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Inhibition of Matrix Metalloproteinase-3 and -13 Synthesis Induced by IL-1β in Chondrocytes from Mice Lacking Microsomal Prostaglandin E Synthase-1

Marjolaine Gosset,* Audrey Pigenet,* Colette Salvat,* Francis Berenbaum,*† and Claire Jacques*

Joint destruction in arthritis is in part due to the induction of matrix metalloproteinase (MMP) expression and their inhibitors, especially MMP-13 and -3, which directly degrade the cartilage matrix. Although IL-1β is considered as the main catabolic factor involved in MMP-13 and -3 expression, the role of PGE2 remains controversial. The goal of this study was to determine the role of PGE2 on MMP synthesis in articular chondrocytes using mice lacking microsomal PGE synthase-1 (mPGES-1), which catalyses the rate-limiting step of PGE2 synthesis. MMP-3 and MMP-13 mRNA and protein expressions were assessed by real-time RT-PCR, immunoblotting, and ELISA in primary cultures of articular chondrocytes from mice with genetic deletion of mPGES-1. IL-1β–induced PGE2 synthesis was dramatically reduced in mPGES-1−/+ and mPGES-1+/− compared with mPGES-1+/+ chondrocytes. A total of 10 ng/ml IL-1β increased MMP-3 and MMP-13 mRNA, protein expression, and release in mPGES-1+/+ chondrocytes in a time-dependent manner. IL-1β–induced MMP-3 and MMP-13 mRNA expression, protein expression, and release decreased in mPGES-1−/+ and mPGES-1+/− chondrocytes compared with mPGES-1−/− chondrocytes from 8 up to 24 h. Otherwise, MMP inhibition was partially reversed by addition of 10 ng/ml PGE2 in mPGES-1−/− chondrocytes. Finally, in mPGES-1−/− chondrocytes treated by forskolin, MMP-3 protein expression was significantly decreased compared with wild-type, suggesting that PGE2 regulates MMP-3 expression via a signaling pathway dependent on cAMP. These results demonstrate that PGE2 plays a key role in the induction of MMP-3 and MMP-13 in an inflammatory context. Therefore, mPGES-1 could be considered as a critical target to counteract cartilage degradation in arthritis.

MMP-3 expression has been described (25), whereas in another study, no effect was obtained (26). By using the selective COX-2 inhibitor NS398, Choi et al. (27) showed that IL-1β induces MMP-2 expression through a PGE2-dependent mechanism. Finally, addition of exogenous PGE2 significantly reduced IL-1β–induced MMP-1, -3, and -13 expression in equine chondrocytes (28). But, in another study, exogenous PGE2 increased IL-1β–induced MMP-13 and decreased IL-1β–induced MMP-1 expression in OA chondrocytes (29). It has been suggested that the chondroprotective or -degradative effect of PGE2 may vary according to the concentration (29, 30). Moreover, catabolic effects of PGE2 could be dependent on the usage of its various E receptors (EP, named EP-1 to EP-4), expressed on chondrocytes, and coupled with distinct signaling pathways (31).

Therefore, up to now, there is no strong evidence on the role of PGE2 in MMP synthesis when chondrocytes are placed in an inflammatory context. In the current study, we demonstrate a critical role for PGE2 in the induction of MMP-3 and MMP-13 in articular chondrocytes using mice lacking mPGES-1.

Materials and methods

Materials

All reagents were purchased from Sigma-Aldrich (St. Quentin Fallavier, France), unless stated otherwise. Recombinant human IL-1β was from PeproTech (Tebu-Bio, Le Perray-en-Yvelines, France). Recombinant PGE2 was from Cayman Chemical (SPI-Bio, Montigny-le Bretonneux, France).

