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Interleukin (IL)-17 Enhances Tumor Necrosis Factor-α-Stimulated IL-6 Synthesis via p38 Mitogen-Activated Protein Kinase in Osteoblasts

Haruhiko Tokuda,[1,2] Yosuke Kanno,[2,4] Akira Ishisaki,[2] Motoki Takenaka,[2] Atsushi Harada,[3] and Osamu Kozawa[2]*

[1]Department of Internal Medicine, Chubu National Hospital, National Institute for Longevity Sciences, Obu, Japan
[2]Department of Pharmacology, Gifu University School of Medicine, Gifu, Japan
[3]Department of Orthopaedics, Chubu National Hospital, National Institute for Longevity Sciences, Obu, Japan
[4]Institute of Biological Science, University of Tsukuba, Tsukuba, Japan

Abstract Inflammatory cytokines are well known to play crucial roles in the pathogenesis of rheumatoid arthritis. Among them, interleukin (IL)-17 is a cytokine that is mainly synthesized by activated T cells and its receptors are present in osteoblasts. The synthesis of IL-6, known to stimulate osteoclastic bone resorption, is reportedly responded to bone resorptive agents such as tumor necrosis factor-α (TNF-α) in osteoblasts. It has been reported that IL-17 enhances TNF-α-stimulated IL-6 synthesis in osteoblast-like MC3T3-E1 cells. We previously showed that sphingosine 1-phosphate (S1-P) mediates TNF-α-stimulated IL-6 synthesis in these cells. In the present study, we investigated the mechanism of IL-17 underlying enhancement of IL-6 synthesis in MC3T3-E1 cells. IL-17 induced phosphorylation of p38 mitogen-activated protein (MAP) kinase. SB203580 and PD169316, specific inhibitors of p38 MAP kinase, significantly reduced the enhancement by IL-17 of TNF-α-stimulated IL-6 synthesis. IL-17 also amplified S1-P-stimulated IL-6 synthesis, and the amplification by IL-17 was suppressed by SB203580. Anisomycin, an activator of p38 MAP kinase, which alone had no effect on IL-6 level, enhanced the IL-6 synthesis stimulated by TNF-α. SB203580 and PD169316 inhibited the amplification by anisomycin of the TNF-α-induced IL-6 synthesis. Taken together, our results strongly suggest that IL-17 enhances TNF-α-stimulated IL-6 synthesis via p38 MAP kinase activation in osteoblasts. J. Cell. Biochem. 91: 1053–1061, 2004.

Key words: IL-17; IL-6; TNF-α; p38 MAP kinase; osteoblast

Rheumatoid arthritis is characterized by a chronic inflammation of the synovial joints and infiltration by activated T cells, macrophages, and plasma cells [Feldmann et al., 1996]. It is well known that bone resorption is increased in patients suffering from rheumatoid arthritis [Flescher et al., 1990], and that inflammatory cytokines play a crucial role in the pathogenesis of rheumatoid arthritis [Feldmann et al., 1996]. Bone metabolism is regulated by two functional cells, osteoblasts and osteoclasts, responsible for bone formation and bone resorption, respectively [Nijweide et al., 1986]. The formation of bone structures and bone remodeling results from the coupling of bone resorption by activated osteoclasts and subsequent deposition of new matrix by osteoblasts. It is recognized that inflammatory cytokines act as autacoids in bone metabolism [Gowen, 1991; Mundy, 1993; Manolagas, 1995]. Among them, interleukin (IL)-17 is a cytokine that is mainly synthesized by activated T cells and its receptors are present in osteoblasts including osteoblast-like MC3T3-E1 cells [Yao et al., 1995; Spriggs, 1997; Bezooijen et al., 1999]. It has recently been reported that IL-17 in synovial fluids from patients with rheumatoid arthritis acts as a

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*Correspondence to: Osamu Kozawa, Department of Pharmacology, Gifu University School of Medicine, Gifu 500-8705, Japan. E-mail: okozawa@cc.gifu-u.ac.jp

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potent stimulator of osteoclastgenesis via osteoblasts [Kotake et al., 1999]. However, the exact mechanism of IL-17 in bone metabolism and osteoblasts has not yet been precisely clarified.

