Cyclo pent enone Prostagland ins Inhibit Cytokine-Induced NF-κB Activation and Chemokine Production by Human Mesangial Cells

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Abstract. In the kidney an uncontrolled inflammatory response to an acute insult may lead to chronic inflammation, permanent tissue damage, and progressive renal insufficiency. Resolution of acute inflammation likely is dependent on endogenous regulatory mechanisms activated in parallel with mediators of renal inflammation. These mechanisms are postulated to attenuate the renal expression of proinflammatory cytokines, including the chemokines responsible for recruiting leukocytes to the kidney, thus facilitating the transition from inflammation to healing. To understand the regulation of the inflammatory response within the kidney, the effects of anti-inflammatory J series cyclo pent enone prostaglandins on chemokine production by human mesangial cells were examined. Treatment of mesangial cells with prostaglandin J3 and 15-deoxy-Δ12,14-prostaglandin J2 blocked interleukin-1β-induced monocyte chemotactic protein-1 mRNA expression and protein production. This correlated with failure of the transcription factor nuclear factor-κB (NF-κB) to translocate to the nucleus and bind to its recognition motif, a step required for cytokine-induced monocyte chemotactic protein-1 gene activation. NF-κB failed to translocate because the cyclo pent enone prostaglandins attenuated degradation of the NF-κB inhibitor IκB-α. These data suggest that certain prostaglandins can limit the extent of renal chemokine expression and thus may have an important role in resolving renal inflammation.

Chemokines, such as monocyte chemotactic protein-1 (MCP-1), are induced in the kidney as a response to various renal insults (1). These chemokines are thought to play a critical role in the pathogenesis of renal inflammation by recruiting leukocytes to the kidney (2). We postulated that resolution of renal inflammation depends in part on intrinsic mechanisms that limit chemokine expression and thereby attenuate further infiltration of leukocytes into the kidney. The identities of such endogenous chemokine regulatory mechanisms are unknown.

Recent work suggests that certain J series prostaglandins derived from prostaglandin D2 (PGD2) may function as intrinsic regulators of inflammation. PGD2 undergoes dehydration to 9-deoxy-Δ9-PGD2 (PGJ2) in aqueous environments and is dehydrated further to 9-deoxy-Δ9,Δ12-dihydroprostaglandin D2 and 15-deoxy-Δ12,14-prostaglandin J2 (15-d-PGJ2) in the presence of protein (3,4). These J series prostaglandins have a cyclopentenone ring that contains a reactive, electrophilic carbon. This ring can bind covalently to intracellular nucleophiles, such as free sulfhydryls and cysteines, and in so doing modify protein function (3).

In addition, the cyclo pent enone prostaglandins (cyPG) are taken up by cells and incorporated into the nucleus (5). The potential anti-inflammatory role of cyPG is illustrated clearly in a mouse model of pleural inflammation. In this model, cyclooxygenase (COX) inhibitors worsened inflammation when used beyond the acute phase of disease (6). This was associated with a decrease in the concentration of PGD2 and 15-d-PGJ2 in the pleural space (6). Replacement of these prostaglandins led to resolution of inflammation (6).

