Induction of Prostaglandin E$_2$ by Human Monocytes Infected with Mycobacterium avium Complex—Modulation of Cytokine Expression

Nandagopal Venkataprasad, Hiroe Shiratsuchi, John L. Johnson, and Jerrold J. Ellner

Prostanoids, including prostaglandin E$_2$ (PGE$_2$), suppress macrophage effector functions against Mycobacterium tuberculosis. PGE$_2$ production by monocytes infected with Mycobacterium avium complex (MAC) and its effects on intracellular mycobacterial growth were examined. Freshly obtained monocytes from healthy subjects were stimulated with lipopolysaccharide or 10$^7$ organisms/mL of 4 MAC strains. PGE$_2$ production in monocyte supernatants peaked at 48 h. Significantly higher levels of PGE$_2$ were produced by monocytes infected with the mixed rough–smooth, flat, and transparent (SmT) morphotype strain 86m2096 (26.8 ± 5.2 ng/mL) than by the more virulent LR114 SmT morphotype strain (2.4 ± 0.6 ng/mL; $P < .05$, paired $t$ test). When infected monocytes were incubated with 1 $\mu$g/mL indomethacin (IM) for 2 days and then further stimulated with interferon-$\gamma$, no effect on intracellular MAC growth was evident. IM increased tumor necrosis factor-$\alpha$ (1.7 ± 0.4 vs. 2.3 ± 0.3 ng/mL; $P = .005$, paired $t$ test) but not interleukin-1$\beta$ (8.2 ± 1.7 vs. 8.7 ± 2.1 ng/mL, $P = .34$) production by monocytes stimulated with lipopolysaccharide. These data suggest that MAC-induced PGE$_2$ expression may modulate cytokine production and intracellular parasitism.

Mycobacterium avium complex (MAC) is a frequent opportunistic pathogen in the late stages of human immunodeficiency virus (HIV) infection. In 3%–4% of AIDS cases, disseminated MAC infection is the initial AIDS-defining condition [1, 2], and 46%–57% of patients with AIDS have evidence of disseminated MAC infection at autopsy [3, 4]. Widely disseminated infection with abundant intracellular growth and mycobacteremia are characteristic.

Mononuclear phagocytes produce arachidonic acid metabolites in response to various stimuli [5–7]. Scra from patients with tuberculosis and the mycobacterial cell wall polysaccharide $\alpha$-arabinoo-$\beta$-galactan have been shown to down-regulate monocyte effector functions in tuberculosis patients [8] and suppress purified protein derivative (PPD)-induced lymphocyte proliferation [9]; these interactions were dependent on the presence of mononuclear phagocytes and reversible with indomethacin (IM). Mice chronically infected with Mycobacterium intracellulare expressed large amounts of serum prostaglandin E$_2$ (PGE$_2$); treatment with interferon-$\gamma$ (IFN-$\gamma$) and IM enhanced mycobacterial killing, suggesting that prostaglandins inhibited the production of IFN-$\gamma$ by lymphocytes and suppressed activation of microbicidal functions [10]. Increased PGE$_2$ production by macrophages was also associated with defective activation and killing of Mycobacterium leprae by macrophages from nude mice [11].

Certain cytokines, such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$), granulocyte-macrophage colony-stimulating factor, and IFN-$\gamma$, have macrophage-activating factor activity and augment intracellular MAC killing by human monocyte-derived macrophages [12–15]. MAC colonial morphology on agar-based media is associated with differences in cell wall structural lipids and capacity for intracellular growth. The degree to which MAC cell wall lipids induced TNF-$\alpha$ and PGE$_2$ production by monocytes also correlated with increased intracellular MAC growth [16–18]. Isogeneic avirulent smooth, domed, and opaque (SmD) and virulent smooth, flat, and transparent (SmT) morphotype MAC strains differentially induced monocyte cytokine expression; avirulent strains consistently induced greater TNF-$\alpha$ and interleukin-1$\beta$ (IL-1$\beta$) production after infection of human monocytes [19, 20].

In this study, we examined the kinetics of PGE$_2$ production by monocytes from healthy human subjects after monocytes were infected with MAC strains of different colonial morphotypes and virulence. We also investigated the modulation of intracellular MAC growth by PGE$_2$ and its reversibility by IM, and the induction of TNF-$\alpha$ and IL-1$\beta$ expression by lipopolysaccharide (LPS)–stimulated monocytes.

