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ANTI-INFLAMMATORY PROPERTIES OF PROSTAGLANDIN E2: DELETION OF MICROSOMAL PROSTAGLANDIN E SYNTHASE-1 EXACERBATES NON-IMMUNE INFLAMMATORY ARTHRITIS IN MICE

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SUMMARY

Prostanoids and PGE2 in particular have been long viewed as one of the major mediators of inflammation in arthritis. However, experimental data indicate that PGE2 can serve both pro- and anti-inflammatory functions. We have previously shown (Kojima, F. et al. 2008 *J. Immunol.* 180, 8361-8368) that microsomal prostaglandin E synthase-1 (mPGES-1) deletion, which regulates PGE2 production, resulted in the suppression of collagen-induced arthritis (CIA) in mice. This suppression was attributable, at least in part, to the impaired generation of type II collagen autoantibodies. In order to examine the function of mPGES-1 and PGE2 in a non-autoimmune form of arthritis, we used the collagen antibody-induced arthritis (CAIA) model in mice deficient in mPGES-1, thereby bypassing the engagement of the adaptive immune response in arthritis development. Here we report that mPGES-1 deletion significantly increased CAIA disease severity. The latter was associated with a significant (~3.6) upregulation of neutrophil, but not macrophage, recruitment to the inflamed joints. The lipidomic analysis of the arthritic mouse paws by quantitative liquid chromatography / tandem mass-spectrometry (LC/MS/MS) revealed a dramatic (~59-fold) reduction of PGE2 at the peak of arthritis. Altogether, this study highlights mPGES-1 and its product PGE2 as important negative regulators of neutrophil-mediated inflammation and suggests that specific mPGES-1 inhibitors may have differential effects on different types of inflammation. Furthermore, neutrophil-mediated diseases could be exacerbated by inhibition of mPGES-1.

Keywords

Nanohybrids; Room-temperature phosphorescence(RTP); Sensor; Rutin

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INTRODUCTION

Prostaglandins (PG) are important lipid mediators known to regulate a broad spectrum of physiological functions, including immunity and inflammation (1,2). PG are produced from arachidonic acid (ARA), which is released from membrane phospholipids by phospholipases (3) or synthesized from linoleic acid (LA) (4). The ARA metabolic cascade includes a rate-limiting step of ARA conversion into PGH₂ which is controlled by the cyclooxygenase (COX) isoforms 1 and 2 (5). PGH₂ is then quickly processed to prostanoids, such as PGF₂, PGD₂, and PGE₂, by their respective PG isomerases (6) (Fig.1).

Prostanoids and PGE₂ in particular, have been shown to play an important role in as mediators of symptoms of inflammatory arthritides including rheumatoid arthritis (RA) as demonstrated by the widespread use of treatments that inhibit PG production (7–10). However, the current strategies to inhibit PGs by using non-steroidal anti-inflammatory drugs (NSAID) and selective COX-2 inhibitors are often inadequate in providing symptomatic relief of pain, swelling, and stiffness (11,12). Setting aside the incomplete blocking efficiency of NSAID and COX-2 selective inhibitors *in vivo*, the limited ability of these agents to curb inflammation could be explained, at least in part, by two-faceted and opposing roles of PG, especially PGE₂, in the regulation of the inflammatory process. Indeed, the lipidomic analysis of animals with Lyme arthritis revealed that among major PG, only PGE₂ production in arthritic joints was undergoing a cyclic change and was rising to a similar level during both the initiation and the resolution stage of the inflammatory disease (13). This would be indicative of a dual role of PGE₂ in regulation of inflammation: it could mediate inflammation at the initiation stage while inhibiting inflammation during resolution or by triggering a resolution program. The requirement of PGE₂ for the resolution of inflammation was confirmed in the recent studies where mice at the chronic stage of the autoimmune arthritis, when treated with the specific COX-2 inhibitor, displayed an increased disease severity which was corrected downwards upon repletion with a synthetic PGE₂ analog (14). A growing body of evidence indicates that PGE₂ apparently exerts its antiinflammatory functions at both a cellular and molecular levels. The cellular mode of PGE₂ antiinflammatory functions includes suppression of neutrophil functions (15), reprogramming the classically activated macrophages (M1) into the alternatively activated macrophages (M2) (16), and skewing monocyte-to-macrophage differentiation toward M2 (17). At the molecular level, PGE₂ has been shown to negatively regulate inflammation by inhibiting CCL5 expression in activated macrophages (18), suppressing macrophage and synovial fibroblast (SF) TNF- α expression induced by T cell-derived IL-17 (19), blocking NF- κ B activation and NF- κ B/DNA binding in response to LPS stimulation (20), as well as by differentially regulating nuclear translocation of p65 and p50 NF- κ B subunits following SF stimulation with IL-1 β and TNF- α . In the latter case, PGE₂ stimulated p50 nuclear accumulation and inhibited that of p65 thereby promoting formation of p50p50 homodimer and reducing p50p65 heterodimer formation thus efficiently blocking expression of the inflammatory genes (21). It should be noted that sustained p50p50 homodimer nuclear accumulation was shown to play a central role in the resolution of inflammation (22–24). Finally, PGE₂ can exert its antiinflammatory function by switching ARA metabolism toward production of anti-inflammatory and pro-resolution lipids, including lipoxin A4 (LXA4) as well as E- and D-series resolvins and protectins (25).

