Regulation of interleukin-1α and tumor necrosis factor-α-induced interleukin-8 production by amnion-derived (WISH) cells

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Summary

Purpose of investigation: It has been reported that interleukin (IL)-8 is produced in the amnion and that its production is enhanced by the initiation of labor. The purpose of this study was to clarify the mechanism of IL-8 production by amnion-derived (WISH) cells. *Methods:* Cells were cultured and treated with various concentrations of interleukin (IL)-1α, IL-1 receptor antagonist (ra), tumor necrosis factor (TNF)- α, C2-, C6-ceramide, mitogen-activated protein (MAP) kinase kinase (MEK) inhibitor (U0126) and pyridinyl imidazole (p38 MAP kinase inhibitor, SB203580). IL-8 in the culture medium was measured by ELISA. *Results:* The production of IL-8 was significantly increased by IL-1α or TNF-α, and the increase of IL-8 stimulated by IL-1α was suppressed by IL-1 ra in a dose-dependent manner. The increase in IL-8 production by IL-1α or TNF-α was further enhanced by simultaneous addition of C2-ceramide. The increase of IL-8 stimulated by IL-1α or TNF-α was also suppressed by treatment with U0126 or SB203580. The results of this study demonstrate that the production of IL-8 induced by IL-1α and TNF-α is enhanced by C2-ceramide, and suppressed by MEK inhibitor or P38 MAP kinase inhibitor. *Conclusion:* The results suggest that ceramide-mediated accumulation and MAP kinase-mediated suppression of inflammatory events in the amnion may play an important role in the maintenance of pregnancy and initiation of labor.

Key words: Interleukin-8, MAP kinase; Amnion; Parturition.

Introduction

It has been hypothesized that inflammatory mediators cause the pathophysiological symptoms associated with infection-induced preterm labor. Neutrophils, which are the predominant type of white blood cell, are found in amniotic fluid, chorionic membranes, and placenta afflicted with chorioamnionitis caused by intraamniotic fluid bacterial infection. However, the detailed mechanism of neutrophil attraction to fetal membrane tissues and amniotic fluid is unclear.

Interleukin (IL)-8, an eight-kirodalton glycoprotein, has been reported to be a chemotactic and activating factor for neutrophils [1]. IL-8 concentration in amniotic fluid has been found to be increased gradually in the third trimester of pregnancy [2]. It has been reported that cultured amnion, chorion, and decidual cells produce IL-8 constitutively and in response to other cytokines [3, 4]. Cherouny et al. demonstrated that spontaneous labor is associated with an increase in the amount of IL-8 in amniotic fluid in women with and without chorioamnionitis [5]. We recently reported that IL-8 concentrations in human cervicovaginal fluid are increased exponentially during the second and third trimesters [6].

Ceramide is the most important of the mediators derived from the sphingolipids produced by the enzymatic breakdown of sphingomyelin by either acidic or neutral sphingomyeliase [7]. Studies have shown that ceramide concentrations are elevated in the amniotic fluid of women who suffer from chorioamnionitis and experience preterm labor [8]. It has been demonstrated that mitogen-activated protein (MAP) kinase and extracellular signal-regulated kinase (ERK) – both of which belong to the family of serine/threonin protein kinases – are widely distributed in lower eukaryotes and mammalian cells [9]. It has also been reported that activation of MAP kinase isoforms is caused by a cascade consisting of sequential activation of Ras, Raf-1 [MAP kinase kinase kinase (MEKK)], and MAP kinase kinase or ERK kinase (MEK) [10-12]. In these studies, MAP kinase was activated by cytokines and growth factors, and it was shown to be involved in the proliferation and differentiation of cells through the stimulation of gene expression. Recent studies have shown that p38 mitogen-activated protein (MAP) kinase, which belongs to the MAP kinase superfamily [13], is involved in cytokine expression [14-18]. p38 MAP kinase also plays a role in gene regulation of cytokines.

