilage is normally avascular and, hence, is secluded from immune surveillance. Nevertheless, aging, longitudinal mechanical stress, or trauma may result in the exposure of cartilage components to the immune system. It was found that these endogenous cartilage components provide a rich source of antigenic determinants in these diseases [Poole, 1992]. After that discovery, it was proposed that exposure of cartilage components to the immune system may consequently induce autoimmune arthritis, initiating the production of inflammatory cytokines, chemokines, nitric

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oxide, and prostaglandins, as well as destructive enzymes. These factors and enzymes then degrade the cartilage matrix, and re-expose cartilage antigens to the immune system, which leads to an autoimmune reaction to cartilage in OA. As a result, such autoimmune reactions cause further destruction of the cartilage and the release of more autoantigens, which can lead to chronic inflammation that damages the articular structure [Yuan et al., 2003]. Immune reactivity to cartilage antigens expectedly plays a crucial role in the pathogenesis of inflammatory arthropathies [Banerjee and Poole, 1992; Poole, 1992].

In addition to the immune-mediated pathway described above in OA patients, another mechanical pathway was proposed which describes the release of interleukin-1β (IL-1β)/tumor necrosis factor-α (TNF-α) or chemokines from synovial cells as a result of induction of synovial inflammation by the direct impact of mechanical forces or by cartilage breakdown products indirectly created by mechanical forces [Yuan et al., 2003]. The release of IL-1β/TNF-α breaks up the normal balance between the catabolic and the anabolic function of chondrocytes and biases it toward the catabolic function [Westacott and Sharif, 1996; Goldring, 2000; Yuan et al., 2003]. The two catabolic cytokines, IL-1β and TNF-α, are well known for their major roles in progressive cartilage disruption by suppressing the syntheses of proteoglycans, type II and IX collagen, and tissue inhibitor of metalloproteinases (TIMPs), and by inducing the expressions of plasminogen activator and proteinases, such as those of the matrix metalloproteinase (MMP) family [Campbell et al., 1988; Goldring et al., 1988; Martel-Pelletier et al., 1991; Sandy et al., 1992] in chondrocytes. However, IL-1β and TNF-α also induce the production of other cytokines and certain chemokines, such as IL-8, growth-regulated oncogene-α (GRO-α), regulated upon activation of normal T cell expression and secretion (RANTES), monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory protein 1α (MIP-1α) [Koch et al., 1992; Rathanaswami et al., 1993; Taub and Oppenheim, 1993; Baggiolini et al., 1994].

Chemokines are a family of small heparin-binding cytokines that are primarily involved in the recruitment of leukocytes to the site of inflammation. According to the juxtaposition of cysteine residues in the chemokine protein's *N*-terminus, four subfamilies can be distinguished and are named C, CC, CXC, and CX3C [Baggiolini et al., 1997; Rollins, 1997]. Among them, CC and CXC chemokines represent two major subgroups. The CC chemokines include RANTES, MCPs, MIP-lα, MIP-1β, eotaxin-l, and eotaxin-2. The CXC chemokines include IL-8, GRO-α, and platelet factor 4 [Sozzani et al., 1996; Lukacs et al., 1999]. Several chemokines have been shown to be overproduced in arthritic joints, including IL-8, MCP-1, MIP-1α, MIP-1β, and RANTES. Another chemokine, eotaxin-1, was first isolated from lung lavage fluid of sensitized guinea pigs following allergen exposure [Jose et al., 1994]. Eotaxin-1 has been demonstrated to selectively induce eosinophil recruitment to the airways and to the skin in vivo [Collins et al., 1995; Rothenberg et al., 1995], and is known to activate eosinophils to release their granule contents. Several cell types express eotaxin-1, including epithelial and endothelial cells, T lymphocytes, macrophages, eosinophils, and fibroblast [Lamkhioued et al., 1997; Mattoli et al., 1997; Ying et al., 1997]. Recent reports indicate that chondrocytes respond to CC and CXC chemokines, such as RANTES and MCP-1, by releasing MMP-3 and *N*-acetyl-β-D-glucosaminidase, thus contributing to cartilage matrix catabolism [Alaaeddine et al., 2001]. However, it is not known whether eotaxin-1 is produced in cartilage or is involved in chondrocyte activation, although the effects of synthesized eotaxin has been examined in parallel with several chemokines in the release of MMP-3 and *N*-acetyl-β-D-glucosaminidase from control and OA chondrocytes [Borzi et al., 2000].

In the present study, we found that eotaxin-1 was induced in cytokine-activated chondrocytes. The production of eotaxin-1 further stimulated expressions of its own receptor of CCR3, CCR5, MMP-3, and MMP-13, which are associated with joint inflammation and cartilage degradation.

MATERIALS AND METHODS

Materials

TNFα, IL-1β, eotaxin-1, and anti-eotoxin-1 monoclonal antibody were purchased from R&D Systems (Minneapolis, MN).

