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Cyclopentenone Prostaglandins PGA_2 and 15-Deoxy- $\Delta^{12,14}$ PGJ_2 Suppress Activation of Murine Microglia and Astrocytes: Implications for Multiple Sclerosis

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Abstract

The cyclopentenone prostaglandin (cPG) 15-deoxy- $\Delta^{12,14}$ - PGJ_2 (15d- PGJ_2) has been identified as a potent antiinflammatory agent that is able to inhibit the activation of macrophages and microglia. Additionally, 15d- PGJ_2 is able to ameliorate the clinical manifestations of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Many biological effects of 15d- PGJ_2 have been attributed to the peroxisome proliferator activated receptor-gamma (PPAR- γ). PGA_2 , like 15d- PGJ_2 , is a cPG. The aim of this study is to compare the relative effectiveness of these two cPGs in inhibiting the inflammatory response of mouse microglia and astrocytes, two cell types that upon activation may contribute to the pathology of EAE and MS. Purified primary mouse microglia and astrocytes were treated with either 15d- PGJ_2 or PGA_2 and then stimulated with either lipopolysaccharide (LPS) or a combination of interferon (IFN)- γ and tumor necrosis factor (TNF)- α . The results show that 15d- PGJ_2 and PGA_2 both potently inhibited the production of nitrite, as well as proinflammatory cytokines and chemokines, from microglia and astrocytes. Generally, regulation of NO production was more sensitive to 15d- PGJ_2 , however, cytokine and chemokine production was more sensitive to PGA_2 treatment. These results demonstrate for the first time that PGA_2 is a potent antiinflammatory mediator.

Keywords

primary cell culture; cytokine; chemokine; mouse

Prostaglandins (PGs) are a family of biologically active molecules that have been implicated in numerous physiologic and pathologic processes (Straus and Glass, 2001). PGs are oxygenated metabolites of arachidonic acid and are divided into two main functional groups. The conventional PGs, including PGE, PGD, and PGF, elicit a biological response after binding to G protein-coupled cell surface receptors. In contrast, the cyclopentenone PGs (cPG), including PGA_1 , PGA_2 , and PGJ_2 , have no cell surface receptor but instead are actively transported into the cell where they are able to interact with signaling molecules and transcription factors. The bioactivity of cPGs has been attributed in part to the chemically reactive α , β -unsaturated carbonyl group located in the cyclopentenone ring that makes these PGs susceptible to undergoing Michael addition reactions with cysteine residues located in cellular proteins (Bui and Straus, 1998; Straus et al., 2000; Straus and Glass, 2001). Additionally, the γ isoform of the peroxisome proliferator-activated receptor (PPAR- γ) has been found to be a specific nuclear receptor for the cPG 15-deoxy- $\Delta^{12,14}$ - PGJ_2 (15d- PGJ_2)

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(Forman et al., 1995; Kliewer et al., 1995). PPAR- γ heterodimerizes with the 9-*cis* retinoic acid receptor, translocates to the nucleus, and recognizes the PPAR response element (PPRE) in the promoters of target genes, suggesting an alternative route of action for the cPG 15d-PGJ₂ (Kielian and Drew, 2003).

Cyclopentenone PGs have a variety of biological actions, including induction of the cellular stress response, inhibition of cell-cycle progression, and suppression of viral replication, cell differentiation, and development (Negishi and Katoh, 2002). Recently, the role of cPGs as antiinflammatory mediators has been identified. The cPG 15d-PGJ₂ has been shown to be effective in ameliorating a variety of inflammatory conditions including arthritis (Kawahito et al., 2000) and inflammatory bowel disease (Su et al., 1999). In addition, elevated serum levels of 15d-PGJ₂ correlate with the resolution of inflammation in a carrageenin-induced pleurisy model (Gilroy et al., 1999). More recently, we and others have demonstrated that 15d-PGJ₂ is effective in inhibiting experimental autoimmune encephalomyelitis (EAE), a rodent model of multiple sclerosis (Diab et al., 2002, 2004; Feinstein et al., 2002; Natarajan and Bright, 2002).

