Prostaglandin E2 modulates TNF-α-induced MCP-1 synthesis in pancreatic acinar cells in a PKA-dependent manner

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Sun L-K, Reding T, Bain M, Heikenwalder M, Bimmler D, Graf R. Prostaglandin E2 modulates TNF-α-induced MCP-1 synthesis in pancreatic acinar cells in a PKA-dependent manner. Am J Physiol Gastrointest Liver Physiol 293:G1196–G1204, 2007. First published October 4, 2007; doi:10.1152/ajpgi.00330.2007.—Cyclooxygenase (COX)-2 is increased in human chronic pancreatitis. We recently demonstrated in a model of chronic pancreatitis (WBN/Kob rat) that inhibition of COX-2 activity reduces and delays pancreatic inflammation and fibrosis. Monocyte chemoattractant protein (MCP-1) mRNA and PGE2 were significantly reduced, correlating with a decreased infiltration of macrophages. MCP-1 plays an important role in the recruitment of macrophages to the site of tissue injury. The aim of our study is to identify mechanisms by which macrophages and acinar cells maintain an inflammatory reaction. The expression profile of E prostanoid receptors EP1-4 and MCP-1 was analyzed by RT-PCR from pancreatic specimens and AR42J cells. MCP-1 secretion was detected by ELISA from rat pancreatic lobuli. We determined EP1-4 mRNA levels in WBN/Kob rats with chronic pancreatic inflammation. Individual isoforms were highly increased in rat pancreas, concurrent with MCP-1 mRNA expression. In supernatants of pancreatic lobuli and AR42J cells, MCP-1 was detectable by ELISA. In the presence of TNF-α, MCP-1 was upregulated. Coincubation with PGE2 enhanced the TNF-α-induced MCP-1 synthesis significantly. Similarly, TNF-α mRNA was synergistically upregulated by TNF-α and PGE2. Furthermore, the synergistic effect of TNF-α and PGE2 was abolished by inhibition of PKA but not of PKC. We conclude that EP receptors are upregulated during chronic pancreatic inflammation. PGE2 modulates the TNF-α-induced MCP-1 synthesis and secretion from acinar cells. This synergistic effect is controlled by PKA. This mechanism might explain the COX-2-dependent propagation of pancreatic inflammation.

chronic pancreatitis; monocyte chemotactic protein-1; tumor necrosis factor-α; E prostanoid receptors 1–4

SEVERAL STUDIES INDICATED that cyclooxygenase-2 (COX-2) is increased in human chronic pancreatitis (CP; Refs. 20, 32). A COX-2 selective inhibitor used in a rat model of CP (WBN/Kob rats) significantly reduced and delayed inflammation and fibrosis. This correlated with markedly decreased levels of PGE2 and decreased infiltration of macrophages. In untreated WBN/Kob rats with chronic inflammation, mRNA levels of both COX-1 and -2 isoforms were highly increased compared with healthy Wistar rats. COX-2 immunoreactivity was localized to inflammatory foci and found in macrophages (29).

COX converts arachidonic acid to prostaglandin G2. Subsequent steps in the cascade lead to the generation of thromboxanes and prostaglandins. It is well known that COX-2 is induced during the inflammatory process. PGE2 is a potent mediator of the inflammatory response and is known to stimulate cell migration and proliferation while inhibiting cell death through four different E prostanoid receptors (EP1-4; Ref. 3). These receptors are coupled to G proteins. EP1 causes an increase of free Ca2+ and activates PKC. EP2 and EP4 are coupled to Gs, which activates adenyl cyclase to increase the cellular cAMP level and activates PKA. The intracellular pathway of EP3 relays its signal through Gαi, which inhibits adenyl cyclase (8). More interestingly, PGE2 induces COX-2 expression via EP2/cAMP response element activation in human pancreatic adenocarcinoma cell (27). A number of reports point out a correlation between inflammation in the pancreas and eicosanoid levels including PGE2 during acute pancreatitis (11, 37). In the early phase of pancreatitis, PGE2 appears to be increased in the tissue (11). In contrast, plasma levels of PGE2 were significantly reduced during necrotizing acute pancreatitis in the rat (37). However, particularly in chronic pancreatic inflammation, the mechanism of the PGE2-mediated reaction, as well as the localization of receptors for PGE2, remains unclear.