Animals

Experiments were performed on mice of the DBA/1 J strain with a deletion of the Pges gene that encodes mPGES-1 [gift from Pfizer, Groton, CT (18)], mPGES-1 knockout mice (n = 8), heterozygous (n = 8), and wild-type DBA/1 J (n = 8) littermates arise from the mPGES-1−/− matings. Genomic DNA from mice tail fragment was prepared following the HotSHOT technique (32) and was used to perform the genotyping PCR. The PCR primer sequences designed to amplify a neomycin-resistance gene fragment were (sense) 5'-CTTCCAGGTGTTGGGATTTAGACG-3' and (antisense) 5'-AGTTGCGCTGACTGTTTGTCG-3'. And those designed to amplify a neomycin-resistance gene fragment were (sense) 5'-CTCCAGGTGTTGGGATTTAGACG-3' and (antisense) 5'-AGTTGCGCTGACTGTTTGTCG-3'. The mPGES-1 targeting vector originally used to disturb the mPGES-1 gene contains a neomycin-resistance gene. All animals were housed individually in a pathogen-free facility at controlled temperature on a 12:12 h light/dark cycle (lights off at 18:00 h) with food and water available ad libitum. All procedures were in accordance with the European Directive (UAR AO4, SAFE, Epinay-sur-Orge, France) and water available ad libitum. All procedures were in accordance with the European Directive (UAR AO4, SAFE, Epinay-sur-Orge, France) and water available ad libitum.

Primary culture of mouse cells

All experiments were performed according to the protocols approved by the French/European ethics committee. Immature mouse articular chondrocytes (iMACs) were isolated by enzymatic digestion of articular cartilage from knockout, heterozygous, or wild-type 2- to 3-old newborn animals from one mouse litter according to the procedure described elsewhere (33, 34). After 6 to 7 d of culture, the cells were placed in serum-free DMEM 1 mg/l glucose-PS glutamine containing 1% BSA for 24 h.

PGE2, MPP-3, TIMP-1, and TIMP-2 assays

PGE2 in the medium was measured with an enzyme immunoassay kit from Cayman Chemical (from SPI-Bio). Total mouse MPP-3 and TIMP-1 and TIMP-2 were measured with an ELISA kit from respectively R&D systems (Lille, France) and RayBiotech (from Tebu-Bio). The limits of detection were 9 pg/ml for PGE2, 5 pg/ml for MPP-3, 3 pg/ml for TIMP-1, and 80 pg/ml for TIMP-2. PGE2, MPP-3, TIMP-1, and TIMP-2 concentrations were analyzed at serial dilutions in duplicate and were read against standard curves.

RNA extraction, reverse transcription, and real-time quantitative RT-PCR

Quantification of specific MMP-3, MMP-13, TIMP-1 to -4, and mPGES-1 mRNA was performed using the iCycler IQ Real Time PCR (Bio-Rad, Marnes-la-Coquette, France) and QuantitTec SYBR PCR kits (Qiagen, Courtaboeuf, France) as previously described (34). mRNA were normalized to those of murine hypoxanthine phosphoribosyltransferase (HPRT). Probe sequences, amplicon lengths, and melting temperatures were: HPRT forward 5'-GCTGGTGAAAAGGACCTCT-3', reverse 5'-CACAGGACCTGAAAACCTCG-3' 249 bp; mPGES-1 forward 5'-CTGCTGTGCACTAAAGGTGATGTA-3', reverse 5'-CCACTGTTGGAACCCAGTGT-3', 58°C, 294 bp; MMP-3 forward 5'-AGAATGAAAGGAGGTCTCCGG-3', reverse 5'-GCAGAGCTCCATACCAGCA-3', 58°C, 108 bp; and MMP-13 forward 5'-TGTGGGACTGCTGACATCCT-3', reverse 5'-TGTAGCCTTGTGAACTGCTT-3', 58°C, 173 bp. Mouse TIMP-1 to -4 primers were from Qiagen.

Protein extraction and Western blotting

Cell lysates were prepared and Western blot was carried out as previously described (8) with anti-mouse mPGES-1 polyclonal Ab (from Cayman Chemical, SPI-Bio), anti-human MMP-3 and MMP-13 polyclonal Ab (from Santa Cruz Biotechnology, Tebu-Bio), and anti-mouse β-actin mAb (Sigma-Aldrich). For densitometry analysis, we used Multi-Gauge version 3.0 software (Fujifilm, Courbevoie, France) (data not shown).