Patients

Patients with OA were selected according to the American College of Rheumatology

TABLE I. Primer Sequences Used for the Reverse-Transcription Polymerase Chain Reaction

Primer sequences[a]	Fragment size (bp)
Eotaxin-1	
Sense: 5′-CCCAACCACCTGCTGCTTTAACCT-3′	
Antisense: 5′-TGGCTTTGGAGTTGGAGATTTTTGG-3′	208
MMP-3	
Sense: 5′-CCTCTGATGGCCCAGAATTGA-3′	
Antisense: 5′-GAAATTGGCCACTCCCTGGGT-3′	440
MMP-13	
Sense: 5′-GACTTCACGATGGCATTGCTG-3′	
Antisense: 5′-GCATCA CCTGCTGAGGATGC-3′	491
TIMP-1	
Sense: 5′-CACCCACAGACGGCCTTCTGCAAT-3′	
Antisense: 5′-AGTGTAGGTCTTGGTGAAGCC-3′	345
CCR3	
Sense: 5′-ATGCTGGTGACAGAGGTGAT-3′	
Antisense: 5′-AGGTGAGTGTGGAAGGCTTA-3′	354
CCR5	
Sense: 5′-CGTCTCTCCCAGGAATCATCTTTAC-3′	
Antisense: 5′-TTGGTCCAACCTGTTAGAGCTACTG-3′	356
GAPDH	
Sense: 5′-CCACCCATGGCAAATTCCATGGCA-3′	
Antisense: 5′-TCTAGACGGCAGGTCAGGTCCACC-3′	598

[a]MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; CCR, C-C chemokine receptor.

criteria [Altman et al., 1986; Arnett et al., 1988]. Plasma samples were obtained from 50 normal donors and 40 patients with OA.

Chondrocyte Isolation and Cell Culture

OA knee cartilage was obtained from patients undergoing total joint replacement surgery. Cartilage slices were cut into pieces ($2 \sim 3$ mm^3), and chondrocytes were released from articular cartilage by sequential enzymatic digestion with 0.1% hyaluronidase (Sigma Chemical, St. Louis, MO) for 30 min, with 0.5% pronase for 1 h, and with 0.2% type II collagenase (Sigma Chemical) for 1 h at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing antibiotics (100 U/ml penicillin, 100 U/ml streptomycin, and 2.5 mg/ml amphotericin B). After filtration through a 100-mesh nylon mesh and centrifugation, chondrocyte residues were washed and seeded at a high density in DMEM supplemented with 10% fetal calf serum (FCS; Gibco BRL, Grand Island, NY) and antibiotics, and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. All experiments were performed with cells between the second and fourth passages. Human SW1353 chondrosarcoma cells were cultured in the same medium as those for primary cultured chondrocytes.

Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from both control and tested cultured cells, and RT-PCR was performed as described previously [Hsieh et al., 2003]. In brief, complementary DNA was synthesized in a 25-μl reaction mixture containing 5 μg of total RNA, 2.5 mM of each dNTP, 1 mM of random hexamer primers, and 10 U of M-MLV reverse transcriptase (Epicentre, Madison, WI), by incubation at 37°C for 90 min. The resulting cDNA (2 μl) was subjected to PCR using Taq DNA polymerase (Epicentre) and specific primers for eotaxin-l, CCR, MMPs, TIMP-1, and GAPDH as shown in Table I. PCR for eotaxin-1 was performed for 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s; and CCR was amplified in a protocol of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. For MMP and TIMP, the PCR protocol was 35 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. In each experiment, amplification of cDNA for the housekeeping gene, *GAPDH*, was used as an internal standard. PCR products were analyzed on 2% agarose gels.

Enzyme-Linked Immunosorbent Assay (ELISA)

Levels of CC chemokines, including RANTES, MCP-1, and eotaxin-1, in plasma and culture

medium were determined by commercially available ELISA kits according to the instructions of the manufacturer (R&D Systems) [Hsieh et al., 2003].

Flow Cytometric Analysis System (FCAS)

For detection of cell surface expression of CC chemokine receptors, cells were placed in 15-ml polyethylene tubes on a rocker plate at 37°C for 6 h to allow the re-expression of receptors cleaved during enzymatic treatment. For permeabilized cell staining, chondrocytes were fixed with freshly prepared 4% paraformaldehyde in PBS at room temperature for 30 min, then washed twice with PBS. Cells (10^5) were processed in 0.05 ml of PBS-10% FCS, and incubated with anti-CCR3 or anti-CCR2 conjugated with phycoerythrin (PE), anti-CCR5 conjugated with fluorescein isothiocyanate (FITC) monoclonal antibodies, and isotype-control mouse IgG conjugated with PE or FITC (R&D Systems) for 45 min at 4°C in the dark. After washing, cells were suspended in 4% paraformaldehyde and immediately analyzed by FCAS.

