Effect of Ciprofloxacin-Induced Prostaglandin E \(_2\) on Interleukin-18-Treated Monocytes

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Ciprofloxacin, a fluorinated 4-quinolone, is useful for the clinical treatment of infections due to its antibacterial properties and also modulates the immune response of monocytes isolated from human peripheral blood mononuclear cells. In the present study, we found that ciprofloxacin induced the production of prostaglandin \(E_2\) in monocytes in a concentration-dependent manner regardless of the presence of interleukin-18 by enhancing the expression of cyclooxygenase-2 protein and that this in turn led to the elevation of intercellular cyclic AMP in monocytes via the stimulation of prostaglandin receptors. The prostaglandin \(E_2\) and cyclic AMP production increased by ciprofloxacin was inhibited by indomethacin, a nonselective cyclooxygenase-2 inhibitor, and NS398, a selective cyclooxygenase-2 inhibitor. In addition, ciprofloxacin suppressed the interleukin-18-induced production of tumor necrosis factor alpha, gamma interferon, and interleukin-12 in peripheral blood mononuclear cells by inhibiting the expression of intercellular adhesion molecule 1, B7.1, B7.2, and CD40 on monocytes, and this effect could be reversed by the addition of indomethacin or NS398. These results indicate that ciprofloxacin exerts immunomodulatory activity via the production of prostaglandin \(E_2\) and imply therapeutic potential of ciprofloxacin for the treatment of systemic inflammatory responses initiated by interleukin-18.

Interleukin-18 (IL-18) requires cleavage at its aspartic acid residue by IL-1β-converting enzyme/caspase-1 to become an active and mature protein (8), and monocytes produce IL-18 while interacting with cognate T cells (10). Furthermore, IL-18 is located upstream of production of Th1 cytokines (8, 12), acts in synergy with IL-12 to induce gamma interferon (IFN-γ) production in CD4 \(^+\) cells via different signaling pathways (2), and along with IL-12 is necessary for Th1 responses. Cell-to-cell interactions brought about via the engagement between intercellular adhesion molecule 1 (ICAM-1), B7.1, B7.2, CD40, and CD40L on monocytes and their ligands on T/NK cells are also involved in the IL-18-induced production of cytokines, including IL-12, tumor necrosis factor alpha (TNF-α), IFN-γ, and IL-10 (20, 21).

A major product of cyclooxygenase (COX)-initiated arachidonic acid metabolism, prostaglandin \(E_2\) (PGE\(_2\)), which is released from antigen-presenting cells, primes naive human T cells and enhances their production of anti-inflammatory cytokines while inhibiting their synthesis of proinflammatory cytokines (6, 9). Among the four PGE\(_2\) receptor subtypes, E-prostanoid 1 (EP\(_1\)), EP\(_2\), EP\(_3\), and EP\(_4\) activation of the EP\(_2\) and EP\(_4\) receptors leads to an increase in cyclic AMP (cAMP) levels and protein kinase A (PKA) activity (3). The stimulation of EP\(_2\) receptors directly inhibits T-cell proliferation, while that of EP\(_3\) and EP\(_4\) receptors regulates antigen-presenting cell functions (11). In a previous study, we found that PGE\(_2\) prevented the IL-18-induced expression of ICAM-1, B7.2, and CD40 on monocytes and the production of IL-12, TNF-α, and IFN-γ in human peripheral blood mononuclear cells (PBMC) (20, 21).

The effects of fluoroquinolone antibacterial agents on immune modulation have been well documented (16), and fluoroquinolones are known to exert their bactericidal activity by inhibiting bacterial type II topoisomerases (TOPO II), a major component of mitotic chromosomes. Ciprofloxacin (CIP), a fluorinated 4-quinolone, may interact with TOPO II in human T cells, because the quinolone derivative CP-115,953, which displays high specificity against mammalian TOPO II, mimics the inducing effect of CIP on the production of IL-2 (5, 17). The synthesis of IL-1β and TNF-α by lipopolysaccharide-stimulated human monocytes is significantly inhibited by CIP (18). However, little is known about the mechanism responsible for CIP activity, including the regulation of adhesion molecule expression.