Statistical analysis

Each result is expressed as the fold induction compared with control (set at 1). All data are reported as the mean ± SD unless stated otherwise. Statistical analysis was performed with the Welch corrected t test to compare mean values between two groups and with one-way ANOVA with Bonferroni post hoc correction to compare mean values among >2 groups using GraphPad Prism software (GraphPad, San Diego, CA). The p values ≤0.05 were considered significant.

Results

Time course of IL-1β–induced mPGES-1 expression in wild-type, heterozygous, and null mPGES-1 mouse articular chondrocytes

We have previously reported the expression of mPGES-1 mRNA and protein in response to IL-1β in primary culture of human OA chondrocytes and in the human cell line T/C-28a2. We notably described a dose-dependent (from 0–10 ng/ml) and a time-dependent (from 0–24 h) overexpression of mPGES-1 in response to IL-1β, paralleled by PGE2 synthesis (8).

In this work, we used iMACs, which allow us to work with mPGES-1 knockout mice. First, we deciphered the time-course expression of mPGES-1 mRNA in the wild-type chondrocytes. We observed an overexpression of mPGES-1 mRNA in the wild-type iMACs from 2 h, peaking at 8 h (26-fold increase compared with the control; p ≤ 0.05) and decreasing progressively up to 24 h (4-fold increase compared with the control; NS; Fig. 1A). At the protein level, mPGES-1 expression was increased from 8 h and sustained up to 24 h (data not shown) and was paralleled with a significative PGE2 overrelease from 18 h (a 70 fold-induction; p ≤ 0.01) up to 24 h (a 136 fold-induction; p ≤ 0.01) compared with the control (wild-type control iMACs released 24 ± 6.3 pg/ml of PGE2; Fig. 1B).

To validate the absence of mPGES-1 expression in null mPGES-1 iMACs, we compared the time course of mPGES-1 mRNA expression in response to 10 ng/ml IL-1β in the three different genotypes (Fig. 1A). As expected, the mPGES-1 expression in null chondrocytes was absent poststimulation by IL-1β at the different times. Interestingly, mPGES-1 expression in heterozygous chondrocytes was significantly reduced after 8 h and 18 h of IL-1β stimulation (respectively, a 72% decrease, p ≤ 0.01, and a 39% decrease, p ≤ 0.05 compared with the wild-type iMACs).

Time course of IL-1β–induced PGE2 production in wild-type, heterozygous, and null mPGES-1 mouse articular chondrocytes

Because mPGES-1 is the final enzyme of the arachidonic cascade leading to PGE2 synthesis, we assayed the consequences of mPGES-1 gene deletion by measuring PGE2 production in the supernatant of the articular chondrocytes treated with IL-1β up to
Effect of mPGES-1 genetic deletion on IL-1β-stimulated MMP-3 expression and secretion

MMP-3 mRNA level was significantly decreased in the heterozygous chondrocytes compared with the wild-type cells at 8 h and sustained up to 24 h (a 65% decrease; p < 0.05; Fig. 2A). In the mPGES-1 null chondrocytes, a tendency to decrease of the IL-1β–induced MMP-3 mRNA release at 24 h was observed (a 60% decrease; p = 0.06; Fig. 2A). At the protein level, IL-1β–induced MMP-3 protein expression was significantly decreased in mPGES-1 heterozygous and null iMACs compared with wild-type chondrocytes from 18 h up to 24 h (at 24 h, 80% decrease between

FIGURE 1. Inhibitory effect of mPGES-1 gene deletion on IL-1β–induced PGE2 release in iMACs. Primary cultures of iMACs from wild-type (+/+), heterozygous (+/−), and null (−/−) mPGES-1 mice were serum starved and then stimulated with 10 ng/ml IL-1β up to 24 h. The PGE2 concentration in the medium was measured, total RNA was extracted, and levels of microsomal PGE synthase type 1 (mPGES-1) mRNA were assayed. A. The mRNA expression of mPGES-1 in chondrocytes in response to IL-1β was determined by real-time PCR. B. The amount of PGE2 released into the medium (pg/ml) in response to IL-1β was measured by enzyme immunoassay. Values are the fold increase compared with wild-type control (set at 1) and are the mean ± SD of three to six independent experiments with n = 1/group/experiment, analyzed in duplicate. *p ≤ 0.05; **p ≤ 0.01 versus wild-type control; †p ≤ 0.05; ‡‡p ≤ 0.01; ‡‡‡p ≤ 0.001 versus wild-type; ††p ≤ 0.05; †††p ≤ 0.01; ††††p ≤ 0.001 versus wild-type.