RESULTS

Patients With OA had High Levels of the Chemokine Eotaxin-1 in Plasma

To investigate whether the CC chemokine, eotaxin-1, was upregulated in patients with OA, concentrations of three CC chemokines in plasma samples from OA patients and normal donors were evaluated by ELISA. As shown in Figure 1, the average concentrations of the chemokines, RANTES (19.84 ± 9.78 ng/ml) and MCP-1α (580.59 ± 277.76 pg/ml), were higher in OA patients than in normal donors (5.34 ± 6.68 ng/ml and 142.74 ± 63.38 pg/ml, respectively). Similar to previous reports, the CC chemokines, RANTES and MCP-1α, were markedly upregulated in OA articular cartilage. In addition, we first found as also shown in Figure 1 that the chemokine, eotaxin-1, was increased in OA samples, and show that the mean concentrations of eotaxin-1 were 24.11 ± 11.11 and 93.84 ± 70.93 pg/ml in normal donors and OA patients, respectively. These results suggest that upregulation of eotaxin-1 might play a role in OA.

Induction of Eotaxin-1 Expression by IL-1β and TNF-α in Articular Chondrocytes

To determine whether chondrocytes express eotaxin-1 in response to stimulation by IL-1β or TNF-α, articular chondrocytes from the cartilage of OA patients were stimulated with IL-1β or TNF-α, and the expression of eotaxin-1 was detected by RT-PCR analysis for its mRNA. As shown in Figure 2A,B, IL-1β and TNF-α significantly induced eotaxin-1 expression in time-dependent manners and reached a maximum at 8 and 6 h, respectively. The sequences of eotaxin-1 RT-PCR products were confirmed and verified by DNA sequencing (data not shown). In addition, secretion of eotaxin-1 stimulated by various amounts of IL-1β (0.3 and 1.0 ng/ml) or TNF-α (1 and 3 ng/ml) was examined by ELISA of cultured supernatants, and results are shown in Figure 2C. Results demonstrate that a low level of eotaxin-1 was detected in the absence of IL-1β or TNF-α. With addition of either IL-1β or TNF-α, a significant amount of eotaxin-1 was released at a concentration as low as 0.3 and 1.0 ng/ml for IL-1β and TNF-α, respectively.

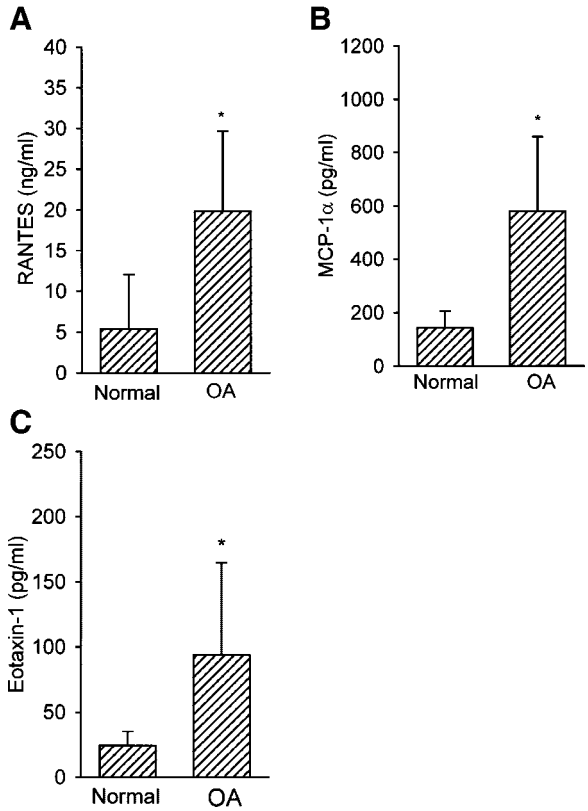
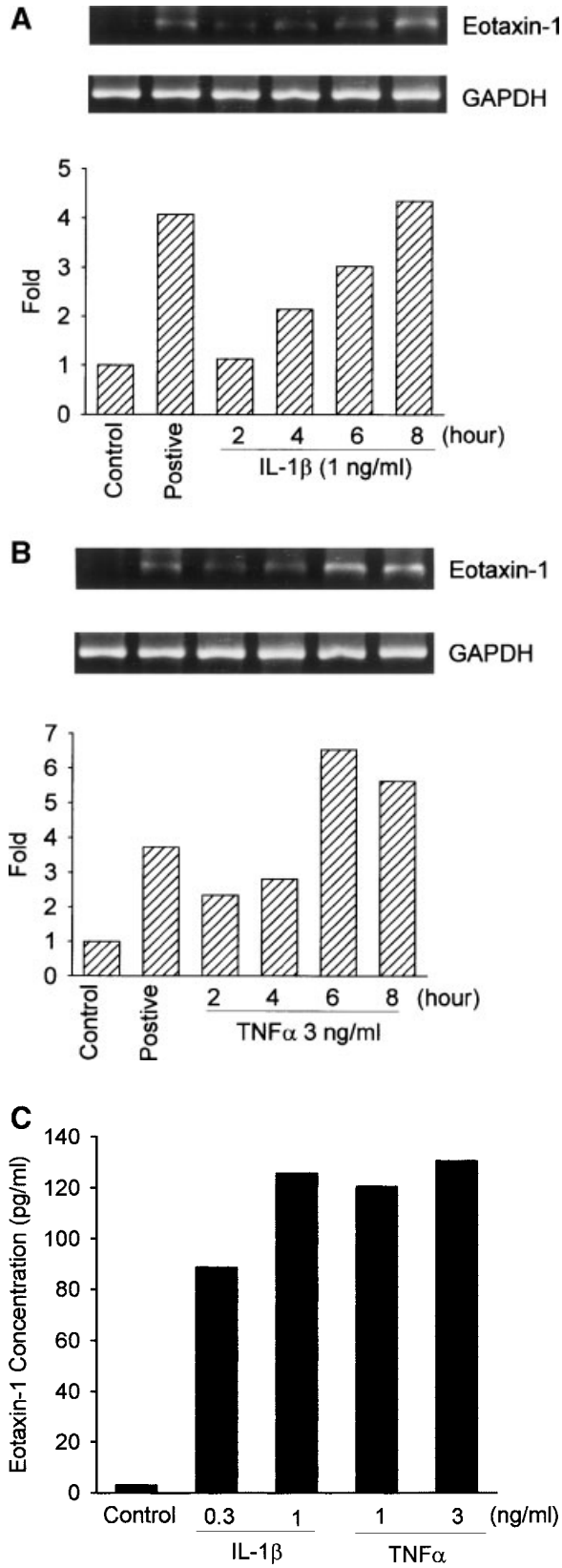