The purpose of this study was to investigate the effects of ceramide on the production of IL-8 in the enhancement of IL-1α or TNF-α by human amnion-derived cells (WISH cells). In addition, we also examined whether IL-1α - and TNF-α-induced IL-8 production was regulated by MAP kinase in these cells.

Materials and Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui (Tokyo, Japan) and fetal calf serum (FCS) from HyClone (Logan, UT, USA). IL-1α, IL-1 ra and TNF-α

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Fig. 1A

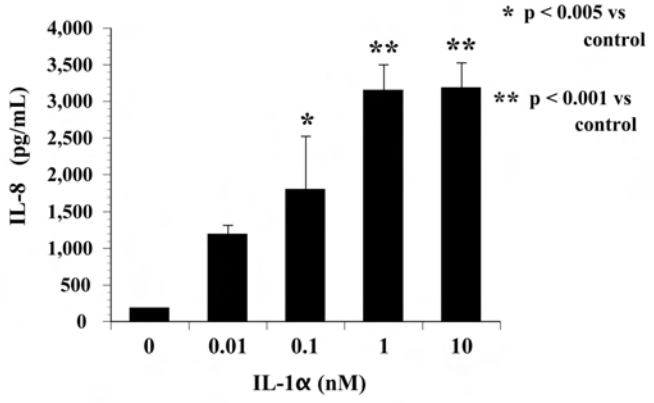

Fig. 1B

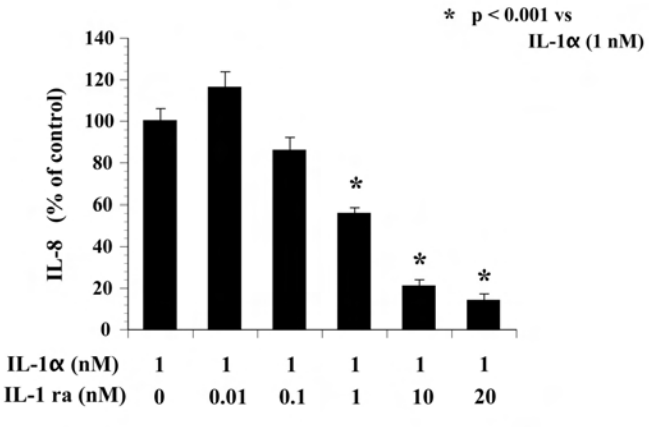

Fig. 1C

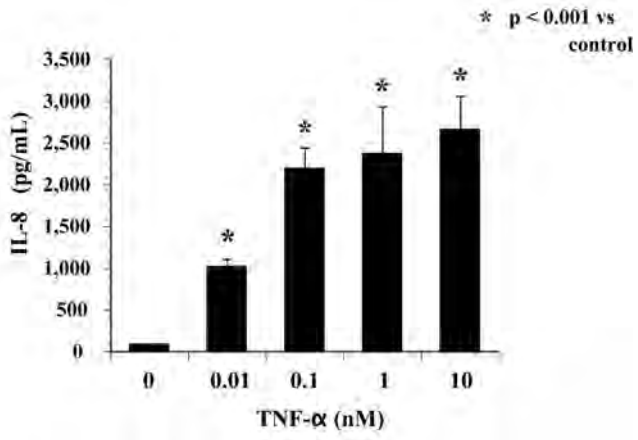

Figure 1. — The levels of IL-8 in culture media of WISH cells after 24 hrs of stimulation with IL-1α. WISH cells were treated with human IL-1α at concentrations of 10 pM to 10 nM (A), with IL-1α (1 nM) and IL-1 ra at concentrations of 10 pM to 10 nM (B), with TNF-α at concentrations of 10 pM to 10 nM (C). Data are expressed as the mean ± SD of triplicate samples from four separate representative experiments.

were purchased from R&D Systems (Minneapolis, MN). N-acetylsphigosine (C2-ceramide) and fumonisin B1 (ceramidase inhibitor) were obtained from Biomol (Plymouth Meeting, PA). U0126 (MEK inhibitor) and pyridinyl imidazole (SB203580) were obtained from Calbiochem (La Jolla, CA).