Experiments using the COX-2 inhibitor in the WBN/Kob rat also demonstrated a significant downregulation of monocyte chemotactic protein-1 (MCP-1) in the pancreas compared with the untreated controls (29). MCP-1 belongs to the C-C subfamily of chemokines and exerts strong chemotactic activity on monocytes, macrophages, and lymphocytes (30). There are a number of cytokines that induce expression of MCP-1 (22, 39). In murine mast cells, PGE2 promotes release of MCP-1 (25). Pancreatic acinar cells produce MCP-1, which is increased after cerulein stimulation (4, 9, 15). Increased expression of MCP-1 has been shown in lesions of several fibrotic diseases including CP (2, 12, 31), while Bindarit, a blocker of MCP-1 synthesis, is partially protective in mice with acute pancreatitis (6). The correlation between the presence of COX-2 transcripts, prostaglandins, and MCP-1 suggests a potential role for PGE2 in the regulation of MCP-1 (29).

In this study, we used the WBN/Kob rat model with or without COX-2 inhibitor treatment, in vitro cell cultures of AR42J cells, and freshly prepared rat pancreatic lobuli to answer the questions: 1) Are PGE2 and EP receptors involved in chronic pancreatic inflammation? 2) Is MCP-1 upregulation in acinar cells PGE2 dependent? 3) Do acinar cells play an active role in the propagation of inflammation?

Here we demonstrate that EP receptors are highly upregulated in chronic pancreatic inflammation with the potential to
mediate PGE₂ signaling. Furthermore, we show that prostaglandins can modulate, via PKA, the induction of proinflammatory cytokines and chemokines such as MCP-1 and TNF-α.

MATERIALS AND METHODS

**Chemicals.** PGE₂ and polyclonal antibodies to EP₁-₄ were purchased from Cayman Chemical (Ann Arbor, MI). Murine recombiant TNF-α was purchased from R&D Systems (Abingdon, UK), and stock solutions were prepared following manufacturer’s recommenda- tions and then diluted in cell culture medium. Cell culture media and supplements were purchased from Invitrogen Life Technologies (Carlsbad, CA). Cell culture plastic wares (TPP) were obtained from Fisher Scientific (Wohlen, Switzerland). Rabbit polyclonal antibodies against rat MCP-1 and normal purified rabbit and goat IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and rabbit monoclonal antibodies against rat cAMP response element-binding protein (CREB) and specific phospho-CREB (Ser133) were purchased from Cell Signaling (Boston, MA). The selective inhibitors for PKA (H-89; PKA: IC₅₀ ~ 50 nM) and PKC (GF109203; PKC: IC₅₀ = 20 nM; PKC: IC₅₀ = 2.0 μM) were purchased from Biomol (Plymouth Meeting, PA). Stock solutions for inhibitors were prepared in DMSO and freshly diluted into cell culture medium before use.

**Cells and cell culture.** The Wistar rat pancreatic acinar cancer cell line AR42J was obtained from American Type Culture Collection (Manassas, VA) and was used to simulate the response of pancreatic acinar cells to cytokines and prostaglandins. AR42J cells were grown in F-12K (Kaighn’s modification) medium supplemented with 20% heat-inactivated FBS, 50 U/ml penicillin G, and 50 μg/ml streptomycin. The cells were cultured at 37°C in a humidified atmosphere (5% CO₂/95% air), and the medium was changed every 3–4 days. After the cells were rested overnight in 1% FBS containing cell culture medium, they were exposed to selected factors as described in the RESULTS. Supernatants were collected for additional analysis, and cells were harvested for RNA isolation or protein extraction, respectively.

**Animals, isolation of rat pancreatic acini, and preparation of rat pancreatic lobuli.** Animal treatment and specimen harvesting were performed according to the guidelines of the German Federation of Laboratory Animal Science (Bundesverband der Deutschen Labormeereinigungen). Details are provided in the following subsections.

