Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase

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Prostaglandin (PG)E₂ is a potent mediator of pain and inflammation, and high levels of this lipid mediator are observed in numerous disease states. The inhibition of PGE₂ production to control pain and to treat diseases such as rheumatoid arthritis to date has depended on nonsteroidal antiinflammatory agents such as aspirin. However, these agents inhibit the synthesis of all prostanoids. To produce biologically active PGE₂, PGE synthases catalyze the isomerization of PGH₂ into PGE₂. Recently, several PGE synthases have been identified and cloned, but their role in inflammation is not clear. To study the physiological role of the individual PGE synthases, we have generated by targeted homologous recombination a mouse line deficient in microsomal PGE synthase 1 (mPGES1) on the inbred DBA/11acJ background. mPGES1-deficient (mPGES1^{-/-}) mice are viable and fertile and develop normally compared with wild-type controls. However, mPGES1^{-/-} mice displayed a marked reduction in inflammatory responses compared with mPGES1^{+/+} mice in multiple assays. Here, we identify mPGES1 as the PGE synthase that contributes to the pathogenesis of collagen-induced arthritis, a disease model of human rheumatoid arthritis. We also show that mPGES1 is responsible for the production of PGE₂ that mediates acute pain during an inflammatory response. These findings suggest that mPGES1 provides a target for the treatment of inflammatory diseases and pain associated with inflammatory states.

arthritis | inflammation | macrophage | knockout | PGE₂

Aspirin and related drugs block the production of all prostaglandins (PGs), and the beneficial effects of these agents in pain and inflammation have provided powerful evidence that the PG pathways are involved in these processes. These drugs inhibit the conversion of arachidonic acid to the unstable intermediate endoperoxide, PGH₂. PGH₂ is the common substrate for a number of different synthases that produce a spectrum of lipid mediators including the major PGs, PGE₂, PGD₂, and PGF_{2α}, as well as thromboxane and prostacyclin (PGI₂). Because direct delivery of PGE₂ into tissues can elicit inflammation and high levels of PGE₂ are observed in inflammatory lesions such as arthritic joints, it has been assumed that at least a portion of the beneficial action of aspirin-like drugs is due to their inhibition of PGE₂ production (1–3).

Recent studies suggest that the regulation of the production of PGE₂ from PGH₂ is complex, and several cytosolic and microsomal synthases capable of carrying out this conversion *in vitro* and *in vivo* have been identified. These enzymes are referred to as microsomal PGE synthase 1 (mPGES1), mPGES2, GSTM2-2, GSTM3-3, and cPGES/p23 (4–9). cPGES/p23 was recently described as constitutively expressed in many tissues with one notable exception in rat brain after lipopolysaccharide (LPS) treatment, where it is up-regulated severalfold (8). Two cytosolic glutathione transferases (GSTM2-2 and GSTM3-3) have been cloned from a human cDNA library and when expressed in *Escherichia coli* found to catalyze the isomerization of PGH₂ to PGE₂ (6). The microsomal PGESs

(mPGES) characterized to date are termed mPGES1 and mPGES2. mPGES2 was originally purified from bovine heart (10), subsequently cloned, and found to be ubiquitously expressed in diverse tissues (9). mPGES1, likely constituting the PGES isolated from sheep and bovine vesicular gland (4, 5), displays a high catalytic activity relative to other PGES isomerases, suggesting a pivotal role for mPGES1 in PGH₂ metabolism (7, 11).

The importance of PGE₂ in inflammation dictates the necessity to understand the role of individual PGES *in vivo*. Expression analyses *in vitro* and *in vivo* suggest that mPGES1 serves as an inducible PGES after exposure to proinflammatory stimuli (7, 12–16) and in inflammatory diseases (13–15). Furthermore, we have recently demonstrated in IL-1β-treated human A549 cells, using antisense treatment targeted against mPGES1, a direct association between mPGES1 expression, its enzymatic activity, and total PGE₂ production after an inflammatory stimulus (17). In mPGES1-deficient (mPGES1^{-/-}) mice generated on a mixed genetic background, LPS-induced PGE₂ release is largely dependent on mPGES1 expression both in an isolated cell culture and *in vivo* (18). This evidence indicates that mPGES1 might be responsible for the elevated PGE₂ during pathophysiological states. To test this hypothesis, we generated mice deficient in this enzyme on an inbred DBA/11acJ genetic background and evaluated the response of these animals against both acute and chronic inflammatory stimuli, including an experimental model of human rheumatoid arthritis.

