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The Cyclopentenone Prostaglandin 15d-PGJ2 Inhibits the NLRP1 and NLRP3 Inflammasomes

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Inflammasomes are cytosolic protein complexes that respond to diverse danger signals by activating caspase-1. The sensor components of the inflammasome, often proteins of the nucleotide-binding oligomerization domain–like receptor (NLR) family, detect stress, danger stimuli, and pathogen-associated molecular patterns. We report that the eicosanoid 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) and related cyclopentenone PGs inhibit caspase-1 activation by the NLR family leucine-rich repeat protein (NLRP)1 and NLRP3 inflammasomes. This inhibition was independent of the well-characterized role of 15d-PGJ2 as a peroxisome proliferator receptor-γ agonist, its activation of NF erythroid 2–related factor 2, or its anti-inflammatory function as an inhibitor of NF-κB. Instead, 15d-PGJ2 prevents the autoproteolytic activation of caspase-1 and the maturation of IL-1β through induction of a cellular state inhibitory to caspase-1 proteolytic function. The eicosanoid does not directly modify or inactivate the caspase-1 enzyme. Rather, inhibition is dependent on de novo protein synthesis. In a mouse peritonitis model of gout, using monosodium urate crystals to activate NLRP3, 15d-PGJ2 caused a significant inhibition of cell recruitment and associated IL-1β release. Furthermore, in a murine anthrax infection model, 15d-PGJ2 reversed anthrax lethal toxin-mediated NLRP1-dependent resistance. The findings reported in this study suggest a novel mechanism for the anti-inflammatory properties of the cyclopentenone PGs through inhibition of caspase-1 and the inflammasome. The Journal of Immunology, 2015, 194: 000–000.
activation of the NLRP3 inflammasome. These PGs inhibit inflammasome-mediated activation of caspase-1 and, as a consequence, prevent maturation and release of IL-1β, both in vitro and in vivo, in a manner independent of effects on NF-κB. Our findings confirm the role of these lipid mediators as anti-inflammatory agents and support their development as therapeutic agents for the treatment of inflammatory diseases.

Materials and Methods

Reagents

15d-PGJ2, CAY10410 (9,10-dihydro-15d-PGJ2), PGD2, PGE2, PGF2α, rosiglitazone, and T0070907 were purchased from Cayman Chemical (Ann Arbor, MI). PAγ1, PAγ2, 4-cyclopetenone-1,3-dione, 2-cyclopentenone, cycloheximide, actinomycin D, indomethacin, buthionine sulfoximine, and N-acetylcysteine and uric acid were from Sigma-Aldrich (St. Louis, MO). Cyclopentanone and cyclopentene were obtained from Tokyo Chemical Industry (Portland, OR). Structures of these prostanoids are shown in Table I. Nigenicin, lactacystin, and ultrapure LPS were purchased from Calbiochem (San Diego, CA).

Toxins

Protective Ag (PA), lethal factor (LF), and FP59 were purified from Bacillus anthracis as described previously (16, 17). FP59 is a fusion protein of the PA binding domain of LF to the ADP-ribosylation domain of Pseudomonas aeruginosa exotoxin A (18, 19). LF-Fla, a toxin also derived by PA, is a similar fusion of the first 254 aa of LF to full-length flagellin from Legionella pneumophila (gift of Dr. Russell Vance, University of California at Berkeley, Berkeley, CA) (20). FlaTox is the combination of LF-Fla and PA. LT is the combination of LF and PA. Concentrations of LT correspond to the concentration of each toxin component (i.e., 1 μg/ml LT is 1 μg/ml PA plus 1 μg/ml LF). Concentrations of FlaTox correspond to the concentration of LF-Fla. Concentration of PA was always twice that of LF-Fla in FlaTox experiments (i.e., 1 μg/ml FlaTox is 2 μg/ml PA plus 1 μg/ml LF-Fla).