PGE₂ production during acute and chronic inflammation is dependent on the enzyme mPGES-1, an inducible enzyme whose expression is upregulated strongly under inflammatory conditions. mPGES-1 functions in the ARA metabolic cascade downstream from COX-2 by catalyzing the isomerization of the endoperoxide PGH₂ into PGE₂ (26,27) (Fig.1). mPGES-1 works alongside two other PGH₂ isomerases, cytosolic PGES (cPGES) and mPGES-2, but the latter two enzymes are constitutively expressed and are mostly

responsible for basal production of PGE₂ (26). Studies on the deletion of mPGES-1 gene in mice revealed its essential role in female fertility, pain, atherosclerosis, tumorigenesis, and inflammation which made this enzyme an attractive therapeutic target (28).

Our group (29) and others (30) by using CIA model have demonstrated a significant reduction in the incidence and the severity of this autoimmune arthritis in mPGES-1 null mice. The CIA model is dependent on developing cellular and humoral immune responses to type II collagen. We found that the mPGES-1 null mice had a profound defect in their ability to generate collagen-II autoantibodies (29). Therefore, we reasoned that an antiinflammatory role of PGE₂ might be masked by a strong effect of PGE₂ in the regulation of humoral immunity. We now test this hypothesis by using microsomal PGE synthase-1 (mPGES-1) KO mice. In order to bypass the autoantibody production step in arthritis development and correctly ascertain a role for PGE₂ in the regulation of systemic and local inflammation, we chose to induce arthritis in mPGES-1 KO mice through direct injection of collagen-II antibody, i.e. utilizing the well established CAIA model (31,32). In this model, the inflammatory response is triggered after the ligation of Fc γ receptors (Fc γ R) on polymorphonuclear cells and macrophages by immune complexes (31,33).

In the present study we confirmed our hypothesis and demonstrated that mPGES-1, and consequently, PGE₂ deficiency, significantly increased the incidence and severity of CAIA in mice. Furthermore, analysis of specific inflammatory cell markers in the arthritic paws pointed toward a major involvement of neutrophils, and not macrophages, in the maintenance of local inflammation in the mPGES-1 KO animals. Lipidomic profiling of the inflamed joints of arthritic mice revealed changes in the ARA metabolic pathway that were not limited to COX-dependent PGE₂ production and could include all branches of the ARA metabolic pathway.

Altogether, our studies further emphasize the pleiotropic activities of mPGES-1-PGE₂ in inflammatory arthritis. The development of mPGES-1 inhibitors for treatment of inflammation and pain will need to account for diversity in the mediators of different forms of arthritis that are likely to influence efficacy of these agents.

MATERIALS AND METHODS

Animals

DBA1/lac J mPGES-1 +/- mice were obtained from Pfizer and were described elsewhere (29). mPGES-1 -/- mice were obtained by mating mPGES-1 +/- animals. Wild type littermates were used as control. Mice were housed in microisolator cages in a pathogen-free barrier facility, and all experiments were performed under the Institutional Animal Care and Use Committee guidelines as set forth by the University of Kentucky, Lexington KY.

Arthritis model

CAIA was induced in 8–12 week old, randomly selected male and female mice using mouse monoclonal anti-type II collagen 5 clone antibody cocktail kit with lipopolysaccharide (LPS) from *E. coli* 0111:B4 (Chondrex) following the manufacturer's instructions. Mice were immunized on day 0 by intraperitoneal (IP) injection of 150 μ g of the antibody cocktail and boosted with 25 μ g of LPS on day 3. The CAIA severity was assessed using Chondrex mouse arthritis scoring system and was shown to reach its peak on day 7 (<http://www.chondrex.com/animal-models/athrogen-cia-arthritis-genic-monoclonal-antibody-cocktail>) (32).