**Preparation of total RNA and real-time PCR analysis.** Total RNA was isolated and used for real-time PCR as described previously (7, 29). TaqMan Gene Expression Assays based on the rat genes coding for EP₁, EP₂, EP₃, and EP₄ were ordered from Applied Biosystems (Rotkreuz, Switzerland). Primers and probes for MCP-1 and TNF-α were used as described previously (24, 40). Real-time PCR was run on a Taqman 7000 (Applied Biosystems) under standard conditions. Transcript levels were quantified using 18S RNA (Applied Biosystems) as a reference.

**Western blot, immunohistochemistry, and ELISA.** After the treatment periods, total protein was extracted and Western blotting was performed as described previously (35). Immunohistochemistry protocols were as described previously (7, 29). Thirty micrograms of total protein were used for detection of target antigen. The ECL detection system (GE Healthcare, Otelfingen, Switzerland) was used for detection. For measurement of rat MCP-1, supernatants of lobuli were collected, total protein was extracted from lobuli homogenized by sonification, and detection was carried out by a commercial rat MCP-1 ELISA assay (BD Biosciences, San Diego, CA) according to manufacturer’s instructions. MCP-1 was normalized to the protein contents in homogenates of pancreatic lobuli.

**Statistical analysis.** Data are means ± SE of three to seven separate experiments. ANOVA was used with the Bonferroni posttest when data were compared within one experiment. Student’s t-test was used for comparisons between two means of repeated experiments. A P value of <0.05 was considered significant.

**RESULTS**

Presence of MCP-1 in the rat pancreas. It has been shown that in murine mast cells PGE₂ promotes release of MCP-1 (25). The observation that MCP-1 along with PGE₂ was strongly reduced in the pancreas of WBN/Kob rats after COX-2 inhibitor treatment (29) led us to determine whether there is a link between PGE₂ and MCP-1 expression.

First, we established that MCP-1 is expressed in the acinar cell of the pancreas as reported earlier (4). An ELISA for MCP-1 detected the chemokine in homogenates of healthy pancreatic lobuli and in medium supernatants of lobuli and AR42J cells. Furthermore, MCP-1 expression was detected in the acinar cancer cell line AR42J using immunocytochemistry (data not shown) and by real-time PCR (see Fig. 5).

Do pancreatic acinar cells express E prostaglandin receptors? In the WBN/Kob rat model of chronic pancreatitis, COX-2 immunoreactivity was localized predominantly in macrophages (29). COX-2 activity leads to the secretion of PGE₂. If PGE₂ targets acinar cells, we would expect them to express EP receptors. To localize these receptors, we performed immunohistochemistry, using antibodies directed against the four known types of EP receptors. Figure 1 demonstrates that EP₁ (A, B) is undetectable, while EP₂ (D, E), EP₃ (G, H), and EP₄ (J, K) are found predominantly in acinar cells, with EP₃ possibly being the most abundant receptor (roughly estimated by immunohistochemistry). Compared with the WBN/Kob rat pancreas (A, D, G, J), the Wistar pancreas (B, E, H, K) exhibited lower staining intensity with the exception of EP₃. To exclude that the absence of EP₁ is a technical failure, we included control sections from kidney that show abundant expression of EP₁ and EP₃ (Fig. 1, F and I). Controls with normal rabbit IgG (Fig. 1C) demonstrate no apparent staining.

The presence of EP receptors in acinar cells was further investigated by real-time PCR using gene-specific probes for the four EP receptors. We compared relative expression levels in healthy Wistar rat pancreas, in freshly prepared pancreatic lobuli, and in the pancreatic acinar cell line AR42J.

mRNA coding for EP₁, EP₂, EP₃, and EP₄, was present in the pancreas of Wistar rats (Fig. 2A), in pancreatic lobuli (Fig. 2B), and in AR42J cells (Fig. 2C). Overall, a very similar expression pattern was observed in all specimens, with EP₃ being the dominant receptor subtype. The expression of EP₂ was nearly undetectable in AR42J cells (Fig. 2C). To compare the expression between the samples, EP₃ mRNA levels are shown in Fig. 2D. Wistar pancreas and
AR42J cells show similar levels, while freshly prepared lobuli exhibited a slightly increased expression. In summary, these data demonstrate the presence of EP receptors in pancreatic acinar cells and strongly suggest that the pancreatic acinar cell is able to mediate PGE2 signaling and actively participate in inflammation.