In the present study, we found that CIP induces the production of PGE\(_2\) in monocytes through the induction of COX-2 protein. Therefore, we analyzed the effect of CIP-induced PGE\(_2\) production on the expression of ICAM-1, B7.1, B7.2, CD40, and CD40L on monocytes and the production of IL-12, TNF-α, IFN-γ, and IL-10 in PBMC using COX inhibitors in the presence and absence of IL-18.

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MATERIALS AND METHODS

**Reagents and drugs.** Recombinant human IL-18 was purchased from MBL (Nagoya, Japan), and CIP [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-
piperazinyl)-3-quinolinocarbonyl acid) was kindly provided by Bayer Yakuhin, Ltd (Osaka, Japan). NS398 and indomethacin were purchased from Cayman Chemical (Ann Arbor, MI), and H-89 was purchased from Sigma Chemical (St. Louis, MO). For flow cytometric analysis, fluorescein isothiocyanate (FITC)-conjugated mouse immunoglobulin G1 (IgG1) monoclonal antibody (MAb) against ICAM-1 and phycoerythrin-conjugated anti-CD14 MAb were acquired from DAKO (Glostrup, Denmark). FITC-conjugated anti-B7.1 MAb (mouse IgG1) was purchased from IMMUNOTECH (Marseille, France), and FITC-conjugated anti-B7.2 and anti-CD40 MAb (mouse IgG1) obtained from Pharmingen (San Diego, CA). Finally, FITC-conjugated IgG1 class-matched control was purchased from Sigma Chemical.

Isolation of PBMC and monocytes. Normal human PBMC were collected from human volunteers after obtaining their oral informed consent. Samples of 20 to 50 ml of peripheral blood were withdrawn from a forearm vein, after which the PBMC were isolated, and monocytes isolated from PBMC were separated by counterflow centrifugal elutriation as previously described (20, 21). The PBMC and monocytes were then suspended at a final concentration of 10^6 cells/ml in the medium as previously described (20, 21).

Flow cytometric analysis. Monocytes at 10^6 cells/ml were incubated with IL-18, CIP, NS398, indomethacin, and H-89 for 24 h at 37°C in a 5% CO2-air mixture under different conditions, for which the reagents were added to the medium at the start of incubation. The cells at 5 × 10^5 cells/sample were prepared as previously described (20, 21) and analyzed using a FACS Calibur (Becton Dickinson Biosciences, San Jose, CA), after which the data were processed using the CELL QUEST program (BD Biosciences). The results were presented as means ± standard errors of the mean (SEM) for five donors.

ELISAs. PBMC at 10^6 cells/ml used to analyze cytokine production and monocytes at 10^6 cells/ml used to analyze PGE2 production were incubated for 24 h at 37°C in a 5% CO2-air mixture under different conditions, for which the reagents were added to the medium at the start of incubation. After culture, IL-12 (p70), TNF-α, IFN-γ, IL-10, and PGE2 proteins in the cell suspensions were prepared as previously described (20, 21) and measured using an enzyme-linked immunosorbent assay (ELISA) kit (IL-12 [p70], TNF-α, IFN-γ, and IL-10 were from R&D Systems, Minneapolis, MN, and PGE2 was from Cayman Chemical), where the detection limits of the kit for IL-12 (p70), TNF-α, IFN-γ, IL-10, and PGE2 were 10 pg/ml. The results were expressed as means ± SEM for five donors.

Measurement of cAMP production in monocytes. Monocytes at 10^6 cells/ml were incubated at 37°C in a 5% CO2-air mixture under different conditions. After 24 h, cells at 2 × 10^5 cells/200 μl/well were supplemented with trichloroacetic acid to a final concentration of 5% and 3-isobutyl-1-methylxanthine, an inhibitor of phosphodiesterase, at 100 μM and frozen at −80°C. Frozen samples were subsequently sonicated and assayed for cAMP using a CAMP enzyme immunoassay kit (Camay Chemical) according to the manufacturer's instructions, for which no acetylation procedures were performed. The results were expressed as means ± SEM for five donors.