Three lines of evidence suggest that cyPG of the J series are reasonable endogenous candidates for modulating renal chemokine expression: (1) J series cyPG are found in the kidney; murine mesangial cells make PGJ2 after treatment with interleukin-1β (IL-1) (7), and 9-deoxy-Δ9,Δ12-dihydroprostaglandin D3 is present in normal human urine (8); (2) 15-d-PGJ2 has been shown to block induction of nuclear factor-κB (NF-κB), a transcription factor necessary for chemokine gene transcription in most renal cell types (9–12); (3) 15-d-PGJ2 is a natural agonist of peroxisome proliferator-activated receptorγ (PPARγ) (13). PPARγ is a nuclear hormone receptor and transcription factor that has a role in regulating fatty acid homeostasis (4,13) and a role in glucose disposition that was realized when it became clear that the thiazolidinedione class of antidiabetic drugs are specific PPARγ ligands (14). Relevant to the theme of this investigation, recent studies have shown that PPARγ also has significant anti-inflammatory activities that include attenuating monocyte cytokine production, promoting macrophage apoptosis, and inhibiting NF-κB activation (15–18).
This investigation was undertaken to examine the effect of the cyPG 15-d-PGJ$_2$ on IL-1–induced production of MCP-1 by cultured human mesangial cells. In addition, because NF-κB is a key regulatory factor for mesangial MCP-1 expression, studies to determine whether 15-d-PGJ$_2$ modulates NF-κB activity were conducted. This work demonstrated 15-d-PGJ$_2$ to be a potent inhibitor of mesangial MCP-1 expression and NF-κB activation. 15-d-PGJ$_2$ acts at the level of IκB-α degradation but is not dependent on PPARγ for its antichemokine effects.

**Materials and Methods**

**Human Mesangial Cell Culture and Treatment**

Human mesangial cells were cultured from kidneys not suitable for transplantation. Mesangial cells from at least three different donors were isolated and characterized as we have previously described (19) and used between passages 4 and 7.

Mesangial cells were grown in RPMI-1640 plus 10% fetal calf serum (BioWhittaker, Walkersville, MD) and changed to RPMI-1640 plus 0.25% bovine serum albumin at the time of use. The cells were treated for the indicated times with 1.1 ng/ml human recombinant IL-1β (R&D Systems, Minneapolis, MN) in the presence or absence of cyPG. All prostaglandins were purchased from Cayman Chemical (Ann Arbor, MI), as solutions in methyl acetate. Ciglitazone, a PPARγ agonist, was purchased from Cayman Chemical (Ann Arbor, MI), as solutions in methyl acetate. Ciglitazone, a PPARγ agonist, was obtained from Biomol (Plymouth Meeting, PA) and dissolved in ethanol. Bisphenol A diglycidyl ether (BADGE), a synthetic PPARγ antagonist, was obtained from Fluka (Milwaukee, WI). In each experiment, the organic solvents were added to control or IL-1–treated cells at the same concentrations as cells treated with prostaglandins or ciglitazone. Mesangial cells were pretreated with prostaglandins or ciglitazone, as indicated in the individual experiments. Reagents used in this study were endotoxin free. The effects of the various treatments on mesangial cell viability were measured by trypan blue exclusion.

**Measurement of Mesangial Cell MCP-1 Expression**

MCP-1 mRNA was measured by Northern blotting of total mesangial cell RNA, as we have previously described (19). The MCP-1 probe was an XhoI fragment from phJE34 (American Type Culture Collection, Rockville, MD). The blots were reprobed for glyceraldehyde-3-phosphate dehydrogenase by use of a full-length human cDNA (Clontech, Palo Alto, CA), to confirm equal RNA loading.

Mesangial cell production of immunoreactive MCP-1 was determined by use of a modification (21) of the double-ligand enzyme-linked immunosorbent assay (ELISA) originally developed by Evanoff et al. (22). The capture antibody was a mouse monoclonal anti-human MCP-1 (R&D Systems, Minneapolis, MN). The capture antibody was a mouse monoclonal anti-human MCP-1 (R&D Systems, Minneapolis, MN). A horseradish peroxidase–conjugated goat anti-rabbit antibody (Zymed, South San Francisco, CA) to confirm specificity of the band identified as IκB-α bands. The blots then were washed and incubated with biotinylated goat anti-rabbit antibody (Zymed) for 45 min. After further washing, the bands of interest were demonstrated by use of 3,3′-diaminobenzidine tetrahydrochloride solution for detection.