IL-6 is a multifunctional cytokine that has important physiological effects such as promoting B cell differentiation, T cell activation, and acute phase proteins induction [Akira et al., 1990; Snick, 1990]. As for bone metabolism, IL-6 has been shown to stimulate bone resorption and induce osteoclast formation [Ishimi et al., 1990; Roodman, 1992]. It has been reported that bone resorptive agents such as tumor necrosis factor-α (TNF-α) and IL-1 stimulate IL-6 synthesis by osteoblasts [Helle et al., 1988; Ishimi et al., 1990; Littlewood et al., 1991]. Thus, accumulating evidence indicates that IL-6 secreted from osteoblasts plays a key role as a downstream effector of bone resorptive agents. In previous studies [Kozawa et al., 1997a,c], we have shown that TNF-α stimulates IL-6 synthesis via sphingosine 1-phosphate (S1-P) produced from sphingomyelin turnover in osteoblast-like MC3T3-E1 cells. It has recently been reported that IL-17, which alone had no effect on basal IL-6 level, increases TNF-α-stimulated IL-6 synthesis in these cells [Bezooijen et al., 1999]. In the present study, we investigated the mechanism of IL-17 in mediating the TNF-α-induced IL-6 synthesis in MC3T3-E1 cells. Herein, we show that IL-17 amplifies TNF-α-induced IL-6 synthesis via p38 mitogen-activated protein (MAP) kinase activation in these cells.

MATERIALS AND METHODS

IL-17 and IL-6 ELISA kit were purchased from R&D Systems (Tokyo, Japan). TNF-α and S1-P were obtained from Funakoshi Pharmaceutical Co. (Tokyo, Japan). SB203580, PD169316, and anisomycin were obtained from Calbiochem-Novabiochem (La Jolla, CA). Phospho-specific p38 MAP kinase antibodies (rabbit polyclonal IgG, affinity purified) and p38 MAP kinase antibodies (rabbit polyclonal IgG, affinity purified) were purchased from New England BioLabs, Inc. (Beverly, MA). The ECL Western blotting detection system was obtained from Amersham Japan (Tokyo, Japan). Other materials and chemicals were obtained from commercial sources. S1-P, SB203580, PD169316, and anisomycin were dissolved in dimethyl sulfoxide. The maximum concentration of dimethyl sulfoxide was 0.1%, which had no effect on the assay for IL-6 or Western blot analysis.

Cell Culture

Cloned osteoblast-like MC3T3-E1 cells derived from newborn mouse calvaria [Sudo et al., 1983] were maintained as previously described [Kozawa et al., 1997b]. Briefly, the cells were seeded into 35 mm (5×10^4) or 90 mm (3×10^5) diameter dishes in α-minimum essential medium (α-MEM) containing 10% fetal calf serum (FCS). After 5 days, the medium was exchanged for 2 ml of α-MEM containing 0.3% FCS. The cells were used for experiments after 48 h. The pretreatment of IL-17 (0, 1, 3, 10, 30, or 100 μM) was performed for 60 min.

Measurement of IL-6

The cultured cells were stimulated by TNF-α (0 or 10 ng/ml) or S1-P (0 or 10 μM) in 1 ml of α-MEM containing 0.3% FCS, and then incubated for the indicated periods. The conditioned medium was collected, and IL-6 in the medium was then measured by an ELISA kit. When indicated, the cells were pretreated with SB203580 (0, 0.1, 0.3, 1, 3, 10, or 30 μM) or PD169316 (0, 0.1, 0.3, 1, 3, 10, or 30 μM) for 60 min. The pretreatment of anisomycin (0, 1, 3, 10, 30, or 100 μM) was performed for 20 min.

Western Blot Analysis

Cultured cells were stimulated by IL-17 (0 or 100 μM) or anisomycin (0 or 100 μM) in serum-free α-MEM for the indicated periods. The cells were washed twice with phosphate-buffered saline and then lysed, homogenized, and sonicated in a lysis buffer containing 62.5 mM Tris/Cl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 50 mM dithiothreitol, and 10% glycerol. The cytosolic fraction was collected as the supernatant after centrifugation at 125,000*g* for 10 min at 4°C. SDS–polyacrylamide gel electrophoresis (PAGE) was performed by the method of Laemmli [Laemmli, 1970] in 10% polyacrylamide gel. Western blotting analysis was performed as previously described [Miwa et al., 1999] using phospho-specific p38 MAP kinase antibodies or p38 MAP kinase antibodies, with peroxidase-labeled antibodies raised in goat against rabbit IgG being used as secondary antibodies. Peroxidase activity on the nitrocellulose sheet was visualized on X-ray film by means of ECL Western blotting detection

system. When indicated, the cells were pretreated with PD169316 (0 or 30 μM) for 60 min.

Determination

The absorbance of ELISA samples was measured at 450 nm with SLT-Labinstruments EAR 340 AT. Absorbance was correlated with various concentrations. The densitometric analysis was performed using Molecular Analyst/Macintosh (Bio-Rad Laboratories, Hercules, CA).

