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Modulatory Effect of Antibiotics on Cytokine Production by Human Monocytes In Vitro

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Some antimicrobial agents have been reported to modify the host immune and inflammatory responses both in vivo and in vitro. Fosfomycin (FOM) and clarithromycin (CAM) have immunomodulatory activity on human lymphocyte function. In the present study, we examined the effects of FOM and CAM on cytokine synthesis by lipopolysaccharide (LPS)-stimulated human monocytes in comparison with that of dexamethasone in vitro. The three drugs demonstrated positive or negative effects on the synthesis of various cytokines by LPS-primed monocytes. They suppressed the synthesis of tumor necrosis factor alpha, interleukin 1α (IL-1α), IL-1β, the IL-1 receptor antagonist, and granulocyte-macrophage colony-stimulating factor in a concentration-dependent manner at concentrations between 1.6 and 40 μg/ml. On the contrary, the drugs showed different actions on the synthesis of IL-6 and IL-10. Namely, FOM enhanced both IL-6 and IL-10 synthesis, CAM enhanced only IL-10 synthesis, but dexamethasone deeply suppressed the synthesis of both cytokines. These data indicate that antibacterial agents may modify acute-phase inflammatory responses through their effects on cytokine synthesis by monocytes.

Cytokines are essential mediators of cell-to-cell signals in physiological and pathological immune responses and in the inflammatory response. Under normal conditions, these cytokines act as crucial signals in the development of appropriate defenses. However, exaggerated or prolonged release can lead to pathological conditions.

Bacterial products or the by-products of opsonins can activate macrophages or monocytes directly. Regardless of the initiating event, monocytes or macrophages are usually the cells that elicit the response cascade in the acute phase of inflammation (4). Thus, these cells play a crucial role in the induction of both immune and inflammatory responses.

Activated monocytes release a broad spectrum of cytokines, which can induce the subsequent cytokine cascade. Interleukin 1 (IL-1), tumor necrosis factor (TNF), and IL-6 are biologically active peptides produced by monocytes, notably in response to endotoxin (4, 6). These soluble factors initiate and maintain the acute phase of the inflammatory response, whereas the IL-1 receptor antagonist (IL-1ra) and IL-10 are anti-inflammatory cytokines, which are also produced by monocytes (6, 12).

Some antibiotics, quinolones and macrolides, have been reported to inhibit the production of IL-1 or TNF in lipopolysaccharide (LPS)-stimulated human monocyte cultures (1–3, 9, 17–19). However, little is known concerning their effects on the production of other cytokines, although many different cytokines, including proinflammatory and anti-inflammatory cytokines, are simultaneously produced by activated monocytes.

We have shown that fosfomycin sodium (FOM) and clarithromycin (CAM) have immunomodulatory activity on human lymphocyte activation in vitro, and this activity is independent of their antibacterial activity (13–15).

In the study described here we examined the effects of these drugs on the synthesis of various kinds of cytokines by human monocytes primed with LPS in vitro. The effects of dexamethasone (DEX) were examined in parallel for comparison.

MATERIALS AND METHODS

Reagents. FOM and the enantiomer of FOM (1*S*,2*R*-1,2-epoxypropyl pyosphonic acid) were supplied by Meiji Seika Ltd. (Tokyo, Japan). CAM and DEX were donated by Dainabot (Tokyo, Japan) and Shionogi Pharmaceutical Co. (Osaka, Japan), respectively. These drugs were dissolved in ethanol and were then diluted to an appropriate concentration in medium and added to the cultures. LPS (*Escherichia coli* O26:B6 LPS) was purchased from Difco Laboratories, Detroit, Mich.

Isolation of monocyte-enriched peripheral blood mononuclear cells. Initially, heparinized peripheral blood obtained from healthy donors was mixed with an equal volume of 3% dextran (Nakalai Tesque, Kyoto, Japan). The mixture was allowed to sediment at room temperature for 40 min until the mononuclear cell-rich supernatant and granulocytes clearly separated from the erythrocytes. The supernatant was collected and centrifuged on a Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) gradient. Peripheral blood mononuclear cells were then washed and resuspended in culture medium (RPMI 1640; GIBCO, Grand Island, N.Y.) supplemented with 2 mM glutamine and 10% heat-inactivated fetal calf serum (Flow Laboratories, McLean, Va.). The cell suspension was incubated for 45 min at 37°C in a plastic dish at a cell density of 2×10^6 cells per ml. Nonadherent cells were then removed, and adherent cells were used as monocyte-enriched peripheral blood mononuclear cells. More than 90% of the adherent cells were monocytes, as determined by esterase staining.

