Biochemical and Biophysical Research Communications **281,** 57–62 (2001)
doi:10.1006/bbrc.2001.4301, available online at http://www.idealibrary.com on IDEAL®

A Cyclopentenone Prostaglandin Activates Mesangial MAP Kinase Independently of PPARγ

William A. Wilmer, Cynthia Dixon, Ling Lu, T. Hilbelink, and Brad H. Rovin[1]
Department of Internal Medicine, Nephrology Division, and Heart Lung Research Institute, Ohio State University School of Medicine and Public Health, Columbus, Ohio 43210

Received December 29, 2000

The mitogen-activated protein (MAP) kinases mediate the response of renal glomerular mesangial cells to a variety of physiologic and pathologic stimuli. This investigation examines the effect of the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) on MAP kinases in human mesangial cells. We show that 15d-PGJ_2 dose-dependently increases the extracellular signal-regulated kinase (ERK) activity of human mesangial cells, but has no effect on Jun-NH_2-terminal kinase or p38 MAP kinase. Despite the fact that 15d-PGJ_2 is a peroxisome proliferator-activated receptor (PPAR) ligand, and PPARγ is shown to be expressed by mesangial cells, the thiazolidinedione PPARγ agonist ciglitazone does not activate ERK. Additionally, a synthetic PPARγ antagonist does not attenuate the activation of ERK by 15d-PGJ_2. 15d-PGJ_2-mediated ERK activation is however blocked by the MEK inhibitor PD 098059, appears to require phosphatidylinositol-3 kinase, but is independent of protein kinase C activation. These results demonstrate a novel effect of 15d-PGJ_2 to induce ERK in human mesangial cells independently of PPARγ. © 2001 Academic Press

Key Words: **cyclopentenone prostaglandin; mitogen-activated protein kinase; PPAR; mesangial cell; glomerulus; extracellular signal-regulated kinase; thiazolidinedione; phosphatidylinositol-3 kinase.**

The mesangial cell plays a central regulatory role in glomerular biology. Induction of mesangial mitogen-activated protein (MAP) kinases is a key step in the pathogenesis of glomerulosclerosis, a severe form of glomerular injury that occurs in response to a number of renal insults, including diabetes and glomerulonephritis. MAP kinases are serine/threonine kinases that regulate a variety of processes, including cell growth, proliferation, apoptosis, and extracellular matrix accumulation (1–8). We and others have shown that extracellular signal-regulated kinase (ERK), Jun-NH_2-terminal kinase (JNK), and p38 MAP kinase can be activated in human mesangial cells by cytokines and growth factors (7, 9, 10). Other (patho)physiologic regulators of mesangial MAP kinases remain to be characterized.

The naturally occurring cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) is derived from prostaglandin D_2 through a series of dehydration steps that occur in aqueous environments (11, 12). Other intermediates in this pathway include PGJ_2 and Δ^{12}-PGJ_2. The J series prostaglandins are unique in having a cyclopentenone ring that contains a reactive, electrophilic carbon. Because cyclopentenone prostaglandins are taken up by cells and incorporated into nuclei (13), the cyclopentenone ring can bind covalently to intracellular nucleophiles such as free sulfhydryls and cysteines, and in so doing modify protein function (11). This may account for the ability of 15d-PGJ_2 to block activation of the transcription factor nuclear factor-kappaB (NF-κB), and thereby the expression of NF-κB-dependent genes (14–16). 15d-PGJ_2 is also a potent agonist of the peroxisome proliferator-activated receptor (PPAR)-γ (17), a nuclear hormone receptor and transcription factor that has a role in regulating fatty acid homeostasis (17, 18). 15d-PGJ_2 can therefore regulate adipocyte differentiation via PPAR responsive genes involved in lipid metabolism (19).

Evidence that 15d-PGJ_2 modulates MAP kinase activity is conflicting. It has been shown that 15d-PGJ_2 activates JNK in untreated Hela cells (16), but blocks IL-1-induced JNK phosphorylation in rodent pancreatic islets (20). Similarly, induction of macrophage apoptosis by 15d-PGJ_2 was shown to depend on the p38 MAP kinase, however 15d-PGJ_2 appeared to decrease phosphorylation of p38 (21), a step necessary for its activity. These data imply that the effects of 15d-PGJ_2 on MAP kinases may be cell-context specific. Although J series prostaglandins are present in human urine, and may be made by mesangial cells (22, 23), there are no data regarding modulation of human mesangial cell

[1] To whom correspondence should be addressed at Nephrology Division, Ohio State University, N210 Means Hall, 1654 Upham Drive, Columbus, OH 43210. Fax: 614-293-3073. E-mail: rovin.1@osu.edu.

0006-291X/01 $35.00

MAP kinase activity by 15d-PGJ_2. The current investigation was undertaken to examine this issue. This report shows that 15d-PGJ_2 specifically activates p42 and p44 ERK in human mesangial cells through a PPARγ-independent mechanism.