Materials and Methods

Gene Targeting. A 335-bp partial cDNA fragment (base pairs 35–369; mPGES1 accession no. AB041997) was used to hybridize a DBA/11acJ genomic λ phage library (Stratagene) and isolate mPGES1 genomic clones. Three clones were restriction mapped and determined to contain 24 kb of the mPGES1 genomic locus, including all three exons. The targeting vector was constructed such that a neomycin-resistance gene would replace 3.0 kb of mPGES1 coding material after homologous recombination of the targeting vector with the endogenous locus. The 3.0-kb fragment that will be deleted in the knockout animal contains part of exon 1 and the entire exon 2 corresponding to base pairs 35–256 of the cDNA sequence. The targeting vector was linearized and electroporated into DBA/252 embryonic stem (ES) cells derived from inbred DBA/11acJ animals (19). Genomic DNA was isolated from neomycin- and ganciclovir-resistant ES cell lines and screened by Southern blotting for homologous recombination. Male chimeras were identified and backcrossed to DBA/11acJ females (The Jackson Laboratory) to generate germ-line heterozygous offspring.

Abbreviations: CIA, collagen-induced arthritis; COX, cyclooxygenase; PG, prostaglandin; mPGES1, microsomal PGE synthase 1; NSAID, nonsteroidal antiinflammatory drug; LPS, lipopolysaccharide; ES cells, embryonic stem cells; EP, PGE₂ receptor.

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Heterozygous *mPGES1*^{+/-} males and females were crossed to generate homozygous *mPGES1*^{-/-} mice, and genotypes were identified by PCR and confirmed by Southern blot analysis. All animals used in our studies were on an inbred DBA/11aJ genetic background.

Microsomal PGES Activity and Western Blot Analysis. Peritoneal macrophages were isolated and cultured as described (2). Microsomal protein fractions were prepared and tested for their ability to metabolize PGH₂ into PGE₂ after a 2-min incubation at 4°C in the presence of 2 mM glutathione (7, 17). PGE₂ levels were measured after the reaction was stopped by the addition of an equal volume of 0.1 M HCl-acidified SnCl₂ (1 mg/ml). For immunodeletions, 20 μg of microsomal protein samples were resolved by SDS/PAGE and probed for *mPGES1* or cyclooxygenase (COX)-2 as described (17).

Macrophage Function. Cells were incubated with arachidonic acid (Biomol, Plymouth Meeting, PA) for 30 min or LPS (*E. coli* O111:B4) for 16 h. At the completion of each experiment, supernatants were isolated and stored at -80°C until assayed. IL-6 levels were measured by ELISA (R & D Systems). The lower limit of detection was 3.1 pg/ml. Media controls for each condition reflected the highest possible amount of solvent used to prepare the corresponding stimulating agent (LPS:PBS; arachidonic acid:ethanol).

Induction and Assessment of Arthritis (Collagen-Induced Arthritis). Native chicken type II collagen (Chondrex, Redmond, WA) was mixed with complete Freund's adjuvant and injected s.c. on days 0 and 20 at the base of the tail of 8- to 12-week-old mice (100 μg of type II collagen in 100 μl of emulsion) (20). The degree of arthritis was determined without knowledge of genotype based on a visual score of 0–3 per paw and summed for all four paws (possible maximum score of 12 per animal) according to the following criteria: 0, no evidence of inflammation, 1, edema and erythema in paw and/or one metatarsal phalange joint or one phalanx; 2, edema and erythema in paw and/or two or more joints or phalanges; and

3, pronounced edema and erythema in the entire paw. Scores were determined twice a week for the entire duration of the experiment. At the completion of each experiment, mice were killed by CO₂ inhalation and blood was collected via caudal vena cava puncture using a gentle vacuum. The serum fraction was isolated and stored at -20°C until assayed. Levels of type II collagen antibodies were determined by ELISA according to the manufacturers' protocols using a mouse IgG type II collagen primary antibody (Chondrex) and a horseradish peroxidase-coupled goat anti-mouse IgG2a secondary antibody (Southern Biotechnology Associates, Birmingham, AL). For the pharmacological characterization of piroxicam in collagen-induced arthritis (CIA), DBA/11aJ mice were immunized as described above and fed a rodent diet supplemented with piroxicam (0.7 mg/kg per day, prepared at Pfizer) starting from day 20 until completion of the study.

Joint Histology. Stifle joints were collected, fixed in 10% neutral buffered formalin, decalcified, and embedded in paraffin, and 5-μm sections were stained with hematoxylin/eosin/safranin O. Sections analyzed by light microscopy were scored without knowledge of genotype by using the modified Mankin method (21) to include cartilage structure, cellularity, proteoglycan staining, synovial inflammation, and hyperplasia according to the following criteria. Cartilage structure scores were: 0, normal; 1, surface irregularities; 2, pannus; 3, clefts to transitional zone; 4, clefts to tidemark; 5, clefts to subchondral bone; and 6, complete disorganization. Cellularity scores were: 0, normal; 1, diffuse hypercellularity; 2, cloning; and 3, hypocellularity (necrosis). Proteoglycan staining scores were: 0, normal; 1, slight reduction to tangential zone; 2, moderate reduction to tidemark; 3, severe reduction to subchondral bone in at least one area; and 4, no dye observed. Synovial inflammation and hyperplasia scores were: 0, normal; 1, mild inflammation with normal synovium or minimal hyperplasia; 2, moderate inflammation with mild villous hyperplasia; 3, moderate inflammation with moderate villous hyperplasia; and 4, marked inflammation with moderate to marked villous hyperplasia. Fibroplasia scores were: 0, normal; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe.