24 h. A total of 10 ng/ml IL-1β–induced PGE2 synthesis was significantly reduced in mPGES-1 null iMACs compared with wild-type cells at 6 h (a 66% decrease; p ≤ 0.05). Indeed, after 24 h of IL-1β stimulation, a 96% inhibition of PGE2 production was observed for null iMACs compared with wild-type cells (Fig. 1B; p ≤ 0.001; IL-1β–induced wild-type chondrocytes released 3267 ± 449 pg/ml of PGE2). Interestingly, in mPGES-1 heterozygous iMACs, inhibition of IL-1β–induced PGE2 production was significant at 18 h (77% decrease; p ≤ 0.01) and sustained up to 24 h (82% decrease; p ≤ 0.001) compared with wild-type cells (Fig. 1B). Therefore, heterozygous mPGES-1 chondrocytes exhibited also an important PGE2 decrease in response to IL-1β. Then, we compared the MMP-3 and MMP-13 expression in chondrocytes from the three genotypes.

Kinetics of MMP-13 and MMP-3 expressions in wild-type mPGES-1 mouse articular chondrocytes in response to IL-1β

Because the matrix breakdown occurring in arthritis is due, at least in part, to MMP activities and especially MMP-3 and MMP-13, we studied the kinetics of their mRNA and protein expression in wild-type mPGES-1 iMACs treated with 10 ng/ml IL-1β. mRNA levels and protein expression and secretion were analyzed using respectively quantitative real-time PCR, immunoblotting, and ELISA. Interestingly, the time-course profile of IL-1β–induced MMP-13 and MMP-3 mRNA and protein expression were different (Supplemental Fig. 1).

FIGURE 2. Effect of mPGES-1 gene deletion on IL-1β–induced MMP-13 expression and release in iMACs. iMACs from wild-type (+/+), heterozygous (+/−), and null (−/−) mPGES-1 mice were serum starved and then stimulated with 10 ng/ml IL-1β up to 24 h. Total RNA or total intracellular proteins were extracted to assay, respectively, MMP-13 mRNA or protein levels. Media from cells incubated were assayed for analyzing MMP-13 release into the media. A. The mRNA expression of MMP-13 in chondrocytes in response to IL-1β was determined by RT-PCR at 8 and 24 h. Values are the mean ± SD of three to six independent experiments with n = 1/group/experiment, analyzed in duplicate. B and C, MMP-13 protein expression and secretion into the medium in response to IL-1β were assessed by immunoblotting. Blots in B and C are representative of three independent experiments. Values are the fold decrease compared with +/+ mPGES-1 chondrocytes. ***p ≤ 0.001 versus wild-type; †p ≤ 0.05 versus wild-type.
FIGURE 3. Effect of mPGES-1 gene deletion on IL-1β–induced MMP-3 expression and release in iMACs. iMACs from wild-type (+/+), heterozygous (+/−), and null (−/−) mPGES-1 mice were stimulated with 10 ng/ml IL-1β up to 24 h in serum-free medium. Total RNA or total intracellular proteins were extracted to assay, respectively, MMP-3 mRNA or protein levels. Media from cells incubated were assayed for analyzing MMP-3 release into the medium. A, The mRNA expression of MMP-3 in chondrocytes in response to IL-1β was determined by RT-PCR at 8 and 24 h. Values are the mean ± SD of three to six independent experiments with n = 1/group/experiment, analyzed in duplicate. Values are the fold decrease compared with +/+ mPGES-1 chondrocytes. B, MMP-3 protein expression in response to IL-1β was assessed by immunoblotting. C and D, Immunoblotting was performed, and the amount of MMP-3 released into the medium (ng/ml) in response to IL-1β was measured by ELISA. Blots in B and C are representative of three independent experiments. Values are the fold increase compared with wild-type control (set at 1) and are the mean ± SD of three to six independent experiments with n = 1/group/experiment, analyzed in duplicate. *p ≤ 0.05; **p ≤ 0.01 versus wild-type control; †p ≤ 0.05; ††p ≤ 0.001 versus wild-type; ‡‡‡p ≤ 0.001 versus wild-type.