Fig. 1. Plasma levels of CC chemokines in patients with osteoarthritis. Plasma samples obtained from patients with osteoarthritis (OA, n = 40) and from normal donors (Normal, n = 50) were analyzed for concentrations of the CC chemokines, RANTES, MCP-1α, and eotaxin-1, by ELISA as described in "Materials and Methods." Values represent the mean ± S.E. *$P < 0.05$ compared with normal donors by Student's *t*-test.



Chemokine Receptor Expression in Chondrocytes

A previous study indicated that eotaxin-1 could activate cells through the CC chemokine receptors, CCR2, CCR3, and CCR5 [Ogilvie et al., 2001]. Furthermore, it was reported that normal chondrocytes express the CCR2, CCR3, and CCR5 receptors [Borzi et al., 2000]. To understand which receptor on chondrocytes is potentially responsive to eotaxin-1, CC chemokine receptor expression by human chondrosarcoma SW1353 cells was analyzed by a flow cytometric method. Results are shown in Figure 3 and demonstrate that CCR3 is normally expressed on cell surfaces of chondrosarcomas with no treatment, whereas both CCR2 (data not shown) and CCR5 are expressed only in trace amounts. Stimulation with 1 ng/ml IL-1β and 3 ng/ml TNF-α expectedly resulted in the increased expression of CCR3 and CCR5 as reported, but not CCR2. With induction using an increasing concentration of eotaxin-1 from 3 to 100 ng/ml, expressions of CCR3 and CCR5 on cell surfaces of chondrosarcoma SW1353 cells reached their respective maximal at 10 and 100 ng/ml of eotaxin-1. However, CCR2 expression showed no change with eotaxin-1 treatment (data not shown). The expression patterns of CCR3 and CCR5 on chondrosarcoma cells were also identical to that expressed by primary cultured chondrocytes (data not shown).

Modulation of MMP-3 and MMP-13 Production by Eotaxin-1

It is well known that MMP and TIMP are involved in cartilage degradation process. To question whether eotaxin-1 modulate expressions of MMPs and TIMPs, RT-PCR analysis was performed to detect the mRNA expressions of MMP-3, MMP-13, and TIMP-1. As shown in Figure 4A–C, eotaxin-1 significantly stimulated about 2.5- and 3-fold increase in MMP-3 and MMP-13 expressions, respectively. In contrast, no significant alteration in the levels of

Fig. 2. Effect of IL-1β and TNF-α on eotaxin-1 expression in chondrocytes. Cells were treated with IL-1β (**A**) or TNF-α (**B**) for indicated times, and mRNA levels of eotaxin-1 and GAPDH were determined by RT-PCR as described in "Materials and Methods." The positive control was obtained from human A549 cells treated with IL-1β for 6 h. Band intensities were quantified by densitometry (IS-1000 Digital Imaging System, Kaiser Alpha Innotech, San Lendro, CA), and the relative multiples of eotaxin-1/GAPDH are shown. **C:** Cells were treated with IL-1β or TNF-α for 24 h, and eotaxin-1 levels in cultured medium were determined by ELISA as described in "Materials and Methods."

