Microsomal Prostaglandin E Synthase-1 Is Overexpressed in Inflammatory Bowel Disease

EVIDENCE FOR INVOLVEMENT OF THE TRANSCRIPTION FACTOR Egr-1*

Kotha Subbaramaiah‡‡, Kazuhiko Yoshimatsu, Ellen Scherl‡, Kiron M. Das, Kenneth D. Glazier, Dragan Golijanin**, Robert A. Soslow‡‡, Tadashi Tanabe‡‡, Hiroaki Naraba‡‡, and Andrew J. Dannenberg‡

From the ‡Department of Medicine, New York Presbyterian Hospital, Weill Medical College of Cornell University and Strang Cancer Prevention Center, New York, New York 10021, the ‡Department of Surgery, Tokyo Women's Medical University Daini Hospital, 2-1-10 Nishiogu Arakawaku, Tokyo, Japan 116-8567, the ‡†Division of Gastroenterology and Hepatology, Crohn's and Colitis Center of New Jersey, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey 08993, the Departments of ‡‡Urology and ‡§Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and the ‡§§Department of Pharmacology, National Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan

Microsomal prostaglandin E synthase-1 (mPGES-1) catalyzes the conversion of cyclooxygenase-derived prostaglandin (PG) H2 to PGE2. Increased amounts of mPGES-1 were detected in inflamed intestinal mucosa from patients with inflammatory bowel disease (IBD). Treatment of mouse colonocytes with TNF-α enhanced guanylate cyclase activity and inhibitors of NO signaling as being important for prostaglandin E2 (PGE2) in IBD. For example, nonsteroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors 4–6), COX-2, and IL-1α, β, and 7, 8), COX-2, and IL-1α, β, and COX-2 inhibitors

This article has been withdrawn by Kotha Subbaramaiah, Kazuhiko Yoshimatsu, Ellen Scherl, Dragan Golijanin, Robert A. Soslow, and Andrew J. Dannenberg. Kiron M. Das, Kenneth D. Glazier, Tadashi Tanabe, and Hiroaki Naraba could not be reached. A portion of Fig. 5A was reused in Fig. 6B and in Subbaramaiah, K., et al. (2003) J. Biol. Chem. 278, 37637-37647. In Fig. 5B, the first two lanes of the actin panel were reused in the last two lanes.

WITHDRAWN

December 2, 2019

Inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis, is characterized by chronic, relapsing inflammation of the gastrointestinal tract. This disease is multifactorial in origin and affects at least 1 in 1000 people in Western countries (1–3). Multiple lines of evidence suggest an important role for prostaglandin E2 (PGE2) in IBD. For example, increased amounts of PGE2 are detected at sites of inflammation in IBD (20). Recent studies suggest that both cytosolic and microsomal enzymes can convert COX-derived PGH2 to PGE2. cPGES/p23 has been reported to be constitutively expressed in many tissues and functionally coupled to COX-1 (21). mPGES-1, an inducible enzyme, is overexpressed in inflamed mucosa in IBD (20). Inflamed joints, atherosclerotic plaques, and neoplastic tissues

---

* This work was supported by the New York Crohn's Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Weill Medical College of Cornell University, 1300 York Ave., Rm. F-203A, New York, NY 10021. Tel.: 212-746-4402; Fax: 212-746-4885; E-mail: ksubba@med.cornell.edu.

* The abbreviations used are: IBD, inflammatory bowel disease; PG, prostaglandin; mPGES, microsomal prostaglandin E synthase; NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; TNF, tumor necrosis factor; Egr, early growth response; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; PC-PLC, phosphatidylcholine-phospholipase C; YC-1, 3-(5-furyl)-1-benzylindazole; PKG, protein kinase G; siRNA, small interfering RNA; cPGES, cytosolic prostaglandin E synthase; PBS, phosphate-buffered saline; GFP, green fluorescent protein; SNAP, S-nitroso-N-acetylimidazole; NONOate, diazeniumdiolate.