Statistical Analysis

The data were analyzed by ANOVA followed by Bonferroni method for multiple comparisons between pairs, and a $P < 0.05$ was considered significant. All data are presented as the mean ± SEM of triplicate determinations.

RESULTS

Effect of IL-17 on p38 MAP Kinase Phosphorylation in MC3T3-E1 Cells

The MAP kinase superfamily mediates intracellularsignaling of a variety of agonists and plays pivotal roles in cellular functions [Widmann et al., 1999]. In order to clarify whether IL-17 activates p38 MAP kinase in MC3T3-E1 cells, we first examined the effect of IL-17 on the phosphorylation of p38 MAP kinase. IL-17 markedly induced the phosphorylation of p38 MAP kinase in a time-dependent manner (Fig. 1). The phosphorylation was clearly detectable after 60 min.

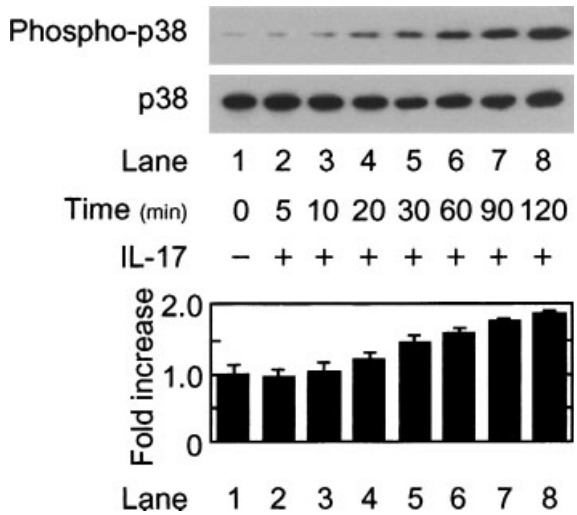

Fig. 1. Effect of interleukin (IL)-17 on the phosphorylation of p38 mitogen-activated protein (MAP) kinase in MC3T3-E1 cells. The cultured cells were stimulated by 100 μM IL-17 for 5 min (**lane 2**), 10 min (**lane 3**), 20 min (**lane 4**), 30 min (**lane 5**), 60 min (**lane 6**), 90 min (**lane 7**), and 120 min (**lane 8**). Cell extracts were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. **Lane 1**, unstimulated cells. The histogram shows quantitative representations of the levels of IL-17-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

Effects of SB203580 or PD169316 on Enhancement by IL-17 of TNF-α-Induced IL-6 Synthesis in MC3T3-E1 Cells

IL-17 reportedly increases TNF-α-induced IL-6 synthesis in MC3T3-E1 cells [Bezooijen et al., 1999]. We confirmed that IL-17 (100 μM), which alone had no effect on IL-6 synthesis, caused about 17-fold amplification in the TNF-α (10 ng/ml)-effect in these cells (Fig. 2). To investigate whether p38 MAP kinase is involved in the enhancement by IL-17 of TNF-α-induced IL-6 synthesis, the effect of SB203580, a specific inhibitor of p38 MAP kinase [Cuenda et al., 1995], on the synthesis of IL-6 was examined. SB203580, which alone had little effect on IL-6 synthesis, significantly suppressed the enhancement by IL-17 of TNF-α-induced IL-6 synthesis (Fig. 2). The inhibitory effect of SB203580 on the TNF-α-induced IL-6 synthesis was dose-dependent between 0.1 and 30 μM, and the maximum effect of SB203580 was observed at 30 μM, a dose that caused about 95% reduction in the TNF-α-effect. The enhancement by IL-17 of the TNF-α-stimulated IL-6 synthesis was also reduced by PD169316, another specific inhibitor of p38 MAP kinase [Kummar et al., 1997], (Fig. 3). PD169316 caused about 95% reduction in the effect of TNF-α.

Effect of PD169316 on IL-17-Stimulated Phosphorylation of p38 MAP Kinase in MC3T3-E1 Cells

We next examined the effect PD169316 on the phosphorylation of p38 MAP kinase induced by IL-17 in MC3T3-E1 cells. PD169316, which alone little affected the phosphorylation of p38 MAP kinase, had little effect on the phosphorylation of p38 MAP kinase induced by IL-17 (Fig. 4).