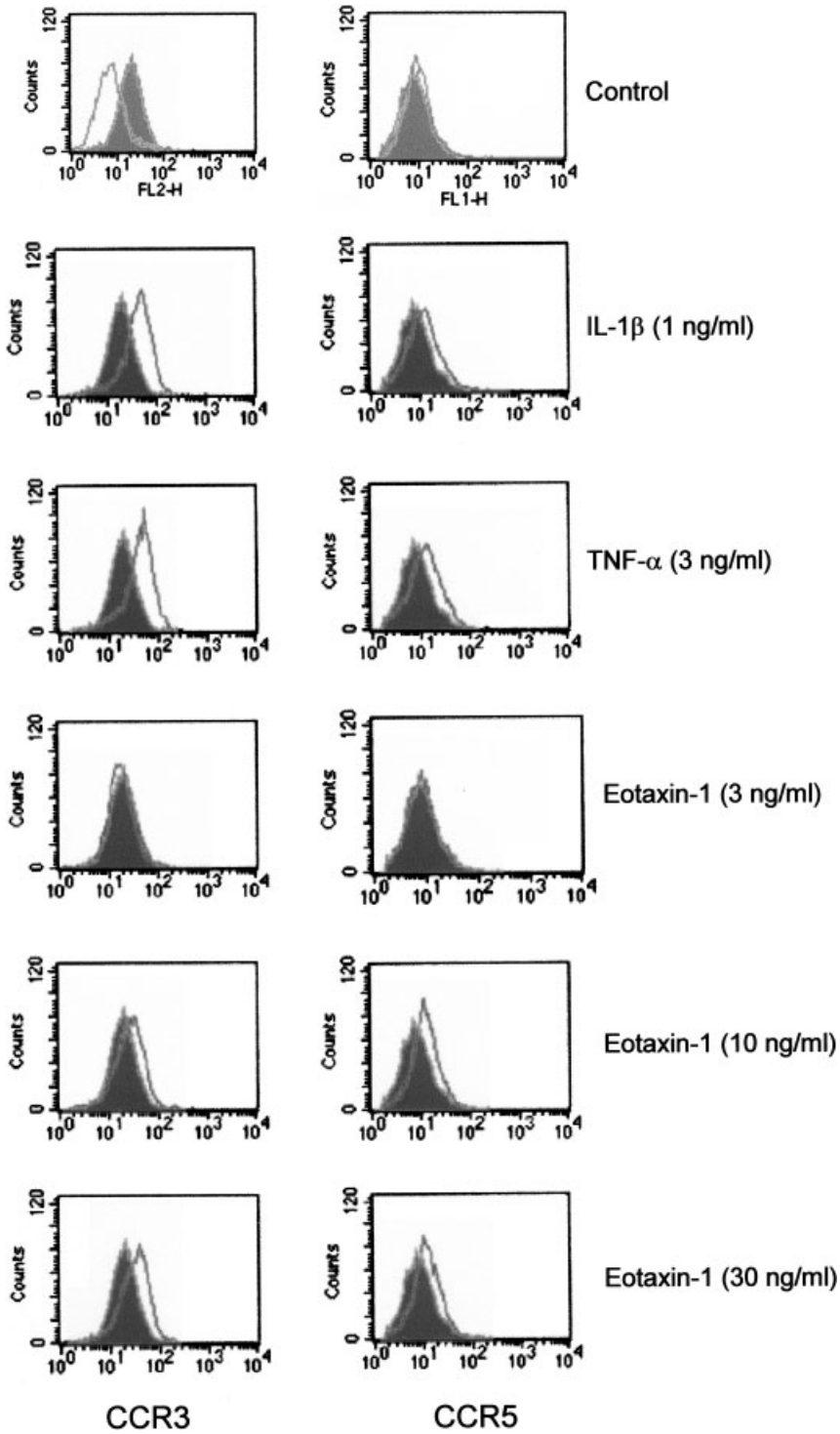


Fig. 3. Effect of eotaxin-1 on chemokine receptor expression in human SW1353 chondrosarcoma cells. Cells were treated with IL-1β (1 ng/ml), TNF-α (3 ng/ml), or various concentrations of eotaxin-1 for 24 h, and levels of CCR3 and CCR5 chemokine receptors were determined by flow cytometry as described in ''Materials and Methods.'' Shaded histograms represent the basal fluorescence levels after staining with the isotype control (anti-CCR antibodies). Open histograms represent expression of the chemokine receptor after stimulation with IL-1β, TNF-α, or eotaxin-1.