This paper is available on line at http://www.jbc.org
activity relative to other PGES isomerases, suggesting a significant role for mPGES-1 in the metabolism of PGH₂. Importantly, treatment of cells with antisense to mPGES-1 attenuated the production of PGE₂ whereas overexpression of mPGES-1 stimulated PGE₂ synthesis (31). Finally, lipopolysaccharide-induced stimulation of PGE₂ synthesis was abrogated in macrophages derived from mPGES-1 null mice (32).

mPGES-1 was evaluated whether mPGES-1 was overexpressed in inflamed intestinal mucosa from patients with IBD. TNF-α plays a central role in mucosal inflammation and is postulated to be at the apex of an inflammatory cascade in IBD (1, 33). Hence, we have defined the signal transduction pathway by which TNF-α-stimulated PGE₂ synthesis was abrogated in mPGES-1 transgenic mice (1).

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium, fetal bovine serum, PKC assay kits, and LipofectAMINE were purchased from Invitrogen. Rabbit polyclonal anti-human mPGES-1 serum, blocking peptide or with preimmune serum. After washing three times with PBS, control sections were incubated with mPGES-1 antiserum preabsorbed with a 100-fold excess of mPGES-1 antibody at a 1:500 dilution for 1 h at room temperature. The slides were then dehydrated with ethanol, rinsed with xylene, and mounted.

Cell Culture—Human colon cancer cell line HCA7 was established from moderately differentiated adenocarcinoma of the colon (34) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin. All treatments were carried out in serum-free medium.

Western Blotting—Frozen tissue was thawed in ice-cold lysis buffer (150 mM NaCl, 100 mM Tris [pH 8.0], 1% Tween 20, 50 mM diethylthiocarbamate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml trypsin inhibitor, and 10 μg/ml leupeptin). The membranes were washed twice with 2× SSC buffer for 1 h at 60°C and dried, and autoradiographed.

Nuclear Run-off Assays—Cells (2.5 × 10⁶) were plated in three 10-cm dishes for each condition. Cells were grown in growth medium until they were ~60% confluent. Nuclei were isolated and stored in liquid nitrogen. For the transcriptional assay, nuclei (1.0 × 10⁷) were thawed and incubated in reaction buffer (10 mM Tris pH 8.5, 5 mM MgCl₂ and 0.5 mM dithiothreitol) for 15 min at 30°C and then incubated for 30 min with [³²P]UTP, ²⁵³P[CTP, and [³²P]ATP (37). The membranes were washed twice with 2× SSC buffer for 1 h at 60°C and dried, and autoradiographed.

Antisense Oligonucleotides—mPGES-1 phosphorothioate antisense oligonucleotides were synthesized. Oligonucleotides used were 5′-CCA-GGCCGCGGAAAGGGATGAGGGGACC-3′ (sense) or Egr-1 consensus site (5′-GGGGCGTTGGGGCGGTGCTG-3′) directed against the region from 0 to 19 of Egr-1 mRNA, and 5′-GCCAGGCCGAGGGGACC-3′, respectively (38).
mPGES-1 and Inflammatory Bowel Disease

RESULTS

mPGES-1 Expression Is Enhanced in Inflammatory Bowel Disease—We compared mPGES-1 expression in inflamed and non-inflamed human colonocytes from patients with Crohn’s disease and ulcerative colitis. Representative immunoblots are shown in Fig. 1. Immunohistochemistry was performed to identify the cell types that expressed mPGES-1 in inflamed colonic mucosa. mPGES-1 was detected in both epithelial cells and inflammatory cells (Fig. 2).

TNF-α Stimulated the Transcription of mPGES-1—TNF-α is widely recognized to contribute to inflammation in IBD (1, 33). It was relevant, therefore, to determine whether TNF-α regulated the expression of mPGES-1 in human colonocytes. As shown in Fig. 3A, TNF-α caused dose-dependent induction of mPGES-1 in HCA7 cells. To exclude the possibility that the inductive effects of TNF-α were unique to HCA7 cells, we also determined whether TNF-α-induced mPGES-1 expression was detected in HCT116 and DLD1 human colonocytes. mPGES-1 was induced by TNF-α in these two cell lines as well (Fig. 3B). In contrast, to mPGES-1, amounts of cPGES were unaffected by treatment with TNF-α (Fig. 3A).

Given the marked increase in amounts of mPGES-1 following treatment with TNF-α, it was important to determine whether mPGES-1 was unique to HCA7 cells. Treatment of HCA7 cells with TNF-α led to a 3-fold increase in synthesis of PGF₂α (Fig. 3C). Importantly, overexpressing antisense to mPGES-1 essentially abrogated the increase in mPGES-1 (data not shown) and PGF₂α production induced by TNF-α (Fig. 3C). To determine whether regulation of mPGES-1 was pretranslational, Northern blot analysis was carried out. TNF-α caused a marked increase in amounts of mPGES-1 mRNA (Fig. 3D).