Cell culturing

The human immortalized amnion epithelial cell line (WISH cells) was maintained in the laboratory and cultured in DMEM supplemented with 10% heat-inactivated FCS, penicillin (100 IU/ml) (Gibco-BRL, Gaithersburg, NY) and streptomycin (100 mg/ml) (Gibco-BRL). WISH cells were grown to confluence in 12-well culture plates (Corning, New York, NY) and in 100 mm diameter wells (Nalge Nunk Int., Naperville, IL) with three replicates per condition. Cells were grown for seven days before each experiment. For the experiments, WISH cells were washed twice with phosphate-buffered saline (PBS) without calcium and magnesium, and then serum-free DMEM was added. Control cells received an equivalent volume of medium alone during incubation. Cultures were incubated at 37°C in air with 5% CO_2.

Stimulation by cytokines, other reagents and measurement of IL-8

To investigate the production of IL-8 by WISH cells, 2×10^5 cells were plated on 12-well culture plates (Corning) in 1 ml of culture medium with 10% FCS and cultured until they were fully confluent. The supernatant was replaced with fresh culture medium containing various concentrations of IL-1α, TNF-α, C2-ceramide, fumonisin B1, U0126 and SB203580 for the desired length of time. The culture medium and cells were removed at specific intervals to establish the time course of IL-8 production. At the end of the culture period, the medium was stored at -80°C until assayed. The vitality of cells was tested and not changed. Experiments were performed in triplicate and were repeated three times. The levels of IL-8 in the supernatant were determined by means of a commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems). The sensitivity of the assay for IL-8 was 10 pg/ml. The inter- and intra-assay coefficient of variance for the ELISA were 9.6% and 5.2%, respectively. Experiments were performed in triplicate and were repeated three times.

Statistical analysis

Data are presented as the mean ± SD. Statistical evaluations were performed using the Student's t-test and Bonferroni/Dunn test with StatView 4.5 software (Abacus Concepts, Berkeley, CA). A level of $p < 0.05$ was accepted as statistically significant.

Results

IL-8 production following treatment with IL-1α, IL-1 ra and TNF-α

The concentration of IL-8 in the culture media without WISH cells was below the level of detection. Small amounts of IL-8 were detected in the supernatant of non-stimulated WISH cells after 24 hrs of incubation (Figure 1). When WISH cells were treated with various concen-

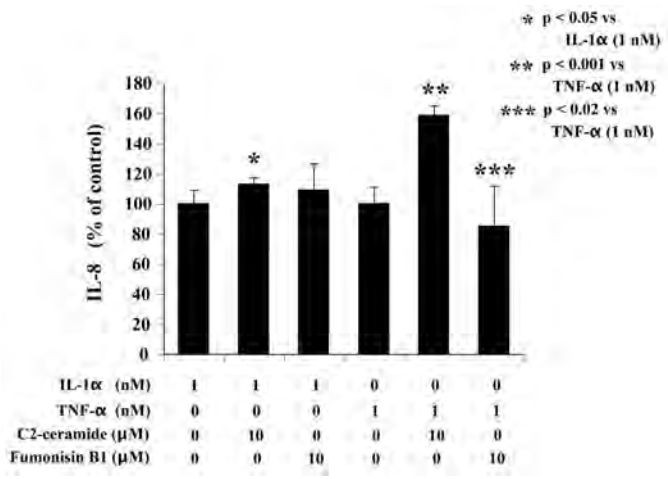

Figure 2. — The levels of IL-8 in culture media of WISH cells after 24 hrs of stimulation with IL-1α, TNF-α, C2-ceramide and fumonisin B1. WISH cells were treated with human IL-1α (1 nM), TNF-α (1 nM), C2-ceramide (10 μM) and fumonisin B1 (10 M). Data are expressed as the mean ± SD of triplicate samples from four separate representative experiments.

trations of IL-1α, the levels of IL-8 were significantly increased in a dose-dependent manner as compared with those of the controls (Figure 1A). When the WISH cells were treated with IL-1α (1 nM) and various concentrations of IL-1 ra, the levels of IL-8 were significantly decreased as compared with those by IL-1 alone (Figure 1B). When WISH cells were treated with various concentrations of TNF-α, the levels of IL-8 were significantly increased in a dose-dependent manner as compared with those of the controls (Figure 1C). The number of cells was counted and was not significantly changed (data not shown).