The mechanisms underlying the ability of cPGs to induce antiinflammatory effects have been studied only recently. The cPG 15d-PGJ₂ has been demonstrated to repress the expression of numerous proinflammatory genes in activated macrophages including those encoding inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF)- α , gelatinase B, thromboxane B₂, and cyclooxygenase (COX)-2 (Jiang et al., 1998; Ricote et al., 1998; Guyton et al., 2001). Activated glia are believed to contribute to the pathogenesis of neuroinflammatory and neurodegenerative diseases including MS. Importantly, 15d-PGJ₂ inhibited the production of nitric oxide (NO) in activated microglia after lipopolysaccharide (LPS) stimulation (Petrova et al., 1999), and was able to inhibit expression of iNOS, TNF- α , interleukin (IL)-1 β , and COX-2 in LPS-stimulated microglia (Bernardo et al., 2000, 2003; Koppal et al., 2000). In addition, we have shown that 15d-PGJ₂ is effective in inhibiting production of potentially cytotoxic molecules, including NO, TNF- α , and IL-12, by microglial cells stimulated with interferon (IFN)- γ and TNF- α , molecules that have been implicated in the pathogenesis of EAE and MS (Drew and Chavis, 2001). The mechanisms by which cPGs alter the expression of proinflammatory genes have not been elucidated completely. Binding of the ligand 15d-PGJ₂ to PPAR- γ can directly modulate gene transcription through receptor binding to the PPRE present in PPAR- γ -responsive genes. Alternatively, 15d-PGJ₂ may also alter gene expression independent of the PPRE, through a mechanism termed receptor-dependent transrepression. In addition, 15d-PGJ₂ is believed to inhibit DNA binding of nuclear factor (NF)- κ B, signal transducers and activators of transcription (STAT), and AP-1, transcription factors that promote transcription of proinflammatory genes, through receptor-dependent transrepression. Furthermore, 15d-PGJ₂ can inhibit NF- κ B activation by suppressing I κ B phosphorylation via PPAR- γ independent mechanisms (Straus et al., 2000; Park et al., 2003). The cyclopentenone ring of 15d-PGJ₂ has been demonstrated to mediate some PPAR- γ -independent effects of this PG (Kielian and Drew, 2003).

The current study aims to examine the ability of the cPG PGA₂ to suppress the activation of microglia and astrocytes. In addition, we directly compare the activity of PGA₂ with that of 15d-PGJ₂. The results show that PGA₂ is effective in inhibiting the production of NO and the expression of proinflammatory cytokines and chemokines from both activated microglia and astrocytes. To our knowledge, this is the first thorough demonstration that PGA₂ may have an antiinflammatory role in the regulation of activated glial cells.

MATERIALS AND METHODS

Materials

The cPGs 15-deoxy- $\Delta^{12,14}$ PGJ₂ and PGA₂ were obtained from Cayman Chemical Company (Ann Arbor, MI). LPS was obtained from Sigma (St. Louis, MO), whereas the cytokines IFN- γ and TNF- α were obtained from R&D Systems (Minneapolis, MN). Fetal bovine serum (FBS) was obtained from Hyclone (Logan, UT).