Nuclear run-offs (Fig. 3E) and transient transfections utilizing mPGES-1 promoter constructs (Fig. 4A) were carried out to determine whether TNF-α induced mPGES-1 by stimulating transcription. Higher rates of synthesis of nascent mPGES-1 mRNA were detected following treatment with
TNF-α (Fig. 3E). Consistent with this result, TNF-α caused approximately a 1.5-fold increase in mPGES-1 promoter activity (Fig. 4A). To define the region of mPGES-1 promoter (Fig. 4A) that responded to TNF-α, transient transfections were performed utilizing different deletions of the human mPGES-1 promoter (Fig. 4B). The inductive effects of TNF-α were lost when the cells were transfected with the deletion construct pGL3/99. There are two GC boxes (−112 to −119 (GC box 2) and −101 to −108 (GC box 1) in the human mPGES-1 promoter. We postulated that either one or both of these GC boxes might be crucial for TNF-α-mediated induction of mPGES-1 promoter activity. As shown in Fig. 4C, mutagenesis of either GC box 1, GC box 2, or both GC boxes (double mutant) abrogated TNF-α-mediated induction of mPGES-1 promoter activity.

Egr-1 has been observed to bind to the GC box of the mPGES-1 promoter and thereby regulate transcription (36). Hence, electrophoretic mobility shift assays were performed to evaluate whether Egr-1 was important for TNF-α-mediated induction of mPGES-1 transcription. As shown in Fig. 5A, treatment with TNF-α led to increased binding of nuclear protein to the GC box region of mPGES-1 promoter. Super-
shift analysis identified Egr-1 in the binding complex. In contrast, c-Jun, CEBP-α, c-Fos, p65, and PEA3 were not identified in the binding complex. Consistent with this finding, binding was prevented by incubating nuclear extract with an excess of GC box cold probe or a consensus Egr-1 oligonucleotide (Fig. 5A). To confirm the involvement of Egr-1 in TNF-α-mediated induction of mPGES-1, we utilized siRNA as well as antisense technologies. Transfection of HCA7 cells with siRNA to Egr-1 inhibited the expression of Egr-1 (data not shown). As shown in Fig. 5B, when cells were transfected with siRNA to Egr-1, TNF-α-mediated induction of mPGES-1 was blocked. In contrast, overexpressing siRNA to GFP did not suppress the induction of mPGES-1 by TNF-α. Additionally, transfection of antisense to Egr-1 suppressed TNF-α-mediated stimulation of mPGES-1 promoter activity (Fig. 5C). Neither antisense nor scrambled Egr-1 oligonucleotides affected basal transcription (data not shown). Taken together, these data clearly show that Egr-1 is crucial for TNF-α-mediated induction of mPGES-1.

