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*J Immunol* 2004; 173:559-565; doi: 10.4049/jimmunol.173.1.559
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*The Journal of Immunology* is published twice each month by
The American Association of Immunologists, Inc.,
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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
Prostaglandin E<sub>2</sub> Inhibits Alveolar Macrophage Phagocytosis through an E-Prostanoid 2 Receptor-Mediated Increase in Intracellular Cyclic AMP<sup>1,2</sup>

David M. Aronoff,*† Claudio Canetti, † and Marc Peters-Golden<sup>3†</sup>

Prostaglandin E<sub>2</sub> is a potent lipid mediator of inflammation that effects changes in cell functions through ligation of four distinct G protein-coupled receptors (E-prostanoid (EP)1, EP2, EP3, and EP4). During pneumonia, PGE<sub>2</sub> production is enhanced. In the present study, we sought to assess the effect of endogenously produced and exogenously added PGE<sub>2</sub> on FcRγ-mediated phagocytosis of bacterial pathogens by alveolar macrophages (AMs), which are critical participants in lung innate immunity. We also sought to characterize the EP receptor signaling pathways responsible for these effects. PGE<sub>2</sub> (1–1000 nM) dose-dependently suppressed the phagocytosis by rat AMs of IgG-opsonized erythrocytes, immune serum-opsonized Klebsiella pneumoniae, and IgG-opsonized Escherichia coli. Conversely, phagocytosis was stimulated by pretreatment with the cyclooxygenase inhibitor indomethacin. PGE<sub>2</sub> suppression of phagocytosis was associated with enhanced intracellular cAMP production. Experiments using both forskolin (adenylate cyclase activator) and rolipram (phosphodiesterase IV inhibitor) confirmed the inhibitory effect of cAMP stimulation. Immunoblot analysis of rat AMs identified expression of only EP2 and EP3 receptors. The selective EP2 agonist butaprost, but neither the EP1/EP3 agonist sulprostone nor the EP4-selective agonist ONO-AE1-329, mimicked the effects of PGE<sub>2</sub> on phagocytosis and cAMP stimulation. Additionally, the EP2 antagonist AH-6809 abrogated the inhibitory effects of both PGE<sub>2</sub> and butaprost. We confirmed the specificity of our results by showing that AMs from EP2-deficient mice were resistant to the inhibitory effects of PGE<sub>2</sub>. Our data support a negative regulatory role for PGE<sub>2</sub> on the antimicrobial activity of AMs, which has important implications for future efforts to prevent and treat bacterial pneumonia. The Journal of Immunology, 2004, 173: 559–565.

Pneumonia is the leading cause of death from infection in the United States (1) and its global mortality is ~4.3 million victims per year (2). This problem is compounded by growing numbers of immunosuppressed patients and multidrug-resistant microorganisms. Developing more effective agents for the prevention and treatment of pneumonia requires a better understanding of how innate pulmonary defense mechanisms are regulated. In the lung periphery the alveolar macrophage (AM)<sup>3</sup> is the resident defender of mucosal stability, patrolling the alveolar epithelial surface and clearing organisms by phagocytosis and intracellular killing (3). The antimicrobial activities of this sentinel of innate immunity are regulated by a number of autocrine and paracrine factors including cytokines, chemokines, and lipids.

Lipid metabolites of arachidonic acid (AA), including PGs and leukotrienes, have emerged as potent endogenous mediators and modulators of innate immunity (4, 5). PGE<sub>2</sub> in particular has been shown to modulate immune and inflammatory responses (5, 6). It is a product of the cyclooxygenase (COX) cascade, which includes two distinct isoforms of COX, the constitutive COX-1 and the (usually) inducible COX-2, as well as constitutive and inducible PGE synthase enzymes. Production of PGE<sub>2</sub> is enhanced at sites of inflammation, in which it demonstrates both pro- and anti-inflammatory effects.