Effect of IL-17 on S1-P-Stimulated IL-6 Synthesis in MC3T3-E1 Cells

We previously reported that TNF-α stimulates IL-6 synthesis via S1-P produced from

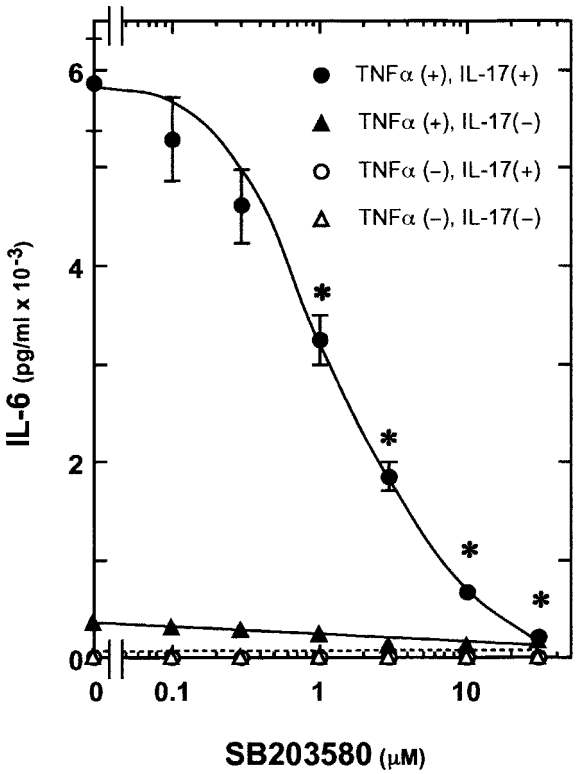

Fig. 2. Effect of SB203580 on the enhancement by IL-17 of TNF-α-induced IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pretreated with various doses of SB203580 for 60 min, and then stimulated by 10 ng/ml TNF-α (closed symbols) or vehicle (open symbols) in the presence of 100 μM IL-17 (circles) or vehicle (triangles) for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $*P < 0.05$, compared to the value of TNF-α with IL-17 without SB203580.

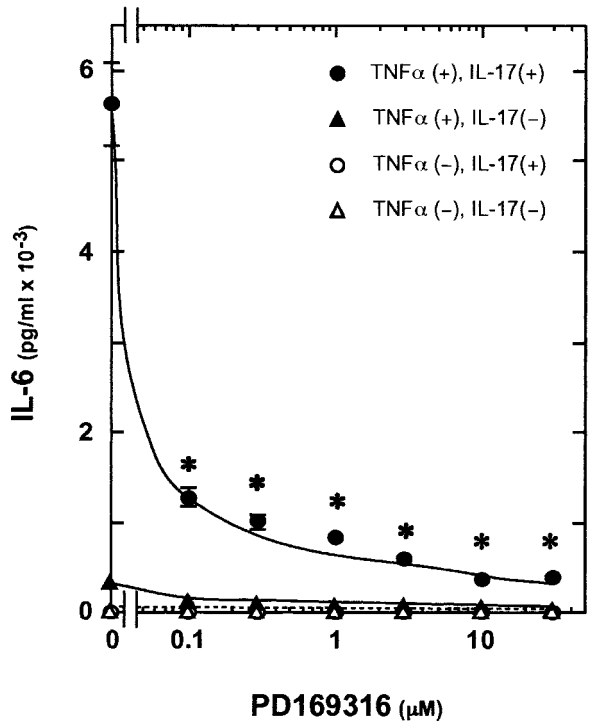

Fig. 3. Effect of PD169316 on the enhancement by IL-17 of TNF-α-induced IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pretreated with various doses of PD169316 for 60 min, and then stimulated by 10 ng/ml TNF-α (closed symbols) or vehicle (open symbols) in the presence of 100 μM IL-17 (circles) or vehicle (triangles) for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $*P < 0.05$, compared to the value of TNF-α with IL-17 without PD169316.

sphingomyelin turnover in MC3T3-E1 cells [Kozawa et al., 1997a,c]. Thus, we examined the effect of IL-17 on the IL-6 synthesis stimulated by S1-P. IL-17, which by itself had little effect on the level of IL-6, significantly amplified the S1-P-induced IL-6 synthesis in a dose-dependent manner in the range between 1 and 100 μM (Fig. 5).

Effect of SB203580 on the Amplification by IL-17 of S1-P-Induced IL-6 Synthesis in MC3T3-E1 Cells

To clarify whether p38 MAP kinase is involved in the amplification by IL-17 or not in MC3T3-E1 cells, we investigated the effect of SB203580 on the IL-17-induced amplification of S1-P-stimulated IL-6 synthesis. SB203580, which alone suppressed the S1-P-stimulated IL-6 synthesis, dose-dependently reduced the amplification in the range between 10 and 30 μM (Fig. 6). The maximum inhibitory effect of SB203580 on the amplification of IL-6 synthesis was observed at 30 μM, which caused about 90% reduction of the amplification.