Deregulated TNF-α signaling plays a central role in IBD. Because mPGES-1 is overexpressed in IBD, we next investigated the expression and binding activity of Egr-1 in IBD. As shown in Fig. 6A, levels of Egr-1 were increased in inflamed mucosa from patients with Crohn’s disease (lanes 1–4) and ulcerative colitis (lanes 5–7) compared with non-inflamed mucosa (lanes 8–10). Notably, enhanced binding of nuclear protein to the GC box region of the mPGES-1 promoter was detected in extracts prepared from inflamed mucosa from patients with Crohn’s disease and ulcerative colitis (Fig. 6B, lanes 2 and 4, respectively). Egr-1 was detected in the binding complex (Fig. 6C). Incubating nuclear extract with an excess of consensus Egr-1 oligonucleotide prevented binding.
TNF-α induces mPGES-1 transcription by enhancing the binding of Egr-1 to the GC box region of the mPGES-1 promoter. A, 5 μg of nuclear protein from HCA7 cells was incubated with a 32P-labeled oligonucleotide containing the GC box region of mPGES-1. Cells were treated with vehicle (lane 1) or TNF-α (10 ng/ml; lane 2) for 30 min. Lanes 3, 4, and 5 represent nuclear extract from TNF-α-treated cells incubated with a 32P-labeled mPGES-1 GC box oligonucleotide and a 100-, 10-, and 50-fold excess of unlabeled oligonucleotide, respectively. Lane 7 represents nuclear extract from TNF-α-treated cells incubated with a 32P-labeled mPGES-1 GC box oligonucleotide and a 100-fold excess of unlabeled Egr-1 consensus oligonucleotide. Lanes 6, 8, and 9 represent nuclear extract from TNF-α-treated cells incubated with antibodies to Egr-1 (lane 6), c-Jun (lane 8), and p65 (lane 9), respectively. The protein-DNA complex that formed was separated on a 4% polyacrylamide gel. B, HCA7 cells were transfected with Egr-1 siRNA or GFP siRNA. Subsequently, cells were treated with vehicle or TNF-α (10 ng/ml) for 24 h. Cellular lysate protein (100 μg/lane) was then loaded onto a 12.5% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. The immunoblot was sequentially probed with antibodies specific for mPGES-1 and β-actin. C, cells were transfected with 0.9 μg of human mPGES-1 promoter construct (pGL3/650) and 0.2 μg of pSVβgal. Bars labeled Egr-1 scrambled and Egr-1 antisense also received 0.9 μg of scrambled or antisense Egr-1 oligonucleotides. The total amount of DNA in each reaction was kept constant at 2 μg by using empty vector. After transfection, cells were treated with vehicle or 10 ng/ml TNF-α. Reporter activities were measured in cellular extracts 24 h later. Luciferase Activity represents data that have been normalized with β-galactosidase. Columns, means; bars, S.D.; n = 6.
The image contains a page from a scientific document discussing the role of TNF-α-mediated induction of mPGES-1 in inflammatory bowel disease (IBD). The text highlights the involvement of NF-κB and PKC signaling pathways in the induction of mPGES-1. It also mentions the importance of cGMP in the regulation of mPGES-1 expression and the role of NO in stimulating the production of NO and cGMP. The study further investigates the role of Egr-1 as a key transcription factor in the induction of mPGES-1.

**Discussion**

In this study, we found increased levels of mPGES-1, an inducible form of PGES, in inflamed mucosa from patients with IBD. Treatment with TNF-α, a cytokine implicated in mucosal inflammation in IBD (1), induced mPGES-1 transcription in human colonocytes. Stimulation of mPGES-1 promoter activity by TNF-α was mediated by tandem GC boxes (101 to –119). Another recent report indicated that these GC boxes play a significant role in regulating the transcription of both human and mouse mPGES-1 genes (36). Several findings support a role for Egr-1 in mediating the induction of mPGES-1 by TNF-α. Increased binding of Egr-1 to the GC boxes of the mPGES-1 promoter was detected in TNF-α-treated cells. The functional importance of Egr-1 was established because TNF-α-mediated induction of mPGES-1 was suppressed with siRNA to Egr-1. Moreover, antisense to Egr-1 inhibited TNF-α-mediated activation of the mPGES-1 promoter. Taken together, these results indicate that Egr-1 is a key transcription factor in regulating the inducible expression of mPGES-1.