PGE<sub>2</sub> promotes inflammation through endothelial cell-mediated vasodilatation (producing warmth, erythema, and edema), facilitating the recruitment of circulating leukocytes to areas of infection (7). In addition, PGE<sub>2</sub> enhances leukocyte production of the inflammatory cytokine IL-6 (8). It is less well appreciated that PGE<sub>2</sub> has potent immunosuppressive properties, including the direct inhibition of leukocyte chemotaxis (9), reactive oxygen intermediate production (10), AA release (11), leukotriene synthesis (12), and the generation of myriad proinflammatory cytokines (13). Conversely, PGE<sub>2</sub> enhances the production of the immunosuppressive cytokine IL-10 (14).

Of interest, PGE<sub>2</sub> production is enhanced in the lung during bacterial pneumonia (15) and overproduction of PGE<sub>2</sub> has also been described in a number of conditions characterized by an increased susceptibility to infection, including cancer, aging, HIV infection, and malnutrition (16–19). That the net effect of PGE<sub>2</sub> is suppressive in the context of infection is supported by studies demonstrating that administration of a COX inhibitor enhanced microbial clearance and survival in animal models of infection (20, 21).

EP2 and EP4 receptors signal predominantly through Gs, increasing cAMP, and the EP3 receptor most often reduces cAMP via Gi coupling after PGE2 ligation (22). PGE2 has been shown to either stimulate or inhibit phagocytosis by macrophages (23–29). Nothing is known about AM expression of EP receptors or the effects of PGE2 on bacterial ingestion by these cells. In the present study, we provide evidence that endogenous and exogenous PGE2 inhibit FcγR-mediated phagocytosis by AMs via EP2-mediated cAMP signaling.

Materials and Methods

Animals

Mice with a targeted disruption of the EP2 gene (30) backcrossed over 10 generations onto a C57BL/6 background (designated as EP2 knockout mice) were obtained from ONO Pharmaceutical (Osaka, Japan) and bred in the University of Michigan Unit for Laboratory Animal Medicine (Ann Arbor, MI). Wild-type C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Pathogen-free 125–150 g female Wistar rats (Charles River Breeding Laboratories, Portage, MI) were also used. Animals were treated according to National Institutes of Health guidelines for the use of experimental animals with the approval of the University of Michigan Committee for the Use and Care of Animals.

Reagents

DMEM without phenol red, RPMI 1640, and penicillin/streptomycin/amphotericin B solution were purchased from Life Technologies-Invitrogen (Carlsbad, CA). Trypsin soy broth was supplied by Difco (Detroit, MI). Aspirin, cytochalasin D, ibuprofen, indomethacin, o-phenylenediamine dihydrolactone, and SDS were from Sigma-Aldrich (St. Louis, MO). AH-6809, butaprost free acid, PGE2, sulprostone, and U-46619 were from Cayman Chemicals (Ann Arbor, MI). ONO-AE1-329 was a generous gift from ONO Pharmaceutical. Forskolin and rolipram were purchased from Calbiochem (San Diego, CA). Compounds requiring reconstitution were dissolved in either ethanol or DMSO. Required dilutions of all compounds were prepared immediately before use, and equivalent quantities of vehicle were added to the appropriate controls.

Cell isolation and culture

Resident AMs from mice and rats were obtained via ex vivo lung lavage as previously described (31) and resuspended in RPMI 1640 to a final concentration of 1 × 10^6 cells/ml. Cells were allowed to adhere to tissue culture-treated slides or plates for 1 h (37°C, 5% CO2) followed by two washes with warm RPMI 1640, resulting in >99% of adherent cells identified as AMs by use of a modified Wright-Giemsa stain (Diff-Quik; American Scientific Products, McGraw Park, IL) (31). Cells were cultured overnight in RPMI 1640 containing 10% FBS and 1% penicillin/streptomycin/amphotericin B before use. The following day cells were washed two times with warm medium to remove nonadherent cells.