METHODS

Human mesangial cell cultured. Human mesangial cells were cultured from kidneys not suitable for transplantation, and characterized as previously described (24). Cells from at least three different donors were used between passages 4 and 7. Cells were grown to confluence in RPMI 1640 containing 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY). Before treatment, cells were washed to remove serum. Experiments were performed in media consisting of serum free RPMI 1640 plus 0.25% bovine serum albumin (SF media).

Pharmacological treatments. To activate MAP kinases, cells were treated with 1.1 ng/ml human recombinant interleukin-1β (IL-1, R & D Systems, Minneapolis, MN) for 30 min, or with 25 nM of the phorbol ester phorbol 12-myristate 13-acetate (PMA) (9, 10). 15d-PGJ_2 was purchased from Cayman Chemical (Ann Arbor, MI) as a methyl acetate solution. Ciglitazone was obtained from Biomol (Plymouth Meeting, PA), and dissolved in ethanol. In each experiment vehicle was added to control or IL-1-treated cells at the same concentration as cells treated with 15d-PGJ_2 or ciglitazone. Mesangial cells were treated for 2 h before harvest, or before the addition of IL-1. In some experiments PPARγ was blocked by the synthetic PPARγ antagonist bisphenol A diglycidyl ether [BADGE (25)]. BADGE was added to cells 2 h before 15d-PGJ_2. In other experiments cells were preincubated for 1 h with PD 098058 (Calbiochem, San Diego, CA), a specific inhibitor of the ERK kinase MEK (9, 10). To test the role of protein kinase C (PKC) and phosphatidylinositol-3 (PI-3) kinase in 15d-PGJ_2-mediated ERK activation, cells were pretreated with GF109203X (GFX, Calbiochem) or LY294002 (Calbiochem), PKC and PI-3 kinase inhibitors, respectively.

Determination of PPARγ expression by human mesangial cells. Mesangial PPARγ expression was examined at the RNA and protein levels. Total RNA was isolated, reverse transcribed to cDNA, and amplified with the following PPARγ primers: 5′-GGCAATTGAATGTCGTGTCTGTGGAGATAA-3′ and 5′-AGCTCCAGGGCTTGTAGCAGGTTGTCTTGA-3′ (expected size: 900 bp). RT-PCR products were separated on agarose gels and visualized by ethidium bromide staining. Nuclear protein (10 μg) was isolated from mesangial cells (26), resolved on 10% SDS–polyacrylamide gels under reducing conditions, and electroblotted onto 0.45-μm nitrocellulose membranes (27). PPARγ was identified with a rabbit polyclonal anti-PPARγ antibody (Affinity BioReagents, Golden, CO). Nonimmune rabbit IgG served as a control. The secondary antibody was a biotinylated goat anti-rabbit IgG (Zymed, San Francisco, CA). Blots were developed using enhanced chemiluminescence (ECL, Amersham, Arlington Heights, IL).

Measurement of MAP kinase activation. MAP kinases must be tyrosine/threonine phosphorylated for activation. We have previously shown that immunoblotting mesangial cell lysates for the tyrosine or tyrosine/threonine phosphorylated forms of ERK, JNK, or p38 MAP kinase correlates with activation of these MAP kinases (9, 10). Thus, immunoblotting for phospho-ERK, JNK, and p38 MAP kinase was used as the initial test of MAP kinase activation. For these experiments mesangial cells were rapidly lysed after treatment using an SDS lysis buffer (2% SDS, 1 mM EDTA, 1 mM PMSF, 20 μM leupeptin and 0.15 u/ml aprotinin). Cell lysates were briefly sonicated at 4°C and equivalent amounts promptly separated on 10% SDS–polyacrylamide gels under reducing conditions. After transfer to nitrocellulose the membranes were probed overnight at 4°C with rabbit polyclonal anti-phospho-ERK, anti-phospho-JNK, or anti-phospho-p38 MAP kinase antibodies, which recognize the epitope of activation of each MAP kinase protein (New England Biolabs, Beverly, MA). Equivalent amounts of lysate were also probed with antibodies that recognize total (phosphorylated and unphosphorylated) ERK, JNK, and p38 MAP kinase (Santa Cruz Biotechnology, Santa Cruz, CA), to verify that any increase in the phosphorylated MAP kinase was not do to an absolute increase in total MAP kinase. The MAP kinases were visualized with ECL as above.

ERK activation was verified by modification of a method we previously used (10). Briefly, p42 and p44 ERK were immunoprecipitated from 250 μg of cell lysate of appropriately treated mesangial cells using a rabbit polyclonal anti-pan-ERK antibody covalently conjugated to Protein A–agarose (Upstate Biotechnology, Lake Placid, NY) for 120 min at 4°C. After several washes with cold PBS, this immunoprecipitate was added to kinase buffer containing 500 μM ATP and 20 μg purified bovine brain myelin basic protein (MBP). The kinase reaction was allowed to proceed for 20 min at 30°C and was halted by the addition of 5 μl SDS loading buffer and boiling for 5 min. The MBP was separated by 12% SDS–PAGE under reducing conditions and transferred to nitrocellulose. Changes in MBP threonine phosphorylation induced by ERK were identified by immunoblotting with an anti-phospho-MBP antibody (Upstate Biotechnology) overnight at 4°C. After the addition of appropriate secondary antibodies, phosphorylated MBP bands were identified by ECL. Densitometry of the phospho-MBP bands was performed using SigmaGel (Jandel Scientific, San Rafael, CA).