Materials and Methods

Microorganisms. MAC LR114, LR542, and LR147 were donated by J. F. Crawford (CDC, Atlanta). LR114 is a serovar 4 and a non–AIDS-associated clinical isolate. LR114 SmT is an isogeneic strain grown from colonies selected from the parent strain on the basis of smooth, flat, and transparent colonial morphology. Strain LR542 has smooth, domed, and opaque (SmD)
morphology; LR147 has rough (Rg) colonies. Strain 86m2096 (mixed Rg-SmT colonial morphology) is a clinical isolate from an AIDS patient treated at University Hospitals of Cleveland. The intracellular growth kinetics of the MAC strains used in this study have been extensively characterized [21–23].

Mycobacteria were grown in Middlebrook 7H9 broth (Difco, Detroit) and incubated at 37°C in 5% CO₂ in air. When the bacterial density was ~1–5 × 10⁸/mL (log-phase growth), mycobacteria were aliquoted and stored at ~70°C. Before being used for infection, bacteria were thawed and sonicated for 10 s using an ultrasonic cell disruptor (W385; Heat-Systems Ultrasonics, Farmingdale, NY) to obtain a single-cell suspension and diluted to 10¹⁰/mL in 5% autologous unheated serum-containing RPMI 1640 (Whittaker Bioproducts, Walkersville, MD).

Monocyte isolation and culture. Peripheral blood was obtained from healthy volunteers (8 men, 9 women; 22–43 years old). Monocytes were isolated and cultured by methods reported previously [14, 21]. Briefly, peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by ficoll-hypaque density-gradient sedimentation and suspended at 10⁷/mL in RPMI 1640 supplemented with 2 mM L-glutamine, 15 mM HEPES buffer, and 2% fresh autologous unheated human serum. Three 50-μL droplets of PBMC suspension were plated in 35-mm petri dishes (Falcon 1008; Becton Dickinson, Lincoln Park, NJ). The dishes were incubated for 60 min at 37°C. Nonadherent cells were gently washed off, and the monocytes were precultured for 2 days in 2 mL of RPMI 1640 with 2% unheated autologous serum at 37°C; 7.5% CO₂. There were ~2 × 10⁷/viable monocytes per plate, as determined by the method of Nakagawara and Nathan [24]. Monocytes were infected with MAC by incubation with 1.5 mL of bacterial suspension (10⁷/mL in medium containing 5% fresh autologous serum) for 60 min at 37°C. The dishes were gently washed four times with prewarmed medium to remove extracellular bacteria. Monocytes were cultured in 2 mL of 2% se­rum-containing medium for up to 8 days in the presence or absence of recombinant human IFN-γ (300 or 3000 U/mL; R & D Systems, Minneapolis) or IM (1 μg/mL; Sigma, St. Louis). PGE₂ (Sigma) was reconstituted as 10 mg/mL in acetone.

Some experiments were done using 96-well flat-bottom plates (Falcon 3072; Becton Dickinson) with a monocyte monolayer preparation and infection method described elsewhere [25]. About 2 × 10⁵ adherent cells per well were present. Monocyte viability and MAC phagocytosis and growth kinetics were similar in experiments using both flat-bottom 96-well plates and 35-mm petri dishes (unpublished data).

Colony-forming unit (cfu) assay. The number of viable MAC in monocyte lysates at each time point was determined using a cfu assay as previously described [14, 21]. Supernatants of cultured monocytes were harvested immediately after infection (time 0) and 5 and 8 days after infection. Plates and supernatants were stored at ~70°C until assay. After thawing, monocytes were lysed with 1.1 mL of 7H9 medium and 0.4 mL of 0.25% SDS in physiologic PBS was added to each plate. The lysates then were transferred to tubes containing 0.5 mL of 20% bovine serum albumin to neutralize the SDS. Cell lysates were sonicated for 10 s to disperse the mycobacteria and were serially 10-fold diluted in 7H9 broth medium. Three 10-μL aliquots of each dilution were plated on Middlebrook 7H-10 agar (Difco). The spots were allowed to absorb onto the surface of the medium to prevent them from running together. The agar plates were incubated for 4–6 days at 37°C in a humidified atmosphere with 5% CO₂ until bacterial colonies were visible. Visible colonies were counted using a stereomicroscope. Results were expressed as mean ± SE cfu/mL of cell lysate, which represented the bacterial cfu associated with ~10⁴ monocytes in 35-mm petri dishes and 10⁴ monocytes in 96-well flat-bottom plates.