Microscopic phagocytosis assay with Klebsiella pneumoniae

K. pneumoniae 43816, serotype 2, was obtained from the American Type Culture Collection (Rockville, MD) and aliquots grown in trypsin soy broth at 37°C (5% CO2) until mid-log phase. The concentration of bacteria in a 30:1 (v/v) dilution of bacteria in warm DMEM was measured by a Coulter counter and adjusted to 5 × 10^9 per well. Cells were then washed twice with warm DMEM and preincubated with compounds of interest, followed by the application of IgG-E. coli (MOI = 30:1). After 30 min incubation in the dark (37°C, 5% CO2), unengested bacteria were washed away with PBS and residual extracellular FITC (representing cell-adherent IgG-E. coli) was quenched with trypan blue (250 μg/ml; Molecular Probes) for 1 min. Fluorescence was determined with a microplate fluorimeter (485/535 nm; SPECTRAFluor Plus; Tecxan, Research Triangle Park, NC). Independent experiments were performed in quadruplicate. Fluorometric data were expressed in arbitrary relative fluorescence units (RFU) and corrected for background fluorescence using wells containing only AMs and medium.

Measurement of intracellular cAMP and PGE2 production

Rat AMs were cultured overnight in six-well plates in RPMI 1640 at concentrations of 3 × 10^6 cells/well. For PGE2 experiments, cultures were then incubated for 15 min in the presence or absence of compounds of interest. Culture supernatants were aspirated and the cells were lysed by incubation with 100 μl 0.1 M HCl (22°C), followed by disruption using a cell scraper. Intracellular PGE2 was determined by enzyme-linked immunoassay kit according to the manufacturer’s directions. For PGE2 determination, cells were incubated with immune serum-epitope conjugate K. pneumoniae (MOI = 1:10) or medium alone for 60 min. Culture supernatants were collected and PGE2 levels quantified by enzyme-linked immunosorbent assay kit according to the manufacturer (Assay Designs, Ann Arbor, MI).

Fluorometric phagocytosis assay with FITC-labeled Escherichia coli

Paraformaldehyde-inactivated, ITC-labeled E. coli BioParticles (3 × 10^8 E. coli/ mg per milligram of solid; Molecular Probes, Eugene, OR) were suspended in PBS at a concentration of 20 mg/ml followed by brief sonication on ice. E. coli were opsonized with specific rabbit polyclonal IgG (Molecular Probes) according to the manufacturer’s directions. Opsonized E. coli (IgG-E. coli) was further diluted in DMEM for use in phagocytosis experiments. Rat AMs were cultured overnight in 96-well tissue culture-treated dishes (Costar, Corning, NY) at a density of 4 × 10^5 cells/well. Murine AMs were cultured in a similar fashion in 384-well plates (Costar) at a density of 5 × 10^5 per well. Cells were then washed two times with warm DMEM and preincubated with compounds of interest, followed by the application of IgG-E. coli (MOI = 30:1). After 30 min incubation in the dark (37°C, 5% CO2), unengested bacteria were washed away with PBS and residual extracellular FITC (representing cell-adherent IgG-E. coli) was quenched with trypan blue (250 μg/ml; Molecular Probes) for 1 min. Fluorescence was determined with a microplate fluorimeter (485/535 nm; SPECTRAFluor Plus; Tecxan, Research Triangle Park, NC). Independent experiments were performed in quadruplicate. Fluorometric data were expressed in arbitrary relative fluorescence units (RFU) and corrected for background fluorescence using wells containing only AMs and medium.

Microscopic erythrocyte phagocytosis assay

The phagocytosis of sheep RBCs (sRBCs) by rat AMs was assessed as previously described (35). Briefly, AMs were plated and cultured overnight in 96-well culture-treated dishes (BD Biosciences) at a density of 2 × 10^5 cells/well. sRBCs (ICN Pharmaceuticals, Costa Mesa, CA) were opsonized with a subagglutinating concentration of polyclonal, rabbit anti-sRBC IgG (Cappel Organon Teknika, Durham, NC) as previously described (36). AMs were then washed twice with warm DMEM and preincubated with cytochalasin D (5 μg/ml; 34). The PI was RFUi - RFUa. The ability of cytochalasin D to completely inhibit phagocytosis was confirmed by fluorescent microscopy.