Cell Culture

Pure mouse microglia and astrocyte cultures were obtained through a modification of the McCarthy and de Vellis (1980) protocol. Briefly, cerebral cortices from 1–2-day-old mouse pups were excised and cortices minced into small pieces. Cells were separated by trypsinization in the presence of DNase and incubated at 37°C for 20 min. After filtration through a 70- μ m strainer, the cells were collected by centrifugation ($153 \times g$ for 5 min at 4°C). The cells were re-suspended in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS, 1.4 mM glutamine, 0.5 ng/ml granulocyte macrophage colony-stimulating factor (GM-CSF) (BD Pharmingen, San Diego, CA), and oxaloacetate pyruvate insulin (OPI) media supplement (Sigma), plated into tissue culture flasks, and allowed to grow to confluence (about 10 days) at 37°C/5% CO₂. Flasks were shaken overnight (200 rpm at 37°C) in a temperature-controlled shaker to loosen microglia and oligodendrocytes from the more adherent astrocytes. These less adherent cells were plated for 2 hr and then lightly shaken to separate oligodendrocytes from the more adherent microglia. Microglial cells were seeded in 96-well plates and incubated overnight at 37°C/5% CO₂. Astrocyte medium contained all the substances described in the defined medium above except GM-CSF. After shaking to remove microglia and oligodendrocytes, astrocytes were recovered by trypsinization, seeded in 96-well plates and incubated overnight at 37°C/5% CO₂. These panning procedures were repeated to obtain primary microglia or astrocytes of greater than 95% purity as determined by immunohistochemistry. Microglial and astroglial cells were treated with either 15d-PGJ₂ or PGA₂ for 1 hr, and then stimulated with LPS or IFN- γ /TNF- α for 24 hr. Finally, tissue culture supernatants were collected to analyze for NO levels and cytokine/chemokine secretion.

NO Production

The production of NO was monitored by measuring the amount of nitrite, one of the end products of NO oxidation, in the supernatant. This assay was accomplished using the Griess reaction, which is based on the diazotization of nitrite by sulfanilic acid. Plates were read on a Molecular Devices Spectromax 190 microplate reader using a test wavelength of 550 nm. Results are reported as the average molar amount of nitrite released into the supernatant. Each experimental condition was run in triplicate.

Cell Viability Assays

Cellular viability was determined by measuring the reduction of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) as described previously (Chang et al., 1998). Briefly, MTT was added during the final 1hr of incubation. The medium was removed, dimethylsulfoxide (DMSO) was added to each well, and the plate shaken for 30 min at room temperature. The plate was then read on a Spectromax 190 microplate reader using a test wavelength of 570 nm. Results are reported as the percent viability as compared to unstimulated microglia.

Enzyme-Linked Immunosorbent Assay

Cytokine (IL-1 β , TNF- α , IL-6) and chemokine (monocyte chemoattractant protein [MCP]-1) levels in tissue culture media were determined by sandwich enzyme-linked immunosorbent

assay (ELISA) as described by the manufacturer (OptEIA mouse ELISA kit; BD Pharmingen). Cytokine and chemokine concentrations in the tissue culture media were determined by spectrophotometer using a test wavelength of 450 nm and calibrated from standards containing known concentrations of the cytokine.

Statistics

Analysis of variance (ANOVA) followed by a Neuman-Keuls post-hoc test at a 95% confidence level were used to analyze the data.

RESULTS

Effects of Cyclopentenone Prostaglandins on NO Production by Microglial Cells After LPS Stimulation

We have demonstrated previously that the prostaglandin 15d-PGJ₂ represses the production of NO by a mouse microglial cell line and primary rat microglia (Drew and Chavis, 2001). In the present studies, we investigated the ability of another cPG to influence the activity of activated microglia. In mouse primary microglial cells, LPS was able to induce the production of NO (Fig. 1A), and 15d-PGJ₂ was able to repress NO production in a dose-dependent manner, without a change in cell viability relative to LPS alone (Fig. 1A,B). Similarly, PGA₂ was able to inhibit the production of NO by mouse primary microglia in a dose-dependent manner (Fig. 1A), without significantly altering the level of cell viability (Fig. 1B). The effective dose of PGA₂ was approximately three- to fivefold higher than that needed for 15d-PGJ₂ to suppress NO production.

