Fatty acid binding protein 5 controls microsomal prostaglandin E synthase 1 (mPGES-1) induction during inflammation

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Keywords: Fatty acid binding protein, FABP, prostaglandin E2, mPGES-1, inflammation, pain

ABSTRACT

Fatty acid binding proteins (FABPs) are intracellular lipid carriers that regulate inflammation, and pharmacological inhibition of FABP5 reduces inflammation and pain. The mechanism(s) underlying the anti-inflammatory effects associated with FABP5 inhibition are poorly understood. Herein, we identify a novel mechanism through which FABP5 modulates inflammation. In mice, intraplantar injection of carrageenan induces acute inflammation that is accompanied by edema, enhanced pain sensitivity, and elevations in pro-inflammatory cytokines and prostaglandin E2 (PGE₂). Inhibition of FABP5 reduced pain, edema, cytokine, and PGE₂ levels. PGE₂ is a major eicosanoid that enhances pain in the setting of inflammation and we focused upon the mechanism(s) through which FABP5 modulates PGE₂ production. Cyclooxygenase-2 and microsomal prostaglandin E synthase-1 (mPGES-1) are enzymes upregulated at the site of inflammation and account for the bulk of PGE₂ biosynthesis. Pharmacological or genetic FABP5 inhibition suppressed the induction of mPGES-1 but not COX-2 in carrageenan-injected paws, which occurred predominantly in macrophages. The cytokine interleukin 1β (IL-1β) is a major inducer of mPGES-1 during inflammation. Using A549 cells that express FABP5, IL-1β stimulation upregulated mPGES-1 expression, and mPGES-1 induction was attenuated in A549 cells bearing a knockdown of FABP5. IL-1β upregulates mPGES-1 via NF-κB, which activates the mPGES-1 promoter. Knockdown of FABP5 reduced the activation and nuclear translocation of NF-κB, and attenuated mPGES-1 promoter activity. Deletion of NF-κB binding sites within the mPGES-1 promoter abrogated the ability of FABP5 to inhibit mPGES-1 promoter activation. Collectively, these results position FABP5 as a novel regulator of mPGES-1 induction and PGE₂ biosynthesis during inflammation.

Pain is a frequent reason for seeking medical care and approximately thirty percent of older adults experience chronic pain (1-3). Nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are mainstay treatments for chronic pain. However, chronic NSAID use is associated with gastrointestinal hemorrhage and even acute NSAID use increases the incidence of myocardial
infarctions (4,5). Chronic opioid use is associated with significant addiction and overdose liability (6-8). Consequently, there is a need to develop novel efficacious non-opioid analgesics.

Fatty acid binding proteins (FABPs) are intracellular carriers for fatty acids and related bioactive lipids such as the endocannabinoid anandamide (9-11). In addition to the cytosolic transport of lipids, FABPs deliver ligands to the nucleus wherein they activate nuclear receptors (10,12-14). There are ten FABP isoforms expressed in mammals and we previously demonstrated that pharmacological inhibition of FABP5 produces analgesic effects by enhancing endocannabinoid signaling (15-18). We also demonstrated that FABP5 is expressed in nociceptors, primary sensory neurons that transmit noxious information from peripheral tissues to the dorsal horn of the spinal cord (18-20). In addition to nociceptors, FABP5 is found in cells of the innate immune system, primarily macrophages and dendritic cells (21,22), raising the possibility that FABP5 may also regulate pain indirectly by suppressing the production of pro-inflammatory mediators. In addition to FABP5, macrophages and dendritic cells also express FABP4, which can likewise regulate inflammation (23).

Tissue inflammation leads to the production of a variety mediators that activate and/or sensitize nociceptors, resulting in enhanced nociceptor activation and an exacerbation of pain termed hyperalgesia (24,25). Proinflammatory cytokines and the eicosanoid prostaglandin E2 (PGE2) are important mediators of inflammatory hyperalgesia (24). PGE2 is synthesized from arachidonic acid through the sequential actions of cyclooxygenase-1 or cyclooxygenase-2 enzymes (COX-1 and COX-2, respectively) followed by microsomal prostaglandin E synthase-1 (mPGES-1) (Fig. 1). During inflammation, COX-2 becomes highly induced, leading to enhanced PGE2 biosynthesis (26). Although there are three enzymes that can catalyze the biosynthesis of PGE2 (Fig. 1), mPGES-1 appears to be the principal isoenzyme that contributes to PGE2 biosynthesis in the setting of inflammation and pain (26,27). mPGES-1 knockout (KO) mice exhibit attenuated inflammatory responses and reduced pain sensitivity (27), positioning this enzyme as a major regulator of PGE2 biosynthesis. In the current study, we explored the mechanism(s) through which FABP5 regulates inflammation. Our findings uncover a novel role for FABP5 as an essential regulator of mPGES-1 induction and PGE2 biosynthesis. These effects are unique to FABP5 because mPGES-1 induction is unaltered in FABP4 KO mice, highlighting a unique feature of FABP5 in controlling inflammation, thus positioning FABP5 as a modulator of mPGES-1 and PGE2 associated pain and inflammation.

Results

Effect of FABP4 and FABP5 inhibition upon inflammation and pain—Administration of carrageenan into the hind paw of WT mice results in the rapid development of edema and hyperalgesia (Fig. 2A and B). We previously demonstrated that FABP5 inhibitors produce antinociceptive (i.e., pain reducing) and anti-inflammatory effects in this model (15,16). Consistent with this, carrageenan-associated edema was significantly reduced in FABP5 KO mice (Fig. 2A). Carrageenan administration also induces hyperalgesia, which manifests as reduced hind paw withdrawal latencies in response to a thermal stimulus (Fig. 2B). Compared to WT mice injected with carrageenan, thermal withdrawal thresholds were significantly higher in FABP5 KO mice, indicative of an antinociceptive phenotype (Fig. 2B). In contrast, FABP4 KO mice displayed normal development of edema and hyperalgesia (Fig. 2A and B).