Effect of Anisomycin on the Phosphorylation of p38 MAP Kinase in MC3T3-E1 Cells

Anisomycin is known to be an activator of p38 MAP kinase [Mahadevan and Edwards, 1991]. Thus, we examined the effect of anisomycin on the phosphorylation of p38 MAP kinase in osteoblast-like MC3T3-E1 cells. Anisomycin time-dependently induced the p38 MAP kinase phosphorylation (Fig. 7). The maximum stimulatory effect of anisomycin on the level of p38 MAP kinase phosphorylation was observed at 20 min after the stimulation of anisomycin.

Effect of Anisomycin on TNF-α-Induced IL-6 Synthesis in MC3T3-E1 Cells

To determine whether the activation of p38 MAP kinase amplifies the IL-6 synthesis

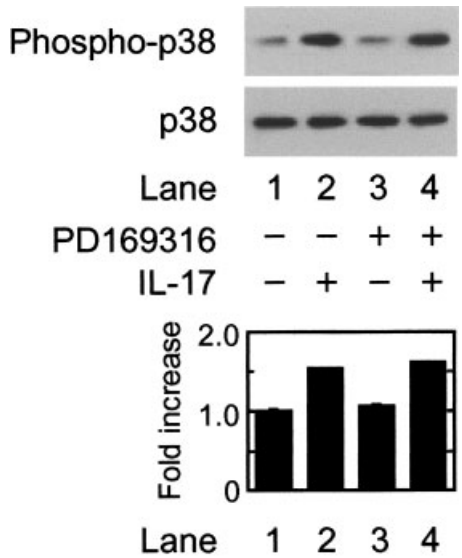

Fig. 4. Effect of PD169316 on the IL-17-induced phosphorylation of p38 MAP kinase in MC3T3-E1 cells. The cultured cells were pretreated with 30 μM PD169316 or vehicle for 60 min, and then stimulated by 100 μM IL-17 or vehicle for 120 min. Cell extracts were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. The histogram shows quantitative representations of the levels of IL-17-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

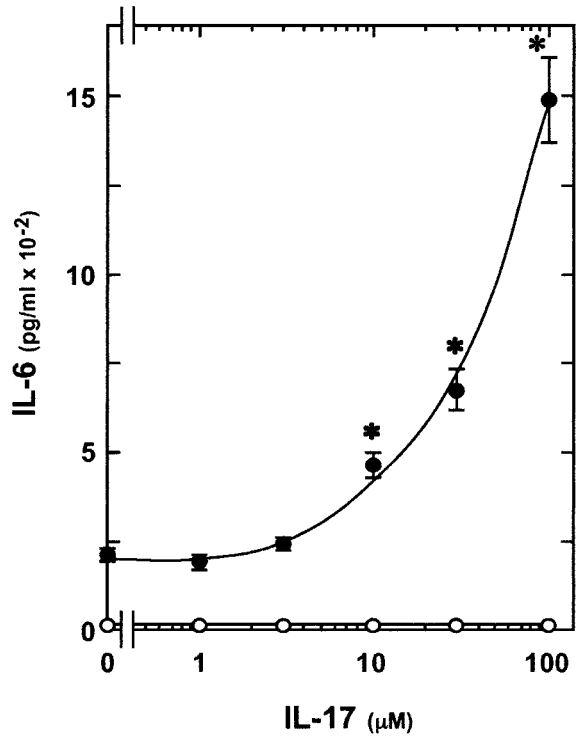

Fig. 5. Effect of IL-17 on the sphingosine 1-phosphate (S1-P)-induced IL-6 synthesis in MC3T3-E1 cells. The cultured cells were stimulated by 10 μM S1-P (closed symbols) or vehicle (open symbols) in the presence of various doses of IL-17 for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *$P < 0.05$, compared to the value of S1-P alone.

induced by TNF-α or not in MC3T3-E1 cells, we tested the effect of anisomycin on the TNF-α-induced IL-6 synthesis. Anisomycin, which alone had little effect on the IL-6 level, significantly enhanced the TNF-α-induced IL-6 synthesis in a dose-dependent manner in the range between 1 and 100 μM (Fig. 8). S1-P-induced IL-6 synthesis, as well as TNF-α-stimulated IL-6 synthesis, was also amplified by anisomycin (data not shown).