Fig. 1. Targeted disruption of the *mPGES1* gene and loss of expression. (a) Strategy to inactivate *mPGES1* by targeted homologous recombination in DBA/11aJ ES cells. Restriction maps depict *mPGES1*-targeting vector (Top), endogenous *mPGES1* (Middle), and the targeted *mPGES1* allele (Bottom). Exons are represented by filled boxes. The 3.0-kb fragment deleted in the knockout animal contains part of exon 1 and the entire exon 2, encoding base pairs 35–256 of the cDNA sequence. Arrowheads indicate PCR primers used to identify homologous recombination. (b) Western blot analysis of *mPGES1* and COX-2 protein expression in microsomes isolated from macrophages. (c) Loss of microsomal glutathione-dependent PGE₂ formation. Filled bars, *mPGES1*^{+/+}; open bars, *mPGES1*^{-/-}. ***, *P* < 0.001 for the comparisons indicated by the brackets at the top.

Group comparisons for histopathology scores were performed by using the Mann–Whitney *U* test.

Delayed-Type Hypersensitivity. On day 0, mice were injected with the same type II collagen emulsion as described above for CIA. On day 17, 10 μ g (15 μ l) of type II collagen prepared in saline was injected into the right paw. The left paw from each animal was used as a control and received 15 μ l of saline in the same location as the contralateral paw. Twenty-four hours later (day 18), paw thickness was determined by volume displacement. After the volume measurements, both paws were removed, decalcified, paraffin embedded, and sectioned (5 μ m). Sections analyzed by light microscopy were scored without knowledge of genotype by grading the inflammatory cell infiltrate on a scale of 0–4 according to the following criteria: 0, absence of cell infiltrate; 1, minimal; 2, mild; 3, moderate; 4, marked. Group comparisons for histopathology scores were performed by using the Mann–Whitney *U* test.

Pain Perception Tests. In both pain perception tests, mice were acclimated to the procedure room for 24 h and acclimated to the apparatus 2 h before the start of the experiment. In the hot plate test, mice were placed on a hot plate (52, 55, or 58°C) and the latency for jumping was determined as described (22). In the writhing test, mice were dosed orally with either vehicle [0.5% methylcellulose] or 10 mg/kg piroxicam followed 1 h later by an i.p. injection of 0.7% acetic acid (16 μ l/g of body weight), and stretch responses were monitored as described (3). Exudates were collected at the end of the experiment and levels of PGE₂ and 6-keto-PGF_{1 α} were determined.

Materials. Unless indicated otherwise, all reagents were obtained from Sigma–Aldrich. Prostanoids were measured by ELISA (Cayman Chemical, Ann Arbor, MI). The lower limit of detection was 15 pg/ml for the PGE₂ and 6-keto-PGF_{1 α} detection assays.

Statistical Analysis. Group comparisons were performed by using ANOVA with a Bonferroni posttest unless otherwise noted.

Results and Discussion

We generated mPGES1-null mice and evaluated their phenotypes in several models of acute and chronic inflammation. To facilitate this comparison, the mutation was introduced into ES cells derived from DBA/1lacJ mice (Fig. 1*a*). The mutation was maintained on this genetic background by mating chimeras with DBA/1lacJ animals. Mice used in these studies, therefore, differ only in expression of mPGES1.

mPGES1^{−/−} mice could not be distinguished from wild-type controls in their general behavior, appearance, body weight, tissue histology (including 39 different tissues), or hematological parameters. We confirmed the inactivation of mPGES1 in homozygous mutants by measuring mPGES1 protein expression by Western blot in LPS-treated macrophages isolated from wild-type and mPGES1^{−/−} mice and subsequently treated with LPS (Fig. 1*b*). Similar levels of COX-2 proteins were detected in +/+ and −/− microsomes, highlighting the specificity of our genetic manipulation (Fig. 1*b*). The loss of functional mPGES1 protein in mPGES1^{−/−} animals was confirmed by the inability of microsomes prepared from LPS-treated macrophages to convert PGH₂ to PGE₂ above baseline levels (Fig. 1*c*). These results support the use of our mPGES1-null mice in subsequent *in vitro* and *in vivo* studies.