B. anthracis spores were prepared from the nonencapsulated, toxigenic B. anthracis Ames 35 (A35) strain (21) by growing the bacteria on sporulation agar at 37°C for 1 d followed by 5 d at 30°C, and inspection by microscopy to verify ≥95% sporulation. Spores were purified from plates by four rounds of centrifugation and sterile water washes, followed by two heat treatments at 70°C for 30 min (to kill any vegetative bacteria). Spore quantification was performed using a Petroff Hauser counting chamber (Hauser Scientific, Horsham, PA) and verified by dilution plating.

Cell culture

RAW264.7 cells and L929 mouse fibroblast cells were grown in DMEM supplemented with 10% FBS, 10 mM HEPES, and 50 μg/ml gentamicin (all purchased from Life Technologies, Grand Island, NY). Selected studies used lower amounts of FBS as indicated in the figure legends. Monolayer bone marrow was cultured in complete DMEM (as above) supplemented with 30% L92 bone-conditioned supernatant and grown 7–9 d to allow time for differentiation to bone marrow–derived macrophages (BMDMs).

Animal studies

Mice were used as source of bone marrow. BALB/cJ (harboring Nlrp1bS/S and LT-responsive macrophages), C57BL/6J (harboring Nlrp1bS/S), and mice were used in experiments as indicated in the figure legends. Monolayer bone marrow was cultured in complete DMEM (as above) supplemented with 30% L92 bone-conditioned supernatant and grown 7–9 d to allow time for differentiation to bone marrow–derived macrophages (BMDMs).

Evaluating caspase-1 sequestration in a high m.w. complex

Sucrose buffer lysates of 15d-PGJ2–treated or heat-shocked (42°C) RAW264.7 cells were incubated with 1 U active recombinant mouse caspase-1 (MBL International, Woburn, MA) per 50 μl PBS for 2 h at 37°C. Western blotting was performed to verify caspase-1 sequestration in the supernatant and pellet.
Results

Cyclopentenone PGs require an α,β-unsaturated carbonyl to prevent NLRP1-dependent pyroptosis

Anthrax LT contains a protease that cleaves the N terminus of rodent NLRP1 proteins, leading to caspase-1 activation (28–30). Activation of caspase-1 leads to a rapid form of cell death known as pyroptosis (for review, see Ref. 31). LT activation of the NLRP1 inflammasome in selected inbred rodent strains that harbor LT-responsive alleles leads to pyroptosis, whereas strains with resistant NLRP1 alleles can undergo apoptosis (32–35). Our earlier studies showed that heat shock inhibits the inflammasome through sequestration of procaspase-1 in a large complex (27), preventing LT-mediated pyroptosis. We tested a number of cyclopentenone PGs that induce HSPs such as HSF70 (36, 37) along with cyclopentanone PGs for the ability to inhibit the NLRP1 inflammasome-dependent pyroptosis of LT-sensitive macrophages. All the cyclopentenone PGs tested inhibited the well-characterized NLRP1-dependent LT-induced pyroptosis. Interestingly, most of the cyclopentanone PGs that were tested failed to inhibit (Fig. 1A, Table I). The exception was the cyclopentanone PG PGD₂, which had low inhibitory activity (Fig. 1A, Table I); however, it has been shown that this PG undergoes nonenzymatic dehydration to the cyclopentenone PG PGJ₂ in aqueous media (38), and many of the purported physiological effects of PGD₂ are actually mediated by PGJ₂ and its downstream dehydration products such as 15d-PGJ₂ (39).