Production of IL-8 following stimulation by IL-1α, TNF-α, C2-ceramide and Fumonisin B1

When the WISH cells were treated with IL-1α and C2-ceramide, IL-8 production was greater than that by IL-1α treatment alone (Figure 2). IL-8 production in response to incubation with IL-1α and fumonisin B1 was not significantly decreased as compared with that by IL-1α treatment alone.

When the WISH cells were treated with TNF-α and C2-ceramide, IL-8 production was greater than that by TNF-α treatment alone (Figure 2). IL-8 production in response to incubation with TNF-α and fumonisin B1 was significantly decreased as compared with that by TNF-α treatment alone.

When WISH cells were treated with C2-ceramide alone, an accumulation of IL-8 was observed, but it was not significantly greater than that in the controls.

Inhibition of IL-8 production following treatment with IL-1α, TNF-α, U0126 and SB203580

When the WISH cells were treated with IL-1α, TNF-α and U0126, we observed an increase in IL-8 production following IL-1α and TNF-α treatment and a suppression of IL-8 production following U0126 treatment (Figure 3A). IL-8 production in response to incubation with IL-1α and U0126 was suppressed as compared with that by IL-1α treatment alone. IL-8 production in response to incubation with TNF-α and U0126 was also suppressed as compared with that by TNF-α treatment alone.

When the WISH cells were treated with IL-1α, TNF-α and SB203580, we observed an increase in IL-8 production following IL-1α and TNF-α treatment and a suppression of IL-8 production following SB203580 treatment (Figure 3B). IL-8 production in response to incubation with IL-1α and SB203580 was suppressed as compared with that by IL-1α treatment alone. IL-8 production in response to incubation with TNF-α and SB203580 was also suppressed as compared with that by TNF-α treatment alone.

Discussion

IL-8, a potent chemokine for neutrophils, has been shown to be involved in host response to microbial invasion of the amniotic cavity [5, 19]. Romero *et al*. [19] reported that the concentrations of this cytokine in amniotic fluid increased during preterm and term parturition. It has been demonstrated that concentrations of IL-8 in amniotic fluid were significantly higher in patients with histologic chorioamnionitis than in those without acute inflammatory lesions of the placenta [5]. In addition, the mean concentration of IL-8 in amniotic fluid obtained from women with positive cultures and in labor was 14-fold higher than that obtained from women at term and in labor [5]. IL-8 induces the activation and migration of cells from vessels into the surrounding uterine cervical tissue and stimulates the release of collagenase [20] and elastase [21] in the tissue, resulting in the digestion of collagen fibers during preterm and term labor.

IL-1 and TNF-α reportedly act by a mechanism that involves the sphingomyelin pathway [7] of signal transduction after binding to their specific receptors on the plasma membrane of the cell to form ligand-receptor complexes. This step initiates a complicated chain of signal transduction events within the cell, including activation of G-proteins and the formation of a variety of second-messenger molecules, including ceramide. This evidence suggests that the signal transduction of inflammatory cytokines within the cells occurs, in part, via ceramide as a second messenger [22].

Hydrolysis of sphingomyelin has been shown to mediate the actions of IL-1β in regard to the induction of prostaglandin H synthase (PGHS)-2 expression [23, 24] and IL-6 production [25] in fibroblasts. Indeed, we previously reported that ceramide enhanced the production of IL-1α-induced PGE2 or PGF2α production in amniotic cells [26] and endometrial stromal cells [24].