Cytokine assay. Adherent cells were seeded at a cell density of 10^6 cells per ml (1 ml per well) and were incubated in 24-well plates in the presence of LPS (100 ng/ml) with or without drugs for 24 h at 37°C in a 5% CO_2 incubator. Cell-free supernatants were recovered by centrifugation and were stored at −20°C until they were assayed. The activity of each cytokine was determined by enzyme-linked immunosorbent assay (ELISA) with commercially available kits. The IL-1β activity in the supernatant was determined with an ELISA kit (Cayman Chemical Company, Ann Arbor, Mich.) with a detection limit of 3.9 pg/ml. IL-6, IL-10, and TNF-α activities were determined with immunoassay kits (BioSource International, Camarillo, Calif.) with a detection limit of 15.6 pg/ml. IL-1α, IL-1ra, and granulocyte-macrophage colony-stimulating factor (GM-CSF) activities were determined with ELISA kits (Amersham International, Buckinghamshire, United Kingdom) with detection limits of 3.9, 31.3, and 7.8 pg/ml, respec-

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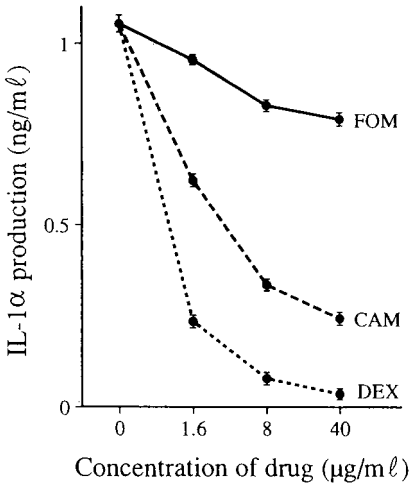

FIG. 1. Effect of study drugs on IL-1α synthesis by LPS-stimulated monocytes. Human monocytes were cultured at a cell density of 10^6 cells per ml in 24-well microplates with LPS (100 ng/ml) in the presence of FOM, CAM, or DEX for 24 h. The supernatant was recovered after centrifugation and was assayed for IL-1α. The content of each cytokine was determined by ELISA with commercially available kits; the concentrations were calculated on the basis of a standard curve derived by linear dilution of the cytokine standards included in the respective kits. Representative data from two different experiments are shown.

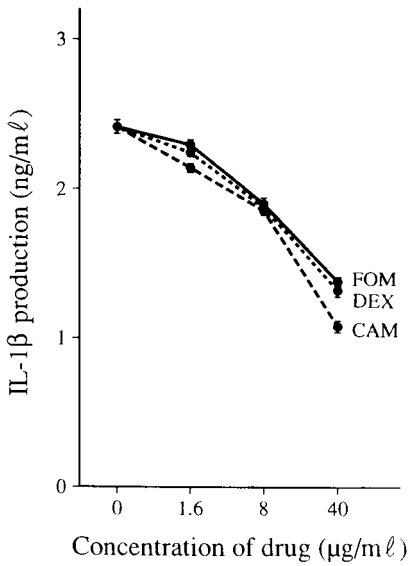

FIG. 2. Effects of study drugs on IL-1β synthesis by LPS-stimulated monocytes. Materials and methods were as described in the legend to Fig. 1.

tively. Some samples were used at an appropriate dilution, which was determined in the preliminary study.

RESULTS

Effects of drugs on the production of cytokines by LPS-stimulated monocytes. In the preliminary study, we examined the amounts of TNF-α and IL-1 in the cultured supernatants of human monocytes primed with LPS using commercially available ELISA kits. The production of the cytokines reached the peak value within 24 h of culture, and the cytokine titer did not increase after further incubation (data not shown). Thus, the effects of the drugs on cytokine synthesis were examined by measuring the amounts of the cytokines secreted in the supernatants of 24-h cultures of LPS-stimulated monocytes.

The LPS-primed monocytes induced the synthesis of different cytokines. FOM, CAM, and DEX at concentrations of between 1.6 and 40 μg/ml affected positively or negatively the production of the cytokines by monocytes, although the degree of the inhibitory or stimulatory potency of these drugs varied among the cytokines examined.