E prostanoid receptors expression profile in the rat model. To examine a potential link between PGE2 and MCP-1, we assessed whether EP receptors are regulated during chronic inflammation in the WBN/Kob rat. Real-time PCR for the four receptor subtypes demonstrated a strong upregulation of EP1, EP2, and EP4 in WBN/Kob rat pancreas compared with Wistar rats (Fig. 3, A, D, G, and J) and Wistar (B, E, H, and K) rat pancreas specimen. A and B: EP1, D and E: EP2, G and H: EP3, J and K: EP4. F and I: sections from kidney using antibodies against EP1 and EP3, respectively. C: Control section stained by using normal rabbit IgG.


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Does PGE2 regulate the expression of MCP-1 in pancreatic acinar cells? To determine whether PGE2 could modify the synthesis and secretion of MCP-1, freshly prepared pancreatic lobuli were used. Two time points were chosen to assess
initial secretion (1 h after stimulation) and synthesis (4 h after stimulation).

TNF-α is highly upregulated in pancreatitis and may mediate the inflammatory reaction in the acinar cell (5, 16, 29). Thus, we examined whether TNF-α alone or in combination with PGE2 can induce MCP-1 expression. After 1 h of stimulation, neither PGE2 (10−9 M) nor TNF-α (100 ng/ml, 6 × 10−8 M) alone had an effect on MCP-1 secretion as determined by MCP-1 ELISA of supernatants. However, in combination, they synergistically increased the expression of MCP-1 significantly (P < 0.05; Fig. 4A).

When the incubation period was prolonged to 4 h, secretion of MCP-1 was significantly enhanced in response to TNF-α (P < 0.05), while PGE2 alone still had no effect. Again, in
combination, PGE2 and TNF-α increased the secretion of MCP-1, with MCP-1 levels higher than in lobuli treated by TNF-α alone (Fig. 4B). The level of MCP-1 in the homogenates of pancreatic lobuli demonstrated a similar profile as in the supernatants after 4 h of treatment with a significant increase in the presence of both TNF-α and PGE2 (P < 0.05 vs. TNF-α alone; Fig. 4C). This suggests that PGE2 plays a modulatory role in TNF-α-induced expression of MCP-1 in freshly prepared pancreatic lobuli.

Freshly prepared pancreatic lobuli consist predominantly of acinar cells but also contain duct cells and other cell types. To ensure that the observation of MCP-1 regulation was acinar cell specific, we sought a source that is not contaminated by other cell types. Although clearly a cancer cell, AR42J cells are capable of producing a number of pancreatic acinar cell products, e.g., amylase and trypsinogen. Therefore, we chose this cell line to recapitulate the experiments.

First, we examined whether either TNF-α or PGE2 alone could induce MCP-1 expression. MCP-1 mRNA was upregulated ~160-fold by TNF-α (P < 0.05), while PGE2 only slightly increased the levels of MCP-1 mRNA. Then, we investigated whether PGE2 could enhance the TNF-α-mediated induction of MCP-1 mRNA. In combination, these factors caused a further enhancement of MCP-1 expression (P < 0.05; Fig. 5A).