Effect of mPGES-1 genetic deletion on forskolin- or PGE2-stimulated MMP-3 secretion

PGE2 has been shown to mediate some of its downstream effects via cAMP-dependent pathways. To delineate the mechanism by which PGE2 regulates MMP-3 expression, we treated wild-type and mPGES-1 null chondrocytes with forskolin (a direct adenyl cyclase activator) or PGE2, and we addressed its effect on MMP-3 protein expression by ELISA. In wild-type mPGES-1 iMACs, we observed an overrelease of MMP-3 in the supernatants of cells stimulated with 10 μM forskolin or 10 ng/ml PGE2 during 24 h compared with control (a 16 fold-increase; p ≤ 0.001; at 24 h, wild-type chondrocytes released 2.8 ng/ml MMP-3). Interestingly, at 24 h, MMP-3 concentration in the supernatant of mPGES-1 null and heterozygous chondrocytes was decreased (respectively, a 72% decrease, p ≤ 0.001, and a 66% decrease, p ≤ 0.001) compared with mPGES-1 wild-type chondrocytes.

Partial reversion of MMP-13 and MMP-3 expression, by addition of exogenous PGE2, in null mPGES-1 mouse chondrocytes stimulated by IL-1β

To confirm the role of PGE2 in IL-1β–induced MMP-3 and MMP-13 expression, we checked if exogenous PGE2 could reverse the inhibition of IL-1β–induced MMP-3 and MMP-13 observed in mPGES-1 null iMACs. Therefore, we costimulated the mPGES-1 null chondrocytes with 10 ng/ml IL-1β and PGE2 at various concentrations (1, 5, and 10 ng/ml) during 24 h. By immunoblotting, we found that MMP-13 inhibition was partially reversed by addition of PGE2 in mPGES-1 null chondrocytes in a dose-dependent manner (Fig. 4A). By ELISA, addition of exogenous PGE2 partially reversed the IL-1β–induced MMP-3 inhibition observed in mPGES-1 null cells. Addition of 1, 5, or 10 ng/ml PGE2 triggered a 2-fold increase (p ≤ 0.05) in IL-1β–treated cells in mPGES-1 null chondrocytes (Fig. 4B).

Effect of mPGES-1 genetic deletion on IL-1β–stimulated TIMP expression and secretion

Because TIMPs were the major natural MMP inhibitor in cartilage, we analyzed their expression in mPGES-1 wild-type iMACs. The time-course profiles of IL-1β–induced TIMP-1 and TIMP-2 to -4 mRNA expressions have shown that IL-1β upregulates TIMP-1 mRNA and protein expressions and downregulates TIMP-2, -3, and -4 mRNA expressions in these cells (Supplemental Fig. 2).
Finally, we compared the IL-1β-induced TIMP-1 to -4 expressions in articular chondrocytes from the three genotypes. TIMP-1 mRNA expression exhibited the same time-course profile in mPGES-1 wild-type, heterozygous, and null chondrocytes treated by 10 ng/ml IL-1β. TIMP-2 mRNA expression exhibited a decrease after 8 h of treatment by IL-1β in heterozygous and null mPGES-1 iMACs compared with the wild-type mPGES-1 iMACs. In control and posttreatment of IL-1β during 24 h, TIMP-2 mRNA expression exhibited the same time-course profile in mPGES-1 wild-type, heterozygous, and null chondrocytes. For TIMP-1 and TIMP-2, these results were confirmed at the protein level (at 24 h of IL-1β induction, wild-type mPGES-1 iMACs released 76 ± 6.2 ng/ml TIMP-1 and 1.39 ± 0.7 ng/ml TIMP-2, whereas null mPGES-1 iMACs released 69 ± 25 ng/ml TIMP-1 and 0.95 ± 0.14 ng/ml TIMP-2; NS, data not shown). TIMP-3 mRNA expression was slightly increased in control null mPGES-1 iMACs compared with the control wild-type mPGES-1 iMACs. In a 2.5-fold increase; p ≤ 0.05), but, IL-1β inhibited TIMP-3 mRNA expression in both genotypes. The profile of TIMP-4 mRNA expression is almost similar as TIMP-3, but the increase in control null mPGES-1 iMACs compared with the control wild-type mPGES-1 iMACs is not significant (Fig. 6).