Statistical analysis. Data are presented as the mean ± standard deviation. Comparisons were made with the Student's *t* test. $P < 0.05$ was considered significant.

RESULTS

15d-PGJ_2 Activates ERK in Human Mesangial Cells

Mesangial cells were treated with 15d-PGJ_2 (1–50 μM) for 2 h. Phosphorylation of ERK, JNK, and p38 MAP kinase was used as an index of MAP kinase activation. As shown in Fig. 1, 15d-PGJ_2 caused a dose-dependent increase in the phosphorylation of both the p42 and p44 isoforms of ERK, but had no effect on JNK or p38 MAP kinase phosphorylation. ERK phosphorylation was apparent beginning at a prostaglandin concentration of 10 μM, and increased to a level comparable to that achieved by IL-1 treatment at a concentration of 50 μM 15d-PGJ_2. This effect appears to be specific for 15d-PGJ_2, as treatment of resting mesangial cells with other 15-deoxy cyclopentenone prostaglandins (15-deoxy-$\Delta^{12,14}$-PGA_1, 15-deoxy-$\Delta^{12,14}$-PGA_2) did not activate ERK (data not shown). Under these experimental conditions 15d-PGJ_2 displayed no cytotoxicity, as assessed by the conversion of a tetrazolium compound to its formazan product, as well as trypan blue exclusion.

The 15d-PGJ_2-mediated increase in ERK phosphorylation correlated with an increase in ERK kinase activity. ERK immunoprecipitated from cells treated with 50 μM 15d-PGJ_2 caused a two-fold increase in the phosphorylation of MBP (Fig. 2). The level of substrate phosphorylation was comparable to the increase in phosphorylation seen after PMA treatment.

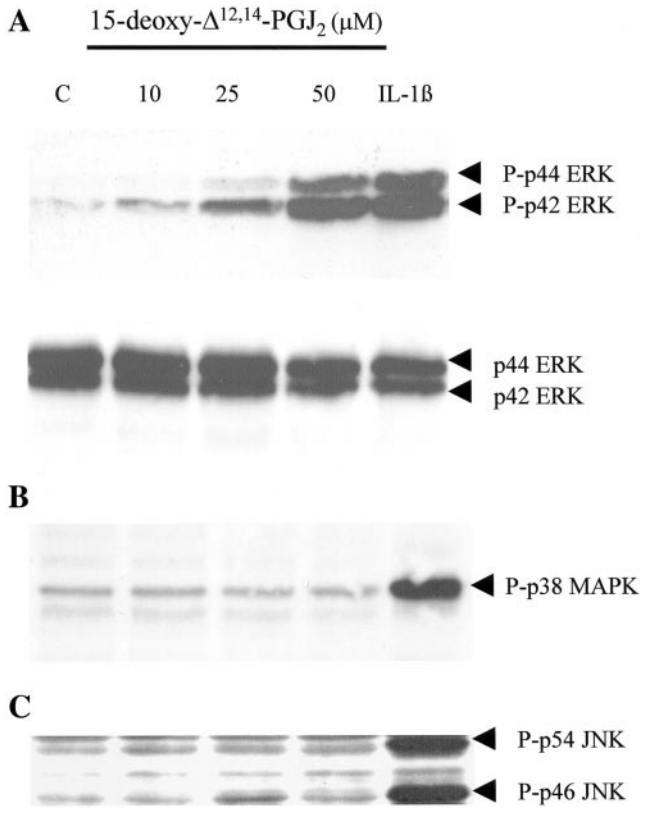

FIG. 1. 15d-PGJ_2 induces the phosphorylation of p42 and p44 ERK. (A) Lysates from vehicle-treated cells (C), or cells treated with the indicated concentrations of 15d-PGJ_2 for 2 h were immunoblotted for tyrosine and threonine phosphorylation of ERK (upper panel). Total ERK remained constant (lower panel). (B, C) The same lysates were also immunoblotted for phospho-p38 MAP (P-p38) kinase and phospho-JNK (P-JNK). IL-1β (1.1 ng/ml for 30 min) was used as a positive control. Representative of four experiments.