The concentration of ceramide in amniotic fluid is strikingly and consistently increased in premature labor in women with chorioamnionitis. In cases of preterm labor without symptomatic chorioamnionitis, the concen-

Fig. 3A

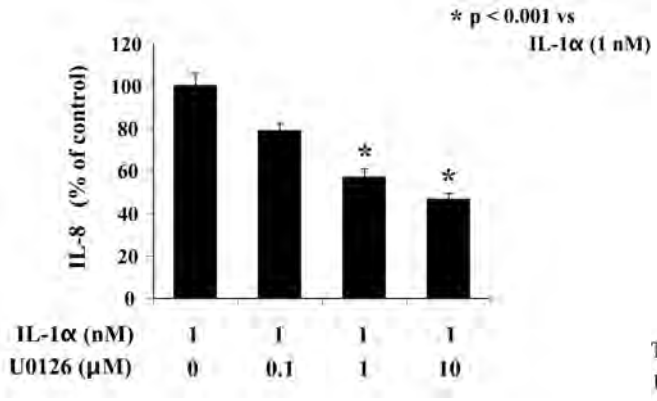

Fig. 3B

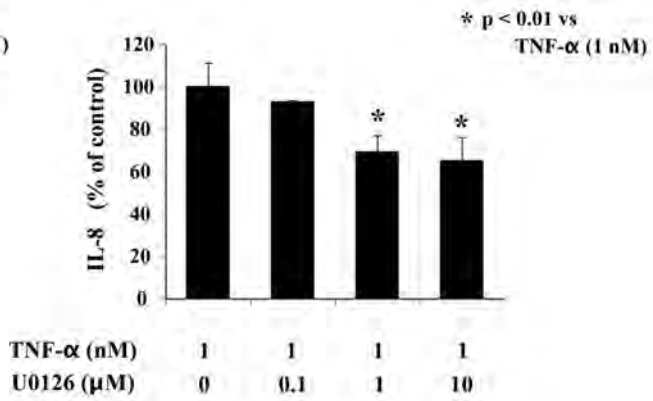

Fig. 3C

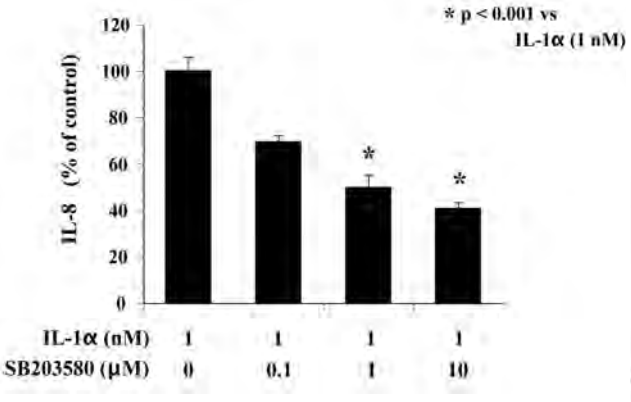

Fig. 3D

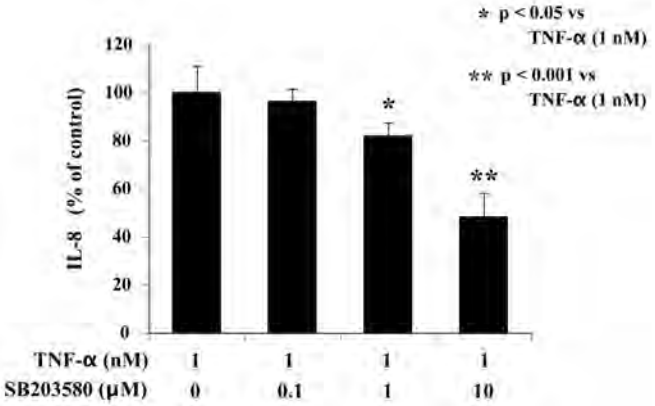

Figure 3. — The levels of IL-8 in culture media of WISH cells after 24 hrs of stimulation with IL-1α, TNF-α, U0126 and SB 203580. WISH cells were treated with human IL-1α (1 nM), TNF-α (1 nM), U0126 and SB 203580 at concentrations of 0.1 μM to 10 μM. Data are expressed as the mean ± SD of triplicate samples from four separate representative experiments.