Generation and assay of PGE₂. To monitor the production of PGE₂ by monocytes, monocyte monolayers in 96-well tissue culture plates were infected with MAC or stimulated with LPS (final concentration, 10 μg/mL) with or without IM (final concentration, 1 μg/mL) or IFN-γ (final concentration, 300 U/mL). Culture supernatants were harvested at 0, 6, 12, 24, 48, and 96 h and separated at 3000 rpm. Supernatants were aspirated and stored at ~70°C until assay. PGE₂ levels in culture supernatants were measured using a commercially available ELISA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions (sensitivity = 200 pg/mL).

Statistical analysis. The significance of differences between groups was calculated using the paired Student’s t test.

Results

PGE₂ production by human monocytes after MAC infection. Monocytes from 6 healthy human subjects were stimulated with 4 MAC strains or 10 μg/mL LPS (final concentration) for up to 96 h. After 48 h, production of PGE₂ by monocytes stimulated with LPS was significantly greater than by unstimulated monocytes (figure 1) (P < .05). Significant strain-to-strain differences were noted in the ability of MAC to induce PGE₂ release. PGE₂ release into culture supernatants increased linearly, peaked at 48 h, and then plateaued over the next 48 h for 3 of 4 strains tested (figure 1). Maximum levels of PGE₂ after MAC stimulation with these 3 strains exceeded those after LPS stimulation alone. The highly virulent, non–AIDS-associated MAC LR114 SmT induced significantly less PGE₂ production than other MAC strains, including an AIDS-associated isolate (P < .05; LR114 SmT vs. 86m2096); however, there was no consistent relationship between source of isolate (AIDS- or non–AIDS-associated) and PGE₂ expression. Colonial morphology, strongly correlated with capacity for intracellural growth in human monocytes, correlated with induction of PGE₂ production. The SmT morphotype strain (LR114) induced less PGE₂ release than did strains with SmD (LR542) or Rg (LR147) colonial morphology.

Effect of exogenous PGE₂ on intracellular MAC growth. On the basis of measured levels of PGE₂ in supernatants of MAC-infected human monocytes and earlier reports [26], we studied the effects of adding exogenous PGE₂ at doses of 0–40 ng/mL immediately after infection to monocytes infected with LR114 SmT. The addition of exogenous PGE₂ in this dose range did not affect intracellular MAC growth (figure
Figure 1. Time-course study of PGE₂ production by monocytes infected with 4 MAC strains or stimulated with lipopolysaccharide (LPS). Monocytes were cultured in flat-bottom 96-well tissue culture plates. Culture supernatants were pooled from identical triplicate or quadruplicate cultures, harvested, and assayed for PGE₂ by EIA. Each data point represents mean ± SE PGE₂ level (ng/mL) in culture supernatants from 3 to 6 independent experiments involving 6 healthy human subjects.

2). Similar results were obtained in experiments using strain 86m2096 (unpublished data). Monocyte viability was not altered by coculture with exogenous PGE₂ (0-80 ng/mL), excluding a direct cytotoxic effect of PGE₂. The addition of PGE₂ at similar concentrations directly to MAC cultures did not alter mycobacterial viability (unpublished data).

Effect of IM and IFN-γ on intracellular growth of MAC in human monocytes. We previously observed significant intracellular growth inhibition of the virulent MAC LR114 SmT when cocultured with both IM and IFN-γ added immediately after infection [22]. Sibley and Krahenbuhl [27] reported earlier that PGE₂ production by M. leprae-infected macrophages was associated with defective IFN-mediated activation against Toxoplasma gondii; this suppression was reversible by IM. In the current study, PGE₂ production by MAC-infected monocytes peaked at 48 h after infection (figure 1). To assess whether IFN-γ had additional macrophage-activating factor activity against MAC following coculture with IM, monocytes were treated with IM immediately after infection, and IFN-γ (300 U/mL and 3000 U/mL, final concentration) was added 2 days after infection (table 1).

![Graph showing PGE₂ production over time](image)

<table>
<thead>
<tr>
<th>MAC strain</th>
<th>Control²</th>
<th>IM alone³</th>
<th>IM + IFN-γ⁴</th>
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<tbody>
<tr>
<td>86m2096</td>
<td>5.96 ± 0.9</td>
<td>5.34 ± 0.1</td>
<td>6.14 ± 0.8</td>
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<tr>
<td>LR114 SmT</td>
<td>4.99 ± 0.7</td>
<td>5.19 ± 0.9</td>
<td>5.30 ± 0.5</td>
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NOTE. Each value represents mean ± SD log₁₀ cfu MAC/mL in monocyte lysates (3-5 donors, 3 independent experiments).