We then determined the impact of this genetic deletion on the immediate and delayed cellular PGE₂ release after treatment of macrophages with either arachidonic acid for 30 min or LPS for 16 h. These data are consistent with an important function for mPGES1 in PGE₂ formation in response to induction by an inflammatory stimulus. After the incubation with 10^{−4} M or 10^{−5} M arachidonic acid, wild-type macrophages secreted more PGE₂ than mPGES1^{−/−} cells, reaching statistical significance at the

higher dose (Fig. 2*a*). Although treatment with LPS for 16 h did not lead to any significant increase in PGE₂ production above baseline in mPGES1^{−/−} cells, wild-type cells displayed a robust release of PGE₂ (Fig. 2*b*). We did not detect any difference in the dose-dependent release of IL-6 between +/+ and −/− cells, consistent with the absence of generalized signal transduction defects or altered cellular viability in mPGES1^{−/−} macrophages (Fig. 2*b*). Our data also suggest that mPGES1 contributes to the immediate release of PGE₂ in response to exogenous arachidonic acid. These findings are consistent with the hypothesis that mPGES1 is functionally coupled to COX-2- and to a lesser degree to COX-1-derived PGH₂ in murine peritoneal macrophages.

PGE₂ plays an important role in acute inflammation, and its administration triggers an acute inflammatory response characterized by pain, edema, and leukocyte infiltration (23). We first examined the role of mPGES1 in a model of inflammatory pain. Immediately after the i.p. injection of a noxious agent, a dilute solution of acetic acid, mPGES1^{+/+} mice responded by writhing $\approx$ 21 times in a 20-min period (Fig. 3*a*). Pretreatment of mPGES1^{+/+} mice with the nonsteroidal antiinflammatory drug (NSAID) piroxicam caused a 40% reduction in the pain response. The response of NSAID-treated control animals was comparable to that of vehicle-treated mPGES1-null mice. We next characterized the *in vivo* levels of the two major inflammatory prostaglandins in this system, 6-keto-PGF_{1 α} (stable metabolite of PGI₂) and PGE₂ (24). Injection of the noxious agent caused a significant elevation above baseline levels of both 6-keto-PGF_{1 α} and PGE₂ (Fig. 3*b*). Interestingly, no detectable difference in 6-keto-PGF_{1 α} levels was

Fig. 2. Immediate and delayed release of PGE₂ from macrophages. (*a*) Arachidonic acid incubation for 30 min. (*b*) LPS incubation for 16 h. PGE₂ and IL-6 levels were measured from the same supernatants. The absence of significant difference in cell number and cellular viability was confirmed at the end of every experiment by determining β -hexosaminidase activity from each well. Filled bars/squares, mPGES1^{+/+}; open bars/squares, mPGES1^{−/−}. Data shown are the mean $\pm$ SEM from triplicate determinations with *n* = 3 per group per experiment. **, *P* < 0.01.

Fig. 3. Inflammatory responses in mPGES1-deficient mice. (a) Inflammatory pain perception in mPGES1^{-/-} mice is decreased to the same extent as in NSAID-treated wild-type mice. Writhing responses were measured in mPGES1^{+/+} and mPGES1^{-/-} mice pretreated with either vehicle or an NSAID (piroxicam) before an i.p. injection of dilute acetic acid. Filled bars, mPGES1^{+/+}; open bars, mPGES1^{-/-}. (b) PGE₂ and 6-keto-PGF_{1α} levels in peritoneal lavages collected at the end of each nociception experiment. One hundred percent is defined as the response in $+/+$ mice dosed with the vehicle solution (6-keto-PGF_{1α}: 13.9 ± 2.4 ng per mouse; PGE₂: 3.8 ± 0.8 ng per mouse). Filled bars, mPGES1^{+/+}; open bars, mPGES1^{-/-}. Data shown are the mean $\pm$ SEM from a representative experiment performed in triplicate with $n = 8-10$ per group per experiment. *, $P < 0.05$; **, $P < 0.01$.

recorded between the two genotypes regardless of treatment. In contrast, PGE₂ levels were reduced by 52% in the $-/-$ group compared with $+/+$ animals, consistent with the mitigated writhing response observed in mPGES1-null animals. PGI and PGE synthases share PGH₂ as a common substrate. One intriguing hypothesis is that in the absence of mPGES1^{-/-}, more PGH₂ would then become available for PGI synthase. However, we didn't detect any divergence of metabolic flow onto the prostacyclin pathway in the mPGES1^{-/-} mice in this model of inflammatory hyperalgesia. Additional studies in isolated cell culture and additional *in vivo* systems are needed to evaluate the potential divergence of PGH₂ onto other prostanoids.

To further characterize the ability of mPGES1 to modulate pain responses such as those at the spinal and supraspinal levels, we measured withdrawal latencies of mPGES1^{-/-} and mPGES1^{+/+} animals in the hot plate assay. Animals from either genotype did not display any difference in their responses to 52, 55, or 58°C (data not shown). The results in these two distinct pain models are consistent with the proposed role of PGE₂ in inflammatory nociception (3, 25) and the distinct contribution of mPGES1 in inflammatory pain.