PPARγ Does Not Mediate ERK Activation by 15d-PGJ_2

Because 15d-PGJ_2 is a potent agonist of PPARγ, we investigated whether the effects on ERK activation were mediated by PPARγ. To this end, mesangial cells were treated with the structurally different PPARγ agonist ciglitazone, a thiazolidinedione (TZD) class anti-diabetic agent (28). Ciglitazone in concentrations of 10–100 μM had no effect on mesangial ERK activity (Fig. 3A). Furthermore, cells were pretreated with bisphenol A diglycidyl ether (BADGE), a synthetic PPARγ agonist (25), followed by 15d-PGJ_2, and then examined for ERK phosphorylation. BADGE had no effect on the intrinsic level of ERK phosphorylation, and was unable to inhibit the 15d-PGJ_2-mediated ERK phosphorylation (Fig. 3B). This apparent lack of involvement of PPARγ is not due to a lack of receptor expression in the mesangial cells. As shown in Fig. 4A, RT-PCR identified PPARγ RNA in mesangial cells. Because these primers crossed introns, and because no product was amplified from RNA samples in which reverse transcriptase was omitted (not shown), it is unlikely that the RNA was contaminated with genomic DNA. The presence of PPARγ was confirmed by immunoblotting mesangial cell nuclear lysates with an antibody to PPARγ. A specific band of molecular mass 51.7 kDa was found in unstimulated cells, as well as cells treated with IL-1 or 15d-PGJ_2 (Fig. 4B). This molecular mass is similar to that described for PPARγ1 (19, 29, 30). Interestingly, there appeared to be more PPARγ1 present in nuclei of cells treated with 15d-PGJ_2, compared to cells treated with vehicle or IL-1. (Fig. 4B).

Upstream Signals in 15d-PGJ_2-Mediated ERK Activation

ERK activation is most often mediated by MAP kinase (MEK). PD 098059, a synthetic compound which inhibits the activation of ERK by MEK, was used to verify that 15d-PGJ_2 activates ERK through MEK. As shown in Fig. 5A, PD 098059 completely prevented the phosphorylation of ERK in cells treated with 15d-PGJ_2.

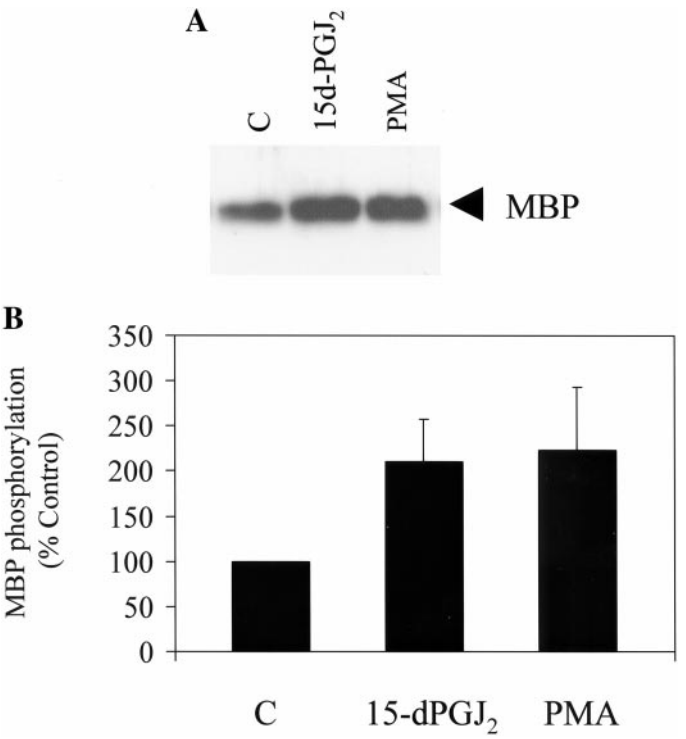

FIG. 2. 15d-PGJ_2 induces ERK activity in human mesangial cells. Cell lysates from vehicle (C) or 15d-PGJ_2-treated mesangial cells (50 μM for 2 h) were immunoprecipitated with anti-ERK antibody and used in an *in vitro* kinase assay with myelin basic protein (MBP) as a substrate. (A) MBP was separated by electrophoresis and immunoblotted for phosphorylated MBP. Phorbol ester treated mesangial cells (PMA, 25 nM for 30 min) served as a positive control. (B) Densitometric analysis of phosphorylated MBP from immunoblots described in A. MBP phosphorylation was significantly higher in response to lysates from cells treated with 15d-PGJ_2 than from cells treated with vehicle alone ($P < 0.02$, $n = 4$).