* Time after infection of monocytes with MAC (2 days coculture with IM alone plus additional 6 days coculture with IFN-γ plus IM).

† Infected with MAC and received no additional stimuli.

‡ Added immediately after infection.

§ IFN-γ (3000 U/mL) was added 2 days after infection.

Figure 2. Effect of exogenous PGE₂ on intracellular MAC growth in human monocytes. Exogenous PGE₂ (0-40 ng/mL) was added to monocyte cultures immediately after infection with MAC LR114 SmT. Each data point represents MAC mean ± SE cfu/mL in monocyte lysates from 3 independent experiments using monocytes from 3 healthy subjects.
Coculture with IM decreased intracellular growth of MAC 86m2096 by one-half log cfu at day 8 compared with control cultures; however, the difference was not statistically significant (table 1). This trend was observed only in experiments with strain 86m2096, which induced high levels of PGE₂ production, and not after infection with strain LR114 SmT, which induced less PGE₂ (figure 1). Intracellular MAC growth decreased after coculture with IM in monocytes from 4 of 5 donors infected with strain 86m2096 and 1 of 3 donors infected with LR114 SmT.

**Effect of IM on cytokine production by monocytes stimulated with LPS.** Next, we examined the effects of IM and IFN-γ on PGE₂ production by monocytes stimulated with LPS. LPS induced significant PGE₂ production by monocytes (figures 1, 3). The addition of IM (1 µg/mL) blocked PGE₂ production by LPS-stimulated monocytes (P < .001). Coculture of monocytes with IFN-γ did not alter PGE₂ release. Spontaneous expression of TNF-α and IL-1β by unstimulated monocytes (negative control) was minimal (data not shown). Production of TNF-α and IL-1β by monocytes stimulated with LPS was higher at 1 day than after 4 days (table 2). Coculture of monocytes with IM significantly increased TNF-α after 1 and 4 days of culture but only transiently increased IL-1β production after 1 day of culture.

**Discussion**

PGE₂ inhibits multiple immune effector cell functions, including tumoricidal activity of T cells, NK cells, lymphokine-activated killer cells, and monocytes and activity against intracellular pathogens such as *Mycobacterium tuberculosis* and *M. leprae* [28, 29]. Tomioka and colleagues [30, 31] demonstrated earlier that arachidonic acid metabolites mediate the immunosuppressive effects of MAC infection on mouse splenic macrophages; these effects were reversible by IM. In the present study, we confirmed that MAC, like other pathogenic mycobacteria, induces release of PGE₂ after infection of human monocytes. MAC strains differ in their capacity for intracellular growth. We observed significant strain-to-strain differences in the capacity of MAC to induce PGE₂ production. Of 4 MAC strains tested, 3 induced greater PGE₂ release from monocytes than did LPS stimulation alone. Higher levels of PGE₂ expression were evident after stimulation of monocytes with the Rg strains 86m2096 and LR147 and SmD strain LR542 than with the SmT strain LR114. Culture with IM had a small inhibitory effect on intracellular MAC growth in monocytes from some donors, suggesting that prostanoid-mediated mechanisms, at least in part, modulate intracellular MAC growth.

**Table 2.** Effect of indomethacin (IM) on cytokine production by monocytes stimulated with lipopolysaccharide (LPS).

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<tr>
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<th>1 day</th>
<th>4 days</th>
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<td>Controls</td>
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<td>.11 ± .03</td>
<td>.08 ± .04</td>
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<tr>
<td>LPS</td>
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<td>IM + LPS</td>
<td>3.4 ± .6</td>
<td>1.7 ± .4</td>
<td>11.9 ± 3.2</td>
<td>8.2 ± 1.7</td>
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NOTE. TNF, tumor necrosis factor; IL, interleukin. Data are ng/mL.

* Final concentration, 1 µg/mL.

† Final concentration, 10 µg/mL.