To examine the role of mPGES1 in edema formation and leukocyte infiltration, we studied the inflammatory responses associated with delayed-type hypersensitivity in mPGES1^{+/+} and mPGES1^{-/-} mice. After local injection of an antigen into one paw and saline into the contralateral paw of previously immunized mice, swelling was monitored 24 h later. mPGES1^{+/+} mice developed significantly more swelling in their antigen-injected paws as compared with their contralateral saline-injected paws (Fig. 4a). The edema was associated with infiltration of white blood cells as determined by histopathological analysis (Fig. 4b). Edema forma-

tion in antigen- or saline-injected mPGES1^{-/-} mice was similar to that of saline-injected mPGES1^{+/+} mice. This deficit in edema was accompanied by a marked reduction in the number of white blood cells infiltrating the injection site, consistent with the role of mPGES1 in inflammation.

The role of mPGES1 was next examined in an experimental model of human rheumatoid arthritis (RA), a chronic inflammatory disease. Recent studies demonstrate that mPGES1 is expressed in joint tissues isolated from arthritic animals and human RA patients (13, 15). Mice of the DBA/1 genetic background, the genetic background into which the mPGES1 mutation was introduced, are sensitive to the induction of CIA. CIA is an experimental animal model of inflammatory arthritis that in many ways resembles human RA (26). Affected animals develop a polyarthritis characterized by clinical signs such as swollen, red, and ankylosed joints and histopathological features such as synovitis, pannus formation, and joint erosion. Because of these similarities, we used CIA as a model system to examine the potential role of mPGES1 in human RA. mPGES1^{+/+} and mPGES1^{-/-} mice were immunized with native chicken type II collagen in complete Freund's adjuvant on day 0, boosted on day 20, and monitored for clinical signs over the next 5 weeks. mPGES1-deficient animals displayed significant reduction in severity and incidence of disease compared with wild-type controls (Fig. 5a and b). The differences in clinical signs were manifested throughout the course of the disease. Total clinical scores, as assessed by area under the curve from days 20 to 59 (terminal day), were reduced in the $-/-$ group by $\approx 89\%$ ($P < 0.001$).

Because clinical scores and joint deteriorations are not necessarily correlated with one another, we investigated the possibility that mPGES1-null mice were also protected from the histopathological deterioration associated with arthritis. mPGES1^{-/-} mice demonstrated a significant reduction in joint damages (Fig. 5c). Specifically, we noted in mPGES1^{-/-} mice, the absence of proteoglycan loss at articular surfaces (Fig. 5d). The number of

Fig. 4. Delayed-type hypersensitivity in response to a local injection of type II collagen in previously sensitized animals. (a) Edema measurements. Filled bars, mPGES1^{+/+}; open bars, mPGES1^{-/-}. (b) Histopathological evaluation of the inflammatory response in a. Filled bars, mPGES1^{+/+}; open bars, mPGES1^{+/+}. Data shown are the mean $\pm$ SEM from a representative experiment performed in triplicate with $n = 8-10$ per group per experiment. *, $P < 0.05$.

Fig. 5. Reduction in incidence and severity of CIA in mPGES1^{-/-} mice. (a) Clinical scores of mPGES1^{+/+} and mPGES1^{-/-} mice as determined by a single-blinded observer according to the following scale: 0, no evidence of inflammation; 1, edema and erythema in paw and/or one metatarsal phalange joint or one phalanx; 2, edema and erythema in paw and/or two or more joints or phalanges; and 3, pronounced edema and erythema in the entire paw. $P < 0.01$ vs. mPGES1^{+/+} after day 29. Filled squares, mPGES1^{+/+}; open squares, mPGES1^{-/-}. (b) Percentage incidence of arthritis in mPGES1^{+/+} and mPGES1^{-/-} mice. Filled squares, mPGES1^{+/+}; open squares, mPGES1^{-/-}. (c) Representative sections of stifle joints isolated from mPGES1^{+/+} (Upper) and mPGES1^{-/-} (Lower) mice on day 56. Joints from mPGES1^{+/+} mice demonstrate hyperplasia, loss of proteoglycan, pannus formation, bone erosion, and destruction of surface cartilage. In contrast to the wild-type group, joints from mPGES1^{-/-} mice presented significantly less structural damage. (Magnification: $\times 10$. Length of the bar is 100 μm .) (d) Scoring of histopathological deterioration associated with CIA. Severe joint arthritis, as defined by combined scores ≥ 10 , was noted in 0.04% of joints isolated from mPGES1^{-/-} mice compared with 36.4% of joints isolated from mPGES1^{+/+} mice. Filled bars, mPGES1^{+/+}; open bars, mPGES1^{-/-}. $P < 0.05$ vs. mPGES1^{+/+} for all categories. Data shown are the mean $\pm$ SEM from a representative experiment performed in quadruplicate with $n = 15$ per group per experiment.

mPGES1^{-/-} animals with severe synovitis, cartilage destruction, and bone erosion [total score >10 and an average score of 2 (mild to moderate) required for all constituting parameters] was considerably less than the wild-type group (mPGES1^{+/+}: 36% vs. mPGES1^{-/-}: 0.04%).