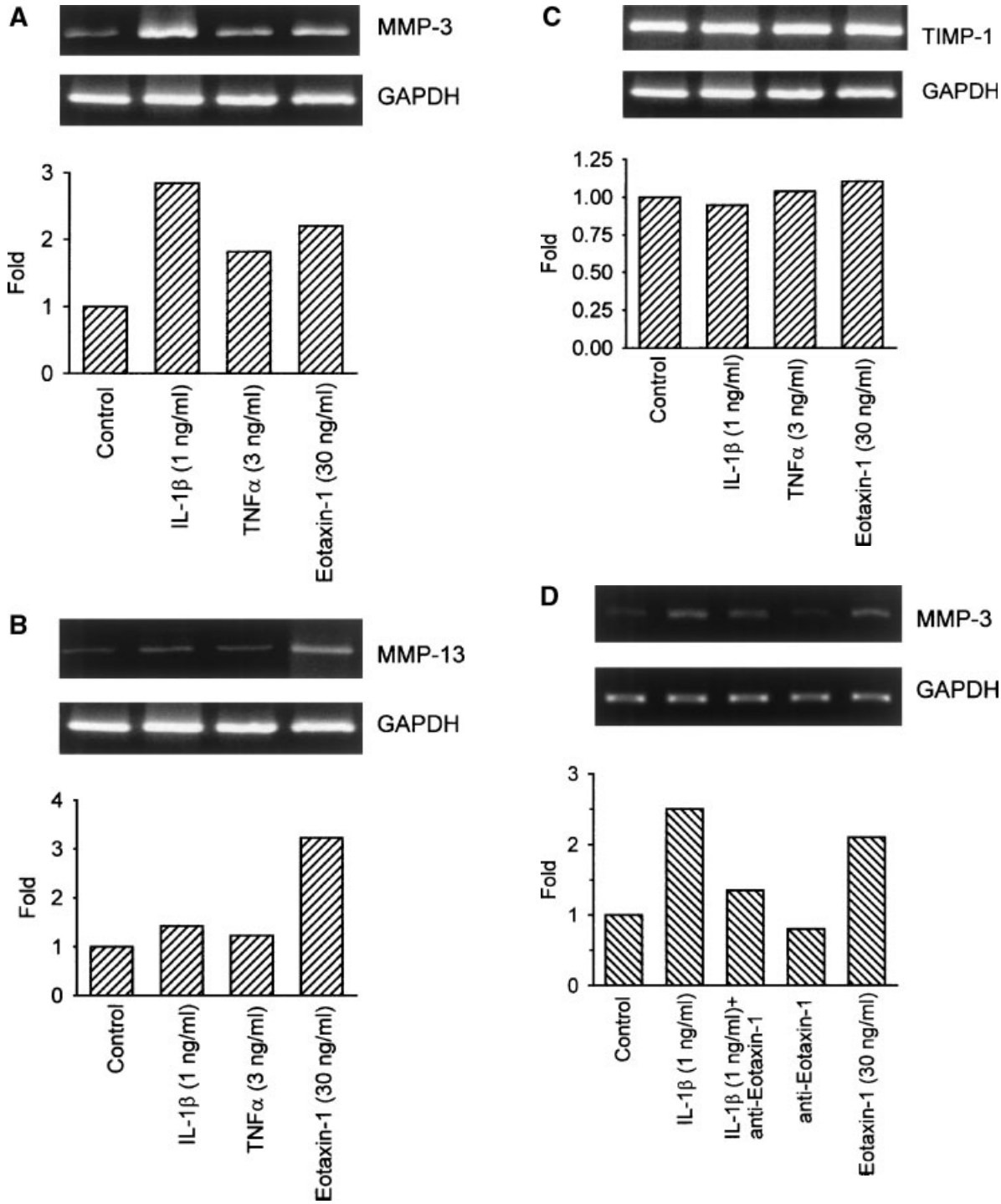


Fig. 4. Effect of eotaxin-1 on the expressions of MMP-3 and MMP-13 in chondrocytes. **A**–**C**: Cells were treated with IL-1β, TNF-α, or eotaxin-1 for 6 h, and the mRNA expressions of MMP-3, MMP-13, TIMP-1, and GAPDH were determined by RT-PCR as described in ''Materials and Methods.'' **D**: Cells were treated with IL-1β, eotaxin-1, and/or anti-eotaxin-1 antibody for 6 h, and the mRNA expression of MMP-3 was determined by RT-PCR as described in ''Materials and Methods.'' Band intensities were quantified by densitometry (IS-1000 Digital Imaging System), and relative multiples of eotaxin-1/GAPDH are shown.

TIMP-1 was found after eotaxin-1 stimulation. To further question how induction of eotaxin-1 by IL-1β modulated MMP-3 expression. Eotaxin-1 antibody was added to cultured medium to block the effect of eotaxin-1 induced by IL-1β. As shown in Figure 4D, the addition of the antibody against eotaxin-1 significantly blocked the induction of MMP-3 by IL-1β treatment. Eotaxin-1 antibody alone diminished the basal level of MMP-3. These results indicated the possibility that IL-1β induced MMP-3 expression, in part, through the induction of eotaxin-1.

DISCUSSION

Several studies have implicated chondrocytes in the expression of chemokines, such as RANTES, IL-8, GRO-α, and MCP-lα, as well as of chemokine receptors, including CCR1, CCR2, CCR3, CCR5, CXCR1, and CXCR2. This study is the first to report that eotaxin-1 is secreted by chondrocytes from OA patients with stimulation by IL-1β and TNF-α. Eotaxin-1 not only enhances the expression of its own receptor, CCR3, on the cell surface of chondrocytes but also induces the expression of CCR5, MMP-3, and MMP-13, but not TIMP-1. Overall, eotaxin-1 expectedly plays an important role in the catabolic process of cartilage expressed by chondrocytes.