As the acinar response to TNF-α resulted in a strong MCP-1 induction, we asked whether TNF-α expression is also modulated by PGE2 in the acinar cells. First, the effect of PGE2 on TNF-α expression was tested (Fig. 5B). PGE2 alone did not influence TNF-α mRNA. When AR42J cells were exposed to TNF-α, a 6,500-fold upregulation of TNF-α mRNA was detected (P < 0.05). Presenting TNF-α in combination with PGE2 at 10⁻⁹ M caused a further significant increase in TNF-α production (35,000-fold; Fig. 5B; P < 0.05). To ensure that TNF-α mRNA was actually translated and secreted, we assessed TNF-α contents in medium supernatants by using a commercial ELISA kit. A significant amount of TNF-α was released, roughly 50% of the TNF-α added as a stimulus (data not shown). Thus, we conclude that the proinflammatory fac-

Fig. 4. Monocyte chemotactic protein (MCP)-1 synthesis after stimulation of freshly prepared rat pancreatic lobuli. Supernatants and homogenates of nonstimulated lobuli and of lobuli stimulated by either TNF-α (100 ng/ml, 6 × 10⁻⁹ M) or by PGE2 (1 × 10⁻⁹ M) or by a combination of both were assayed for MCP-1 contents by ELISA. A: supernatants of pancreatic lobuli after a 1-h treatment. B: supernatants after a 4-h treatment. C: homogenates of pancreatic lobuli after a 4-h treatment. MCP-1 was normalized to the protein contents in homogenates. (n = 3; *significant difference, t-test, P < 0.05). Data are means ± SE.

Fig. 5. Determination of mRNA levels of MCP-1 and TNF-α in AR42J cells after stimulation with TNF-α and PGE2. Cells were stimulated for 4 h before harvesting and analysis by real time PCR A: mRNA levels of MCP-1 in nonstimulated cells and in cells stimulated by TNF-α, by PGE2, or by a combination of both. B: TNF-α transcript levels in the same experiment. (n = 7; *significant difference, t-test, P < 0.05). Data are means ± SE.
tors TNF-α and PGE2 act on the acinar cell in a synergistic fashion.

Which EP-receptor-dependent pathway mediates the synergistic effect of PGE2 and TNF-α? In both freshly prepared pancreatic lobuli and AR42J cells, we demonstrated a synergistic effect of PGE2 and TNF-α on MCP-1 expression. The four EP receptors (EP1-4) signal via different G proteins, either by activation or inhibition of adenyl cyclase or by the inositol triphosphate pathway. EP2 and EP4 are known to mediate their signals through PKA while EP1 uses PKC (8). To examine which EP-dependent receptor pathway is activated, we exposed pancreatic lobuli to a selective PKA inhibitor (H-89, 30 μM) or to a selective PKC inhibitor (GF109203, 0.5 μM) in presence of TNF-α at 100 ng/ml and PGE2 at 10⁻⁹ M. As a positive control, lobuli were stimulated by TNF-α alone (P < 0.05) and by TNF-α combined with PGE2 (P < 0.05) in the absence of inhibitors. After 4 h of stimulation, MCP-1 in supernatants was determined by ELISA. The synergistic effect of PGE2 and TNF-α on MCP-1 expression was abolished by PKA inhibition (H-89, P < 0.05) but not by PKC inhibition (GF109203; Fig. 6A) where MCP-1 was even slightly increased. Inhibition of PKA resulted in an MCP-1 expression equal to that after TNF-α stimulation alone, strongly suggesting that PKA may be responsible for the PGE2 effect on MCP-1 secretion using either EP2 and/or EP4.

Finally, we explored whether a substrate of PKA, cAMP responsive element binding protein (CREB), is phosphorylated. In untreated AR42J cells, phospho-CREB was practically absent with only a minor induction after TNF-α stimulation. In combination with PGE2, phospho-CREB was strongly enhanced and this effect was not abolished by the addition of an inhibitor of PKC. However, addition of a PKA inhibitor caused a reduction of phospho-CREB (Fig. 6B). This further supports the observation that there is a crosstalk between E prostanoid receptors and TNF-α signaling leading to activation of CREB via PKA to modulate the synthesis/secretion of MCP-1, which plays a critical role in macrophage recruitment during CP development.

**DISCUSSION**

In this study, we have shown that the pancreatic acinar cell is highly responsive to TNF-α, PGE2, a product of the COX-controlled prostaglandin pathway, caused only minor induction of MCP-1. However, in the presence of TNF-α, PGE2 further enhanced the synthesis and secretion of MCP-1 in freshly prepared pancreatic lobuli as well as in the pancreatic acinar cancer cell line AR42J. We found that both EP receptors and MCP-1 are highly upregulated in chronic pancreatic inflammation in rats. EP receptors are differentially expressed with respect to their subtypes. PGE2 modulated MCP-1 synthesis in pancreatic acinar cells through EP receptors by a PKA-dependent mechanism. Therefore, we propose that the acinar cell has an active role in the propagation of inflammation.