High levels of IgG2a anti-type II collagen (CII) antibodies are essential for disease onset (27). Both $+/+$ and $-/-$ mice were capable of mounting a robust humoral response against CII as assessed by measuring serum IgG2a anti-CII antibody levels. We could not measure a significant difference in circulating serum levels of anti-CII IgG2a; however, lower antibody titers were detected in mPGES1^{-/-} compared with control mice ($+/+$, $2.6 \pm 0.4 \times 10^5$ units/ml; $-/-$, $1.5 \pm 0.4 \times 10^5$ units/ml; $P = 0.06$). Antibody levels do not necessarily correlate with onset and severity of arthritis in diseased animals (data not shown and ref. 28). These findings suggest that the absence of mPGES1 expression in mice does not cause any gross immunological abnormalities *in vivo*. PGE₂ has been implicated in dendritic cell maturation, macrophage activation, B cell function and T cell polarization (29, 30). Therefore, with the potential impact of PGE₂ on these diverse immunological functions, one cannot exclude, on the basis of the present data, participation of the immune response to our arthritis phenotype. Future studies designed to dissect the relative contributions of the local inflammatory and immunological responses (such as

Th1/Th2 polarization) could provide insights into the underlying mechanisms of our phenotype.

Diverse experimental approaches have been used to demonstrate the involvement of COX metabolites in CIA. Pharmacological inhibition of COX1/2 results in a phenotype similar to that observed with mPGES1-deficient mice (Table 1). Administration of SC-046 (COX-2 inhibitor) (31) or piroxicam (NSAID) reduces the severity of disease by $>90\%$ throughout the duration of the experiment. COX-2^{-/-}, but not COX-1^{-/-}, mice display a signif-

Table 1. Disruption of the PG pathway in CIA

Manipulation	Impact on disease outcome	
	AUC (disease progression)	Clinical score (terminal day)
COX-1 ^{-/-} (32)	Not effective	Not effective
COX-2 ^{-/-} (32)	Effective	Effective
mPGES1 ^{-/-}	Effective	Effective
NSAID	Effective	Effective
COX-2 inhibitor (31)	Effective	Effective

Area under the curve (AUC) and terminal scores were obtained from the published literature [COX-1^{-/-} and COX-2^{-/-} (32); COX-2 inhibitor (SC-046) (31)] and from experiments with the NSAID piroxicam as described above.

icant reduction in both clinical and histological signs of CIA (32). Pharmacological and genetic evidence suggests a link *in vivo* between COX-2 (and its metabolite PGH₂) and mPGES1 (and its substrate PGH₂) in this disease model.

A review of the literature describing the kinetics of mPGES1 and COX-2 expression revealed intriguing differences between these two proteins in terms of the up-regulation and maintenance of steady-state expression levels (13, 14, 33, 34). Differences in regulatory sequences contained in the mPGES1 gene are associated in IL-1 β -treated human orbital fibroblasts with an increase in the mRNA stability over time of mPGES1 compared with COX-2 expression (33). The cell may be presented under different circumstances with an imbalanced ratio of COX-2 to mPGES1. It is difficult to reconcile the idea of an exclusive interaction between COX-2 and mPGES1 considering these observations and the relative solution instability of PGH₂ (35). In cultured peritoneal macrophages, COX-2^{-/-} cells lose their ability to generate PGE₂ in response to a 6-h incubation with LPS (36), whereas COX-1^{-/-} cells and to a lesser degree COX-1^{+/-} cells lose their ability to generate PGE₂ in response to a 30-min incubation with arachidonic acid (37). Our results demonstrate that mPGES1 can be involved in both the immediate and delayed formation of PGE₂ and suggest that mPGES1^{-/-} macrophages display PGE₂ biosynthetic attributes of both COX-2^{-/-} and COX-1^{+/-} macrophages.

In the acetic acid writhing model of inflammatory hyperalgesia, using COX-1- and COX-2-deficient mice, COX-1 appears to be a major contributor of the response, with COX-2 contributing a relatively minor component (38). In this present study, PGE₂ levels were significantly reduced in the acetic acid-treated mPGES1^{-/-} animals compared with controls. Our results are consistent with a previous report demonstrating the importance of PGE₂ and the PGE₂ receptor (EP)1 in mediating this type of visceral pain response (3). One possible interpretation of these findings is that PGE₂ generated by mPGES1 binds to EP1, in turn mediating the nociceptive response.