Effects of Cyclopentenone Prostaglandins on NO Production by Microglial Cells After Cytokine Stimulation

The proinflammatory cytokines TNF- α and IFN- γ are capable of stimulating microglial cells and are considered to be important modulators in MS. We thus wanted to examine the ability of cPGs to alter microglial activity after cytokine-induced activation. Administration of IFN- γ and TNF- α (IT) resulted in the production of NO in mouse primary microglial cells (Fig. 2A). The cPG 15d-PGJ₂ was able to repress the production of NO in a dose-dependent manner (Fig. 2A). There was no effect on cell viability with these treatments (Fig. 2B). Additionally, administration of PGA₂ also resulted in the dose-dependent suppression of NO levels when compared to IT alone (Fig. 2A). There was no effect on cell viability when compared to IT stimulation alone after PGA₂ treatment (Fig. 2B). The effective dose of PGA₂ in suppressing NO levels was again three- to fivefold higher than the dose of 15d-PGJ₂ required to suppress NO production.

Effects of Cyclopentenone Prostaglandins on Cytokine and Chemokine Production by Microglial Cells

To test the ability of cyclopentenone prostaglandins to alter the production of proinflammatory cytokines or chemokines, we utilized ELISA to examine the release of soluble factors from primary mouse microglia after stimulation with LPS (Fig. 3). Stimulation of microglia with LPS was able to induce the secretion of the cytokines TNF- α , IL-6, IL-1 β , and the chemokine MCP-1 (Fig. 3). Pretreatment of microglial cells with either 15d-PGJ₂ or PGA₂ was able to suppress the production of all cytokines examined. Additionally, both prostaglandins were effective in inhibiting the secretion of the chemokine MCP-1. In contrast to the dose parameters observed in the nitrite studies, PGA₂ was generally two- to threefold more effective as an inhibitor of both cytokine and chemokine secretion relative to 15d-PGJ₂.

Effects of Cyclopentenone Prostaglandins on NO, Cytokine, and Chemokine Production by Astrocytes

Activated astrocytes, like activated microglia, have also been implicated in the course of MS as they are able to produce NO and many proinflammatory cytokine and chemokines that may contribute to disease progression. We examined the ability of cPGs to modulate the inflammatory activity of activated primary mouse astrocytes. Stimulation of astrocytes with LPS was able to induce NO production (Fig. 4). Treatment with 15d-PGJ₂ or PGA₂ resulted in dose-dependent suppression of NO production. Similar to the effects in microglia, 15d-PGJ₂ was more effective than PGA₂ in suppressing NO production by stimulated astrocytes. There was no decrease in cell viability detected after LPS stimulation or after treatment with 15d-PGJ₂ or PGA₂ (data not shown).

Furthermore, LPS stimulated the production and release of the proinflammatory cytokines TNF- α , IL-1 β , IL-6, and the chemokine MCP-1 from primary mouse astrocytes as determined by ELISA analysis (Fig. 5). Similar to the effects on primary mouse microglia, both 15d-PGJ₂ and PGA₂ potently suppressed the production of these cytokines and chemokine (Fig. 5) from LPS-stimulated astrocytes. Additionally, PGA₂ was more effective than 15d-PGJ₂ in inhibiting cytokine and chemokine release from activated astrocytes.

DISCUSSION

MS is characterized by demyelination and neuronal injury that are a result of an immune response directed at antigens of the CNS (Lutton et al., 2004). Activated myelin-specific T cells are believed to play a role in the induction of this presumed autoimmune disease (Martin et al., 1992; Stinissen et al., 1998; Xiao and Link, 1999; Steinman, 2001; Yan et al., 2003). A major effector role for resident glial cells, including microglia and astroglia, however, has been identified in the pathology of MS (Benveniste, 1997; De Keyser et al., 2003). Inflammation leads to the activation of resident microglia and astrocytes and results in the production of reactive oxygen species, proinflammatory cytokines, and chemokines that can be directly or indirectly toxic to oligodendrocytes and neurons, perpetuate immune cell activation, and facilitate extravasation of immune cells into the CNS. Actions that result in the suppression of microglial and astroglial activation may thus provide potential avenues for the treatment of MS.