The restriction of inhibitory activity to only those PGs with cyclopentenone rings suggested a requirement for an α,β-unsaturated carbonyl. We further evaluated this requirement by examining the inhibitory activity of cyclopentene, cyclopentanone, 2-cyclopentenone, and various structural analogs (Table I). Cyclopentene and cyclopentanone, which have no α,β-unsaturated carbonyls, had no effect on LT-induced pyroptosis. However, 2-cyclopentenone, which has one carbonyl, did demonstrate inhibitory activity. Furthermore, 4-cyclopentene-1,3-dione, which has two α,β-unsaturated carbonyls, had a lower EC₅₀ than did 2-cyclopentenone. Finally, the 15d-PGJ₂ did demonstrate inhibitory activity. Furthermore, 4-cyclopentene-1,3-dione, which has two α,β-unsaturated carbonyls, had a lower EC₅₀ than did 2-cyclopentenone. Finally, the 15d-PGJ₂ does not inhibit LT cleavage of MEKs

To determine whether 15d-PGJ₂ protects against LT pyroptosis by inhibiting toxin translocation to the cytosol or enzymatic activity, we examined the cleavage of the cytosolic MEK substrates of LT. LT rapidly cleaves the N terminus of multiple MEK proteins, and this cleavage can be monitored by loss of an epitope (MEK1) and by altered mobility of the MEK3 protein (41, 42). We found that cleavage of the MEK proteins was uninhibited by treatment of the cells with 15d-PGJ₂, thus confirming that, in the presence of the drug, active LT translocated to the cytosol (Fig. 1C). Thus 15d-PGJ₂ does not impact the binding, endocytosis, translocation, or activity of LT.

15d-PGJ₂ inhibits cytokine processing of multiple inflammasomes

Inflammasome activation causes rapid autoproteolysis of caspase-1, which leads to processing and secretion of the proinflammatory cytokine IL-1β (1). 15d-PGJ₂ inhibits NF-κB phosphorylation by IC₅₀ as well as binding of NF-κB to DNA (5–7). NF-κB signaling is important for priming of the inflammasome. For all studies of inflammasome activation and cytokine processing, cells were primed with LPS before exposure to 15d-PGJ₂ to avoid any NF-κB signaling inhibitory effects that could lead to differences in priming and IL-1β levels. Using this method, no differences in inflammasome priming, indicated by pro–IL-1β levels, were observed in PG-treated cells in any experiments (as demonstrated by all Western blots). 15d-PGJ₂ inhibited caspase-1 autoproteolysis (monitored by generation of the p10 fragment) as well as maturation and secretion of IL-1β in LT-treated cells at a step subsequent to NF-κB signaling (Fig. 2A). Early studies have established that MEK signaling pathways that led to NF-κB activation (41, 42) are inactivated by LT, and, furthermore, inhibitors of NF-κB such as BAY11-7082, BAY11-7085, SN-50, and knockdown of p65 have previously been shown to have no effect on LT-mediated activation of NLRP1 (data not shown). Thus, the inhibitory effects of LT on caspase-1 were independent of effects on NF-κB. Interestingly, application of the drug up to 45 min after LT caused significant inhibition of cytokine processing, further confirming an...
effect independent of inhibition of NF-κB signaling. Inhibition of pyroptosis by 15d-PGJ2 required earlier application of the drug (Supplemental Fig. 1B, 1C). Importantly, the ability of 15d-PGJ2 to inhibit the inflammasome was not limited to NLRP1 or linked to pyroptosis. 15d-PGJ2 also inhibited cytokine processing and secretion in response to treatment with nigericin, an ionophore that activates the NLRP3 inflammasome (Fig. 2B). 15d-PGJ2 demonstrated only modest inhibition of the NLR family caspase-1 recruitment domain–containing protein 4/NLR family apoptosis inhibitory protein 5 inflammasome after stimulation with FlaTox (Fig. 2C). However, previous work has similarly found that FlaTox is more difficult to inhibit (43), possibly due to its ligand binding–based mechanism of activation (44, 45). These data indicate that 15d-PGJ2 is capable of inhibiting multiple inflammasomes with diverse activating stimuli.