Each drug suppressed the synthesis of IL-1α by LPS-stimulated monocytes in a concentration-dependent manner. CAM and DEX strongly inhibited synthesis, but FOM weakly suppressed synthesis even at the highest concentration used (40 μg/ml) (Fig. 1). The monocytes induced the production of more TNF-α and IL-1β than IL-1α. The three drugs suppressed the synthesis of these cytokines in a similar concentration-dependent manner (Fig. 2 and 3). The amount of GM-CSF production by LPS-primed monocytes was relatively small, and the inhibitory effects of the three drugs at a concentration of 1.6 μg/ml were very weak (Fig. 4). LPS-stimulated monocytes induced more IL-1ra than IL-1α in the present study. FOM and CAM hardly suppressed the synthesis of IL-1ra when they were used at concentrations of between 1.6 and 8 μg/ml, and they finally suppressed IL-1ra synthesis at the highest concentration tested (40 μg/ml). DEX strongly suppressed IL-1ra synthesis even at the lowest concentration tested (1.6 μg/ml) (Fig. 5). The effects of these drugs on the production of IL-6 and IL-10 were quite different from those that they exerted on the cytokines mentioned above. LPS-primed monocytes induced the largest amounts of IL-6 among the cytokines tested in the present study. DEX suppressed IL-6 production in a concentration-dependent manner, whereas the suppressive effect of CAM was very weak and FOM enhanced the production of this cytokine (Fig. 6). The level of synthesis of IL-10 by the monocytes was very low compared with the level of synthesis of the other cytokines tested in this experiment. FOM and CAM, but not DEX, enhanced the production of IL-10 in a concentration-dependent manner (Fig. 7). The effects of the drugs at concentrations of 40 μg/ml on the synthesis of various cytokines are summarized in Table 1. The data in Table 1 demonstrate that FOM and CAM are apparently

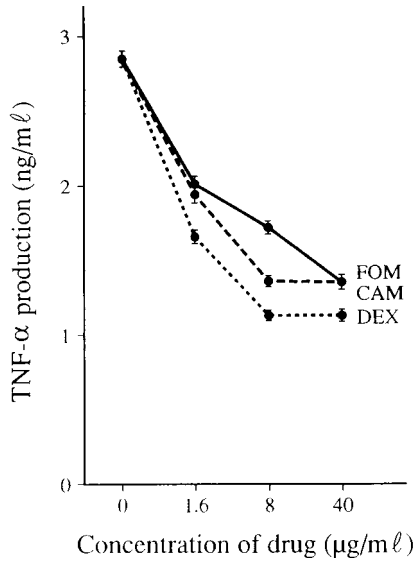

FIG. 3. Effects of study drugs on TNF-α synthesis by LPS-stimulated monocytes. Materials and methods were as described in the legend to Fig. 1.

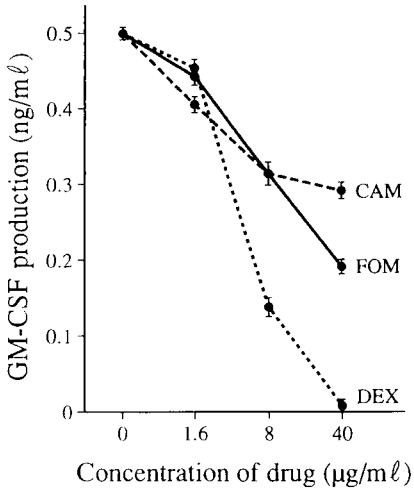

FIG. 4. Effects of study drugs on GM-CSF synthesis by LPS-stimulated monocytes. Materials and methods were as described in the legend to Fig. 1.

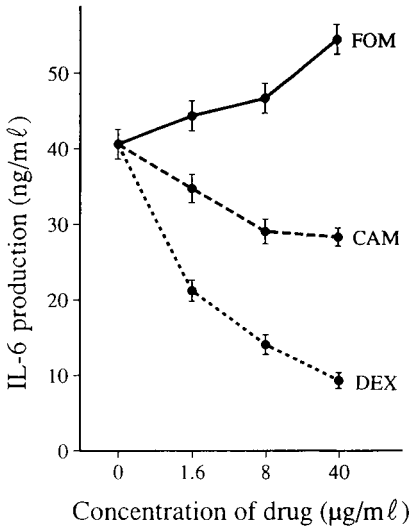

FIG. 6. Effects of study drugs on IL-6 synthesis by LPS-stimulated monocytes. Materials and methods were as described in the legend to Fig. 1.

alike in the influence that they exert on the synthesis of all cytokines except IL-6 and IL-1, whereas the action of DEX was different from those of FOM and CAM.