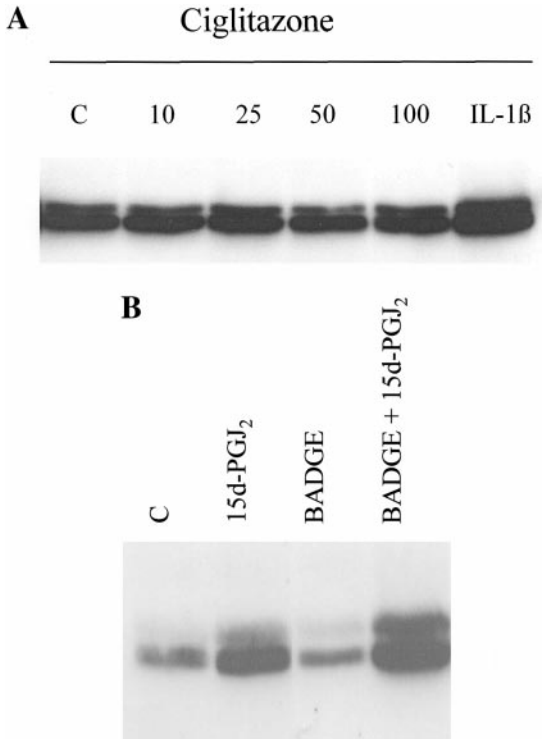

FIG. 3. 15d-PGJ_2-mediated ERK phosphorylation is not PPARγ-mediated. (A) Lysates from vehicle-treated cells (C), or cells treated with the indicated concentrations of ciglitazone for 2 h were immunoblotted for phospho-ERK. IL-1β (1.1 ng/ml for 30 min) was used as a positive control. Representative of four experiments. (B) Cells were pretreated with the PPARγ antagonist BADGE (100 μM) 2 h prior to 15d-PGJ_2 (50 μM). After an additional 2 h cell lysates were harvested and immunoblotted for phospho-ERK. Representative of three experiments.

ERK pathway activation can be PKC-dependent or PI-3 kinase-dependent (18, 31–33). PKC is probably not involved in 15d-PGJ_2-mediated ERK activation,

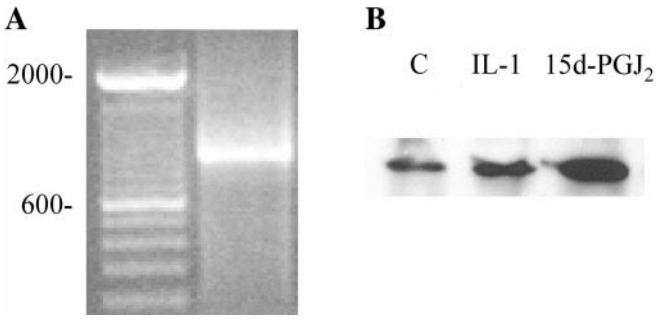

FIG. 4. Expression of PPARγ in human mesangial cells. (A) Total RNA was isolated from unstimulated mesangial cells and probed for PPARγ mRNA by RT-PCR. The expected product size of 900 bp is demonstrated. Representative of four experiments. (B) Mesangial cell nuclear protein was isolated and immunoblotted for PPARγ. Cells were either vehicle-treated (C), or treated with 1.1 ng/ml IL-1 or 10 μM 15d-PGJ_2 for 18 h before harvest. These preparations (n = 3) showed a single specific band with a molecular mass of 51.7 kDa.

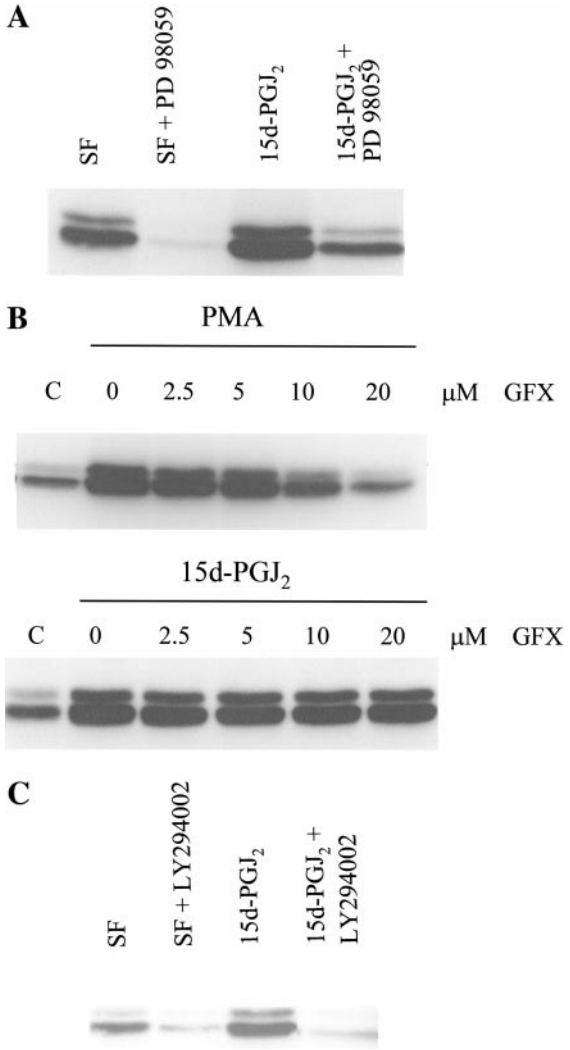

FIG. 5. Upstream signals in 15d-PGJ_2-mediated ERK activation. (A) Cells were treated with vehicle alone (SF) or PD 98059 (25 μM) for 60 min. 15d-PGJ_2 (50 μM) was added as indicated for 2 h. Cell lysates were subsequently immunoblotted for phospho-ERK. Representative of two experiments. (B) Mesangial cells were pretreated for 60 min with the indicated concentrations of the specific PKC inhibitor GFX. The effect of GFX on PMA phosphorylation of ERK (25 nM for 30 min) is shown in the upper panel. The same concentrations of GFX were used to determine the effect of PKC inhibition on 15d-PGJ_2-mediated ERK phosphorylation (lower panel). Representative of three experiments. (C) Mesangial cells were treated with vehicle alone (SF) or the PI-3 kinase inhibitor LY294002 (25 μM) for 60 min. 15d-PGJ_2 (50 μM) was added as indicated for 2 h. Cell lysates were immunoblotted for phospho-ERK. Representative of three experiments.