**Electrophoretic Mobility Shift Analysis**

Nuclear proteins were harvested from mesangial cells as described previously (10). Ten μg of nuclear protein were incubated with 0.2 ng of double-stranded oligonucleotide probe containing a tandem repeat of the consensus NF-κB motif (5’-GATCCAGGGGATTCGCGTG-3’; Life Technologies BRL, Grand Island, NY). Probes were 32P-end–labeled with T4 polynucleotide kinase (Life Technologies BRL) plus γ-32P-ATP (3000 Ci/mmol; New England Nuclear, Boston, MA). Binding of the nuclear proteins to the NF-κB oligonucleotide was performed at 27°C in 25 μl of buffer containing 10 mM Tris (pH 7.5), 100 mM NaCl, 1 mM dithiothreitol, 5 mM ethylenediaminetetraacetic acid, 4% glycerol, and 2 μg poly(dI-dC) for 20 min. Oligonucleotide bound by NF-κB protein was separated from unbound oligonucleotide by electrophoresis on a 6% nondenaturing polyacrylamide gel in 0.25× Tris-borate-ethylenediaminetetraacetate buffer at 4°C. Transcription factors bound to the radiolabeled probes were identified by the appearance of bands with retarded mobility relative to unbound probe in autoradiographs of these gels. Specificity of the binding reaction was confirmed by use of excess (100-fold) unlabeled oligonucleotide to compete with labeled probes binding to nuclear proteins.

**Western Blot Analyses**

Prostaglandin-treated mesangial cells were lysed in 10 mM sodium dodecyl sulfate at specific times after addition of IL-1. Fifty μl of cell lysate were separated by electrophoresis on 15% polyacrylamide gels and transferred to nitrocellulose with the use of a semidyne technique (23). The nitrocellulose was dried and quenched in phosphate-buffered saline, 0.1% Tween-20 with 2% bovine serum albumin for 120 min. After washing, the nitrocellulose was blotted overnight at 4°C with a 1:1000 dilution of rabbit polyclonal antibodies that recognize either native IκB-α or IκB-α that had been phosphorylated on the serine residue at position 32 (New England Biolabs, Beverly, MA). Control blots (not shown) were probed with nonspecific rabbit IgG (Zymed, South San Francisco, CA) to confirm specificity of the band identified as IκB-α bands. The blots then were washed and incubated with biotinylated goat anti-rabbit antibody (Zymed) for 45 min. After further washing, the bands of interest were demonstrated by use of 3,3′-diaminobenzidine tetrahydrochloride solution for detection.

**Figure 1.** 15-deoxy-Δ12,14-prostaglandin J$_2$ (15-d-PGJ$_2$) attenuates interleukin-1 (IL-1)–induced monocyte chemotactic protein-1 (MCP-1) mRNA expression. Mesangial cells were pretreated with the indicated concentrations of 15-d-PGJ$_2$ for 2 h, followed by IL-1 (1.1 ng/ml) for 3 h. Total cellular RNA was isolated and probed for MCP-1 mRNA by Northern blot analysis. Blots were reprobed for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to ensure equal RNA loading. This blot is representative of five independent experiments.
enhanced chemiluminescence (Amersham Life Sciences, Inc., Arlington Heights, IL).

Nuclear proteins isolated from mesangial cells were immunoblotted with a rabbit polyclonal anti-PPARγ antibody (Affinity BioReagents, Golden, CO). Nonimmune rabbit IgG served as a control. Blots were developed by use of enhanced chemiluminescence, as described above.

Statistical Analyses

Data are presented as mean ± SEM. Comparisons were made with the use of the paired t test. P < 0.05 was considered significant.