Chronic pancreatic inflammation, e.g., chronic pancreatitis, is characterized by a succession of inflammatory flares, leading to the destruction of the parenchyma (19). To circumvent the difficulty to study early phases of CP in humans, rats or mice are often used as a model (26, 38). Although these animal models do not reflect exactly the pathophysiology of human CP in every aspect, they still provide insightful information on the mechanism of chronic pancreatic inflammation. In the current experiments, we have studied WBN/Kob rats in the presence and absence of a COX-2 inhibitor, as well as AR42J cells and Wistar rat pancreatic lobuli, to examine the response of the acinar cell to proinflammatory mediators known to be secreted by macrophages. Activated macrophages induce COX-2, thereby enhancing the synthesis and secretion of prostaglandins (17, 34). We have focused on PGE2, a known proinflammatory molecule (23), that acts locally in a paracrine- or autocrine manner.

![Fig. 6](http://ajpgi.physiology.org/) Effects of PKA- or PKC-inhibitors on MCP-1 expression levels and on cAMP response element binding protein (CREB) phosphorylation. A: Supernatants of freshly prepared rat pancreatic lobuli: MCP-1 after a 4-h treatment with TNF-α or in combination with PGE2, PKC inhibitor (Inh.) GF109203 (0.5 μM) in parallel wells with the combination treatments (n = 4; *significant difference, t-test, P < 0.05). B: AR42J cells were treated for 4 h using the same scheme as shown in A. The cells were then harvested and subjected to Western blot analysis of phospho-(p)-CREB (top) and CREB (bottom). Cells were treated for 4 h with TNF-α alone (T), a combination of TNF-α and PGE2 (T+P), with the combination and a PKC-inhibitor (T+P+G) or a PKA-inhibitor (T+P+H). C: Densitometric analysis of phospho-CREB and CREB on the Western blots shown in B. Data are means ± SE.
Our group (29) has recently shown that the application of a selective COX-2 inhibitor in WBN/Kob rats decreased the infiltration rate of macrophages in the rat pancreas in correlation with a decreased expression of MCP-1. In a model of acute pancreatitis, it was shown that application of Bindarit, a MCP-1 inhibitor, suppressed MCP-1 production and attenuated tissue injury in the pancreas (6, 41). MCP-1 was also significantly increased in a rat model of CP induced by dibutyltin dichloride, supporting the hypothesis that this chemokine is an important mediator of inflammation (6, 41). MCP-1 expression is also known to be increased in human CP (31). We confirmed the presence of MCP-1 freshly prepared pancreatic lobuli and in the acinar cell line AR42J. These observations support a previous study (31) using in situ hybridization. TNF-α has also been localized to pancreatic acinar cells after exposure to cytokines (16).

**TNF-α and PGE2 crosstalk.** The role of PGE2 as a modulator of the expression of MCP-1 was demonstrated in freshly prepared pancreatic lobuli after a 1-h treatment. Secretion of MCP-1 was only observed when PGE2 and TNF-α were applied simultaneously, as either PGE2 or TNF-α alone had no effect. When the incubation period was prolonged to 4 h, the effect was still observed; however, the role of PGE2 now appeared to be modulatory. PGE2 appears to sensitize the pancreatic acinar cell to respond to cytokines such as TNF-α during inflammation. The difference between the early response (1 h) and the late (4 h) may be that initially PGE2 acts on secretion, while later de novo synthesis may be induced. The data from AR42J cells confirmed the modulatory effect of PGE2 on TNF-α-induced expression of MCP-1 in pancreatic lobuli. Thus, the combined presence of PGE2 and TNF-α might play a critical role in COX-2-dependent pancreatic inflammation.