### Discussion

This study using iMACs from mPGES-1-deficient mice clearly draws out two main conclusions. First, we confirm that a genetic deletion of mPGES-1 results in a dramatic decrease in IL-1β–induced PGE2 production, not only in mPGES-1 null chondrocytes but also in heterozygous mice. This result demonstrates that mPGES-1 is critical for PGE2 production in murine articular chondrocytes. Second, the striking point of this study is the demonstration that mPGES-1 gene deletion and resultant decrease in PGE2 production results in a differential expression profile of MMP-13 and MMP-3 in both heterozygous and null mPGES-1 chondrocytes compared with wild-type cells. This is clearly shown by the partial reversion of MMP-13 and MMP-3 expression in response to exogenous PGE2 added to IL-1β induced null mPGES-1 iMACs. Therefore, these results demonstrate that PGE2 plays a crucial role in the induction of MMP-13 and MMP-3 in chondrocytes in response to IL-1β.

In this study, we decided to analyze MMP-3 and MMP-13 expressions, two MMPs mainly involved in cartilage degradation occurring in arthritis. In fact, MMP-13 and MMP-3 level expression increases considerably in arthritic cartilage and synovium, resulting in aberrant destruction of cartilage tissue (35–39). MMP-13 and MMP-3 are involved in cartilage degradation occurring in several murine models of arthritis, therefore validating mouse species as a good tool to study MMP-13 and MMP-3 regulation. Moreover, we analyzed mRNA expression of MMP-1, -2, and -9, three MMPs that could be involved in matrix degradation of cartilage. At 8 h and 24 h, IL-1β–induced MMP-1 mRNA expression was not modified in mPGES-1 null chondrocytes compared with wild-type chondrocytes. Concerning MMP-2 and MMP-9, there was a tendency to a decrease of mRNA level at 8 h in mPGES-1 null chondrocytes compared with wild-type chondrocytes, but it was not statistically significant. This tendency became significant at 24 h for MMP-2, but the percentage of decrease was 37% (compared with 60% for MMP-3 and 46% for MMP-13).

So, these results suggest that MMP-3 and MMP-13 are the MMPs that are strongly regulated between mPGES-1 null chondrocytes and wild-type chondrocytes even if MMP-2 and MMP-9...
were also able to be regulated, but the percentage of decrease was less important at 24 h between the two genotypes (data not shown).

We also decided to study TIMP expression in iMACs from mPGES-1 knockout mice. Four TIMPs named TIMP-1–4 have been cloned. These small proteins bind to MMPs (ratio 1:1) to inhibit their activity. They exhibit a specific tissue expression profile and affinity for the various MMPs, characterizing their activities. Their expressions have been studied in the arthritic joint (40–43) but remain unclear. In our study, we originally described the time-course of TIMP-1, -2, -3, and -4 expressions in murine articular chondrocytes stimulated by IL-1β. We found that IL-1β upregulates TIMP-1 mRNA and protein expressions and down-regulates TIMP-2, -3, and -4 mRNA expressions in these cells. We did not find any modifications in IL-1β–induced TIMP expression in null or heterozygous mPGES-1 iMACs compared with wild-type cells. Only the TIMP-3 mRNA basal expression was increased in control null mPGES-1 compared with control wild-type mPGES-1 iMACs. Interestingly, TIMP-3 knockout mice naturally developed with age a more severe OA than TIMP-3 wild-type mice (44). Therefore, the overexpression of TIMP-3 observed in mPGES-1 null mice could represent a protection against cartilage degradation in arthritis. It may explain, at least in part, the persistance of cartilage degradation in surgically induced OA in mPGES-1 null mice (45).