tration of ceramide has been shown to be higher than in case of other term labor or no labor [8]. These findings suggest that the level of ceramide in the amniotic fluid may serve as an indicator of the imminence of preterm labor. However, the relationship between ceramide and the specific events leading to the onset of preterm labor is unclear. Keelan and Mitchell reported that the TNF-α-induced production of PGE2 was amplified by treatment with C2-ceramide [27]. It is suggested that IL-1 and TNF-α also act by a mechanism that involves the sphingomyelin pathway in amniotic cells. When we investigated the effect of treatment with C2-ceramide in addition to IL-1α or TNF-α, the accumulation of IL-8 was significantly increased. On the other hand, the increase of IL-8 was significantly decreased by treatment with TNF-α and fumonosin B1, but not by IL-1α and fumonisin B1. A differing affinity for the cells or in the effective concentrations of IL-1α or TNF-α may, perhaps, explain these differences. In our data, it is suggested that ceramide may contribute to increase the levels of IL-8 cooperated with IL-1α or TNF-α resulting to cause chorioamnionitis.

IL-1α and TNF-α have also been shown to induce the phosphorylation and acivation of p38 MAP kinase in various cells [13, 28-31]. SB203580, a specific inhibitor of p38 MAP kinase activity, has been used to investigate the signal transduction pathway regulating IL-8 production in TNF-α-, and IL-1α-stimulated human pulmonary vescular endothelial cells [14, 32]. A class of pyridinyl imidazole compounds, which are inhibitors of p38 MAP kinase, block LPS-stimulated IL-1 and TNF-production at the translational level [32]. SB203580, which has recently been shown to be highly specific for kinase [14], completely inhibited p38 MAP kinase activity and IL-8 production in TNF-α- and IL-1α-stimulated human pulmonary vascular endothelial cells. These facts indicate that p38 MAP kinase plays an important role in the TNF-α- and IL-1α-activated signalling pathways which regulate IL-8 expression.

In the present study, treatment with U0126 and SB203580 markedly inhibited IL-1α- and TNF-α-induced IL-8 production. In a study by Song *et al*., ERK was activated by treatment with IL-1β and TNF-α, and this activation was suppressed by an MEK inhibitor in human airway epithelial cells [33]. Taken together, these results suggest that IL-8 production induced by IL-1α and TNF-α might be regulated by the mechanism involving the ERK pathway or p38 MAP kinase pathway from a point downstream of the IL-1 and TNF-α receptors in amniotic cells.

IL-1 activates a number of protein kinase pathways including those of the three types of MAPK (p42/p44, p54/JNK, and p38), and a β-casein kinase [30, 34-37]. These kinases may cause the activation of transcriptional factor resulting in increased expression of cytokines such as IL-8. In the present study, we judged that the inhibitory effects of SB203580 or U0126 at 0.1 M to 10 M were due to p38 MAPK inhibition. SB203580 inhibits the catalytic activity of p38 MAP kinase by binding to the ATP site and subsequently phosphorylating its substrate [38]. SB203580 might exert an inhibitory effect on the induced p38 MAP kinase activity and subsequent IL-8 production, but not on the basal levels of p38 MAP kinase activity and IL-8 production under our experimental conditions. These results may indicate that SB203580 could be used to control p38 MAP kinase-mediated IL-8 production in inflammatory disease.

In conclusion, the present study demonstrated that IL-8 production induced by TPA is regulated by MAP kinase inhibitors and ceramide in WISH cells. The results of this study suggest that MAP kinase inhibitors and ceramide-mediated regulation of inflammatory events in the amnion may play an important role in the maintenance of pregnancy and initiation of labor. Further studies will be necessary to evaluate the regulatory mechanism of IL-8 production by other mediators.

Acknowledgement

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