P < .005 compared to IM + LPS, paired t test.
These observations are consistent with the reports of Hines et al. [32] and Barrow and colleagues [17, 18]. Differences in MAC colonial morphology during culture on agar-based medium correlated with capacity for intracellular growth in murine, chicken, and human monocyte/macrophages [14, 33]. SmT morphotype strains showed greater capacity for intracellular growth than did SmD strains; Rg strains had intermediate capacity. These differences in colonial morphology appear to be due to variations in the structure of essential cell wall glycopepitidolipids that also can modulate immune effector cell functions. Glycolipid extracts of MAC cell walls inhibited intracellular killing of Candida albicans by bovine peripheral blood–derived macrophages [32]. Purified MAC cell wall lipid fractions also differentially induced PGE₂ expression by human monocytes from healthy subjects. MAC cell wall lipid fractions that induced PGE₂ expression were associated with increased intracellular MAC growth, whereas lipid fractions that did not induce PGE₂ production did not enhance mycobacterial replication [17, 18].

It is of interest that the less virulent SmD MAC strain induced the highest levels of PGE₂ production. Infection with Rg strains induced intermediate levels of PGE₂ production, whereas minimal levels were seen after infection with the virulent SmT morphotype. In earlier work [20], we demonstrated an association between colonial morphotype and expression of MAC growth-modulating cytokines by infected human monocytes. Infection with less virulent SmD strains induced more TNF-α and IL-1 expression than did infection with virulent SmT strains. Therefore, the direct signal supplied by avirulent MAC strains for the up-regulation of cytokine expression was not entirely counterbalanced by the expected inhibitory effects of PGE₂. In our present study, blocking PGE₂ production resulted in a significant and sustained increase in TNF-α, but not IL-1β, production. In vitro studies suggest that infection with some MAC strains may down-regulate expression of TNF-α, a key cytokine in host defenses against mycobacterial disease [20, 26]. TNF-α augmented intracellular MAC growth inhibition in human monocyte–derived macrophages [12]. Recently, Gan et al. [16] reported that MAC infection of human macrophages decreased TNF-α mRNA expression and induced unresponsiveness to subsequent restimulation with MAC. This monocyte hyporesponsiveness was abrogated by IM and restored by the addition of exogenous PGE₂. Therefore, under certain conditions, modulation of TNF-α production by IM could promote intracellular containment of MAC.

The production of PGE₂ by infected macrophages also may alter IFN-γ–induced macrophage-activating factor activity and intracellular mycobacterial killing. We and others have shown that IFN-γ decreases intracellular replication of some MAC strains in monocytes from healthy subjects and patients with AIDS [14, 23, 34]. We also observed significant decreases in intracellular growth of the highly virulent MAC LR114 SmT when 2-day-precultured monocytes were infected with MAC and then cocultured with IFN-γ and IM immediately after infection [22]. In the current study, addition of IFN-γ in doses comparable to those used in other earlier experiments 2 days after MAC infection did not enhance intracellular mycobacterial killing, suggesting that synergistic effects of IFN-γ and IM may only occur shortly after infection. Administration of IFN-γ within 24 h after infection fully activated macrophages against M. leprae, whereas addition at 3–5 days after infection did not [27]. The modest effects of IM on intracellular MAC growth may not, however, be entirely due to modulation of PGE₂. Administration of exogenous IFN-γ and IM augmented M. intracellulare killing by murine macrophages despite the production of large amounts of PGE₂ [10].

Modulation of mycobacterial-induced prostaglandin expression may be relevant for the treatment of human disease. Disseminated MAC infection in patients with AIDS is characterized by chronic fever, weight loss, fatigue, and other symptoms. Nonsteroidal antiinflammatory drugs such as naproxen were often used to alleviate symptoms in AIDS patients with disseminated MAC disease before effective chemotherapy became available. Cyclooxygenase inhibition by IM or related agents is an inexpensive and practical possible therapeutic adjunct to specific antimycobacterial drugs that also may augment host immune defenses and relieve symptoms. Blockade of prostaglandin metabolism with IM has been shown to enhance in vitro T lymphocyte proliferation in patients with atypical mycobacterial disease [35, 36]. When patients with nontuberculous mycobacterial disease were treated with oral IM in addition to antimycobacterial drugs, in vitro T lymphocyte responses to PPDs of M. tuberculosis (PPD-S) and M. intracellulare (PPD-B) and phytohemagglutinin and delayed cutaneous hypersensitivity reactions to PPD-S improved [36]. Further studies with monocytes or macrophages from HIV-infected persons would be valuable in clarifying the role of prostaglandin metabolism in MAC disease in patients with AIDS and the potential impact of adjunctive cyclooxygenase inhibitor therapy.

References