Results

15-d-PGJ2 Inhibits Mesangial MCP-1 Expression

Induction of MCP-1 mRNA by IL-1 was measured in human mesangial cells treated with 15-d-PGJ2. As shown in Figure 1, 15-d-PGJ2 caused a dose-dependent inhibition of MCP-1 mRNA expression, with complete inhibition occurring between 25 and 50 μM 15-d-PGJ2. Treatment of mesangial cells with the prostaglandin alone had no effect on baseline MCP-1 mRNA levels or the expression of the glyceraldehyde-3-phosphate dehydrogenase housekeeping gene (not shown). Consistent with blocking MCP-1 mRNA expression, 15-d-PGJ2 also inhibited the secretion of immunoreactive MCP-1 by mesangial cells with an IC50 of approximately 14.5 μM (Figure 2). Under these experimental conditions, 15-d-PGJ2 had no effect on cell viability assessed by trypan blue exclusion.

15-d-PGJ2 attenuates IL-1–induced nuclear factor-κB (NF-κB) activation. Mesangial cells were pretreated with the indicated concentrations of 15-d-PGJ2 for 2 h. IL-1 (1.1 ng/ml) was then added for 1 h. Nuclear proteins were harvested, and binding to a radiolabeled oligonucleotide containing the consensus NF-κB binding motif was determined by use of electrophoretic mobility shift analysis. Specificity of binding was confirmed by use of a 100-fold excess of unlabeled oligonucleotide to compete for binding (com). Unbound oligonucleotide migrated to the bottom of the gel (arrow), whereas migration of oligonucleotide bound to NF-κB was retarded. Representative of five experiments.

Figure 1. 15d-PGJ2 attenuates IL-1–induced nuclear factor-κB (NF-κB) activation. Mesangial cells were pretreated with the indicated concentrations of 15-d-PGJ2 for 2 h. IL-1 (1.1 ng/ml) was then added for 1 h. Nuclear proteins were harvested, and binding to a radiolabeled oligonucleotide containing the consensus NF-κB binding motif was determined by use of electrophoretic mobility shift analysis. Specificity of binding was confirmed by use of a 100-fold excess of unlabeled oligonucleotide to compete for binding (com). Unbound oligonucleotide migrated to the bottom of the gel (arrow), whereas migration of oligonucleotide bound to NF-κB was retarded. Representative of five experiments.
15-d-PGJ2 Inhibits NF-κB Activation in IL-1–Treated Mesangial Cells

In mesangial cells, the transcription factor NF-κB seems to be necessary for activation of the MCP-1 gene by IL-1, and most manipulations that downregulate mesangial MCP-1 also prevent NF-κB activation (10,24,25). This was found to be true for 15-d-PGJ2 as well. Mobility shift analysis of nuclear proteins from mesangial cells treated with IL-1 plus varying concentrations of 15-d-PGJ2 demonstrated a dose-dependent decrease in nuclear protein binding to a consensus NF-κB recognition motif (Figure 3). This effect was apparent with 10 μM 15-d-PGJ2, and 25 μM caused complete inhibition. The effects of 15-d-PGJ2 were not limited to cells activated by IL-1; a similar inhibition of NF-κB induction after phorbol ester treatment also was observed (data not shown). 15-d-PGJ2 alone had no effect on mesangial NF-κB activation.

To examine the mechanism of 15-d-PGJ2-mediated NF-κB inhibition, we looked at the effects of this prostaglandin on the NF-κB inhibitory protein IκB-α. Degradation of IκB-α by the 26S proteasome complex is necessary for NF-κB nuclear translocation and occurs after phosphorylation of IκB-α serine residues at positions 32 and 36 and ubiquitination of the protein (26). As shown in Figure 4A, IL-1 induced a rapid (within 2 to 5 min) phosphorylation of serine 32 of IκB-α, followed by a decline in the phospho–IκB-α level within 10 min that was progressive and consistent with degradation of this protein. In cells treated with IL-1 plus 15-d-PGJ2, the phosphorylation of IκB-α was slower, occurring within 5 to 10 min, and there was no tendency for the level of phospho–IκB-α to decline after peak phosphorylation (Figure 4A). Levels were maintained for 20 min. To confirm an impairment in IκB-α degradation by 15-d-PGJ2, we immunoblotted cells for total IκB-α (Figure 4B). Cells treated with IL-1 alone showed a near absence of IκB-α 10 to 20 min after IL-1 was added. IκB-α levels began to recover by 30 to 60 min (not shown), as we documented previously (10). In contrast, this rapid and profound decline in IκB-α levels was not observed in cells treated with IL-1 plus 15-d-PGJ2, which showed only a mild decrease in the level of IκB-α after 15 to 20 min of incubation (Figure 4B).