15d-PGJ2 induces a cellular state inhibitory to caspase-1

Given the ability of this compound to inhibit multiple inflammasomes, we hypothesized that 15d-PGJ2 could inhibit caspase-1 directly. The α,β-unsaturated carbonyl makes cyclopentenone PGs highly electrophilic and capable of conjugation to cysteine residues on various proteins, altering their functions (4). Caspase-1, a cysteine protease, contains catalytically important thiol groups that could be modified. However, using recombinant active caspase in

Table I. EC50 values of structural variants of 15d-PGJ2

<table>
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<tr>
<th>Compound</th>
<th>EC50 (μM)</th>
<th>Structure</th>
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<tr>
<td>CAY10410</td>
<td>&gt;1000</td>
<td><img src="image11" alt="Structure" /></td>
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*Raw264.7 cells were treated 30 min with variable concentrations of compound. Cells were challenged with LT (1 μg/ml, 1.5 h) and cell viability was measured by MTT staining. At least two experiments represented by a single shown study were used to generate an EC50 value for each compound’s ability to inhibit LT-induced pyroptosis.

FIGURE 2. 15d-PGJ2 inhibits caspase-1 autoproteolysis and cytokine maturation of multiple inflammasomes. BALB/cJ BMDMs were primed with LPS (1 μg/ml, 2 h). Cells were then exposed to 15d-PGJ2 (50 μM, 30 min) followed by (A) LT (1 μg/ml), (B) nigericin (50 μM), or (C) FlaTox (1 μg/ml) for indicated times. Western blotting of cell lysates and culture supernatants was performed with Abs against the p10 fragment of caspase-1 or IL-1β. The asterisk (*) indicates a cross-reactive protein band that acts as an internal equal loading control. Results shown in these Western blots are representative of between 4 and up to 16 similar experiments per treatment.
activity was induced by 15d-PGJ2 in intact cells. A cellular state or protein that could inhibit caspase-1 enzymatic activity was induced by 15d-PGJ2 in intact cells.

Inflammasome inhibition by 15d-PGJ2 is dependent on protein translation

We previously reported that both heat shock and arsenical compound inhibition of inflammasome activation did not require de novo protein synthesis (27, 43). In the case of heat shock, procaspase-1 is trapped in a high m.w. complex that can be centrifuged away from the cytosolic cellular fraction (27). We found that 15d-PGJ2, a potent inducer of heat shock proteins (8), did not induce sequestration of caspase-1 into a high m.w. complex (Supplemental Fig. 2).

In contrast to the response to both heat shock and arsenical compounds, the transcription inhibitor actinomycin D and the translation inhibitor cycloheximide reversed the protective effects of 15d-PGJ2 in a dose-dependent manner (Fig. 4A, 4B). This requirement for transcription further supported the fact that 15d-PGJ2 manifested its inhibitory effects on the inflammasome in a manner independent of the eicosanoid’s inhibitory effect on NF-κB transcription. Similar results were found upon an even more complete enzymatic inhibition of translation using FP59, a fusion protein of an ADP-ribosylating enzyme that targets elongation factor 2 (Fig. 4C). Furthermore, inhibition of translation partially restored cytokine processing in 15d-PGJ2–treated cells (Fig. 4D). This evidence indicated that inflammasome inhibition by 15d-PGJ2 required de novo protein translation, either to induce a cytoprotective protein or to maintain a certain threshold level of a caspase-1 inhibitory protein with normally rapid turnover (the levels of which diminish after inhibition of protein synthesis). Interestingly, inhibition of NLRP3-mediated cytokine processing was not affected by cycloheximide treatment (data not shown), suggesting that the lipid mediator could have multiple inhibitory mechanisms.

Inhibition is independent of PPARγ, NRF2, or COX-1

15d-PGJ2 is a ligand of the nuclear receptor PPARγ (10, 11), which is known to have anti-inflammatory roles (14). We hypothesized that the eicosanoid could mediate its effects through this receptor. However, the PPARγ agonist rosiglitazone was unable to inhibit LT-induced pyroptosis (Fig. 5A). Furthermore, despite loss of the α,β-unsaturated bond in the ring, the 15d-PGJ2 analog CAY10410 retains the ability to activate PPARγ (46). This drug does not protect cells from LT-induced pyroptosis (Table 1). Similarly, these compounds were unable to prevent LT-induced (Fig. 5B) or nigericin-induced (data not shown) cytokine processing. Additionally, the PPARγ inhibitor T0070907 did not reverse the ability of 15d-PGJ2 to inhibit the inflammasome (Fig. 5C, 5D). Taken together, these data suggest that PPARγ signaling and transcriptional processes downstream of this receptor are not required for the effects of 15d-PGJ2 on the inflammasome.