PGE2, SUPPRESSION OF AM PHAGOCYTOSIS
Immunohistochemical analysis

Western blots were performed as previously described (37). Briefly, whole cell protein extracts were obtained by lysing freshly harvested AMs in a buffer (50 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM MgCl2, 0.2% Nonidet P-40) supplemented with protease inhibitors (Roche Diagnostics, Mannheim, Germany). Protein samples (20 μg) were resolved on 10% Tris-HCl polyacrylamide gels and subsequently transferred to a nitrocellulose membrane. Membranes were probed with commercially available rabbit polyclonal EP receptor Abs (Alpha Diagnostic International, San Antonio, TX, and Cayman Chemicals) followed by HRP-conjugated anti-rabbit secondary Abs and ECL Plus detection reagents (Amersham Biosciences, Piscataway, NJ).

Cell viability

All experimental compounds and vehicles showed no adverse effects on rat AM viability using a commercially available MTT assay kit (Sigma-Aldrich; data not shown).

Statistical analysis

Data are represented as mean ± SE and were analyzed with the Prism 3.0 statistical program (GraphPad Software, San Diego, CA). Comparisons between two experimental groups were performed with Student’s t test. Comparisons among three or more experimental groups were performed with ANOVA followed by either Dunnett’s or Tukey’s multiple comparison tests as indicated. Differences were considered significant if p ≤ 0.05. All experiments were performed on at least three separate occasions unless otherwise specified.

Results

PGE2 inhibits FcR-mediated phagocytosis

Exogenous PGE2 has been shown to inhibit FcR-mediated phagocytosis of IgG-opsonized sRBCs by murine AMs (27). We sought to confirm this finding with rat AMs, which were pretreated for 5 min with increasing concentrations of PGE2 before incubation with IgG-opsonized sRBCs. PGE2 showed a concentration-dependent suppression of FcR-mediated phagocytosis that reached ~54% at 1 μM (Fig. 1). Because the effect of PGE2 on AM phagocytosis of bacterial pathogens has not been determined previously, we examined the ingestion of live, immune serum-opsonized K. pneumoniae bacteria following exposure to PGE2. Phagocytosis, as measured by light microscopy, was inhibited by PGE2 with maximal inhibition (71 ± 5%) found at 1 μM (Fig. 1). The percentage of AMs ingesting bacteria was reduced to a greater degree than was the mean number of bacteria per cell (61 ± 7% vs 27 ± 3% maximal inhibition, respectively). The specificity of this effect was demonstrated by the fact that an alternative prostanooid, the thromboxane A2 receptor agonist U-46619, failed to inhibit phagocytosis at concentrations up to 1 μM (data not shown). As illustrated (Fig. 1), PGE2 also inhibited AM ingestion of IgG-E. coli in a dose-dependent manner.

Endogenous PGE2 restricts AM phagocytosis

Because AMs are capable of PGE2 biosynthesis, it was of interest to ascertain whether levels of PGE2 generated endogenously influenced phagocytosis. We initially assessed the effects of a 60 min challenge with immune serum-opsonized K. pneumoniae on PGE2 synthesis by rat AMs (MOI = 10:1). Under these conditions, basal production of PGE2 by AMs (183.8 ± 4.2 pg/million AM) was only marginally enhanced following infection (205.5 ± 4.5 pg/million AM; p < 0.05, n = 3). Greater enhancement of AM PGE2 production was observed with either a larger MOI (100:1) or a longer incubation time for bacterial/AM cultures (data not shown). When endogenous PGE2 production was inhibited with the COX-1/COX-2 inhibitor indomethacin, phagocytosis of K. pneumoniae was enhanced by 43 ± 4.9% (p < 0.05) and the ingestion of IgG-opsonized sRBCs by 44 ± 5.8% (p < 0.01; Fig. 2). Because indomethacin has been found to have effects on cell function that are independent of its COX inhibitory action (38), we examined the influence of two other nonspecific COX inhibitors (aspirin and ibuprofen) on FcR-mediated phagocytosis of IgG-sRBCs (Fig. 2). As illustrated, similar effects were seen with these diverse COX inhibitors.