Real-time RT-PCR

Total RNA was obtained from the synovial capsules that were isolated on day 7 from the front paws of arthritic mice with an arthritis score of 3. The RNA isolation was performed with TRIzol reagent (Ambion) following the manufacturer's instructions. Total RNA was quantitatively converted into the single stranded cDNA by using High Capacity cDNA Archive Kit (Applied Biosystems). Particular genes were detected using the respective TaqMan Gene Expression Assays (Applied Biosystems) on a 7300 Real-Time RT-PCR system from the same manufacturer by relative quantitation employing glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference gene.

Lipid extraction

Lipids were extracted from pulverized front mouse paws as described (13) with the following modifications. After pulverization, the respective frozen powder was immediately placed into the Pyrex 10 ml screw cap analytical tube with the 3 ml of ice-cold 50% ethanol and weighted. The sample wet weight was later used for the normalization of eicosanoid levels. 10 μ l of antioxidant mixture (0.2 mg/ml butylated hydroxytoluene and 0.2 mg/ml EDTA in 2:1:1 methanol:ethanol:H₂O solution) was added to each tube and samples were kept at -80°C for a few days prior to their overnight shipment on dry ice to the lipid analytical facility.

Analysis of eicosanoids by LC/MS/MS

This was carried out as previously described in detail (34). Briefly, an Agilent 1200 SL liquid chromatography series (Agilent Corporation, Palo Alto, CA) with an Agilent Eclipse Plus C18 2.1 \times 150 mm, 1.8 μ m column were used for the eicosanoid separation. The mobile phase A was water with 0.1% acetic acid while the mobile phase B was composed by acetonitrile/methanol (80/15, v/v) and 0.1% acetic acid. Gradient elution was performed at a flow rate of 250 μ L/min. The injection volume was 10 μ L and the samples were kept at 4 °C in the auto sampler. Analytes were detected by negative MRM mode using a 4000 QTrap tandem mass spectrometer (Applied Biosystems) equipped with an electrospray ionization source (Turbo V). Calibration curves were generated by 10 μ L injections of seven standards containing each analyte, internal standard I (d4-PGE₂, d11-14,15-DiHETrE, d8-5-HETE, and d11-11(12)-EpETrE), and internal standard II (1-cyclohexyl-dodecanoic acid urea, CUDA) for quantification purpose.

RESULTS

CAIA is exacerbated in mPGES-1KO mice

Using the described protocol, CAIA in mice is a highly reproducible experimental arthritis model the inflammation reaches a maximum at D7 and persists for two weeks (32). Because we were interested in examining the effect of mPGES-1 deficiency on inflammation when the latter reaches its maximum, we did not extend our analysis of experimental animals with CAIA beyond D7 (Fig.2). As shown in this Figure, two major indicators of arthritis, its incidence and clinical score, were significantly higher in mPGES-1 KO compared to WT mice on D7 (Fig.2 A&B). It is worth noting that arthritis was numerically though not statistically more severe in mPGES-1 KO mice throughout the course of the experiment (Fig.2 A&B). The animal body weights, however, were not different comparing mPGES-1 null and WT mice (Fig.2C). The data would be consistent with the requirement of mPGES-1, and consequently PGE₂, primarily for the suppressive effect on local, and not systemic, inflammation in the CAIA model of inflammatory arthritis.

Increased neutrophil infiltration into the inflamed joints of mPGES-1 KO mice with CAIA

It was previously shown that CAIA was driven by the recruitment of neutrophils and macrophages to the inflamed joints (31,33). Therefore, in order to elucidate a mechanism which is responsible for the observed increased CAIA severity in mPGES-1 mice (Fig.2), we assessed the infiltration of neutrophils and macrophages into the arthritic joints of WT and mPGES-1 KO mice with CAIA at its peak by measuring the expression of the respective specific markers on D7 by real-time RT-PCR. We used myeloperoxidase (MPO) as the neutrophil specific marker (35,36) as well as IL-6 (37,38) and PTGS2 (COX-2) (39,40) as the dual neutrophil/macrophage inflammatory markers. We detailed further the phenotype of activated macrophages in the arthritic joints by probing the expression of the classically activated (M1, inflammatory) and alternatively activated (M2, antiinflammatory) specific markers. We used CCL3 and NOS2 to detect M1 macrophages as well as CD163 and CD206 for the M2 macrophage identification (38,41). The respective data are presented in the Figs.3–5. As it is seen from the Figure 3, CAIA in mPGES-1 KO mice at its peak on D7 is characterized by significantly increased MPO (Fig.3A, ~ 3.6-fold) and PTGS2 (Fig.3C, ~ 2.5-fold) expression as compared to the WT mice. Expression of IL-6 demonstrated a trend toward upregulation (Fig.3B, ~ 2.5-fold), although this change did not reach statistical significance. Given that PTGS2 could be expressed by both neutrophils and macrophages, the above data while being clearly indicative of the increased infiltration of neutrophils into the arthritic joints of mPGES-1 KO mice with CAIA, leave in question the respective macrophage infiltration. We addressed the latter by measuring expression of the M1 and M2 macrophage specific markers. (Figs.4&5). As in these Figures, neither M1 (Fig.4) nor M2 (Fig.5) macrophage markers demonstrated any change, thereby efficiently ruling out upregulated macrophage infiltration as a dominant force for the increased severity of the inflammatory arthritis in mPGES-1 KO mice.