15d-PGJ2 also activates the cap’n’collar family transcription factor NRF2 (12) by binding to cysteine residues of the NRF2 repressor KEAP1. NRF2 controls the expression of a large and diverse group of cytoprotective and anti-inflammatory genes (13), which we hypothesized could be inhibitory to inflammasome function. However, this seemed unlikely as NRF2 is stabilized and activated by proteasome inhibition (47–50), and treatment of macrophages with the proteasome inhibitor lactacystin does not prevent nigericin-based NLRP3-dependent cytokine processing (data not shown and Ref. 35). We tested the effects of 15d-PGJ2 in BMDMs lacking NRF2 and found that the drug was capable of inhibiting nigericin-induced cytokine processing, indicating that the effects of this eicosanoid on the inflammasome did not involve this transcription factor (Fig. 5E).

Recent studies have linked inflammasome activation with release of a variety of lipid mediators described as an “eicosanoid storm” (20). We hypothesized that modulation of this prostanoid release by exogenous 15d-PGJ2 could alter inflammasome function. However, 15d-PGJ2 inhibited LT-mediated pyroptosis and cytokine processing independent of COX-1, a central enzyme in the PG biosynthetic pathway (Fig. 6). Additionally, the cyclooxygenase inhibitor indomethacin did not reverse 15d-PGJ2–mediated protection from pyroptosis (Supplemental Fig. 3A).

Glutathione modulation does not alter inhibition

Cyclopentenone PGs form conjugates with cellular glutathione that can alter the physiological effectiveness of the PG (51). Increasing the levels of cellular glutathione using the glutathione precursor N-acetylcysteine or decreasing cellular levels of glutathione using the glutathione synthesis inhibitor buthionine sulfoximine had no effect on the protection of cells from LT-induced pyroptosis by 15d-PGJ2 (Supplemental Fig. 3B, 3C).

Cyclopentenone PGs are also potent inducers of intracellular reactive oxygen species (52), and oxidative stress is a potential mechanism of inflammasome regulation (53). However, the failure of N-acetylcysteine, a potent antioxidant, to alter 15d-PGJ2 protection suggests that induction of reactive oxygen species does not

**FIGURE 3.** 15d-PGJ2 does not inhibit caspase-1 enzymatic activity directly but induces a cellular state inhibitory to caspase-1. (A) RAW264.7 cells were primed with LPS (1 μg/ml, 2 h). Sucrose lysates were incubated with recombinant active caspase-1 (1 U/50 μl, 3 h, 37°C) in the presence or absence of 15d-PGJ2 at indicated concentrations or positive control caspase-1 inhibitor Boc-Asp(OBzI)-chloromethylketone (400 μM). (B) RAW264.7 cells were primed with LPS (1 μg/ml, 2 h) followed by treatment with 15d-PGJ2 (50 μM, 1 h). As above, sucrose lysates were incubated with recombinant active caspase-1. The asterisk (*) indicates a cross-reactive protein band that acts as an internal equal loading control. Western blot results are representative of at least three similar experiments.
play a role in the effects of 15d-PGJ2 on the inflammasome. Furthermore, alterations of oxidative state by 15d-PGJ2 are usually coupled with direct modification of proteins. We used a biotin-labeled 15d-PGJ2 analog coupled with immunoprecipitation and did not find any evidence that 15d-PGJ2 directly bound to NLRP3 (data not shown), suggesting that a novel inhibitory mechanism is operating that does not involve covalent adduct formation to NLRP3.