PGE2 enhances intracellular cAMP in AMs

PGE2 acts through four distinct G protein-coupled receptor subtypes (EP1, EP2, EP3, and EP4), with distinct signaling pathways, but neither the PGE2 signaling mechanisms nor the EP receptor expression profile of AMs is known. Because the Gs-coupled EP2 and EP4 receptors activate, and the Gi-coupled EP3 receptor suppresses adenylate cyclase activity (22), we measured changes in

![FIGURE 1. Inhibition of phagocytosis by PGE2. Rat AMs were harvested by lung lavage and cultured as described in Materials and Methods. Cells were pretreated with PGE2, or vehicle control for 5 min and then challenged with IgG-opsonized sRBCs (●), 2% immune serum (IS)-opsonized, live K. pneumoniae (□), or FITC-labeled, IgG-opsonized E. coli (○). PIs were calculated as described and expressed as a percentage of the control value to which no PGE2 was added. *, p < 0.05; **, p < 0.01 compared with control, by ANOVA followed by Dunnett’s multiple comparison test (n = 3–8 independent experiments performed in quadruplicate).](http://www.jimmunol.org/content/ji/173/9A/F1.large.jpg)

![FIGURE 2. Effect of endogenous prostanoids on phagocytosis. Rat AMs were harvested by lung lavage and cultured as described in Materials and Methods. A. Cells were pretreated with the COX inhibitor indomethacin (10 μM) or vehicle for 30 min and then challenged with 2% immune serum (IS)-opsonized, live K. pneumoniae. Data represent the mean of three independent experiments performed in quadruplicate. B. Cells were pretreated with indomethacin (10 μM), ibuprofen (100 μM), aspirin (200 μM), or vehicle for 30 min followed by IgG-opsonized sRBCs. Data represent the mean of six independent experiments (indomethacin) and a single experiment (ibuprofen and aspirin) performed in septuplet. The PI was calculated as described and expressed as a percentage of control to which no drug was added. *, p < 0.05, by Student’s t test (A) or ANOVA followed by Dunnett’s multiple comparison test (B).](http://www.jimmunol.org/content/ji/173/9A/F2.large.jpg)
intracellular cAMP in response to PGE$_2$ (Fig. 3A). Rat AMs treated for 15 min with PGE$_2$, showed a concentration-dependent increase in intracellular cAMP, suggesting the participation of EP2 and/or EP4 receptors. Using the highly selective EP2 and EP4 receptor agonists butaprost and ONO-AE1-329, respectively, we found that only the EP2 receptor agonist stimulated cAMP production at concentrations as high as 1 µM (Fig. 3B). The basal intracellular concentration of cAMP in AMs (138.1 ± 11.0 fmol/10$^6$ cells) was not reduced by the EP1/EP3 receptor agonist sulprostone, however sulprostone (1 µM) limited the increase in intracellular cAMP stimulated by butaprost (1 µM) by ~25% (data not shown).

**AMs express EP2 and EP3 receptors**

The expression of all four EP receptors in rat AMs was examined using commercially available polyclonal Abs. Protein preparations from lysates of freshly harvested, unstimulated rat AMs were subjected to Western blot analysis for the detection of EP receptor protein. Neither EP1 nor EP4 receptors were detected by this method; however, bands of 68 kDa and 62 kDa were seen for EP2 and EP3, respectively (Fig. 4).

**Pharmacological activation of adenylate cyclase suppresses phagocytosis**

Our results support the findings of other investigators (28, 39) that elevations in intracellular cAMP are associated with suppression of phagocytic function. We explored the causal relationship between PGE$_2$-induced cAMP generation and depressed phagocytosis in AM pharmacologically. We used forskolin, a direct activator of adenylate cyclase, and rolipram, an inhibitor of the phosphodiesterase IV enzyme that degrades cAMP. Preliminary dose-response experiments demonstrated that 100 µM forskolin enhanced intracellular cAMP production in rat AMs to approximately the same degree as 1 µM PGE$_2$ (data not shown). A standard concentration of rolipram was used (10 µM) (39). Both PGE$_2$ (Fig. 5A) and forskolin (Fig. 5B) suppressed phagocytosis. The effects of both compounds were enhanced when cells were pretreated with rolipram. Furthermore, inhibition of phagocytosis directly correlated with measured levels of cAMP.