Lipidomic profiling of CAIA in mPGES-1 KO mice

Although a role of ARA metabolites, such as lipoxins, resolvins, and protectins in resolution of inflammation is well established (25), very little is known regarding the function of eicosanoids as antiinflammatory agents in arthritis (13). In order to gain more insight into the role of ARA metabolites, and particularly PGE₂, in inflammatory arthritis, we performed lipidomic analysis of front paws from the unelicited (non-immunized) WT and mPGES-1 KO mice as well as from the mice with CAIA when the latter reached its peak on D7. The results are presented in the Figs.6&7 where the ARA metabolites are grouped according to their respective biosynthetic pathways (Fig.1),

COX-dependent pathway—As seen in the Figure 6A, genetic deletion of mPGES-1 did not result in the statistically significant differences in the basal production of COX-dependent ARA metabolites. At the same time, there was a trend toward ~ 2.2-fold reduction in the basal PGE₂ production with the concomitant trends toward ~2.7-fold upregulation of PGD₂ and ~ 2-fold reduction of 15-deoxy-PGJ₂ (Fig. 6A). More importantly, in the setting of inflammatory arthritis, mPGES-1 KO as compared to WT animals displayed a dramatic (~59-fold) and statistically significant reduction in PGE₂ production, whereas the sizable downregulation of PGD₂ (~3.2-fold), 6-keto-PGF₁α (~2.2-fold), and PGF₂α (~19-fold) levels can be only viewed as a trend (Fig.6A). We were unable to measure a change in 15-deoxy-PGJ₂ levels during arthritis induction in either the WT or mPGES-1 null mice (Fig.6A). These data are consistent with mPGES-1 being a dominant PGH₂ isomerase which is responsible for PGE₂ production in both the naïve and arthritic mouse joints.

LOX-dependent pathway—There was no significant difference in 5-HETE, 12-HETE, and 15-HETE levels between naïve WT and mPGES-1 KO mice, whereas their production

in arthritic mPGES-1 KO mice vs WT mice with arthritis showed a trend toward reduction by respectively ~3.4, 3.8, and 2.6-fold (Fig.6B).

CYP450-dependent pathway—This data set revealed that only ARA metabolites, 14(15)-EET and 11(12)-EET, and not the LA derivatives, 12,13DiHOME, 9,10-DiHOME, 9(10)-EpOME, and 12(13)-EpOME could have been affected by mPGES-1 deletion in arthritic animals. As compared to the WT mice with arthritis, the respective 14(15)-EET and 11(12)-EET displayed a tendency toward reduction in arthritic mPGES-1 KO mice by ~2.9 and ~2.0-fold (Fig.6C).

Non-enzymatic pathway—While the 8-HETE level displayed no difference when it was measured in WT and mPGES-1 under the basal and arthritic conditions, 11-HETE production in the naïve mPGES-1 KO vs WT mice showed a trend toward reduction by ~1.7-fold and this disparity had reached ~5.6-fold difference when the average 11-HETE levels were compared between arthritic WT and mPGES-1 KO animals (Fig.7A).

sEH pathway—The strong antiinflammatory properties of EETs are well-established and their physiological levels are mainly regulated by their conversion into inactive or less active DHETs which is catalyzed by sEH (42). We have already noticed that m-PGES-1 KO mice with arthritis were characterized by a significantly lower average levels of 11,12-EET and 14,15-EET as compared to their WT counterparts (Fig.6C). Since this apparent reduction could be attributable to the upregulated sEH activity, we have measured the levels of the respective EET metabolites, 11,12-DHET and 14,15-DHET (Fig.7B). The data in the Figure 7B show a tendency toward reduction of 11,12-DHET and 14,15-DHET levels in the arthritic mPGES-1 KO mice vs WT mice with arthritis by respectively ~2.0 and 3.4-fold. The calculated ratios of 11,12-DHET/11,12-EET for the arthritic WT and mPGES-1 KO were quite similar (~0.41 and ~0.44) so were the respective 14,15-DHET/14,15-EET values (~0.55 and ~0.55). This supports the hypothesis that sEH activity remains intact in the arthritic mPGES-1 KO mice.