NO has a wide range of biological actions and has been implicated in numerous aspects of the inflammatory response (Willenborg et al., 1999). The role for NO in EAE and MS is somewhat controversial. Chronically activated glia are believed to produce high levels of NO, which is believed to contribute to the pathogenesis associated with these disorders; however, mice in which the iNOS gene is inactivated are more susceptible to EAE (Fenyk-Melody et al., 1998; Sahrbacher et al., 1998; van der Veen et al., 2003; Kahl et al., 2004). In the course of EAE or MS, NO may thus be protective or alternatively destructive.

The current study demonstrates that the cPGs 15d-PGJ₂ and PGA₂ are effective in inhibiting NO production by mouse microglia after stimulation with either LPS or IFN- γ /TNF- α . The ability of 15d-PGJ₂ to inhibit NO production has been described previously in mouse and rat macrophages (Ricote et al., 1998; Guyton et al., 2001; Alleva et al., 2002) and also in rat primary microglia (Bernardo et al., 2000; Drew and Chavis, 2001). Both PGA₂ and 15d-PGJ₂ had similar patterns of inhibition, but 15d-PGJ₂ was more potent, illustrated by the lower concentration required to inhibit NO production. This difference may be explained in part by the alternate pathways of action that exist between these two cPGs. Both cPGs are actively transported into the cell and are able to elicit biological actions via the reactive carbonyl group in the cyclopentenone ring structure; however, 15d-PGJ₂ is known to be a specific ligand for PPAR- γ , a nuclear receptor that is able to alter gene transcription after binding to specific

response elements in the promoter of target genes. In addition, 15d-PGJ₂ may also inhibit NO production by PPAR- γ independent mechanisms. For example, 15d-PGJ₂ was demonstrated previously to inhibit the activity of NF- κ B independently of PPAR- γ , but the potency of 15d-PGJ₂ increased when these cells were induced to express PPAR- γ (Ricote et al., 1998). Additionally, higher concentrations of 15d-PGJ₂ were required to inhibit iNOS expression in cells that lacked PPAR- γ when compared to cells that did express PPAR- γ (Petrova et al., 1999). Numerous studies have demonstrated that 15d-PGJ₂ is able to inhibit the expression of iNOS (Petrova et al., 1999; Bernardo et al., 2000; Koppal et al., 2000; Drew and Chavis, 2001; Chen et al., 2003); however, the ability of PGA₂ to alter this enzyme has not been studied to date.

Activated microglial cells are also the source of numerous proinflammatory cytokines that may contribute to the pathology of several inflammatory disorders in the CNS, including MS (Brosnan et al., 1995; Cannella and Raine, 1995; Benveniste, 1997). In MS, the expression of numerous cytokines, including IL-1 β , TNF- α , and IL-6 are found at very high levels in the area of active lesions (Cannella and Raine, 1995; Samoilova et al., 1998). These cytokines are involved in the induction and effector phases of EAE, notably in the recruitment of cells into the CNS and localized inflammatory and acute-phase responses in the brain (Merrill and Benveniste, 1996; Korner et al., 1997; Wang et al., 2002). The current study demonstrates that both 15d-PGJ₂ and PGA₂ are able to inhibit the production of proinflammatory cytokines including TNF- α , IL-6, and IL-1 β . Many studies have shown already that 15d-PGJ₂ is very effective in attenuating the expression of a number of proinflammatory cytokines from numerous sources, including rodent and human monocyte/macrophages (Jiang et al., 1998; Ricote et al., 1998; Thieringer et al., 2000; Guyton et al., 2001; Zhang et al., 2001; Alleva et al., 2002; Hinz et al., 2003) and rodent microglia (Bernardo et al., 2000; Koppal et al., 2000; Drew and Chavis, 2001). This is the first demonstration, however, of PGA₂ inhibition of proinflammatory cytokine production. In contrast to the relative effects on microglial NO production, PGA₂ seems to be more effective than 15d-PGJ₂ in downregulating proinflammatory cytokine production from activated microglial cells. This is surprising, considering the multifaceted actions that 15d-PGJ₂ has on gene expression. For example, it is known that 15d-PGJ₂ can alter DNA-binding activity of NF- κ B and STAT-1, transcription factors that regulate the expression of proinflammatory genes, via PPAR- γ -independent mechanisms or via receptor-dependent transrepression (Kielian and Drew, 2003). It is possible that the actions of both cPGs on microglial cytokine expression are largely through the reactive carbonyl group. The mechanisms of action of PGA₂ on cytokine expression are not known at this time, however, and represent an important avenue of further investigation.