**An EP2 receptor agonist inhibits AM phagocytosis**

To characterize the participation of the EP2 receptor in determining the effect of PGE$_2$ on AM function, we assessed the ingestion of IgG-E. coli in the presence or absence of PGE$_2$, the EP2 agonist butaprost, and the DP1/EP1/EP2 receptor antagonist AH-6809. Butaprost and PGE$_2$ demonstrated equivalent inhibition of phagocytosis at a concentration of 1 µM (Fig. 6). The effect of butaprost was completely abrogated by AH-6809 (100 µM). The ability of
AH-6809 to prevent the inhibitory action of butaprost was dose-dependent (data not shown). Inhibition by PGE₂ was not entirely prevented by AH-6809, but the residual degree of inhibition (16.9 ± 4.1%) was not statistically significant as compared with the control value (p > 0.05). EP2 receptor blockade with AH-6809 did not significantly enhance the phagocytic activity of AMs. Neither the EP1/EP3 agonist sulprostone (1 μM) nor the EP4 agonist ONO-AE1-329 (1 μM) affected phagocytosis significantly compared with control (Fig. 6).

Murine AMs lacking the EP2 receptor are protected from PGE₂ inhibition

A complementary approach to determining the role of the EP2 receptor in mediating the effects of PGE₂ on phagocytosis used mice genetically deficient in the EP2 receptor. AMs harvested from EP2 knockout mice and their wild-type strain controls were incubated in the presence or absence of PGE₂ (1 μM) 5 min before the administration of IgG-E. coli (Fig. 7). PGE₂ inhibited phagocytosis by wild-type AMs by 24.6 ± 3.0% (p < 0.05; n = 3) but had no effect on EP2 knockout cells. Inhibition of AM phagocytosis by forskolin was equivalent in wild-type and EP2 knockout mice, demonstrating that the receptor null cells had intact postreceptor signaling.

Discussion

The present study confirms that PGE₂ has important immunosuppressive effects on AM antimicrobial function (27, 40, 41) and enhances our understanding of the mechanisms whereby these effects occur. PGE₂ has previously been shown to either stimulate or inhibit the phagocytic capacity of monocytes/macrophages, with the majority of studies demonstrating inhibition (23–29). In 1991, Canning et al. (27) demonstrated that exogenously added PGE₂ suppressed the phagocytosis of IgG-opsonized sRBCs by murine AMs. However, neither the effects of PGE₂ on AM phagocytosis of bacterial pathogens nor the expression profile of EP receptors by AMs is known.

Our study focused on the effects of PGE₂ on phagocytosis mediated by the FcRγ because rat AMs, as compared with AMs from other species or from peritoneal macrophages, exhibit little or no phagocytosis of complement-opsonized targets (42). The effects of PGE₂ on AM phagocytosis via FcR-independent pathways await further investigation. Using three different in vitro models of FcR-mediated phagocytosis, we have demonstrated a dose-dependent inhibitory effect of exogenous PGE₂ on rat AM ingestion of serum-opsonized or IgG-opsonized targets (Fig. 1). Using a microscopic assay of phagocytosis we found ~70% inhibition of phagocytosis of live, immune serum-opsonized K. pneumoniae with 1 μM PGE₂. Because microscopy is labor intensive and susceptible to subjective human error, we adopted and optimized a fluorometric assay with greater throughput capacity and objectivity (43). Although this assay required a higher MOI (30:1) than we used for microscopy (MOI 10:1), we still found significant inhibition with PGE₂. It is notable that the maximal degree of PGE₂ inhibition observed varied among the three models used. The reasons for this variability are unclear but may reflect differences among the phagocytic targets used, different target to AM ratios, the source/type of opsonin used, and differences in assay sensitivity. Consistent with our experience, the maximal inhibitory effect of PGE₂ on phagocytosis in other in vitro monocyte/macrophage systems has ranged from ~25% to ~85% (23, 27–29).