Many MMPs are regulated at the transcriptional level by a variety of growth factors, cytokines, and chemokines (46). Interestingly, MMP promoters can be assigned to one of three groups: 1) those that contain TATA boxes at around 70 bp with AP-1 sites (MMP1, MMP3, MMP7, MMP9, MMP10, MMP12, MMP13, MMP19, and MMP26); 2) those that contain a TATA box, but no AP-1 site in the proximal promoter (MMP8, MMP11, and MMP21); and 3) those with neither TATA boxes nor proximal AP-1 sites (MMP2, MMP14, and MMP28) (46). Interestingly, MMP-3 and MMP-13 belong to the same group.

Regulation of MMP13 gene expression by runt-related transcription factor 2 and AP-1 sites has been described in osteoblasts, hypertrophic chondrocytes, and stably transfected chondrocytic cell lines (47–50). Moreover, recent studies have shown that estrogen receptor-α can regulate the expression of MMP-13, primarily through the AP-1 transcriptional regulatory site (51) in the HIG-82 cells.

Recently, chromatin immunoprecipitation experiments showed nucleolin binding in the vicinity of the AP-1 site within the MMP13 promoter. Moreover, overexpression of nucleolin can repress transactivation of the MMP13 promoter transiently transfected into HeLa cells (52). So, there are many levels to regulate MMP expression, and the way in which PGE2 is able to regulate MMP-3 and MMP-13 needs to be clarified.

The main objective of our study was to decipher the role of PGE2 in MMP-3 and MMP-13 expression in iMACs in an inflammatory context. Many studies described an overrelease of PGE2 in the synovial fluid of RA and OA patients. But, although it has been appreciated for a decade that chondrocytes synthesized and are sensitive to PGE2 (6), consequences of PG release on cartilage homeostasis remain unclear. Some studies indicated that the effect of PGE2 may be anabolic or catabolic according to its concentration. For example, addition of a low dose of PGE2 (10 pg/ml) downregulated collagen cleavage and expression of genes associated with collagen cleavage, such as MMP-13, IL-1β, and TNF-α (30). But, at a higher dose, exogenous PGE2 (10 µM equivalent to 3.5 µg/ml) increased IL-1β–induced MMP-13 expression in cartilage explants obtained from patients undergoing knee replacement surgery for advanced OA (29). Other arguments come from studies using IL-1β–stimulated chondrocytes. It is well known that IL-1β is a potent inducer of PGE2 release (5). In these cells, opposite effects on the regulation of MMPs expression in response to COX inhibitor were published. Indomethacin inhibited IL-1β–increased MMP-3 expression in human articular chondrocytes (25), whereas IL-1β–induced MMP-13 expression was significantly inhibited by the COX-2 selective inhibitor celecoxib in human OA articular cartilage explants (29). However, celecoxib inhibited IL-1β–induced MMP-3 in human normal OA and RA chondrocytes but in a PGE2-independent manner (26). In contrast, neither indomethacin nor celecoxib and any other nonsteroidal anti-inflammatory drugs were able to inhibit IL-1β–induced MMP-3 production in human chondrocytes in three-dimensional cultures, whereas these nonsteroidal anti-inflammatory drugs fully blocked PGE2 production (53). Because our study used chondrocytes coming from mice lacking the mPGES-1 gene, we ruled out the question of dosage by naturally depleting these cells of IL-1β–induced PGE2 release.
The reasons for the dual effects of PGE₂, chondroprotective or chondrodegradative according to the dose, may be related to receptor usage. In chondrocytes, expression of the four subtypes of PGE₂ receptors, named EP1, EP2, EP3, and EP4, was described. They are coupled to distinct signaling pathways involving activation of phospholipase C (EP1), activation of adenylate cyclase (EP2 and EP4), and inhibition of adenylate cyclase (EP3), indicating their potential for divergent responses. It is well established that EP1 and EP2 receptors for PGE₂ are low-affinity receptors, whereas EP3 and EP4 are high-affinity receptors (54). Activation of the low-affinity PGE₂ receptors is likely to be important in mediating the actions of much higher concentrations of PGE₂ found in various inflammatory responses such as OA. Sato et al. (55) have found that suppression of EP2 expression enhances MMP-13 induction in human OA chondrocytes. Moreover, selective stimulation of the PGE2 signal through EP2 receptors is a specific agonist-promoted cartilage regeneration, characterized by an enhanced collagen type II expression in chondral defects of rabbit knee joints (56). Therefore, EP2 is a low-affinity receptor that could be implicated in anabolic response to PGE₂ in inflammatory processes. At the opposite end, the catabolic effects of PGE₂ in cartilage that may occur in arthritis could be due to the EP4 receptors. Notably, receptor deletion studies have shown that homozygous deletion of the EP1, EP2 or EP3 receptors does not affect the development of arthritis, whereas EP4 receptor-deficient mice showed decreased incidence and severity of the disease (57). Interestingly, Attur et al. (29) have shown that only EP4 was upregulated in OA versus normal cartilage, suggesting that EP4 is implicated in the mediation of PGE₂ catabolic effects in OA. Moreover, blocking EP4 receptor inhibited MMP-13 expression induced by PGE₂ (29). Therefore, it seems that in inflammatory conditions, both EP2 and EP4 receptors (58, 59) are implicated in chondrocytes in response to PGE₂. As the first one can trigger anabolic responses, the second one, which is the only one up-regulated in arthritis cartilage, may mainly be implicated in transducing the catabolic processes of PGE₂. Our results using forskolin stimulation suggest that PGE₂ regulates MMP-3 expression via a signaling pathway dependent of cAMP and therefore probably by binding to EP4 receptors. Surprisingly, mPGES-1 null iMACs do not make similar amounts of MMP3 as wild-type cells when the PGE₂ signal is provided directly with either exogenous PGE₂ or forskolin. This difference could be due to p eroxisome proliferator-activated receptor γ (PPARγ) differences (16).