Based on the above findings, we also evaluated whether either the amounts or binding activity of Egr-1 were deregulated in IBD. Notably, both an increase in amounts and binding activity of Egr-1 were detected in inflamed mucosa. Although Egr-1 is rapidly activated by tissue injury (44), to the best of our knowledge, neither of these findings has been reported previously in IBD. Recently, NO was reported to enhance Egr-1 expression (45). Increased levels of NO synthesizing enzymes occur in IBD (46). It is reasonable to postulate, therefore, that NO played a role in stimulating the expression of Egr-1, which contributed, in turn, to the elevated levels of mPGES-1 in inflamed mucosa.
FIG. 7. Induction of mPGES-1 by TNF-α is mediated by PC-PLC and PKC signaling. A, PC-PLC activity was measured from HCA7 cells treated with vehicle (Control) or 10 ng/ml TNF-α for 15, 30, and 60 min as described under “Experimental Procedures.” B, cells were treated with vehicle (C), 0.2 unit/ml or 0.5 unit/ml of PC-PLC for 24 h. C, cells were treated with vehicle (C), TNF-α (10 ng/ml), or TNF-α plus D609 (50, 100 μM) for 24 h. In B and C, cellular lysate protein (100 μg/lane) was loaded onto a 12.5% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. The immunoblot was sequentially probed with antibodies specific for mPGES-1 and β-actin. D, PKC activity was measured (total, cytosol, membrane) in HCA7 cells treated with vehicle (Control) or TNF-α (10 ng/ml) for 30 and 60 min. E, HCA7 cells were treated with vehicle (C), TNF-α (10 ng/ml), or TNF-α plus calphostin C (1, 2 μM) for 24 h. Cellular lysate protein (100 μg/lane) was loaded onto a 12.5% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. The immunoblot was sequentially probed with antibodies specific for mPGES-1 and β-actin.
Fig. 8. TNF-α-mediated induction of mPGES-1 is NO-dependent in HCA7 cells. A, cells were treated with vehicle (C) or TNF-α (10 ng/ml) for 24 h. B, cells were treated with vehicle (C), TNF-α, or TNF-α plus 100 μM D609 for 24 h. C, cells were treated with vehicle (C), TNF-α, or TNF-α plus 2 μM calphostin C for 24 h. In A–C, nitrite concentration was measured in culture supernatant. D, cellular lysate protein was isolated from cells treated with vehicle (C) or the indicated concentration of SNAP for 24 h. E, cellular lysate protein was isolated from cells treated with vehicle (C) or the indicated concentration of spermine NONOate for 24 h. F, cellular lysate protein was isolated from cells treated with vehicle, TNF-α, or TNF-α plus the indicated concentration of diphenyleneiodonium chloride for 24 h. In D–F, cellular lysate protein (100 μg/lane) was loaded onto a 12.5% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. The immunoblots were sequentially probed with antibodies specific for mPGES-1 and β-actin.
Egr-1 can also induce TNF-α/H9251 (47). PGE2, the catalytic product of mPGES-1, can induce Egr-1 (48). Hence, a complex feedback loop appears to exist contributing to the observed increase in levels of Egr-1 and mPGES-1 in inflamed mucosa.

In contrast to COX-2 (49), very little is known about the signal transduction pathway(s) that regulate the expression of mPGES-1. We show that TNF-α/H9251 induced mPGES-1 by a signaling pathway comprising PC-PLC → PKC → NO → cGMP and PKG (Fig. 10). TNF-α can stimulate the hydrolysis of phospholipids (50–52). TNF-α induced the activity of PC-PLC but not phosphatidylinositol-phospholipase C (data not shown). Treating cells with purified PC-PLC induced mPGES-1 whereas an inhibitor of PC-PLC activity suppressed TNF-α-mediated induction of mPGES-1. These results indicate that TNF-α induced mPGES-1 by a signaling pathway that includes PC-PLC. Notably, PC-PLC has already been reported to play a role in TNF-α-mediated induction of COX-2 (52). Diacylglycerol, a product of PC-PLC-mediated hydrolysis of phospholipids, activates PKC (50). We showed that TNF-α stimulated PKC activity, whereas a prototypic inhibitor of PKC blocked TNF-α-mediated induction of mPGES-1. Presumably, diacylglycerol was responsible at least, in part, for activating PKC. Activation of PKC can stimulate p38 and ERK1/2 MAPK activity (53). Others have suggested that MAPKs can mediate the
induction of mPGES-1 (54–56). Additional experiments were carried out to establish a causal link between the observed increase in NO production mediated by TNF-α and the induction of mPGES-1. Support of this mechanism, treatment with NO donors induced mPGES-1, whereas an inhibitor of NO synthase blocked TNF-α-mediated induction of mPGES-1. Nitric oxide is a potent transcriptional regulator influencing a variety of genes either by cGMP-dependent or -independent mechanisms (58, 59). cGMP appears to be important for TNF-α-mediated induction of mPGES-1. This conclusion was supported by evidence that an inducer of cGMP production caused an increase in amounts of mPGES-1 whereas two inhibitors of guanylate cyclase activity blocked TNF-α-mediated induction of mPGES-1.

Fig. 10. Schematic of signaling cascade by which TNF-α-mediated induction of mPGES-1 by NO donors mimics the role of PKC activity in colonocytes.

REFERENCES
1. Fiocchi, C. (1998) Gastroenterology 115, 182–205
2. Blumberg, R. S., Saubermann, L. J., and Strober, W. (1999) Curr. Opin. Immunol. 11, 648–656
3. Logan, R. F. (1998) Gastroenterology 115, 309–311
4. Sharon, P., Liguinsky, M., Rachmilewitz, D., and Zor, U. (1978) Gastroenterology 75, 638–640
5. Wiercinska-Drapalo, A., Flisiak, R., and Prokopowicz, D. (1999) Prostaglandins