The concurrent presence of TNF-α and PGE2 is conceivably possible in a situation where macrophages and acinar cells are in close proximity. Interestingly, in the presence of PGE2, TNF-α significantly enhanced its own expression, although PGE2 alone had no effect on the production of TNF-α. This amplification of chemokines will possibly result in further attraction of inflammatory cells. The observed and discussed data imply that the pancreatic acinar cell can adopt an “inflammatory” state, which in itself propagates the inflammatory reaction. A summary of this concept is integrated in Fig. 7. Acinar cell damage or impaired function leads to a chemokine release (e.g., MCP-1) that attracts monocytes. Concurrent release of TNF-α activates the monocyte to a macrophage. Activation leads to induction of COX-2, which results in increased PGE2 secretion. TNF-α and PGE2 together enhance the production of more MCP-1 (and TNF-α) of acinar cells.

In contrast, PGE2 has an inhibitory effect on chemokine production in macrophages. Several studies have shown that LPS-activated macrophages secrete less TNF-α (28) and macrophage inflammatory protein 1α and 1β in the presence of PGE2 (18, 36). This modulatory effect may lead to a balanced reaction of the macrophages to the extent of tissue injury. In the concept shown in Fig. 7, destruction of acinar cells will eventually lead to a reduction of acinar cell derived TNF-α. Therefore, activated macrophages are exposed to PGE2, which they are able to secrete, and less TNF-α. This shift of the balance between PGE2 and cytokines may indicate that macrophage activity is not required and hence macrophage activation is subdued.

To examine the mechanism of PGE2 signaling in pancreatic acinar cells, we investigated the expression of EP receptors in Wistar and WBN/Kob rat pancreas, in freshly pancreatic acini, and in the acinar cancer cell line AR42J. In contrast to pancreatic tissue and lobuli and AR42J cells, isolated pancreatic acini exhibited an ~10-fold higher expression of EP receptor mRNA (data not shown). Since the acinar cell is the predominant cell type, making up 80–90% of the cell volume in the pancreas, the increase in EP receptor mRNA levels in pancreatic acini cannot be explained by the selective accumulation of acinar cells in this preparation. Although freshly isolated acini responded to and produced TNF-α (16), the modulatory effect of PGE2 on TNF-α, showing the same trend, was not significant. Hence, the increase in EP-receptor mRNA levels and lack of responsiveness are presumably the result of the method of acinar cell isolation, particularly due to collagenase digestion. This convinced us to use the more gentle production of pancreatic lobuli.

In WBN/Kob rats, we demonstrated that specific subtypes of EP receptors are significantly upregulated during CP. Particularly EP2- and EP3-mRNA were 15- to 25-fold more abundant in the pancreas of WBN/Kob rats compared with Wistar rats. However, EP3, the receptor most abundant in acinar cells, was hardly affected. The expression of the four EP receptors is organ specific; therefore, the extent as well as the combination of receptor isoforms may vary considerably. In the mouse, EP3 is highly expressed in the kidney and uterus, while EP4 is dominant in the ileum, uterus and thymus (33). Similarly, in human tissue, the expression of EP3 is most prominent in the kidney, pancreas, and uterus (21). Since EP3 is highly ex-
pressed in the normal pancreas, we assume that it is responsible for homeostasis and normal physiological roles, while the regulated receptors EP3 and EP4 may be involved in pathophysiological processes.

PKA inhibition, but not PKC inhibition, abolished the synergistic effect of TNF-α and PGE2 on MCP-1 expression in freshly prepared rat pancreatic lobuli. As it is known that the signal transduction pathway involving PKA is mediated predominantly by EP3 and/or EP4 (8, 14), it is likely that these two receptors are responsible for the PGE2 modulated TNF-α-stimulated MCP-1 expression in pancreatic acini. Further support for a PKA-mediated effect is the phosphorylation of CREB. After activation of Gs by the EP2,4 receptor(s) cAMP is increased and subsequently PKA converts CREB to phospho-CREB. As it is known that the cAMP response element-binding protein after activation of EP2 and EP4 prostaglandin receptors by prostaglandin E2. Mol Pharmacol 68: 251–257, 2005.


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