Effects of SB203580 or PD169316 on the Enhancement by Anisomycin of TNF-α-Induced IL-6 Synthesis in MC3T3-E1 Cells

We further examined the effects of SB203580 or PD169316 on the anisomycin-induced enhancement of TNF-α-stimulated IL-6 synthesis in MC3T3-E1 cells. SB203580 dose-dependently reduced the enhancement by anisomycin in the range between 10 and 30 μM (Fig. 9). The maximum inhibitory effect of SB203580 on the enhancement was observed at 30 μM, which caused about 70% reduction. PD169316 also inhibited the enhancement of IL-6 synthesis (Table I). PD169316 (30 μM) caused about 60% reduction in the anisomycin-effect.

DISCUSSION

In the present study, we showed that IL-17 induced phosphorylation of p38 MAP kinase in osteoblast-like MC3T3-E1 cells. The MAP kinase superfamily exists in ubiquitous cells, and plays crucial roles in cellular functions [Widmann et al., 1999]. It is well recognized that MAP kinases are activated by phosphorylation of threonine and tyrosine residues by dual specificity MAP kinase kinase [Raingeaud et al., 1995; Widmann et al., 1999]. Therefore, our findings suggest that IL-17 activates p38 MAP kinase in MC3T3-E1 cells. It has been reported that IL-17 activates MAP kinases in human chondrocytes and macrophages [Shalom-Barak et al., 1998; Martel-Pelletier et al., 1999; Laan et al., 2001]. To our knowledge, we are the first to report the activation of p38 MAP

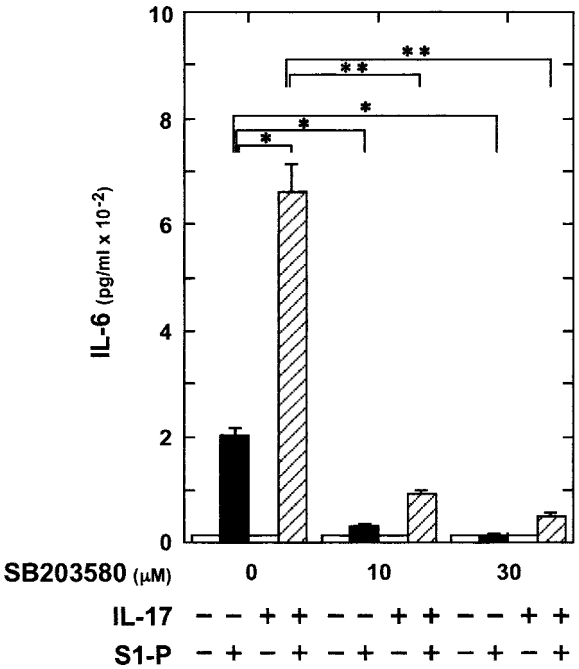

Fig. 6. Effect of SB203680 on the enhancement by IL-17 of S1-P-induced IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pretreated with 30 μM SB203580 or vehicle for 60 min, and then stimulated by 10 μM S1-P or vehicle in the presence of 100 μM IL-17 or vehicle for 24 h. $^*P < 0.05$, compared to the value of S1-P alone, $^*P < 0.05$, compared to the value of S1-P with IL-17.

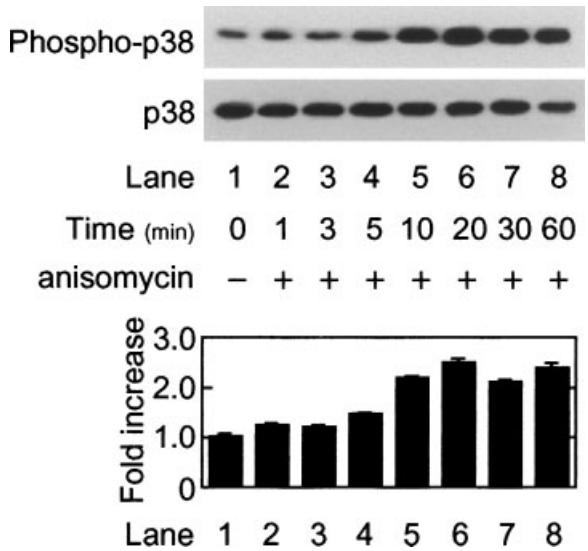

Fig. 7. Effect of anisomycin on the phosphorylation of p38 MAP kinase in MC3T3-E1 cells. The cultured cells were stimulated by 100 μM anisomycin for 1 min (**lane 2**), 3 min (**lane 3**), 5 min (**lane 4**), 10 min (**lane 5**), 20 min (**lane 6**), 30 min (**lane 7**) and 60 min (**lane 8**). Cell extracts were subjected to SDS–PAGE with subsequent Western blotting analysis with antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. **Lane 1**, unstimulated cells. The histogram shows quantitative representations of the levels of anisomycin-induced phosphorylation obtained from laser densitometric analysis of three independent experiments. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

kinase by IL-17 in osteoblasts. It is possible that the activation of p38 MAP kinase plays a role in the modulation of osteoblast cell functions by IL-17.