Effect of Other Prostaglandins on Mesangial Cells

To determine whether the modulation of mesangial cell activation is specific to 15-d-PGJ2, we incubated cells with other 15-deoxy-Δ12,14-prostaglandin derivatives, or PGJ2, the
parent prostaglandin of 15-d-PGJ₂. Like 15-d-PGJ₂, 15-d-PGA₁ and 15-d-PGA₂ are cyPG, formed by the dehydration of PGE₁ and PGE₂, respectively. As shown in Figure 5, 15-d-PGA₁, 15-d-PGA₂, and 15-d-PGD₂ had little effect on IL-1–induced NF-κB activation or MCP-1 mRNA expression at concentrations that were effective for 15-d-PGJ₂. Consistent with these results, 15-d-PGA₁, 15-d-PGA₂, and 15-d-PGD₂ did not block MCP-1 protein production by mesangial cells (data not shown). PGJ₂ did, however, cause a dose-dependent inhibition of NF-κB activation similar to that of 15-d-PGJ₂ (Figure 6). PGJ₂ also blocked IL-1–induced MCP-1 mRNA expression (Figure 5), and incubation with 10 μM PGJ₂ overnight reduced mesangial MCP-1 secretion by 45% (32.7 ± 2.5 ng/ml versus 58.9 ± 14.9 from cells treated with IL-1 alone). Although PGD₂ is not a cyPG, it was predicted that it could inhibit NF-κB activation if it were metabolized to J series prostaglandins. Although the brief administration (2 h) of PGD₂ in concentrations up to 50 μM had little effect on IL-1–induced NF-κB activation, sustained exposure (up to 18 h) did inhibit NF-κB (Figure 6), consistent with a requirement for the metabolism of PGD₂. The 18-h treatment with PGD₂ had no effect on cell viability, assessed by trypan blue exclusion and MTT assay.

**Effects of 15-d-PGJ₂ Are Not Mediated through PPARγ**

15-d-PGJ₂ is a potent ligand and activator of PPARγ. In some cell types, such as monocytes, activation of PPARγ by 15-d-PGJ₂ seems to block the expression of NF-κB–dependent cytokine genes. Two approaches were used to determine whether the effects of 15-d-PGJ₂ on mesangial MCP-1 production were PPARγ dependent. First, mesangial cells were treated with IL-1 in the presence or absence of ciglitazone, a thiazolidinedione and specific PPARγ agonist (14). As shown in Figure 7, A and B, treatment of mesangial cells with ciglitazone in concentrations as high as 100 to 200 μM had no effect on IL-1–induced MCP-1 mRNA expression or NF-κB expression. This was not because of a lack of expression of PPARγ by human mesangial cells. PPARγ was found to be constitutively present in nuclear protein extracts of untreated mesangial cells and seemed to increase after ciglitazone (10 μM) treatment (Figure 7C). The apparent molecular mass of the protein identified by the anti-PPARγ antibody is 51.7 kD (n = 5), consistent with the molecular mass of the PPARγ1 isoform (27–29).