**FIGURE 4.** 15d-PGJ2–mediated inhibition of the NLRP1 inflammasome requires protein synthesis. (A) BALB/cJ BMDMs or (B) RAW264.7 cells were incubated with variable concentrations of cycloheximide or actinomycin D for 1 h. Cells were then exposed to 15d-PGJ2 (50 μM, 30 min) followed by LT (1 μg/ml, 1.5–2 h). (C) RAW264.7 cells were treated with 10 ng/ml PA with or without 100 ng/ml FPS9 for 2 h. PA receptor saturation in this cell type occurs at doses 20-fold higher than that used here (69). Thus, sufficient receptor remained on the cell surface to mediate LT intoxication. Unbound toxin was then washed from the cells, and the cells were incubated with 15d-PGJ2 and LT as above. Cell viability was measured by MTT staining. Each point was assayed in triplicate. Shown results are for a representative of two identical experiments. In (C), error bars represent SEM. (D) BALB/cJ BMDMs were treated with FPS9 (1 μg/ml, 3 h). Cells were then allowed to rest in LPS-free media for 1 h before cycloheximide treatment (2 μg/ml, 1 h). 15d-PGJ2 (50 μM, 30 min) followed by LT (1 μg/ml, 75 min) were then applied. Cell lysates were analyzed for IL-1β maturation by Western blot. The asterisk (*) indicates a cross-reactive protein band that acts as an internal equal loading control. Western blot results are representative of three similar experiments.

**FIGURE 5.** 15d-PGJ2 inflammasome inhibition is independent of PPARγ and NRF2. (A) RAW264.7 cells were incubated with variable concentrations of PPARγ agonist rosiglitazone for 1.5 h. Cells were exposed to LT (1 μg/ml, 2 h) and cell viability was assessed by MTT staining. (B) BALB/cJ BMDMs were primed with LPS (1 μg/ml, 2 h) followed by 15d-PGJ2, CAY10410, or rosiglitazone (50 μM, 30 min) and LT (1 μg/ml, 75 min). IL-1β in cell lysates and supernatants was assessed by Western blot. (C) RAW264.7 cells were treated with variable concentrations of PPARγ antagonist T0070907 for 1 h before treatment with 15d-PGJ2 (50 μM, 30 min). Following LT treatment (1 μg/ml, 2 h), cell viability was assessed by MTT staining. (D) RAW264.7 cells were primed with LPS (1 μg/ml, 2 h) followed by treatment with T0070907 (30 μM, 1 h). Cells were then incubated with 15d-PGJ2 (50 μM, 30 min) followed by LT (1 μg/ml, 1.5 h). (E) C57BL/6J wild-type and Nrf2−/− BMDMs were primed with LPS (1 μg/ml, 2 h) followed by 15d-PGJ2 (50 μM, 30 min) and then with nigericin (50 μM, 30 min). Cell lysates and culture supernatants were analyzed for IL-1β maturation by Western blot. In (A) and (C), each condition was assayed in duplicate. Curves are representative of at least two similar experiments. The asterisk (*) indicates a cross-reactive protein band that acts as an internal equal loading control.
15d-PGJ2 inhibits the inflammasomes in mice

To investigate the effects of 15d-PGJ2 in vivo, we used two models. The MU-induced peritonitis model used to replicate the inflammation associated with gout requires NLRP3 activation and caspase-1-dependent release of IL-1β for cell recruitment (24). We used this model in mice treated with vehicle or 15d-PGJ2 to assess the relative cell numbers recruited to the peritoneum that was significantly inhibited by the PG treatment (Fig. 7A). Similarly, the IL-1β recruitment of cells to the peritoneum was significantly decreased at the 6JNTac congenic mice, which are identical at 99% of loci but differ at the Nlrp1b locus, have striking different susceptibility to anthrax spore infection compared with strains (such as C57BL/6 mice) that express the Nlrp1bR variant (23). Similarly, C57BL/6NTac congenic mice with 15d-PGJ2 led to a rapid and striking sensitization to spore infection and a susceptibility comparable to mice harboring the Nlrp1bR/R allele (Fig. 7C, 7D). Because the effects of LT on susceptibility to spore are uniquely independent of NF-kB signaling, the 15d-PGJ2 effects in this model, unlike the MU cell recruitment model, are likely to be on a downstream inflammasome activation event required for IL-1β release.