We next examined whether the modulatory effect of FOM is related to its bactericidal activity. The enantiomer of FOM, which is structurally an isomer of FOM but which has no bactericidal activity, was evaluated for its effect on the synthesis of IL-1β and TNF-α by LPS-primed monocytes in a parallel experiment for a direct comparison of the two drugs. The enantiomer of FOM showed a suppressive effect on the synthesis of these cytokines at levels similar to those at which FOM exerts a suppressive effect (data not shown). These results indicate that FOM-induced modulation of cytokine synthesis is not linked to its antimicrobial activity.

DISCUSSION

In the present study, we examined the effects of FOM, CAM, and DEX on cytokine synthesis by LPS-stimulated human monocytes. LPS-stimulated monocytes induced the production of many different cytokines. The drugs suppressed the synthesis of IL-1α, IL-1β, TNF-α, and GM-CSF in a concentration-dependent manner. The monocytes synthesized more IL-1ra than IL-1α and IL-1β. DEX strongly suppressed the production of IL-1ra, while FOM and CAM weakly inhibited IL-1ra production. The amount of IL-6 synthesized was greater than the amounts of the other cytokines synthesized. While FOM enhanced the production of IL-6, CAM and DEX had a mild and a strong inhibitory effect on IL-6 production, respectively. In contrast, the amount of IL-10 production was very small, and both FOM and CAM, but not DEX, enhanced IL-10 production.

Cytokines of the IL-1 and TNF families appear to be uniquely important in initiating a series of events within the cytokine cascade. Naturally occurring antagonists, such as IL-1ra and the soluble TNF receptor, are extremely potent regu-

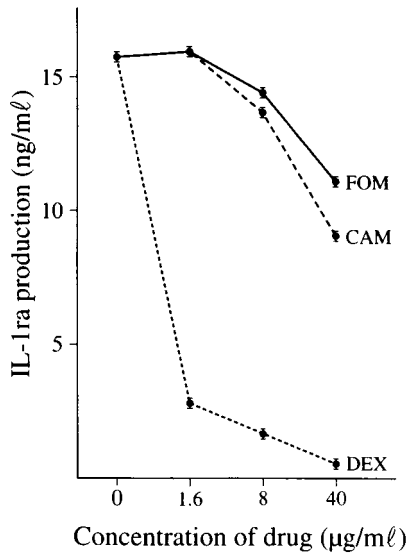

FIG. 5. Effects of study drugs on IL-1ra synthesis by LPS-stimulated monocytes. Materials and methods were as described in the legend to Fig. 1.

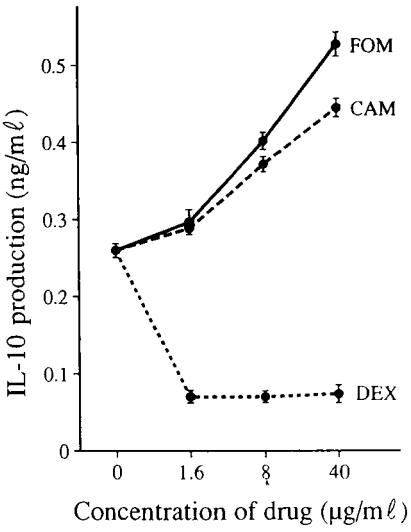

FIG. 7. Effects of study drugs on IL-10 synthesis by LPS-stimulated monocytes. Materials and methods were as described in the legend to Fig. 1.