The current study also shows that both 15d-PGJ₂ and PGA₂ are effective in downregulating the expression of the β chemokine MCP-1 by microglia. MCP-1 is primarily chemoattractant for T cells and monocytes (Smeltz and Swanborg, 1998; Kennedy and Karpus, 1999); however, MCP-1 is chemoattractant for dendritic cells, natural killer cells, and microglia as well (Mahad and Ransohoff, 2003). MCP-1 has been implicated in MS based on its localization to astrocytes in active lesions, cerebrospinal fluid, and serum of affected patients (McManus et al., 1998; Simpson et al., 1998; Sorensen et al., 1999; Van Der Voorn et al., 1999; Franciotta et al., 2001). The inactivation of MCP-1 has demonstrated the importance of this chemokine in directing monocytes to areas of inflammation and its importance in amplification rather than initiation of EAE (Hulkower et al., 1993; Fuentes et al., 1995; Glabinski et al., 1995; Kennedy et al., 1998). Both cPGs were effective in inhibiting MCP-1 secretion; however, the effect of PGA₂ on MCP-1 expression was greater than that of 15d-PGJ₂. This correlates with the greater effect of PGA₂ on inhibiting expression of the cytokines that we have examined. Cyclopentenone PGs are thus able to regulate the inflammatory response in part by regulating the expression of specific cytokines and chemokines from activated microglia.

In addition to the contribution of activated microglia, activated astrocytes also produce proinflammatory cytokines and NO that can lead to the pathology associated with MS (Becher et al., 2000; Dong and Benveniste, 2001; De Keyser et al., 2003). Additionally, the activation of astrocytes in the course of MS leads to the formation of astrocytic scarring in the chronic MS plaques that can produce an inhibitory environment and ultimately impede tissue repair (Zeinstra et al., 2000, 2003; Holley et al., 2003; Morcos et al., 2003). We thus examined the ability of the cPGs to alter the production of NO and proinflammatory cytokines and chemokines from activated astrocytes. We discovered that both 15d-PGJ₂ and PGA₂ were able to inhibit the production of NO, IL-1 β , TNF- α , IL-6, and MCP-1 from astrocytes stimulated with LPS. Astrocytes express PPAR- γ , suggesting that 15d-PGJ₂ may act at least in part through a PPAR- γ -dependent mechanism (Chattopadhyay et al., 2000; Cristiano et al., 2001; Klotz et al., 2003). Additionally, the ability of 15d-PGJ₂ to alter cytokine gene expression of both microglia and astrocytes has been linked to the regulation of the JAK-STAT pathway via the upregulation of suppressor of cytokine signaling (SOCS)-1 and -3 (Park et al., 2003). One previous study demonstrated that the administration of 15d-PGJ₂ was able to upregulate the expression of the neurotrophic factors nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) by mouse primary astrocytes (Toyomoto et al., 2004), suggesting multiple avenues of therapeutic action of cPGs on astroglial cells.

The current studies indicate that 15d-PGJ₂ inhibited NO production by microglia and astrocytes more potently than did PGA₂. PGA₂ is more potent than 15d-PGJ₂, however, in suppressing production of the cytokines and chemokine that we investigated in these glial cells. This may suggest that these cPGs differentially regulate the expression of genes encoding iNOS and the cytokines and chemokine studied; however, further studies are required to determine the molecular mechanisms resulting in the differential expression of these proinflammatory molecules.