Western immunoblotting. For Western immunoblotting, monocytes at 10^6 cells/ml were incubated with and without IL-18 or CIP for between 0 and 24 h at 37°C in a 5% CO2-air mixture. After incubation, the cells were washed twice in phosphate-buffered saline before the addition of 60 ml of ice-cold lysis buffer (HEPES-buffered Hanks' balanced salt solution, pH 7.4, 0.5% Triton X-100, 10 mg/ml leupeptin, 10 mg/ml aprotinin) and 60 μl of 2× sample buffer (0.125 M Trizma base, pH 6.8, 20% glycerol, 4% sodium dodecyl sulfate, 10% 2-mercaptoethanol). The samples were then heated at 95°C for 7 min before being stored at −20°C. Sample proteins (50 ml/lane) were separated on 9% acrylamide gel and transferred onto Trans-Blot membranes at 4°C for 16 h at 300 mA, after which the membranes were blocked for 1 h at 25°C in Tris-buffered saline (25 mM Tris-HCl, 0.2 M NaCl, 0.15% Tween 20, pH 7.6) containing 5% dried milk (wt/vol). Next, the membranes were treated with horseradish peroxidase-conjugated rabbit polyclonal Ab against human COX-1 and COX-2 (Cayman Chemical) and β-actin (Sigma Chemical).

Statistical analysis. Statistical significance was evaluated using analysis of variance followed by Dunnet's test, where a probability value less than 0.05 was considered to indicate significance.

RESULTS

The effect of CIP on COX-1 and COX-2 protein expression in monocytes. The effect of 100 μg/ml CIP on COX-1 and COX-2 protein expression in monocytes in the presence and absence of 100 ng/ml IL-18 was determined by Western blot analysis after 24 h of incubation (Fig. 1). COX-1 and COX-2 expression in monocytes cultured in the medium was marginal, but the addition of CIP in the presence and absence of IL-18 remarkably induced the expression of COX-2 24 h after the start of the incubation.

The effect of CIP on PGE2 production in monocytes. The effect of 0 to 100 μg/ml CIP on PGE2 production in medium from incubated monocytes in the presence and absence of 100 ng/ml IL-18 was determined by ELISA after 24 h of incubation (Fig. 2A). IL-18 had no effect on the production of PGE2, but production increased by 100 μg/ml CIP in a time-dependent manner and reached a maximum level after 24 h. The CIP concentration directly elicited the production of PGE2 in both the presence and absence of IL-18. At 100 μg/ml, CIP induced 30 nM PGE2 production irrespective of the presence of IL-18. The 50% effective doses for the effect of CIP on the production of PGE2 in the presence and absence of IL-18 were 2 and 20 μg/ml, respectively.

The effect of indomethacin and NS398 on the CIP-induced production of PGE2 in monocytes. The effects of different concentrations ranging between 10^{-7} and 10^{-4} M indomethacin, a nonselective COX-2 inhibitor, and NS398, a selective COX-2 inhibitor, on the CIP-enhanced production of PGE2 in monocytes in the presence and absence of 100 ng/ml IL-18 were determined by ELISA after 24 h of incubation (Fig. 2B). NS398 and indomethacin inhibited the production of PGE2 irrespective of the presence of IL-18 in a concentration-dependent manner.

The effect of CIP on cAMP production in monocytes. The effect of 100 μg/ml CIP and 30 nM PGE2 on the elevation of intercellular cAMP in monocytes in the presence and absence of 100 ng/ml IL-18 was determined by ELISA (Fig. 3). IL-18 had no effect on the production of cAMP, but CIP and PGE2 elicited production irrespective of the presence of IL-18. Also, NS398 at 10^{-5} M blocked the inhibitory effect of CIP on the production of cAMP irrespective of the presence of IL-18.
The effect of CIP on the expression of ICAM-1, B7.1, B7.2, CD40, and CD40L on monocytes. The effects of 0 to 100 ng/ml CIP on the changes in expression of ICAM-1, B7.1, B7.2, CD40, and CD40L on monocytes in the presence and absence of 100 ng/ml IL-18 were determined by flow cytometry after 24 h. In the absence of IL-18, CIP inhibited the expression of ICAM-1, B7.1, B7.2, and CD40 (data not shown) but had no effect on the expression of CD40L (data not shown). The 50% inhibitory concentrations for the inhibitory effect of CIP on the expression of ICAM-1, B7.1, B7.2, and CD40 in the presence of IL-18 were estimated as 2, 3, 2, and 2 µg/ml, respectively.