DISCUSSION AND CONCLUSIONS

Prostanoids and particularly PGE2 have often been viewed as simply proinflammatory mediators in arthritis (7–10). This is somewhat surprising because an important role of PGE2 in the resolution of inflammation where it switches ARA metabolism toward production of anti-inflammatory and pro-resolving lipoxins, resolvins, and protectins is well established (25). Moreover, exogenous PGE1 was shown to curb nephritis and adjuvant arthritis in experimental animals (43,44). However, there is a growing body of clinical data (11,12) and experimental studies (13,14) pointing toward a more complicated and sophisticated role of PGE2 in regulation of inflammatory arthritis. A recent study (14) identified an important role for PGE2 in resolving inflammation in the autoimmune (CIA) model of experimental arthritis through regulation of the antiinflammatory lipid, lipoxin A4, thereby serving as pro-resolving lipid molecule. There is a growing understanding of differences between antiinflammatory and pro-resolving modes of action. The former mainly targets neutrophil recruitment to the site of inflammation whereas the latter promotes the removal of apoptotic cells and infectious microorganisms by macrophages (25). Therefore, we designed our study with the aim of examining the antiinflammatory functions of PGE2 using mPGES-1 KO mice and CAIA as a model of inflammatory arthritis.

The current study provides several important insights into the regulation of inflammatory arthritis by mPGES-1 and its product, PGE2. We established that mPGES-1 deficiency increased both the incidence and the severity of CAIA in mice. These results are in the agreement with those of Zurier & Quagliata (44) who demonstrated the antiinflammatory

effect of PGE1 in the adjuvant model of inflammatory arthritis. However, our results disagree with those of Kamei et al (45) who reported on the suppression of CAIA in the mPGES-1 KO mice. The above discrepancy could be explained by the different genetic background of mPGES-1 KO animals, pure DBA1/*lac J* background used in our study and a mixed C57BL/6 × 129/SvJ background used by Kamei et al (45). Yet the different approach for mPGES-1 gene deletion and CAIA induction could also add to the observed discrepancy. The fact that both arthritis score and incidence were increased in the PGES-1 KO animals with arthritis, but the loss of the body weight in arthritic mPGES-1 KO animals was similar to that of arthritic WT mice, would be consistent with local and not systemic inflammation being primarily affected by the deletion of mPGES-1 gene.

The above conclusion is reinforced by our observation that as compared to the WT mice, the peak of CAIA in the mPGES-1 KO animals coincided with significantly (~ 3.6-fold) upregulated neutrophil, and not macrophage infiltration. Therefore, in considering antiinflammatory versus pro-resolving modes of action (25), mPGES-1 and consequently PGE2, appear to exert primarily antiinflammatory effects in the CAIA model of inflammatory arthritis. That said, mPGES-1 deletion results in other changes in eicosanoid metabolism in addition to a dramatic reduction in PGE2 production. PGD2 and 6-keto-PGF1 α increased in mPGES-1-deficient arthritic mice compared with control, albeit lower than levels in arthritis WT mice. Nevertheless, the ratio of PGE2 and other PG are dramatically altered by mPGES-1 deficiency and it is certainly plausible that altered relative levels of eicosanoids could account for the increased arthritis severity in this model.

Finally, the involvement of PGE2 in promoting the antiinflammatory action of mPGES-1 is supported by our lipidomics data that demonstrated a staggering (~ 59-fold) PGE2 reduction at the peak of inflammatory arthritis. It should be noted, that mPGES-1 is constitutively expressed at high level in neutrophils and is strongly upregulated by inflammatory stimuli such as LPS (46). More importantly, mPGES-1 expression level correlates strongly with PGE2 production (46). These data provide a strong support to the notion that PGE2 might exert its antiinflammatory effect through the intracrine/autocrine negative regulation of neutrophil recruitment and function, including the suppression of the neutrophil-mediated phagocytosis (15). The antiinflammatory mode of PGE2 could be masked in the autoimmune model of arthritis (CIA) (14,29) by its strong positive regulation of autoantibody formation (29). Indeed, PGE2 may play a critical role at the intersection of innate and acquired immunity.