In conclusion, we have demonstrated a novel function for PGA₂ as a potent mediator of antiinflammatory activity in microglia. Furthermore, we have shown that the cPGs PGA₂ and 15d-PGJ₂ also inhibit production of inflammatory cytokines and chemokines by astrocytes. These results emphasize the importance of cPGs in the regulation of the immune response, and further suggest that these PGs may be effective in the treatment of neuroinflammatory disorders including MS.

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Fig. 1.

The cyclopentenone prostaglandins 15d-PGJ₂ and PGA₂ inhibit LPS induction of NO by microglial cells. Primary microglia were plated at 40,000 cells/well in 96-well plates. The next day cells were pre-treated for 1 hr with the indicated concentrations of 15d-PGJ₂ or PGA₂ (in μM). LPS (0.5 μg/ml) was added as indicated and 24 hr later, the concentration of nitrite in the culture medium was determined (A). Cell viability was determined by MTT assay (B). Shown are the results of a representative experiment, and each experiment was conducted independently at least three times. Values represent the mean ± SEM for triplicate cultures.

**P* < 0.01 vs. LPS-treated cultures.

Fig. 2.

The cyclopentenone prostaglandins 15d-PGJ₂ and PGA₂ inhibit IFN- γ plus TNF- α induction of NO by microglial cells. Primary microglia were plated at 40,000 cells/well in 96-well plates. The next day cells were pretreated for 1 hr with the indicated concentrations of 15d-PGJ₂ or PGA₂ (in μM). IFN- γ (250 U/ml) and TNF- α (500 U/ml) were added as indicated and 24 hr later, the concentration of nitrite in the culture medium was determined (**A**). Cell viability was determined by MTT assay (**B**). Shown are the results of a representative experiment, and each experiment was conducted independently at least three times. Values represent the mean $\pm$ SEM for triplicate cultures. * $P < 0.01$ vs. IT-treated cultures.

Fig. 3.

The cyclopentenone prostaglandins 15d-PGJ₂ and PGA₂ inhibit cytokine and chemokine production by microglial cells. Primary microglia were plated at 40,000 cells/well in 96-well plates. The next day cells were pretreated for 1 hr with the indicated concentration of 15d-PGJ₂ or PGA₂ (in μM), then LPS (0.5 μg/ml) was added as indicated, and 24 hr later, IL-6 (A), IL-1β (B), TNF-α (C), and MCP-1 (D) levels were determined in culture media by ELISA. Shown are the results of a representative experiment, and each experiment was conducted independently at least three times. Values represent the mean ± SEM for triplicate cultures.

**P* < 0.01 vs. LPS-treated cultures.

Fig. 4.

The cyclopentenone prostaglandins 15d-PGJ₂ and PGA₂ inhibit LPS induction of NO by astrocytes. Primary astrocytes were plated at 50,000 cells/well in 96-well plates. The next day cells were pretreated for 1 hr with the indicated concentration of 15d-PGJ₂ or PGA₂ (in μM), then LPS (2 μg/ml) was added as indicated, and 24 hr later, the concentration of nitrite in the culture medium was determined. Shown are the results of a representative experiment, and each experiment was conducted independently a minimum of three times. Values represent the mean ± SEM of triplicate cultures. *P < 0.01 vs. LPS-treated cultures.

Fig. 5.

The cyclopentenone prostaglandins 15d-PGJ₂ and PGA₂ inhibit cytokine and chemokine production by astrocytes. Primary astrocytes were plated at 50,000 cells/well in 96-well plates. The next day cells were pretreated for 1 hr with the indicated concentration of 15d-PGJ₂ or PGA₂ (in μ M). LPS (2 μ g/ml) was added as indicated, and 24 hr later, IL-6 (**A**), IL-1 β (**B**), TNF- α (**C**), and MCP-1 (**D**) levels were determined in culture media by ELISA. Shown are the results of a representative experiment, and each experiment was conducted independently at least three times. Values represent the mean $\pm$ SEM of triplicate cultures. * P < 0.01 vs. LPS-treated cultures.