Actually, an upregulation of anti-inflammatory molecules in embryonic fibroblasts from null mPGES-1 mice has been described. Kapoor et al. (16) reported a basal elevation of PPARγ expression (protein and mRNA) in these cells. Interestingly, PPARγ reduces in vivo the severity of OA in a guinea pig model in part due to the reduction of MMP-13 protein expression in cartilage (60). Moreover, PPARγ agonist inhibited IL-1β-induced MMP-13 expression in human chondrocytes at the transcriptional level, probably by interfering with the activation of AP-1 (61). Finally, this effect was also described for MMP-3 because PPARγ inhibited MMP-3 production and subsequent proteoglycan degradation in rat cartilage explants (62). Therefore, besides the down-regulation of PGE₂ release in mPGES-1 null iMACs, an increased release of PPARγ may be implicated in downregulation of IL-1β-induced MMP-3 and MMP-13 expression.

mPGES-1 is functionally coupled to COX-2, being responsible for excessive PGE₂ generation by converting PGH₂ into PGE₂ in an inflammatory context. Blockade of mPGES-1 allows a shift of the substrate PGH₂ to other PG synthases, resulting in overproduction of other PGs. Some of them could have anti-inflammatory activities (16), but we cannot rule out an overexpression of other prodegradative PGs explaining the partial and not the full inhibition of MMP-3 and MMP-13 expression in iMACs from mPGES-1-/- mice.

Recent clinical trials using selective COX-2 inhibitors have suggested that specific inhibition of COX-2 could be associated with increased incidence of cardiovascular events (63–65). One hypothesis is based on the concomitant inhibition of the antithrombotic prostacyclin (PGI2) (66). Thus, pharmacological inhibition of mPGES-1 in inflammatory arthritis may avoid this cardiovascular deleterious effect of COX-2 inhibitors.

In summary, our study clearly demonstrates that PGE₂ involved in IL-1β induced MMP-3 and MMP-13, but not in TIMP-1 to -4, expressions in murine articular chondrocytes. mPGES-1 represents an attractive target for disease-modifying drug development because its inhibition would specifically diminish PGE₂ production while preserving the production of other PGs. Specific inhibitors of mPGES-1 are actually tested in in vivo studies (67, 68). Interestingly, in accord with our results, the use of such inhibitors could improve arthritis treatment by interacting with two major MMPs implicated in cartilage degradation. This study highlights potential mechanisms explaining why the inhibition of mPGES-1 protects cartilage degradation in experimental arthritis. Therefore, these results show that mPGES-1 could be considered as a critical target to counteract cartilage degradation in arthritis (69, 70).

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Disclosures

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References