These conclusions are supported by the work of Serhan and colleagues who demonstrate that early events in an inflammatory response set the resolution program in motion. In this model, specialized pro-resolving mediators, including lipoxins, resolvins, and protectins, are actively generated and act on neutrophils and macrophages to promote resolution of inflammation (25). PG, including PGE2, involved in the initiation phase of inflammation activate the translation of mRNAs encoding enzymes that are necessary for production of these immunoresolvents. Inhibitors of COX-2, in fact, delay the resolution of inflammation (47,48). In summary, all of these data point toward mPGES-1 as a pivotal regulator in the CAIA model of arthritis by inhibition of neutrophil function. When developing and using selective mPGES-1 inhibitors, attention must be paid to the specific mediators of different forms of arthritis in evaluating potential clinical targets for these agents.

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ABBREVIATIONS

ARA	arachidonic acid
RA	rheumatoid arthritis
MPO	myeloperoxidase
IL-6	interleukin 6
LC/MS/MS	liquid chromatography/tandem mass spectrometry
PGE2	prostaglandin E2
PGD2	prostaglandin D2
PGF2α	prostaglandin F2 α
5-HETE	5-Hydroxyeicosatetraenoic acid
9-HETE	9-Hydroxyeicosatetraenoic acid
11-HETE	11-Hydroxyeicosatetraenoic acid
15-HETE	15-Hydroxyeicosatetraenoic acid
9,10-DiHOME	(\pm)9,10-dihydroxy-12Z-octadecenoic acid
14,15-DiHETrE	(\pm)14,15-dihydroxy-5Z,8Z,11Z-eicosatrienoic acid
9,10-EpOME	(\pm)9(10)-epoxy-12Z-octadecenoic acid
14,15-EpETrE	(\pm)14(15)-epoxy-5Z,8Z,11Z-eicosatrienoic acid.

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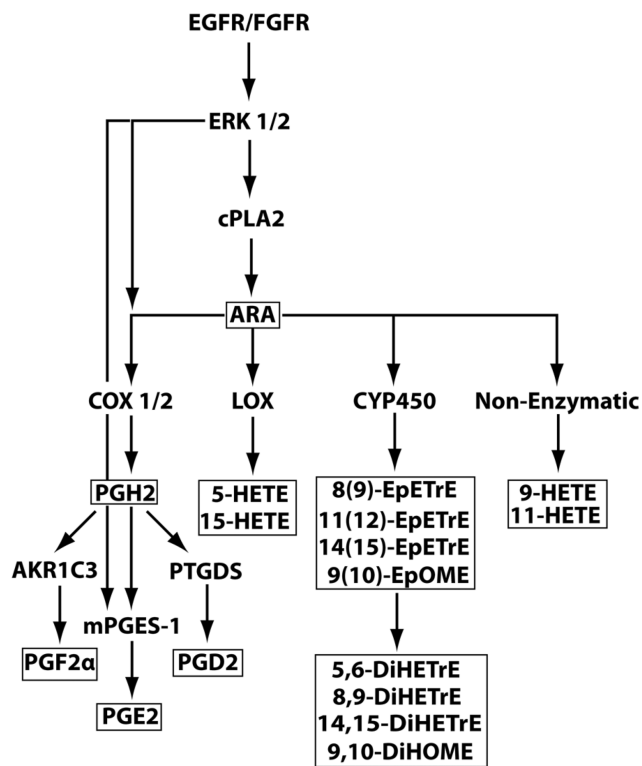
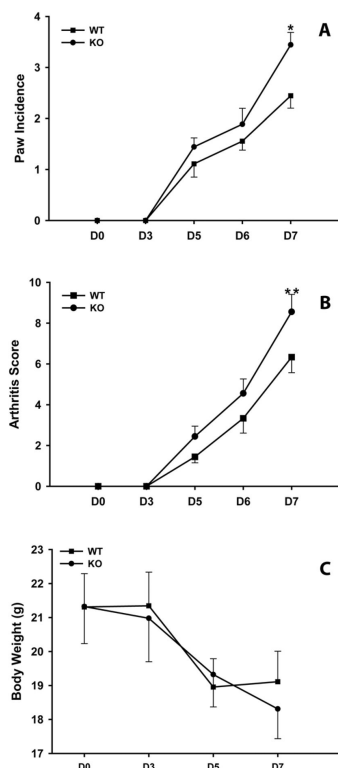
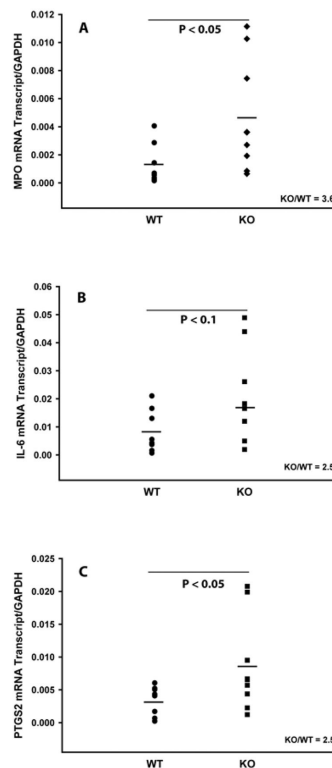