TABLE 1. Effects of study drugs on synthesis of cytokines by LPS-primed human monocytes

Cytokine	Effect[a]		
	FOM	CAM	DEX
IL-1α	↓ (+)	↓ (+++)	↓ (+++)
IL-1β	↓ (++)	↓ (++)	↓ (++)
IL-1ra	↓ (++)	↓ (++)	↓ (+++)
TNF-α	↓ (++)	↓ (++)	↓ (++)
GM-CSF	↓ (++)	↓ (++)	↓ (+++)
IL-6	↑ (+)	↓ (+)	↓ (+++)
IL-10	↑ (++)	↑ (++)	↓ (++)

[a] Symbols denote the enhancement (↑) or suppression (↓) of the cytokine synthesis by the drugs at a concentration of 40 μg/ml. The degree of potency is indicated on the basis of cytokine levels in the absence of drug, from + to +++: +, <25%; ++, 25 to 75%; +++, >75%.

lators of the acute-phase response (10). It is interesting that FOM and CAM demonstrated a weak inhibitory effect on IL-1ra production, whereas DEX had a strong inhibitory effect.

IL-6 is a pleiotropic cytokine involved in the regulation of the immune response, the acute-phase reaction, and hematopoiesis. IL-6 inhibits the production of LPS-induced TNF-α and IL-1β by cultured human monocytes and in mice in vivo (20). IL-6 is also known to increase the amounts of TNF receptors in hepatocytes. Thus, IL-6 belongs to the category of anti-inflammatory cytokines (20). The enhancement of IL-6 production by FOM may be involved in the suppression of TNF and IL-1 synthesis.

IL-10 is an autocrine inhibitor of cytokine production for monocytes (7, 12). IL-10 inhibits the synthesis of IL-1, TNF, IL-6, IL-8, and colony-stimulating factors by LPS-stimulated monocytes and upregulates the synthesis of IL-1ra by these cells. It seems that the IL-10 cytokine is sufficient to regulate the termination of the acute-phase response. Indeed, recent data indicate that exogenous administration of IL-10 can protect mice from the lethal effects of endotoxin shock (8). IL-10 also exerts its anti-inflammatory effects in part by suppressing IL-1 and TNF synthesis by activated T cells. Interestingly, both FOM and CAM similarly enhanced IL-10 production (Fig. 6). Considering that the synthesis of IL-1, TNF, and IL-6 is negatively regulated by IL-10, the enhanced level of IL-10 synthesis in the presence of CAM may be involved in the inhibition of the synthesis of these cytokines, whereas the inhibition of IL-1, TNF, and GM-CSF synthesis by FOM may not be related to it, since FOM enhanced the production of IL-6, in addition to that of IL-10. These results suggest that the regulatory effects of these drugs on cytokine production seem to be exerted through different mechanisms.

DEX suppressed the synthesis of all of the cytokines (IL-1, TNF, IL-6, IL-1ra, and IL-10) examined in the study. Inhibition of LPS-induced production of cytokines by corticosteroids has been well-documented (6, 11, 16, 21).

It was shown (Table 1) that there are close similarities in the actions of two antibiotics, FOM and CAM, on the synthesis of several cytokines, but the effect of DEX was distinct from those of FOM and CAM at a concentration of 40 μg/ml. Our data suggest that the mechanism by which the antimicrobial agents FOM and CAM exert their anti-inflammatory effects is different from that of DEX.

We additionally examined the effects of these drugs on the phagocytic activity, superoxide-generating activity, and level of HLA-DR expression of monocytes. These functions of monocytes were not affected in the presence of these drugs in culture (data not shown). Therefore, the modulatory actions of the drugs on cytokine synthesis appeared to be unrelated to these functions of monocytes.

FOM-induced modulation of cytokine production seems to be unrelated to its bactericidal activity, because the enantiomer of FOM, an isomer of FOM which does not have antimicrobial activity, showed inhibitory effects similar to those of FOM on IL-1β and TNF-α production by monocytes.

The results of the present study also suggest that the anti-inflammatory effects of the antimicrobial agents FOM and CAM may be exerted, at least in part, through the inhibition of cytokine production by monocytes.

These findings could be of physiological relevance at sites of infection where the antibiotics may accumulate. The peak concentration of FOM in serum when it was administered intravenously at a dose of 50 mg/kg of body weight was 276 μg/ml (5). Thus, the concentration of FOM required to modify the synthesis of various cytokines by monocytes is attained in serum after therapeutic FOM administration. In contrast, at concentrations usually attained in serum after therapeutic administration, CAM may be unable to exert its action on cytokine synthesis, because the peak concentration of CAM in serum is 2.42 μg/ml after the oral administration of 400 mg of CAM per ml (14).

In conclusion, the present study showed that some antibacterial drugs may modify the acute-phase inflammatory response by disturbing the cytokine cascade.

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