**Discussion**

In this study, we show that cyclopentenone PGs inhibit activation of caspase-1 and maturation of IL-1β by the NLRP1 and NLRP3 inflammasomes. This inhibition is shown to be independent of the drugs’ inhibitory effects on NF-kB signaling and transcription and not due to interference with inflammasome-activating stimuli or direct modification of caspase-1. Inflammasome inhibition occurred instead through induction of a cellular state inhibitory to caspase-1 enzymatic activity. This cellular state was dependent on de novo protein translation but independent of the PPARγ or NLRP3 signaling and transcription pathways often associated with the actions of these PGs. Our results suggest that a novel mechanism and transcription pathway are responsible for the anti-inflammatory effects of these PGs.

There is substantial evidence that the reactive α,β-unsaturated carbonyl group is necessary for many of the biological activities of cyclopentenone PGs (4). The α,β-unsaturated carbonyl imparts a reactive electrophilic character to carbon 9 of the molecule. This allows the compound to form adducts with cellular thiols such as glutathione or cysteine residues on proteins (4). The α,β-unsaturated carbonyl appears to be necessary for inflammasome inhibition, as PGs lacking the cyclopentenone ring were inactive. Similarly, 2-cyclopentenone, but not the related compounds cyclopentene and cyclopentanone, was able to inhibit the inflammasome. This requirement for a chemically reactive center suggests that the mechanism of inflammasome inhibition is through conjugation to exposed thiol residues on a key inflammasome regulatory component. However, we found no evidence of direct inactivation of caspase-1 or NLRP3. The finding that de novo protein synthesis is required for the compound’s effects suggests that direct modification of the inflammasome is not likely, but we cannot rule out the possibility of 15d-PGJ2-mediated modification of an unknown yet important inflammasome component. Our finding that inhibition of NLRP3 was not dependent on protein synthesis suggests that the mechanism of 15d-PGJ2 inhibition differs across inflammasomes. This may be due to the varied biochemical mechanisms of inflammasome activation. NLRP1, for example, is activated in rodent macrophages through direct proteolytic cleavage by LT (28–30). NLR family caspase-1 recruitment domain–containing protein 4, in contrast, acts as an adapter for a number of NAIP proteins that directly bind to flagellin and flagellin-like proteins (44, 45), and NLRP3 responds to a wide range of danger signals through an as yet unresolved mechanism (1).

PPARγ and NRF2 are the primary transcriptional pathways activated by 15d-PGJ2 (15), and we hypothesized that one of them may play a role in the ability of this PG to inhibit the inflammasome. 15d-PGJ2 was the first identified endogenous ligand of PPARγ, and many of the biological effects of this PG result from PPARγ activation (10, 11). However, the compound also induces PPARγ-independent reactions. In fact, prior studies have demonstrated that 15d-PGJ2 has anti-inflammatory effects on macrophages deficient in PPARγ (54), and we found that inflammasome inhibition is also independent of PPARγ. A recent study demonstrated that NRF2-deficient BMDMs are impaired in inflammasome function (55). Although we also found a slight reduction of cytokine processing in NRF2-deficient BMDMs relative to wild-type
was used to assess impact of 15d-PGJ2 on the NLRP3 inflammasome. A well-established mouse monosodium urate induced peritonitis model PGJ2 or vehicle (10% DMSO in PBS) at 5 min prior and 4 h after ad-