Macrophages are an important source of PGE₂ at sites of inflammation, and endogenously produced PGE₂ can regulate macrophage function in an autocrine fashion. In support of this, we found that inhibition of COX activity with indomethacin, ibuprofen, or aspirin revealed a suppressive effect of endogenous prostaglandins on the phagocytic activity of AMs. It would be expected that this role of endogenous PGE₂ could be magnified in circumstances characterized by overproduction of PGE₂, such as cancer, aging, HIV infection, and malnutrition (16–19).

Despite the observation that PGE₂ tends to suppress phagocytosis, very little is known about the receptor-mediated intracellular signaling mechanisms responsible for this effect. PGE₂ has been shown to stimulate AM adenylate cyclase activity (44) and agents that enhance intracellular cAMP production generally inhibit phagocytosis (28, 39, 45, 46). In human peripheral blood monocyte-derived macrophages, for example, PGE₂ suppression of phagocytosis was associated with increased intracellular cAMP generation (28). These cells express both the EP2 and EP4 receptor as evidenced by pharmacological and genetic analysis (47, 48).

That intracellular cAMP production in AMs was stimulated in a dose-dependent manner by PGE₂ (Fig. 3) suggested this mechanism for its inhibitory effect. This possibility was strongly supported by the finding that inhibition of phagocytosis by PGE₂ and forskolin was augmented when cAMP degradation was prevented by the phosphodiesterase inhibitor rolipram. A cAMP-dependent mechanism of PGE₂ immunosuppression suggests the participation of the EP2 and/or EP4 receptors and a possible counter-regulatory role for the G₄-coupled EP3 receptor. Using pharmacological agonists for all four EP receptors, we showed a significant increase in cAMP only with the EP2 specific agonist butaprost, suggesting that the EP4 receptor does not mediate PGE₂ regulation in these cells. Sulprostone, an EP1/EP3 agonist, failed to suppress basal cAMP production, although it blunted the cAMP increase evoked by butaprost, suggesting the presence and modest functional participation of this receptor. The role of EP2 in the suppression of AM phagocytosis was confirmed using butaprost in the presence or absence of AH-6809, an effective but relatively nonspecific EP2 receptor antagonist. This compound, which at the concentration used in this study blocked the PGE₂-induced production of cAMP in COS cells transfected with the human EP2 receptor (49), entirely prevented inhibition of phagocytosis by butaprost and largely abrogated the response to PGE₂.
The pharmacological evidence for PGE2-EP2 signaling was supported by experimental data using AMs harvested from EP2 knockout mice. As demonstrated in Fig. 7, EP2 knockout macrophages demonstrated phagocytic ability comparable to wild-type controls. Whereas the ingestion of IgG-E. coli by wild-type AMs was significantly inhibited by PGE2, AMs from EP2 knockout mice were not affected by exogenous PGE2. Importantly, forskolin suppressed phagocytosis of wild-type and EP2 knockout cells equivalently, demonstrating intact cAMP signaling pathways in both groups of mice.

Our data suggest that endogenously generated PGE2 suppresses AM phagocytosis via the EP2 receptor. However, neither pretreatment of rat AMs with AH-6809 nor deletion of the EP2 receptor in murine AMs enhanced phagocytosis in a similar manner to COX inhibitor treatment. The reasons for this are unclear. It is possible that the suppressive effect of COX inhibitors on prostanooids other than PGE2 is responsible for the enhanced phagocytosis seen with these agents. Indeed, prostacyclin-induced cAMP production inhibited phagocytosis by rat peritoneal macrophages (50); however, AM capacity for prostacyclin synthesis is quite limited (51). COX inhibition might also augment phagocytosis by shunting AA away from COX enzymes and into alternative metabolic pathways such as the 5-lipoxygenase cascade. This is important because products of the 5-lipoxygenase pathway (namely leukotriene B4 and the cysteinyl leukotrienes) have been found to enhance FcR-mediated phagocytosis in AMs (33). However, we have previously reported that shunting of AA from the COX cascade into the 5-lipoxygenase pathway did not occur when indomethacin-treated AMs were stimulated with calcium ionophore (52). When we measured PGE2 and leukotriene B4 by AMs challenged with immune serum-opsonized K. pneumoniae, we similarly did not see AA shunting following indomethacin exposure (data not shown). In the absence of measurable AA shunting, it could still be hypothesized that blocking the endogenous suppressor compound PGE2 increases the ability of leukotrienes to promote FcR-mediated ingestion. Studies to address potential interactions between PGE2 and leukotrienes and the relative contribution of these immunomodulators in regulating AM antimicrobial function are ongoing.