because the specific PKC inhibitor GFX had no effect on 15d-PGJ_2-induced ERK phosphorylation (Fig 5B). In contrast, GFX caused a dose-dependent inhibition of phorbol ester-induced ERK phosphorylation, as expected (Fig. 5B). Pretreatment of mesangial cells with LY294002, an inhibitor of PI-3 kinase, completely prevented induction of ERK phosphorylation by 15d-PGJ_2 (Fig. 5C), indicating a dependence on PI-3 kinase for ERK activation.

DISCUSSION

The cyclopentenone prostaglandin 15d-PGJ_2 has recently received increasing attention because it is a potential regulator of diverse processes such as cell growth and differentiation (34) and inflammation (35). The present study adds activation of MAP kinase to this growing list of regulatory functions. We found that 15d-PGJ_2 induces the phosphorylation and activation of p42 and p44 ERK in human mesangial cells, but has no effect on JNK or p38 MAP kinase. This specificity for ERK is interesting, as many of the stimuli that activate mesangial MAP kinases activate multiple MAP kinases (7, 9, 10, 18, 36). This effect also appears to be specific for 15d-PGJ_2, as other 15-deoxy cyclopentenone prostaglandins do not activate ERK. The effective concentrations of 15d-PGJ_2 for ERK induction are higher than the range classically associated with prostaglandin activity. This however, is likely dependent on cell type. For example, in some cell lines the effects of 15d-PGJ_2 on cytokine expression were observed at concentrations less than 10 μM, whereas other cell types required concentrations up to 30 μM to achieve similar effects (16, 20, 37, 38). Given the evidence that J series prostaglandins may be inducible within the renal parenchyma (22), ERK activation by 15d-PGJ_2 may play a role in glomerulosclerosis after renal injury.

Although 15d-PGJ_2 is a potent PPARγ ligand, the effect of 15d-PGJ_2 on mesangial ERK activity cannot be reproduced by ciglitazone, a TZD PPARγ agonist, and is not prevented by BADGE, a synthetic PPARγ antagonist. These data suggest that activation of ERK by 15d-PGJ_2 is independent of PPARγ, and are consistent with the observation that in other cell types several biologic activities of 15d-PGJ_2 are PPARγ-independent (15, 16, 21). This is not because human mesangial cells lack PPARγ. Under our cell culture conditions, human mesangial cells constitutively express PPARγ, and nuclear expression appears to increase in cells treated with 15d-PGJ_2. Similar expression of PPARγ has been described in rodent mesangial cells (39, 40).

The upstream signals that mediate mesangial ERK activation in response to 15d-PGJ_2 are presently unclear. PKC can activate ERK (18, 31), but the failure of GFX to inhibit 15d-PGJ_2-induced ERK activation does not support a role for PKC in this case. PI-3 kinase has also been implicated in ERK activation (32, 33), and may play a role in the mesangial 15d-PGJ_2-ERK pathway, given the observation that the PI-3 kinase inhibitor LY294002 abrogates the effect of 15d-PGJ_2 on ERK. Mechanistically, 15d-PGJ_2 may directly alter the activity of intermediates in the ERK activation pathway through covalent modification of critical residues by its reactive cyclopentenone ring, as has been shown for other proteins (14–16).

In summary, this investigation demonstrates that the J series prostaglandin 15d-PGJ_2 induces mesangial ERK activity in a PKC-independent, and PI-3 kinase-dependent manner. This effect is specific for ERK, as JNK and p38 MAP kinases are not activated. Despite evidence that 15d-PGJ_2 is a PPARγ ligand, the induction of ERK appears to be PPARγ-independent. These data suggest 15d-PGJ_2 may modulate mesangial cell survival, production of extracellular matrix proteins, or other processes classically regulated by the ERK pathway. The relationship of 15d-PGJ_2-mediated ERK activation to glomerular injury is currently under investigation.

ACKNOWLEDGMENTS

This work was supported in part by United States Public Health Service Grant DK46055 (B.H.R.), grants from the National Kidney Foundation of Ohio (B.H.R., W.A.W., T.H.), a grant from the Baxter Extramural Research Program (W.A.W.), and a grant from the Juvenile Diabetes Foundation-International (W.A.W.). Portions of this work were presented in abstract form at the 2000 American Society of Nephrology Meetings, Toronto, Ontario.