Fig.1. Eicosanoid biosynthetic pathway. The plain symbols depict proteins and biosynthetic pathways. The boxed symbols identify ARA and LA as well as their metabolites that were measured in the current study.

**Fig.2.**

Increased severity of inflammatory arthritis in mPGES-1 KO mice. WT and mPGES-1 KO mice were injected with mouse monoclonal anti-type II collagen 5 clone antibody cocktail kit with lipopolysaccharide (LPS) from *E. coli* 0111:B4 on D0 and boosted with LPS on D3. The arthritis severity was assessed by arthritis score (A) and by the number of inflamed paws (B). The animal body weight (C) was used as a surrogate, whole-body inflammation marker. Data shown are mean \pm SE. (*) depicts $P < 0.05$ as compared to control (Student *t*-test, $n=9$). (**) depicts $P < 0.05$ as compared to control (Mann-Whitney rank sum test, $n=9$).

**Fig.3.**

Analysis of inflammatory markers in arthritic paws of WT and mPGES-1 KO animals by real-time RT-PCR. Total RNA was obtained from a synovial capsule isolated from the front arthritic paw on D7. Relative mRNA expression of MPO (A), IL-6 (B), and PTGS2 (C) was measured using the respective *TaqMan* Gene Expression Assays from Applied Biosystems. Each datum point represents a single animal. Two animal groups were compared by Student *t*-test with the $P = 0.05$ considered to be significant ($n=9$).

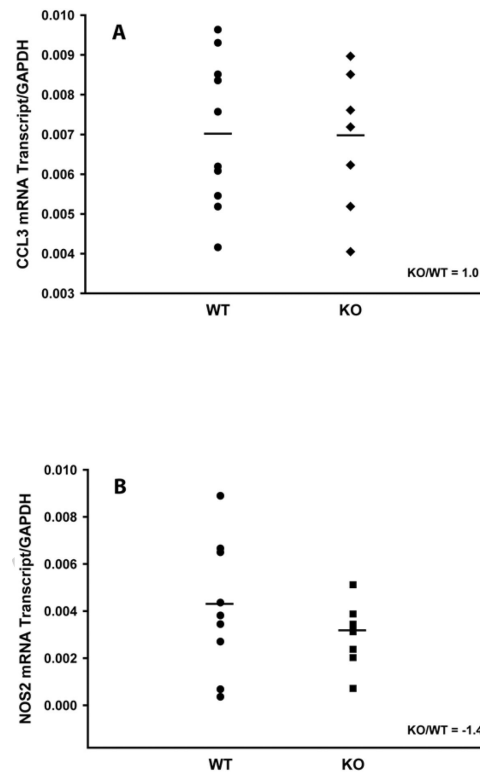
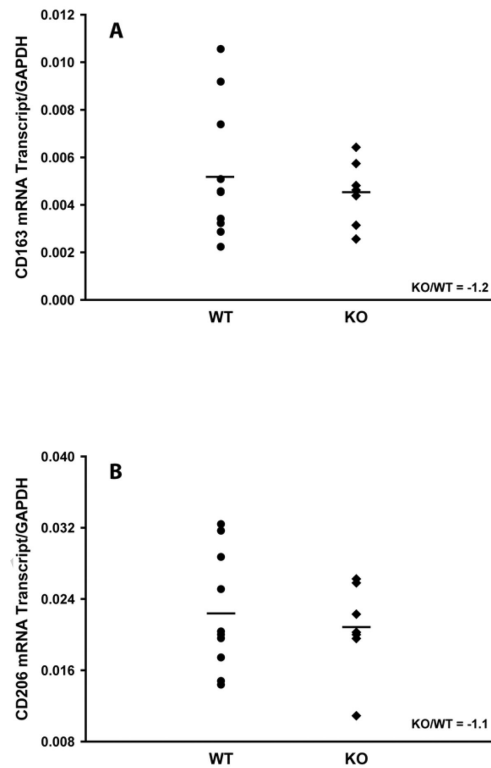