Yet another potential mechanism whereby COX inhibitors might influence AM function includes COX-independent actions of these drugs (such as effects on transcription factors and cytokine production) (38, 53). We attempted to limit this potential confounder by 1) using chemically diverse COX inhibitors, 2) using production) (38, 53). We attempted to limit this potential confounder by (1) using chemically diverse COX inhibitors, 2) using concentration of these compounds below those at which nonspecific effects arise, and 3) exposing AMs to these drugs for periods of time short enough to minimize changes in cellular gene expression.

Conversely, the failure of AH-6809 to enhance phagocytosis may result from its lack of specificity for the EP2 receptor, particularly at the concentration used in this study (100 μM) (22). AMs from EP2 knockout mice tended to phagocytose IgG-E. coli better than did wild-type cells, but the difference was not significant. Unexpected results with the EP2 null macrophages could occur if compensatory changes in PGE2 production or EP1, EP3, or EP4 expression are present; this remains to be investigated.

Detection of EP receptor proteins in whole cell lysates prepared from rat AMs was performed using immunoblot analysis with commercially available polyclonal Abs directed against each of the four EP receptors. Our results demonstrated evidence for the presence of the EP2 and EP3 receptors. We were unable, however, to identify either the EP1 or EP4 receptor proteins. Our findings were similar using EP receptor Abs from two different commercial vendors (data not shown). The apparent mobilities for the EP2 and EP3 receptors (~68 kDa and ~62 kDa, respectively) were different from the theoretical molecular masses predicted from the primary amino acid sequences (40 kDa and 40–45 kDa, respectively) (22, 54). This discrepancy between predicted and apparent molecular masses by SDS-PAGE has been reported previously with membrane receptors, including rat, bovine, and human EP receptors (55–57), and may result from receptor oligomerization (58) or posttranslational modifications such as glycosylation and/or phosphorylation (55, 56). In agreement with our results, apparent mobilities of 68 and 62 kDa have also been reported for the human EP2 and EP3 receptors (55).

Our findings are important given the critical role AMs play in the innate immune defense against bacterial pneumonia. Impairments in the phagocytic ability of AMs increase the risk of pulmonary infection or its resultant morbidity and mortality (59). PGE2 is a prominent metabolite of AA whose production is dramatically up-regulated at sites of infection and inflammation, including the lung (15, 60). Sources of this PGE2 include both AMs themselves as well as neighboring cells such as epithelial cells (61). That AM function may be negatively regulated by PGE2 fits with the emerging concept of PGE2 as a suppressor of macrophage function (5, 6, 60).

In summary, we have shown that exogenously supplied and endogenously generated PGE2 play an inhibitory role in FcR-mediated AM phagocytosis of bacterial pathogens. This immunosuppressive action is mediated by increased cAMP levels following ligation of the transmembrane EP2 receptor. These results have important implications for future efforts to prevent and treat bacterial pneumonia, particularly in those subsets of immunosuppressed individuals that may have exaggerated PGE2 production. Variations among individuals in PGE2 production as well as EP2 expression might influence the susceptibility to and outcome of respiratory infections. Moreover, EP2 antagonism may represent a strategy for immunostimulation.

Acknowledgments

We thank Dr. Shuh Narumiya (Kyoto University, Kyoto, Japan) for availability of the EP2 knockout mice, Dr. Takayuki Maruyama (Minase Research Institute, ONO Pharmaceutical, Osaka, Japan) for provision of EP2 knockout mice, and Dr. Takayuki Maruyama (Minase Research Institute, ONO Pharmaceutical, Osaka, Japan) for provision of EP2 antagonist. We thank Dr. Bethany Moore for assistance with mouse breeding, and Susan Phare and Teresa Marshall for technical assistance.

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