REFERENCES

1. Robinson, M. J., and Cobb, M. H. (1997) Mitogen-activated protein kinase pathways. *Curr. Opin. Cell Biol.* **9,** 180–186.
2. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* **270,** 1326–1331.
3. Berra, E., Milanini, J., Richard, D. E., Le Gall, M., Vinals, F., Gothie, E., Roux, D., Pages, G., and Pouyssegur, J. (2000) Signaling angiogenesis via p42/p44 MAP kinase and hypoxia. *Biochem. Pharmacol.* **60,** 1171–1178.
4. Parameswaran, N., Nambi, P., Brooks, D. P., and Spielman, W. S. (1999) Regulation of glomerular mesangial cell proliferation in culture by adrenomedullin. *Eur. J. Pharm.* **372,** 85–95.
5. Brauchle, M., Gluck, D., Di Padova, F., Han, J., and Gram, H. (2000) Independent role of p38 and ERK1/2 mitogen-activated kinases in the upregulation of matrix metalloproteinase-1. *Exp. Cell. Res.* **10,** 135–144.
6. Amemiya, T., Sasamura, H., Mifune, M., Kitamura, Y., Hirahashi, J., Hayashi, M., and Saruta, T. (1999) Vascular endothelial growth factor activates MAP kinase and enhances collagen synthesis in human mesangial cells. *Kidney Int.* **56,** 2055–2063.
7. Hayashida, T., and Poncelet, A.-C. (1999) TGF-beta1 activates MAP kinase in human mesangial cells: A possible role in collagen expression. *Kidney Int.* **56,** 1710–1720.
8. Ishida, T., Haneda, M., Maeda, S., Koya, D., and Kikkawa, R. (1999) Stretch-induced overproduction of fibronectin in mesangial cells is mediated by the activation of mitogen-activated protein kinase. *Diabetes* **48,** 595–602.
9. Wilmer, W. A., Tan, L. C., Dickerson, J. A., Danne, M., and Rovin, B. H. (1997) Interleukin-1B induction of mitogen-activated protein kinases in human mesangial cells. *J. Biol. Chem.* **272,** 10877–10881.
10. Rovin, B. H., Wilmer, W. A., Danner, M., Dickerson, J. A., Dixon, C. G., and Lu, L. (1999) The mitogen-activated protein kinase p38 is necessary for interleukin-1 beta-induced monocyte chemoattractant protein-1 expression by human mesangial cells. *Cytokine* **11,** 118–126.