It has recently been reported that IL-17 enhances TNF-α-induced IL-6 synthesis in osteoblasts-like MC3T3-E1 cells [Bezooijen et al., 1999]. We previously reported that TNF-α induces sphingomyelin hydrolysis resulting in the formation of S1-P in MC3T3-E1 cells, and S1-P subsequently stimulates IL-6 synthesis [Kozawa et al., 1997a,c]. Thus, we investigated the involvement of p38 MAP kinase in the IL-17-induced amplification of the IL-6 synthesis in these cells. Herein, we showed that SB203580 or PD169316 reduced the enhancement by IL-17 of TNF-α-induced IL-6 synthesis. In addition, we demonstrated that IL-17 amplified the S1-P-induced IL-6 synthesis in these cells, and that the S1-P-stimulated IL-6 synthesis and the amplification by IL-17 were also inhibited by SB203580. Therefore, our results suggest that p38 MAP kinase activated by IL-17 positively affects TNF-α-induced IL-6 synthesis at the point downstream from the sphingomyelin hydrolysis in MC3T3-E1 cells. SB203580 or PD169316 is a specific inhibitor not for the upstream kinase of p38 MAP kinase, but for p38 MAP kinase itself [Cuenda et al., 1995; Kummar et al., 1997]. This is a probable reason why PD169316 failed to affect the phosphorylation of p38 MAP kinase induced by IL-17 in MC3T3-E1 cells. Furthermore, we showed here that anisomycin enhanced both TNF-α- and S1-P-induced IL-6 synthesis, and that SB203580 or PD169316 reduced the enhancement by anisomycin of TNF-α-induced IL-6 synthesis in these cells. We also demonstrated that anisomycin induced the phosphorylation of p38 MAP kinase in MC3T3-E1 cells. These results suggest that activation of p38 MAP kinase by itself successfully enhanced the IL-6 synthesis stimulated by TNF-α in osteoblast-like MC3T3-E1 cells. Based on our findings as a whole, it is most likely that IL-17 enhances TNF-α-induced IL-6 synthesis through the activation of p38 MAP kinase in osteoblasts.

Under the pathological conditions characterized by the presence of activated T cells, such as rheumatoid arthritis, it is generally known to be

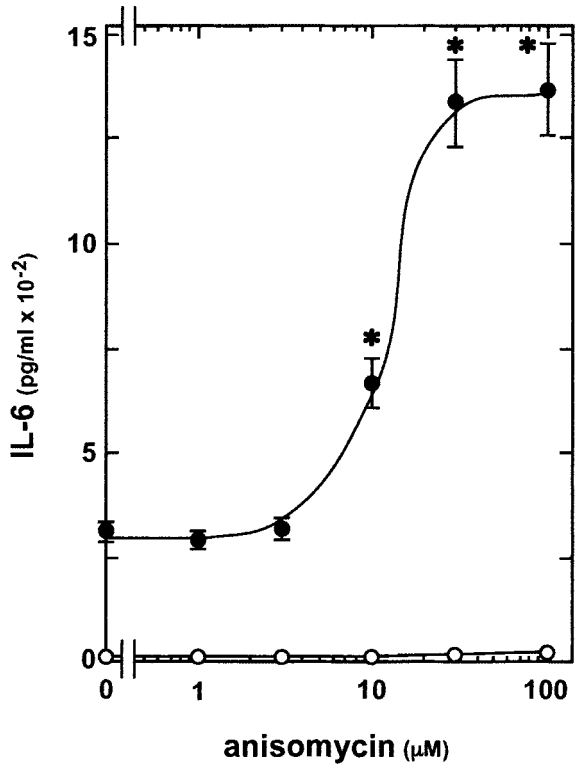

Fig. 8. Effect of anisomycin on the TNF-α-induced IL-6 synthesis in MC3T3-E1 cells. The cultured cells were stimulated by 10 ng/ml TNF-α (closed symbols) or vehicle (open symbols) in the presence of various doses of anisomycin for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $*P < 0.05$, compared to the value of TNF-α alone.