The effect of indomethacin, NS398, and H-89 on CIP-inhibited ICAM-1, B7.1, B7.2, and CD40 expression on monocytes. The effects of indomethacin, NS398, and H-89, a PKA inhibitor, between 0 and 10^-4 M on 100 ng/ml CIP-inhibited ICAM-1, B7.1, B7.2, and CD40 expression on monocytes in the presence and absence of 100 ng/ml IL-18 were determined by flow cytometry after 24 h of incubation (Fig. 4B and C). NS398 and indomethacin abolished the inhibitory effect of CIP on the expression of ICAM-1, B7.2, and CD40 but had no effect on the expression of B7.1. The rates of ICAM-1 expression recovered by indomethacin, NS398, and H-89 at 10^-4 M were 68, 65, and 60%, respectively. In absence of IL-18, H-89 also had no effect on the CIP-initiated expression of ICAM-1, B7.1, B7.2, and CD40. However, these inhibitors had no effect in the absence of CIP (data not shown).

The effect of CIP on cytokine responses in PBMC. The effect of 0 to 100 µg/ml CIP on the production of IL-12, IFN-γ, TNF-α, and IL-10 in PBMC incubated in medium in the presence and absence of IL-18 was determined by ELISA after 24 h. In IL-18-treated PBMC, CIP prevented the production of IL-12, IFN-γ, and TNF-α but induced IL-10 production. The 50% inhibitory concentrations for the inhibitory effect of CIP on the production of IL-12, IFN-γ, and TNF-α in the presence of IL-18 were estimated as 3, 3, and 2 µg/ml, respectively.
CIP-inhibited production of IL-12, IFN-γ, TNF-α, and IL-10 in PBMC treated with 100 ng/ml IL-18 were determined by ELISA after 24 h of incubation (Fig. 5B). NS398, indomethacin, and H-89 blocked CIP-initiated production of TNF-α, IL-12, IFN-γ, and IL-10. The rates of TNF-α production recovered by indomethacin, NS398, and H-89 at 10^{-4} M were 72, 63, and 61%, respectively. These inhibitors had no effect in the absence of CIP (data not shown).

DISCUSSION

The present study examined for the first time the effects of CIP on the immune response of IL-18-treated monocytes. CIP induced the expression of COX-2 protein in monocytes treated with IL-18 or not treated (Fig. 1). In the absence and presence of IL-18, an unexpectedly large concentration 30 nM PGE₂ was detected in the medium of 100 μg/ml CIP-treated monocytes (Fig. 2A). CIP-initiated endogenous PGE₂ production was inhibited by the nonselective and selective COX-2 inhibitors indomethacin and NS398 (Fig. 2B), respectively, indicating that the increase in endogenous PGE₂ production might have depended on the enhancement of COX-2 expression. CIP as well as exogenous PGE₂ induced the elevation of intercellular cAMP in monocytes irrespective of the presence of IL-18 (Fig. 3). Also, CIP-enhanced cAMP expression was abolished by NS398. These results suggest that endogenously produced PGE₂ and the elevation of cAMP are associated with the CIP-induced enhancement of COX-2 expression.