PGE₂ is well recognized as an important contributor of the febrile response. The up-regulation and localization of mPGES1 in the brain after LPS or IL-1 β exposure provides circumstantial evidence of its involvement in pyresis (12, 34, 39). Interestingly, in these experiments, mPGES1 and COX-2 colocalized to the same

brain vascular cells. With pharmacological and genetic evidence pointing to the primordial participation of COX-2 in fever (40, 41), the concurrent expression of mPGES1 and COX-2 suggests that mPGES1 may also have an important role in fever. The third step in the pathway involves binding of PGE₂ to individual EP receptors. Ushikubi *et al.* showed that EP3 receptor-deficient mice are resistant to IL-1 β or PGE₂-induced pyresis (42). These observations raise the intriguing hypothesis that an additional signaling pathway has been identified *in vivo* between COX-2, mPGES1 and EP3 in the context of fever.

In conclusion, we are proposing that mPGES1 is involved in both acute and chronic PGE₂-dependent experimental models of inflammation: acetic acid-induced writhing (mPGES1 $\rightarrow$ PGE₂ $\rightarrow$ EP1) and CIA (COX-2 $\rightarrow$ mPGES1 $\rightarrow$ PGE₂). We show here that inactivation of mPGES1 in mice results in a decrease in writhing, an indicator of inflammatory pain, that is indistinguishable in magnitude from that observed in mice treated with an NSAID. In addition, we show that loss of mPGES1 reduces the inflammation associated with antigen-induced inflammation, both in a model of delayed type hypersensitivity and in a model of arthritis. To our knowledge this is the first published report to demonstrate the importance of mPGES1 in inflammatory pain and in a model of chronic inflammation. The complexity of the enzymatic and non-enzymatic pathways that are responsible for the conversion of the metabolite of cyclooxygenases into specific PGs has until now made it difficult to identify other possible targets for intervention in prostaglandin-mediated inflammation (43, 44). Taking these findings together, we suggest that mPGES1 represents a target for the treatment of inflammatory disease such as arthritis that will spare important physiological systems in which other PGs participate.