11. Fukushima, M. (1992) Biological activities and mechanisms of action of PGJ2 and related compounds: An Update. *Prost. Leuk. Essential Fatty Acids* **47,** 1–12.
12. Dussault, I., and Forman, B. M. (2000) Prostaglandins and fatty acids regulate transcriptional signaling via the peroxisome proliferator activated nuclear receptors. *Prostaglandins Lipid Mediators* **62,** 1–13.
13. Chen, Y., Morrow, J. D., and Roberts, L. J. (1999) Formation of reactive cyclopentenone compounds *in vivo* as products of the isoprostane pathway. *J. Biol. Chem.* **274,** 10863–10868.
14. Straus, D. S., Pascual, G., Li, M., Welch, J. S., Ricote, M., Hsiang, C.-H., Sengchanthalangsy, L. L., Ghosh, G., and Glass, C. K. (2000) 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappaB signaling pathway. *Proc. Natl. Acad. Sci. USA* **97,** 4844–4849.
15. Castrillo, A., Diaz-Guerra, M. J. M., Hortelano, S., Martin-Sanz, P., and Bosca, L. (2000) Inhibition of I kappaB kinase and I kappaB phosphorylation by 15-deoxy-delta 12,14-prostaglandin J2 in activated murine macrophages. *Mol. Cell. Biol.* **20,** 1692–1698.
16. Rossi, A., Kapahl, P., Natoli, G., Takahashi, T., Chen, Y., Karin, M., and Santoro, M. G. (2000) Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of I kappa B kinase. *Nature* **403,** 103–108.
17. Bishop-Bailey, D. (2000) Peroxisome proliferator-activated receptors in the cardiovascular system. *Brit. J. Pharm.* **129,** 823–834.
18. Haneda, M., Araki, S., Togawa, M., Sugimoto, T., Isono, M., and Kikkawa, R. (1997) Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells culture under high glucose condition. *Diabetes* **46,** 847–853.
19. Fajas, L., Auboeuf, D., Raspe, E., Schoonjans, K., Lefebvere, A.-M., Saladin, R., Najib, J., Laville, M., Fruchart, J.-C., Deeb, S., Vidal-Plug, A., Flier, J., Briggs, M. R., Staels, B., Vidal, H., and Auwerx, J. (1997) The organization, promoter analysis, and expression of the human PPAR gamma gene. *J. Biol. Chem.* **272,** 18779–18789.
20. Maggi, L. B., Sadeghi, H., Weigand, C., Scarim, A. L., Heitmeier, M. R., and Corbett, J. A. (2000) Anti-inflammatory actions of 15-deoxy-delta 12,14-prostaglandin J2 and trioglitazone. *Diabetes* **49,** 346–355.
21. Hortelano, S., Castrillo, A., Alvarez, A. M., and Bosca, L. (2000) Contribution of cyclopentenone prostaglandins to the resolution of inflammation through the potentiation of apoptosis in activated macrophages. *J. Immunol.* **165,** 6525–6531.
22. Reilly, C. M., Oates, J. C., Cook, J. A., Morrow, J. D., Halushka, P. V., and Gilkeson, G. S. (2000) Inhibition of mesangial cell nitric oxide in MRL/lpr mice by prostaglandin J2 and proliferator activation receptor gamma agonists. *J. Immunol.* **164,** 1498–1504.
23. Gervois, P., Torra, I. P., Chinetti, G., Grotzinger, T., Dubois, G., Fruchart, J. C., Fruchart-Najib, J., Leitersdorf, E., and Staels, B. (1999) *Mol. Endo.* **13,** 1535–1549.
24. Rovin, B. H., Yoshiumura, T., and Tan, L. (1992) Cytokine induced production of monocyte chemoattractant protein-1 by cultured human mesangial cells. *J. Immunol.* **148,** 2148–2153.
25. Wright, H. M., Clish, C. B., Mikami, T., Hauser, S., Yanagi, K., Hiramatsu, R., Serhan, C. N., and Spiegelman, B. M. (2000) A synthetic antagonist for the peroxisome proliferator-activated receptor gamma inhibits adipocyte differentiation. *J. Biol. Chem.* **275,** 1873–1877.
26. Rovin, B. H., Dickerson, J. A., Tan, L. C., and Hebert, C. A. (1995) Activation of nuclear factor-kappaB correlates with MCP-1 expression by human mesangial cells. *Kidney Int.* **48,** 1263–1271.
27. Kyhse-Andersen, J. (1984) Electroblotting of multiple gels: A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J. Biochem. Biophys. Meth.* **10,** 203–209.
28. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M., and Kliewer, S. A. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma. *J. Biol. Chem.* **270,** 12953–12956.
29. Camp, H. S., Whitton, A. L., and Tafuri, S. R. (1999) PPAR gamma activators down-regulate the expression of PPAR gamma in 3T3-L1 adipocytes. *FEBS Lett.* **186,** 186–190.
30. Yee, L. D., Sabourin, C. L. K., Liu, L., Li, H. M., Smith, P. J., Seewaldt, V., and Kniss, D. A. (1999) Peroxisome proliferator-activated receptor gamma activation in human breast cancer. *Int. J. Onc.* **15,** 967–973.
31. Grammer, T. C., and Blenis, J. (1997) Evidence for MEK-independent pathways regulating the prolonged activation of the ERK-MAP kinases. *Oncogene* **14,** 1635–1642.
32. Yamaki, K., Yonezawa, T., and Ohuchi, K. (2000) Signal transduction cascade in staurosporine-induced prostaglandin E(2) production by rat peritoneal macrophages. *J. Pharmacol. Exp. Ther.* **293,** 206–213.
33. Pandey, S. K., Theberge, J. F., Bernier, M., and Srivastava, A. K. (1999) Phosphatidylinositol 3-kinase requirement in activation of the ras/C-raf-1/MEK/ERK and p70(s6K) signaling cascade by the insulinomimetic agent vanadyl sulfate. *Biochemistry* **38,** 14667–14675.
34. Clay, C. E., Namen, A. M., Fonteh, A. N., Atsumi, G., High, K. P., and Chilton, F. H. (2000) 15-deoxy-delta 12,14, PGJ2 induces diverse biological responses via PPAR gamma activation in cancer cells. *Prostaglandins Lipid Mediators* **62,** 23–32.
35. Gilroy, D. W., Colville-Nash, P. R., Willis, D., Chivers, J., Paul-Clark, M. J., and Willoughby, D. A. (1999) Inducible cyclooxygenase may have anti-inflammatory properties. *Nat. Med.* **5,** 698–701.
36. Igarashi, M., Wakasaki, H., Takahara, N., Ishii, H., Jiang, Z.-Y., Yamauchi, T., Kuboki, K., Meier, M., Rhodes, C. J., and King, G. L. (1999) Glucose or diabetes activates p38 mitogen-activated protein kinase via different pathways. *J. Clin. Invest.* **103,** 185–195.
37. Jiang, C., Ting, A. T., and Seed, B. (1998) PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* **391,** 82–86.
38. Su, C. G., Wen, X., Bailey, S. T., Jiang, W., Rangwala, S. M., Keilbaugh, S. A., Flanigan, A., Murthy, S., Lazar, M. A., and Wu, G. D. (1999) A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit epithelial inflammatory response. *J. Clin. Invest.* **104,** 383–389.
39. Iwashima, Y., Eto, M., Horiuchi, S., and Sano, H. (1999) Advanced glycation end product-induced peroxisome proliferator-activated receptor gamma gene expression in culture mesangial cells. *Biochem. Biophys. Res. Comm.* **264,** 441–448.
40. Asano, T., Wakisaka, M., Yoshinari, M., Iino, K., Sonoki, K., Iwase, M., and Fujishima, M. (2000) Peroxisome proliferator-activated receptor gamma1 expresses in rat mesangial cells and PPARgamma agonists modulate its differentiation. *Biochim. Biophys. Acta* **1497,** 148–154.