Recently, we found that PGE₂ prevented IL-18-enhanced ICAM-1, B7.1, B7.2, and CD40 expression through stimulation of the EP₂/EP₄ receptor (20). As shown in Fig. 4A, CIP suppressed IL-18-enhanced ICAM-1, B7.2, and CD40 expression on monocytes. Whereas the inhibitory effect of 100 μg/ml CIP on the expression of ICAM-1 was 50% (Fig. 4A), that of 30 nM
exogenous PGE2 was 35% (20). The inhibitors of COX-2 and PKA partially blocked the effect of CIP on IL-18-initiated adhesion molecule expression (Fig. 4B). Therefore, there might exist endogenous PGE2-dependent and -independent pathways associated with the effects of CIP activity on adhesion molecule expression in the presence of IL-18. On the other hand, whereas PGE2 had no effect on the adhesion molecule expression in the absence of IL-18 (20), CIP inhibited the expression of ICAM-1, B7.1, B7.2, and CD40 (Fig. 4A). The COX-2 inhibitors, but not the PKA inhibitor, abolished the adhesion molecule expression-suppressing effect of CIP in the absence of IL-18 (Fig. 4B), suggesting that the endogenous PGE2 might not be involved in the effect of CIP in the absence of IL-18.

Previously, we reported that the inhibition of ICAM-1, B7.2, and CD40 expression on monocytes contributed to the suppression of IL-18-initiated cytokine production in PBMC (20, 21). PGE2 inhibited the production of IL-12, IFN-γ, and TNF-α but induced the production of IL-10 in PBMC treated with IL-18 (20). We found here that CIP mimicked the effect of PGE2 on IL-18-initiated cytokine production (Fig. 5A). The inhibitors of COX-2 and PKA also blocked the effect of CIP on IL-18-initiated cytokine production (Fig. 5B). However, the rates of cytokine production as well as those of adhesion molecule expression recovered by indomethacin, NS398, and H-89 (10^-4 M) were similar and reached only between 60 and 70%. Therefore, the suppressive effect of CIP on IL-18-initiated cytokine production might depend on the inhibition of ICAM-1, B7.2, and CD40 expression.

IL-18 plays a role in inflammatory conditions, such as graft-versus-host disease (15) and Crohn’s disease (14), and antimicrobial chemotherapy targeted against intestinal anaerobic bacteria significantly reduces the severity of the acute stage of these diseases (4, 13). CIP significantly ameliorates the severity of graft-versus-host disease and Crohn’s disease by reducing the number of intestinal bacteria, some of which induce the lipopolysaccharide-initiated production of TNF-α (1, 4, 19). In a randomized crossover study, the mean maximum concentration of CIP in the serum of normal human volunteers who received a single oral dose of 500 mg for up to 24 h was 2.46 μg/ml (7), which is within the range of the concentration noted in the present study. Therefore, the effects of CIP on immune responses may indicate new therapeutic potential for IL-18-induced diseases.

TOPO II-targeting drugs are apoptosis-inducing drugs, and both isoforms of TOPO II, alpha and beta, are inhibited by the chemotherapeutic agent etoposide (22). We found that etoposide had no effect on COX expression, adhesion molecule expression, and cytokine production in the absence and presence of IL-18 (data not shown). Cell viabilities of monocytes

![FIG. 5. The effect of CIP on cytokine response of PBMC. (A) PBMC at 10⁶ cells/ml were treated with between 0 and 100 μg/ml CIP in the presence and absence of 100 ng/ml IL-18 for 24 h. After the treatment, IL-12, TNF-α, IFN-γ, and IL-10 production was determined by ELISA. Filled circles and filled squares represent the results obtained with medium and IL-18, respectively. ##, P < 0.01 compared with the value for IL-18. (B) PBMC (10⁶ cells/ml) were incubated with between 0 and 0.1 mM indomethacin, NS398, and H-89 in the presence and absence of 100 ng/ml IL-18 or 100 μg/ml CIP for 24 h. Open squares, filled circles, and filled squares represent the results obtained with indomethacin, H-89, and NS398, respectively. ##, P < 0.01 compared with the value for IL-18 and CIP. The results are the means ± SEM for five donors. When an error bar was within a symbol, the bar was omitted.](http://aac.asm.org/2012/10/issue)

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and lymphocytes in the presence of CIP and/or IL-18 were almost the same and were estimated to be 90% after 24 h of incubation. Therefore, the effect of CIP might be independent of the inhibition of TOPO II, and the regulation of cytokine production and adhesion molecule expression was not due to a reduction in cell viability. It is still unclear what the primary target or binding site of CIP in monocytes is for regulating reduction in cell viability. It is still unclear what the primary

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