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- Vane, J. R. (1971) *Nat. New Biol.* **231**, 232–235.
- McCoey, J. M., Wicks, J. R. & Audoly, L. P. (2002) *J. Clin. Invest.* **110**, 651–658.
- Stock, J. L., Shinjo, K., Burkhardt, J., Roach, M., Toniguchi, K., Ichikawa, T., Coffman, T. M., McNeish, J. D. & Audoly, L. P. (2001) *J. Clin. Invest.* **107**, 325–331.
- Ogino, N., Miyamoto, T., Yamamoto, S. & Hayaishi, O. (1977) *J. Biol. Chem.* **252**, 890–895.
- Tanaka, Y., Ward, S. L. & Smith, W. L. (1987) *J. Biol. Chem.* **262**, 1374–1381.
- Beuckmann, C. T., Fujimori, K., Urade, Y. & Hayaishi, O. (2000) *Neurochem. Res.* **25**, 733–738.
- Jakobsson, P. J., Thorén, S., Morgenstern, R. & Samuelsson, B. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 7220–7225.
- Tanioka, T., Nakatani, Y., Semmyo, N., Murakami, M. & Kudo, I. (2000) *J. Biol. Chem.* **275**, 32775–32782.
- Tanikawa, N., Ohmiya, Y., Ohkubo, H., Hashimoto, K., Kangawa, K., Kojima, M. & Watanabe, K. (2002) *Biochem. Biophys. Res. Commun.* **291**, 884–889.
- Watanabe, K., Kurihara, K. & Suzuki, T. (1999) *Biochim. Biophys. Acta* **1439**, 406–414.
- Lazarus, M., Kubata, B. L., Eguchi, N., Fujinami, Y., Urade, Y. & Hayaishi, O. (2002) *Arch. Biochem. Biophys.* **397**, 336–341.
- Ek, M., Engblom, D., Saha, S., Blomqvist, A., Jakobsson, P.-J. & Ericsson-Dahlstrand, A. (2001) *Nature* **410**, 430–431.
- Stichtenoeth, D., Thorén, S., Bian, H., Peters-Golden, M., Jakobsson, P. & Crofford, L. (2001) *J. Immunol.* **167**, 469–474.
- Kojima, F., Naraba, H., Sasaki, Y., Okamoto, R., Koshino, T. & Kawai, S. (2002) *J. Rheumatol.* **29**, 1836–1842.
- Mancini, J. A., Blood, K., Guay, J., Gordon, R., Claveau, D., Chan, C. C. & Riendeau, D. (2001) *J. Biol. Chem.* **276**, 4469–4475.
- Thorén, S. & Jakobsson, P. (2000) *Eur. J. Biochem.* **267**, 1–8.
- Sweeney, F. J., Wachtmann, T. S., Eskra, J. D., Verdries, K. A., Lambalot, R. H., Carty, T. J., Perez, J. R. & Audoly, L. P. (2002) *Mol. Cell. Endocrinol.*, in press.
- Uematsu, S., Matsumoto, M., Takeda, K. & Akira, S. (2002) *J. Immunol.* **168**, 5811–5816.
- Roach, M., Stock, J., Byrum, R., Koller, B. & McNeish, J. (1995) *Exp. Cell Res.* **221**, 520–525.
- Griffiths, R., Smith, M., Roach, M., Stock, J., Stam, E., Milici, A., Scampolliti, D., Eskra, J., Byrum, R., Koller, B. & McNeish, J. (1997) *J. Exp. Med.* **185**, 1123–1129.
- Mankin, H., Dorfman, H., Lippicello, L. & Zarins, A. (1971) *J. Bone Jt. Surg. Am. Vol.* **53**, 523–527.
- Piercey, M. F. & Schroeder, L. A. (1981) *Eur. J. Pharmacol.* **74**, 135–140.
- Griffiths, R. (1999) In *Inflammation: Basic Principles and Clinical Correlates*, eds. Gallin, J. & Snyderman, R. (Lippincott Williams & Wilkins), pp. 349–360.
- Berkenkopf, J. & Weichman, B. M. (1988) *Prostaglandins* **36**, 693–709.
- Schäible, H.-G., Ebersberger, A. & Von Banchet, G. (2002) *Ann. N.Y. Acad. Sci.* **966**, 343–354.
- Wooley, P. H., Luthra, H. S., Stuart, J. M. & David, C. S. (1981) *J. Exp. Med.* **154**, 688–700.
- Watson, W. C. & Townes, A. (1985) *J. Exp. Med.* **162**, 1878–1891.
- Campbell, I. K., Hamilton, J. A. & Wicks, I. P. (2000) *Eur. J. Immunol.* **30**, 1568–1575.
- Rocca, B. & FitzGerald, G. A. (2002) *Int. Immunopharmacol.* **2**, 603–630.
- Harris, S. G., Padilla, J., Koumas, L., Ray, D. & Phipps, R. P. (2002) *Trends Immunol.* **23**, 144–150.
- Obukowicz, M. G. & Ornberg, R. L. (1999) In *Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury*, ed. Honn, E. A. (Kluwer Academic/Plenum, New York), Vol. 4, pp. 145–150.
- Myers, L. K., Kang, A. H., Postlethwaite, A. E., Rosloniec, E. F., Morham, S. G., Shlopov, B. V., Hoorha, S. & Ballou, L. R. (2000) *Arthritis Rheum.* **43**, 2687–2693.
- Han, R., Tsui, S. & Smith, T. J. (2002) *J. Biol. Chem.* **277**, 16355–16364.
- Ivanov, A. I., Pero, R. S., Scheck, A. C. & Romanovsky, A. A. (2002) *Am. J. Physiol.* **283**, R1104–R1117.
- Nugteren, D. H. & Christ-Hazelhof, E. (1980) *Adv. Prostaglandin Thromboxane Res.* **6**, 129–137.
- Morham, S., Langenbach, R., Loftin, C., Tian, H., Vouloumanos, N., Jennette, J., Mahler, J., Kluckman, K., Ledford, A., Lee, C. & Smithies, O. (1995) *Cell* **83**, 473–482.
- Langenbach, R., Morham, S., Tian, H., Loftin, C., Ghanayem, B., Chulada, P., Mahler, J., Lee, C., Goulding, E., Kluckman, K., *et al.* (1995) *Cell* **83**, 483–492.
- Ballou, L., Bottling, R., Goorha, S., Zhang, J. & Vane, J. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 10272–10276.
- Yamagata, K., Matsumura, K., Inoue, W., Shiraki, T., Suzuki, K., Yasuda, S., Sugiura, H., Cao, C., Watanabe, Y. & Kobayashi, S. (2001) *J. Neurosci.* **21**, 2669–2677.
- Li, S., Wang, Y., Matsumura, K., Ballou, L. R., Morham, S. G. & Blatteis, C. M. (1999) *Brain Res.* **825**, 86–94.
- Cao, C., Matsumura, K., Yamagata, K. & Watanabe, Y. (1997) *Am. J. Physiol.* **272**, R1712–R1725.
- Ushikubi, F., Segi, E., Sugimoto, Y., Murata, T., Matsuoka, T., Kobayashi, T., Hizaki, H., Tuboi, K., Katsuyama, M., Ichikawa, A., *et al.* (1998) *Nature* **395**, 281–284.
- Tilley, S. L., Audoly, L. P., Hicks, E. H., Kim, H.-S., Flannery, P. J., Coffman, T. M. & Koller, B. H. (1999) *J. Clin. Invest.* **103**, 1539–1545.
- Coggins, K. G., Latour, A., Nguyen, M. S., Audoly, L., Coffman, T. M. & Koller, B. H. (2002) *Nat. Med.* **8**, 91–92.