OA is a non-inflammatory type of arthritis, and lymphocyte infiltration was not significant, while concentrations of several chemokines, including IL-8, GRO-α, RANTES, and MCP-1α, were higher than those of normal controls. These results imply that chemokines do not always accompany inflammation. In this study, higher plasma concentrations of eotaxin-1 in OA patients than in normal controls were demonstrated, which is also true for two known chemokines of RANTES and MCP-1α. Only low levels of eotaxin-1 were detected in the absence of IL-1β or TNF-α. Both IL-1β and TNF-α further induced the release of eotaxin-1 in significant amounts. The expression of eotaxin-1 mRNA on chondrocytes stimulated with IL-1β and TNF-α was confirmed to occur in time- and concentration-dependent manners. This finding somewhat differs from those known chemokines, including MCP-1, RANTES, MIP-1α, and MIP-1β, as reported by Yuan et al. [2001].

Chemokines express their physiologic or pathologic functions by binding to the corresponding G protein-linked cell surface receptors [Rollins, 1997]. Among the three CC chemokine receptors (CCR2, CCR3, and CCR5) examined, this study found that CCR3 is normally expressed on the cell surface of SW1353 chondrosarcoma cells, and stimulation with IL-1β or TNF-α increased the surface expression of CCR3 and CCR5, with the former occurring to a greater extent than the latter. However, eotaxin-1 induced the expression of CCR3 and CCR5 to reach their respective maximal at 10 and 100 ng/ml of eotaxin-1. CCR2 expression showed no change for all treatments. The expression patterns of CCR3 and CCR5 by chondrosarcoma cells were also identical to the results from primary cultured chondrocytes (data not shown). Previously, Yuan et al. [2001] confirmed the existence of the surface expression of CCR2 and CCR5 by a small percentage of chondrocytes and intracellular CCR in relatively large numbers of chondrocytes. They did not demonstrate the expression of CCR3 as shown in this study. However, Borzi et al. [2000] described the expression of a wide variety of chemokine receptors (CCR1, CCR2, CCR3, CCR5, CXCR-1, and CXCR-2) in chondrocytes from normal and OA cartilage. The differences may be attributed to various cell sources or culture conditions used.

Moreover, in this study, we found that stimulation with eotaxin-1 not only increased the surface expression of its own receptor, CCR3, on chondrosarcoma cells but also the production of CCR3 mRNA in a concentration dependent manner. This may indicate the existence of an autocrine/paracrine pathway by which eotaxin-1 increases the expression of its own CCR3 receptor. This autocrine/paracrine pathway was also reported to exist for the chemokine, RANTES, and its own receptor, CCR5, on chondrocytes. The novel biologic and pathogenetic activities of chondrocyte activation and cartilage degradation identified for the chemokine, RANTES, might be applicable to describe similar functions of eotaxin-1 [Alaaeddine et al., 2001]. Furthermore, eotaxin-1 also increased the surface expression of the CCR5 receptor, while the expression of CCR5 mRNA was not detectable by RT-PCR. It is possible that eotaxin-1 is not a ligand but an agonist for the CCR5 receptor. Since eotaxin-1 is not a true ligand for the CCR5 receptor, the binding of eotaxin-1 to the CCR5 receptor to induce expression of CCR5 mRNA might occur over too short of a time to be