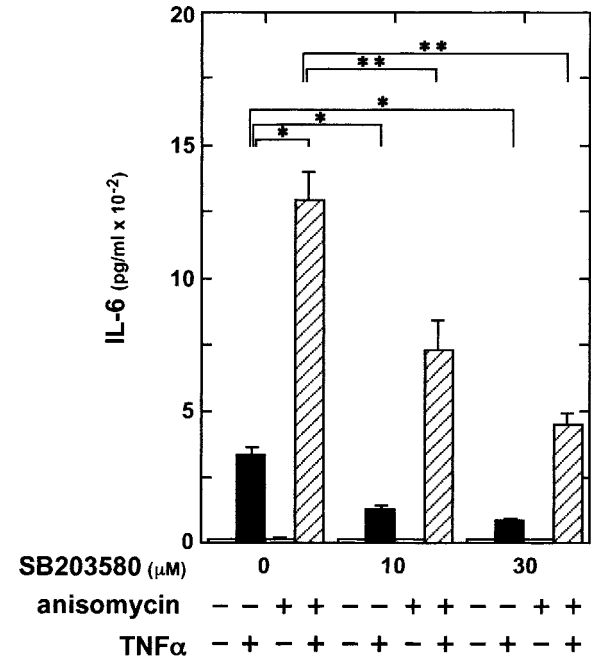

Fig. 9. Effect of SB203680 on the enhancement by anisomycin of TNF-α-induced IL-6 synthesis in MC3T3-E1 cells. The cultured cells were pretreated with SB203580 or vehicle for 60 min, and then stimulated by 10 ng/ml TNF-α or vehicle in the presence of 100 μM anisomycin or vehicle for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $*P < 0.05$, compared to the value of TNF-α alone, $**P < 0.05$, compared to the value of TNF-α and anisomycin without SB203580.

associated with increased osteoclastic bone resorption [Flescher et al., 1990]. Activated T cells produce multiple cytokines including TNF-α, which promotes bone resorption, and they are also the exclusive source of IL-17 [Yao et al., 1995; Fossiez et al., 1996]. It seems that the IL-17-induced enhancement of TNF-α-stimulated IL-6 synthesis by osteoblasts is a worsening cycle promoting bone resorption in inflammatory bone diseases. The levels of IL-17 in synovial fluids are reportedly elevated in rheumatoid arthritis patients [Kotake et al., 1999]. Thus, it is probable that the enhancement by IL-17 of TNF-α-induced IL-6 synthesis through the activation of p38 MAP kinase in osteoblasts shown here plays a crucial role in pathological bone resorption in inflammatory bone diseases such as rheumatoid arthritis. The regulation of p38 MAP kinase activation in osteoblasts might be an important molecular target of therapeutic agent for inflammatory bone resorption.

It is well known that receptor activator of nuclear factor κB ligand (RANKL) expressed on osteoblastic cells responding to bone resorbing agents binds to its specific receptor RANK,

TABLE I. Effect of PD169316 on the Enhancement by Anisomycin of TNF-α-Induced IL-6 Synthesis in MC3T3-E1 Cells

PD169316	Anisomycin	TNF-α	IL-6 (pg/ml)
−	−	−	<15.6
−	−	+	322 ± 25
−	+	−	22 ± 3
−	+	+	1415 ± 113*
+	−	−	<15.6
+	−	+	42 ± 5*
+	+	−	<15.6
+	+	+	513 ± 81**

The cultured cells were pretreated with 30 μM PD169316 or vehicle for 60 min, and then stimulated by 10 ng/ml TNF-α or vehicle in the presence of 100 μM anisomycin or vehicle for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $*P < 0.05$, compared to the value of TNF-α alone, $**P < 0.05$, compared to the value of TNF-α and anisomycin without PD169316.

expressed on osteoclast precursor, providing the signals necessary for osteoclast differentiation [Suda et al., 1999]. A combination of IL-6 and soluble IL-6 receptor reportedly induces RANKL expression in UAMS-32 stromal/osteoblastic cell line [O'Brien et al., 1999]. In has been reported that RANK expression is markedly increased by TNF-α in bone marrow cells containing osteoclast precursors [Komine et al., 2001]. Furthermore, it has recently been reported that overexpression of IL-17 promotes RANKL and RANK expression in synovium, resulting in increase of osteoclastic bone resorption and bone erosion in collagen-induced arthritis [Lubberts et al., 2003]. Taking these findings into account, it is likely that the enhancement by IL-17 of TNF-α-induced IL-6 synthesis in osteoblasts acts as a potent positive regulating mechanism of osteoclastic bone resorption cooperatively with RANKL/RANK pathway in the inflammatory bone resorption. Further in vivo and ex vivo investigations using osteoprotegerin, a decoy receptor of RANK which blocks the RANKL signals to osteoclast, or the antibodies for RANKL would be required to clarify the exact mechanism of pathological bone resorption in inflammatory bone diseases.

In conclusion, our present results strongly suggest that IL-17 stimulates TNF-α-induced IL-6 synthesis via p38 MAP kinase activation in osteoblasts.

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