In a second set of experiments, mesangial cells were pretreated with BADGE, a synthetic PPARγ antagonist (20), to determine whether the inhibitory effect of 15-d-PGJ₂ could be reversed. BADGE is a competitive inhibitor of PPARγ, binding to but not activating this receptor (20). As shown in Figure 8, BADGE was unable to reverse the effect of 15-d-PGJ₂ on NF-κB activation. Although it seems that NF-κB binding was reduced further in cells treated with both BADGE and 15-d-PGJ₂, compared with 15-d-PGJ₂ alone (Figure 8), BADGE had no effect on IL-1–induced NF-κB activation (data not shown).
Chemokines are potent proinflammatory agents that contribute to the development of renal inflammation after immune and nonimmune renal injury (1). We postulated that to limit parenchymal damage from inflammation and prevent progressive renal insufficiency, intrinsic mechanisms are activated during the inflammatory response to modulate inflammation by attenuating chemokine expression. COX-2 products, including cyPG, are considered to have anti-inflammatory effects (6,30). The data from the present study support a role for J series cyPG in the endogenous regulation of renal chemokine expression. This conclusion is based on our observation that 15-d-PGJ2 and PGJ2 block mesangial cell MCP-1 expression and NF-κB activation in response to proinflammatory stimuli, along with published reports indicating that J series prostaglandins can be produced by renal cells (7) and are found in human urine (8). That PGJ2 and 15-d-PGJ2 also prevent nuclear translocation of NF-κB suggests that these prostaglandins may downregulate not only MCP-1 expression but also the transcription of other chemokines and cytokines that require NF-κB.

15-d-PGJ2 seems to interfere directly with IkB-α processing. Although IL-1–induced IkB-α phosphorylation was slower in 15-d-PGJ2–treated cells, the main inhibitory effect was at the level of IkB-α degradation. Preventing the degradation of IkB-α provides a mechanistic explanation for 15-d-PGJ2–mediated inhibition of NF-κB nuclear translocation. These data suggest further that 15-d-PGJ2 interferes with either the process of IkB-α ubiquitination after its phosphorylation, a step necessary for proteasomal degradation, or the function of the 26S proteasome complex, which is responsible for digesting IkB-α (26). Which of these novel activities is to be added to the growing list of ways that 15-d-PGJ2 interferes with the NF-κB activation cascade remains to be determined. At present, it seems that, in some cells, the reactive cyclopentenone ring may alkylate cysteine residues in the p50 and p65 subunits of NF-κB and thus block DNA binding (12). Alternatively, a number of recent reports demonstrated that IkB-α phosphorylation is prevented by 15-d-PGJ2, because 15-d-PGJ2 directly blocks the activity of IkB kinases (11,12,31). This presumably occurs via covalent modification of critical residues of IkB kinase by the cyclopentenone ring. It is conceivable that cyPG could modify the structure of the proteasome in this manner and alter its function. The exact mechanism(s) involved in NF-κB inhibition may depend on the specific cell type. Indeed, one investigation showed that despite clear inhibition of IkB kinase activity in a macrophage cell line, 15-d-PGJ2 had no effect on IkB kinase activity in HeLa cells (12). Studies to evaluate proteasome function in 15-d-PGJ2–treated mesangial cells are under way in our laboratory.

Although 15-d-PGJ2 is a potent agonist of PPARγ and human mesangial cells express this receptor, this study found no evidence supporting a role for PPARγ in the modulation of MCP-1 or NF-κB by 15-d-PGJ2 in mesangial cells. In contrast, there is a report that the thiazolidinedione troglitazone attenuates MCP-1 production in mesangial cells (32). Many effects of troglitazone may, however, be drug specific rather than class specific and do not necessarily involve PPARγ (33). In our system, the thiazolidinedione cigitazone did not reproduce the effects of 15-d-PGJ2, nor were the effects of 15-d-PGJ2 inhibited by a PPARγ antagonist. Similar conclusions have been reached by other investigators (11,31,34), although it seems clear that PPARγ can modulate NF-κB activity and cytokine expression in at least some types of cells (17,18,35). This may depend on the level of PPARγ expression in a given cell type.