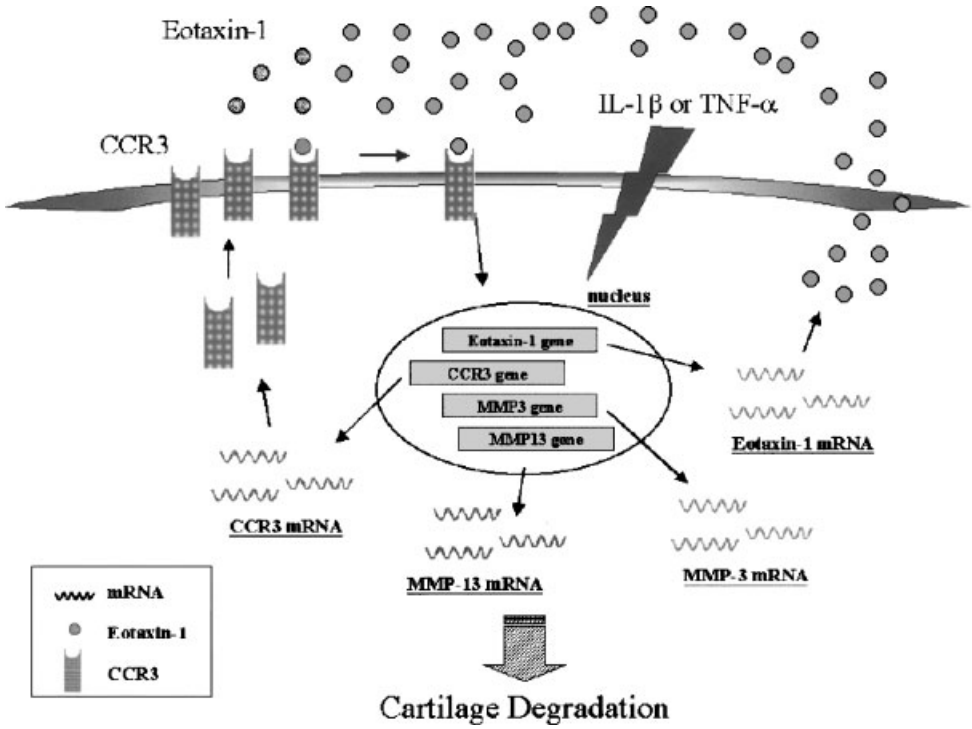


Fig. 5. Hypothesis for the positive feedback regulation of MMP and chemokine receptor expressions by eotaxin-1 in OA. Stimulation of chondrocytes by cytokines (IL-1β or TNF-α) directly and indirectly activates productions of eotaxin-1, CCR, and MMPs. The production of eotaxin-1, in turn, further activates the expression of it own receptors, which results in amplification of the eotaxin-1 signal, and MMP production, which result in destruction of articular cartilage.

detectable in a 24-h incubation, but the increase in the surface expression of CCR5 receptor was consistently observed.

Regarding cartilage homeostasis, maintaining the balance between catabolic and anabolic functions of chondrocytes is very important, and is regulated by various cytokines and growth factors. It is well established that cartilage-derived MMPs are involved in the degradation of cartilage collagen and proteoglycan in OA. Cartilage degradation produced by MMPs is irreversible. MMP-3 is particularly effective, since it cleaves a wide variety of matrix molecules, such as proteoglycans, fibronectins, and several collagens [Woessner, 1991; Knauper et al., 1996; Hornung et al., 2000; Neuhold et al., 2001]. On the other hand, MMP-13 has been shown to degrade type II collagen more rapidly and effectively than MMP-1 or MMP-8 [Gonzalo et al., 1996; Mitchell et al., 1996]. Animal experiments have also demonstrated that blockage of MMP-13 activity results in decreasing degradation of type II collagen [Rothenberg et al., 1996; Neuhold et al., 2001]. Those results strongly suggest that MMP-13 plays an important role in the cartilage degradation cascade. In the present study, we demonstrate that eotaxin-1 not only markedly increases MMP-3 expression in chondrocytes from OA patients to an extent similar to that by TNF-α and to a smaller extent than by IL-1β, but also increases MMP-13 expression that is insignificantly induced by both IL-1β and TNF-α. The effects of eotaxin-1 on MMP-3 expression are consistent with the results reported by Borzi et al. [2000], who showed that the release of MMP-3 from chondrocytes is induced by MCP-1, RANTES, MIP-1β, and other C-X-C chemokines at a concentration of 100 nM. Yuan et al. [2001] further reported that rHuMCP-1 (recombinant human MCP-1), and especially rHuRANTES, at a low concentration of ~3.0 nM (25 ng/ml), were able to induce MMP-3 mRNA expression and protein production by chondrocytes. However, no effects on the production of MMP-9, MMP-13, or TIMP-1 were detected. Together with the effect on MMP-3, the effect of eotaxin-1 on MMP-13 expression might potentially be to induce greater cartilage degradation than that induced by other chemokines.

Different from other chemokines, eotaxin-1 is constitutively expressed in some animal tissues without eosinophil infiltration [Gonzalo et al.,

1996; Rothenberg et al., 1996; Hornung et al., 2000]. This finding suggests that eotaxin-1 not only regulates the immune system but also influences certain physiological functions. The protein and DNA sequences of eotaxin-1, a CC chemokine, closely resemble those of the MCP family, and the expression of another CC chemokine, RANTES, was induced by IL-1β treatment in chondrocytes. In the present study, we found that concentrations of eotaxin-1 in sera of OA patients were higher than those in normal subjects, and that eotaxin-1 was upregulated in chondrocytes by IL-1β and TNF-α stimulation. These findings support an understanding of how the inflammatory cytokine and the chemokine systems interact, resulting in degradation of the cartilage proteoglycan structure. Moreover, eotaxin-1 significantly increased the expressions of its own receptor, CCR3, indicating that a positive feedback regulation loop exists to amplify eotaxin-1's action, thus enhancing MMP expression and cartilage degradation (Fig. 5).

Overall, it was confirmed that the increased secretion of eotaxin-1 from chondrocytes in cartilage of OA patients was inducible in response to the stimulation by cytokines, IL-1β and TNF-α, without infiltration of eosinophil. The additional effect of eotaxin-1 on MMP-13 expression might induce greater cartilage degradation than that induced by other chemokines, thus amplifying the destruction of articular cartilage (Fig. 5). Since this greater influence of eotaxin in the destruction of articular cartilage, it is worthy of studying what role of genetic polymorphism related to eotaxin playing in OA progression?

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