Fig.4. Analysis of the classically activated (M1) macrophage markers in arthritic paws of WT and mPGES-1 KO animals by real-time RT-PCR. Total RNA was obtained from a synovial capsule isolated from the front arthritic paw (arthritis score = 3) on D7. Relative mRNA expression of CCL3 (A) and NOS2 (B) was measured using the respective *TaqMan* Gene Expression Assays from Applied Biosystems. Each data point represents a single animal. Two animal groups were compared by Student *t*-test with the $P < 0.05$ considered to be significant (n=9).

**Fig.5.**

Analysis of the alternatively activated (M2) macrophage markers in arthritic paws of WT and mPGES-1 KO animals by real-time RT-PCR. Total RNA was obtained from a synovial capsule isolated from the front arthritic paw (arthritis score = 3) on D7. Relative mRNA expression of CD163 (A) and CD206 (B) was measured using the respective *TaqMan* Gene Expression Assays from Applied Biosystems. Each datum point represents a single animal. Two animal groups were compared by Student *t*-test with the $P < 0.05$ considered to be significant (n=9).

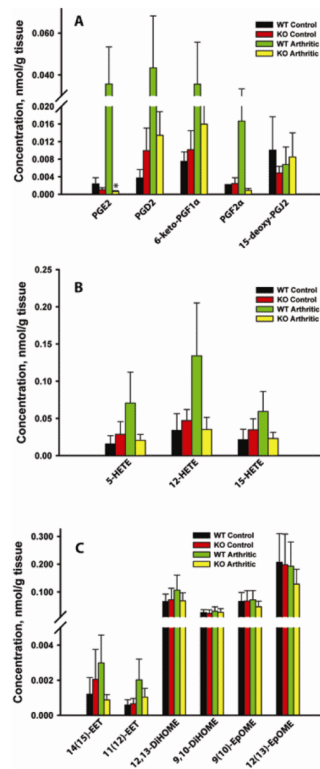


Fig.6. Quantitative eicosanoid profiling of arthritic paws from WT and mPGES-1 KO animals by LC/MS/MS. Lipids were extracted from the front paws of WT control, WT arthritic (arthritis score 3 on D7), mPGES-1 KO control, mPGES-1 KO arthritic (arthritis score 3 on D7). The lipid analytes were grouped based on their respective metabolic pathways: COX (A), LOX (B), and CYP450 (C) (Fig.1). Data shown are mean \pm SE. (*) depicts $P < 0.05$ as compared to WT arthritic (n=3–5).

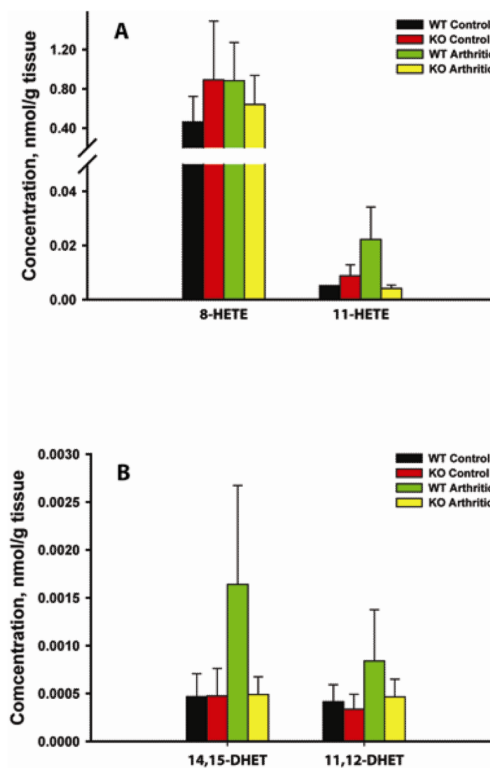


Fig.7. Quantitative eicosanoid profiling of arthritic paws from WT and mPGES-1 KO animals by LC/MS/MS. Lipids were extracted from the front paws of WT control, WT arthritic (arthritis score 3 on D7), mPGES-1 KO control, mPGES-1 KO arthritic (arthritis score 3 on D7). The lipid analytes were grouped based on their respective metabolic pathways: Non-Enzymatic (A) and sEH (B) (Fig.1). Data shown are mean \pm SE (n=3–5).