4) or drug (\(\text{DMSO}\)) groups to MSU (plus 15d-PGJ2) groups are

assessed (\(\text{IL-1}\beta\)). The \(p\) values (unpaired \(t\) test) comparing the MSU (plus drug) and IL-1\(\beta\) levels in vehicle and infected with 2 \(\times 10^7\) A35 spores (C) or 4 \(\times 10^7\) A35 spores (D). Spore-sensitive NLRP1b\(^{R/R}\)-expressing C57BL/6\(l\) mice (\(n = 4\)) infected with the same spore dose served as controls. For (C), the \(p\) value comparing vehicle-treated, spore-infected BALB/c\(l\) to the 15d-PGJ2–treated infected BALB/c\(l\) group or infected C57BL/6\(l\) mice is \(<0.0001\). There is no significant difference between the infection susceptibility for drug-treated BALB/c\(l\) and genetically susceptible C57BL/6\(l\) mice infected with \(B.\ anthracis\) spores. For (D), the \(p\) value comparing vehicle-treated, spore-infected C57BL/6\(l\) Nlrp1b\(^{R/R}\) to 15d-PGJ2–treated infected group is 0.0065. The \(p\) value comparing vehicle-treated, spore-infected C57BL/6\(l\) Nlrp1b\(^{R/R}\) mice to similarly infected C57BL/6\(l\) Nlrp1b\(^{R/R}\) mice is 0.00429. The log-rank test was demonstrated that upon dilution into culture media containing FBS, most (97–99%) of the PG is inactivated and does not enter cells (40). Thus, the levels actually available for the cells in vitro are likely at physiological concentrations. Additionally, it can be postulated that systemic measurements of free PG in vivo are an underesti-
mation of true levels found within localized pockets of inflammation or in the cell vicinity, given the high reactivity of these eicosanoids with serum and intracellular proteins (60).

Exogenous administration of 15d-PGJ2 also has efficacy as an anti-inflammatory treatment (15). In the present study we show that 15d-PGJ2 can inhibit MSU-mediated neutrophil recruitment in an NLRP3-dependent inflammatory peritonitis model. Owing to the reactivity and resulting instability of cyclopentenone PGs, most experimental studies rely on administration of relatively high doses of 15d-PGJ2. Thus, alternative delivery mechanisms for the PG may increase its therapeutic potential. Loading of poly-
glycolide nanocapsules with the PG increased serum availability and improved the anti-inflammatory activity of 15d-PGJ2 in a peritonitis model (61). Retroviral overexpression of the PG synthase responsible for 15d-PGJ2 production has also been successful in a murine air-pouch model of acute inflammation, as well as in bleomycin-induced lung injury and scleroderma models (62–64). Synthetic derivatives of 15d-PGJ2 with similar biological activities have also been reported (65, 66).

Administration as an anti-inflammatory treatment in animal models of inflammation, including ischemic brain injury, LPS-
induced fever, spinal cord injury, chronic obstructive pulmonary disease, colitis, myocarditis, pancreatitis, and arthritis, has been reported (15). The mechanism of action of the prostanoi in these diverse inflammatory models may not be uniform. For example, tritoglaizone, a synthetic PPARγ ligand, was equally effective as 15d-PGJ2 at improving adjuvant-induced arthritis (67), whereas the protective effect of 15d-PGJ2 in a carrageenan-induced acute lung injury model was found to be NRF2-dependent (68). The inflammasome is known to play a role in a number of the diseases that this compound may be acting through its ability to inhibit IL-1β processing via the inflammasome. In this study, in addition to establishing the inhibitory effects of 15d-PGJ2 in a NLRP3-dependent peritonitis model, we were also able to cause a striking reversal of NLRP1 and caspase-1–dependent resistance of mice to anthrax spore infection by treatment with a single dose of the PG. These findings demonstrate that the anti-inflammatory effects of 15d-PGJ2 in vivo may be partially mediated through its impact on caspase-1 and the inflammasome pathways. In conclusion, we demonstrate that the cyclopentenone PGs inhibit the inflammasome. Most studies to date have explained the anti-inflammatory effects of 15d-PGJ2 by its effects on transcriptional pathways such as PPARγ, NRF2, and NF-κB. We suggest that a full explanation of the anti-inflammatory effects of 15d-PGJ2 must include its modulation of IL-1β release through inhibition of the inflammasome.

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Disclosures

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References