**Figure 6.** The effect of PGJ2 and PGD2 on IL-1–induced NF-κB activation. Mesangial cells were pretreated with the indicated concentrations of PGJ2 or PGD2 for 2 h (A and B) or 10 μM PGD2 (C) for the indicated times (hours). IL-1 (1.1 ng/ml) was added for 1 h before harvest of nuclear proteins. NF-κB binding was measured by electrophoretic mobility shift analysis, as in Figure 3. Representative of three experiments.
This also raises the interesting possibility that therapeutically increasing PPARγ levels in a tissue compartment may augment the intrinsic anti-inflammatory effects of the cyPG.

The exact J series prostaglandins that are functional in vivo in the kidney remain to be determined. Although there is some evidence that 15-d-PGJ2 is produced at sites of inflammation (6), other investigators have questioned whether PGJ2 is metabolized to 15-d-PGJ2 in vivo (36). In addition, although the effects of 15-d-PGJ2 seem to be due to its reactive cyclopentenone ring (12), it is clear from our data that not all cyPG are equally active, at least in human mesangial cells. 15-d-PGA1 and 15-d-PGA2 were less effective inhibitors of IL-1–induced NF-κB activation and MCP-1 expression than 15-d-PGJ2. It is interesting that other studies also showed J series cyPG to be more potent when compared with A series cyPG (34,37).

Despite this selectivity, the concentration of 15-d-PGJ2 required to inhibit cytokine expression seems to be cell specific. In some cells, cytokine expression and NF-κB activation were inhibited by 15-d-PGJ2 with IC50s of <5 μM (11,17). In other cells, the concentration of 15-d-PGJ2 for NF-κB and cytokine inhibition was found to be in the 5- to 30-μM range (35,38), similar to that of the mesangial cell.

The data from this study may help explain the observation that COX inhibitors can worsen renal inflammation in experimental models of nephritis. In anti–glomerular basement membrane antibody nephritis and anti–Thy-1 nephritis, administration of nonselective COX inhibitors increased glomerular leukocyte infiltration and MCP-1 expression (39,40). Timing of the administration of COX inhibitors may determine whether these agents treat or exacerbate inflammation. For example, in the model of pleural inflammation discussed previously (6), two peaks of COX-2 activity were observed. An early peak was associated with production of proinflammatory prostaglandins, whereas a delayed rise in COX-2 activity was associated with the production of PGD2 and cyPG. Appropriate use of COX inhibition in humans may require a detailed analysis of the time course of appearance of specific prostaglandins.

Activation of cyPG production in the kidney thus may be an endogenous mechanism that contributes to the resolution of the renal inflammation by attenuating NF-κB–dependent proinflammatory gene expression. It is tempting to speculate that clinically observed variations in tissue injury in response to inflammatory stimuli are determined by the level of J series prostaglandins that an individual is able to produce. Supporting the feasibility of this idea, a defect in the production of PGJ2 has been described in mesangial cells from lupus-prone mice (7). Such defects may lead to a more severe disease phenotype because of uncontrolled inflammation. Understanding how to augment the production of cyPG in human disease therefore may prove therapeutically useful in treating renal inflammation.
IL-1 | - + + + + + + + +
15dPGJ2 (10μM) | - - + + - - - - - -
15dPGJ2 (25μM) | - - - - + - - - - - -
BADGE (100 μM) | - - - + + + + + + +
Competitor | - - - - - - - - - - -

NF-κB

Figure 8. The PPARγ antagonist bisphenol A diglycidyl ether (BADGE) does not reverse the inhibitory effects of 15-d-PGJ2 on IL-1–induced NF-κB activation. Mesangial cells were pretreated with BADGE for 2 h, followed by the indicated concentrations of 15-d-PGJ2 for 2 h. IL-1 (1.1 ng/ml) was added, and, after 1 h, nuclear proteins were isolated, and NF-κB binding activity was measured by electrophoretic mobility shift analysis. Representative of two experiments. In a third experiment (not shown), BADGE did not reverse the effect of 15-d-PGJ2 even after pretreating for 24 h.

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