To confirm the behavioral data, we examined the release of calcitonin gene-related peptide (CGRP) from lumbar spinal cord sections of WT and FABP5 KO mice that received an intraplantar injection of saline or carrageenan. CGRP is released from nociceptors into the dorsal horn of the spinal cord and its release is enhanced in sensitized nociceptors, such as during inflammatory hyperalgesia in rodents and humans (28,29). We injected mice with carrageenan and collected lumbar spinal cords, which were bisected along the midline to yield ipsilateral (carrageenan-injected) and contralateral (saline-injected) sections. Similar levels of CGRP release were observed in contralateral sections of WT and FABP5 KO mice (Fig. 2C). Compared to the contralateral sections, spinal CGRP release was elevated in ipsilateral spinal sections of WT but
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not FABP5 KO mice (Fig. 2C). Importantly, compared to ipsilateral spinal sections obtained from WT mice, CGRP release was significantly lower in ipsilateral sections obtained from FABP5 KO mice (Fig. 2C), indicative of reduced sensitization of nociceptors after inflammation in FABP5 KO mice. We recently reported that FABP5 is expressed in nociceptors that co-express CGRP and the transient receptor potential vanilloid 1 (TRPV1) ion channel (18). TRPV1 mediates thermal hyperalgesia, is activated by the exogenous ligand capsaicin, and its activation enhances CGRP release (28,30,31). To determine whether FABP5 deletion alters capsaicin-evoked CGRP release, we incubated ipsilateral spinal cord sections with 1 μM capsaicin for 30 min and quantified CGRP release. Capsaicin induced a significant increase in spinal CGRP release in WT sections, and a smaller but significant increase in sections from FABP5 KO mice (Fig 2D). These data indicate that FABP5 inhibition reduces nociceptor sensitization after inflammation, consistent with the behavioral phenotypes of FABP5 KO mice.

Effect of FABP5 inhibition upon cytokines and PGE₂—Peripheral inflammation induces the recruitment of innate immune cells, predominantly neutrophils and macrophages, which secrete pro-inflammatory cytokines that sensitize nociceptors (32,33). As expected, carrageenan administration elevated the levels of tumor necrosis factor alpha (TNFα), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in the paws of WT mice (Fig. 2E). Compared to WT mice, levels of these cytokines were significantly lower in paws of WT mice treated with the FABP5 inhibitor SBFI26 (15) and in FABP5 KO mice. Of note, IL-1β contributes to inflammatory pain by upregulating the expression of COX-2 and mPGES-1 and enhancing inflammatory PGE₂ biosynthesis (34-36). To determine whether FABP5 inhibition reduces PGE₂ levels in inflamed paws, we quantified PGE₂ in paws of WT, SBFI26-treated, and FABP5 KO mice. As expected, PGE₂ was elevated in the paws of WT mice treated with carrageenan (Fig. 2F). PGE₂ levels were significantly reduced in WT mice treated with SBFI26 and in FABP5 KO mice (Fig. 2F), confirming that FABP5 regulates PGE₂ biosynthesis during inflammation. We examined the expression of FABP5 in cross-sections of mouse paws before and after carrageenan injection using a validated FABP5 antibody (18). At baseline, FABP5 staining was predominantly observed in the epidermis and in resident macrophages (Fig. 3). Carrageenan administration resulted in the recruitment of FABP5-expressing macrophages to the site of inflammation (Fig. 3). Collectively, these results indicate that FABP5 regulates PGE₂ biosynthesis in inflamed tissue, possibly within macrophages.

FABP5 is a cytosolic transport protein that binds to arachidonic acid, the precursor for PGE₂ (37). Therefore, it is possible that FABP5 may regulate PGE₂ biosynthesis in immune cells by shuttling arachidonic acid to COX-2, which is localized on the endoplasmic reticulum. To test this experimentally, human THP-1 monocyte cells, which express FABP5 but not other FABP isoforms (38), were differentiated into macrophages using phorbol myristate acetate and subsequently activated with lipopolysaccharide (LPS) to ensure robust PGE₂ biosynthesis as described (39). Treatment with LPS for 24 h was required to induce COX-2 expression (Fig. 4A). After activation with LPS for 24 h, the cells were washed and incubated for 2 h in the presence or absence of SBFI26 to determine whether acute FABP5 inhibition in fully activated cells can reduce PGE₂ production. Indeed, FABP5 inhibition modestly but significantly suppressed PGE₂ levels in activated THP-1 cells (Fig. 4B). Treatment of cells with the nonselective COX-1/2 inhibitor diclofenac robustly inhibited PGE₂ production (Fig. 4B). To rule out the possibility that SBFI26 directly inhibits COX enzymes, we confirmed that SBFI26 did not inhibit the activity of purified COX-1 or COX-2 (Fig. 4C). To differentiate the effects of SBFI26 upon arachidonic acid transport from direct effects upon COX enzymes, we lysed cells and isolated membrane fractions from activated THP-1 cells. The membranes were incubated with arachidonic acid to stimulate PGE₂ biosynthesis (Fig. 4D). Co-incubation of membranes with arachidonic acid in the presence of diclofenac significantly reduced PGE₂ production (Fig. 4D), confirming the presence of COX-mediated PGE₂ biosynthesis. In contrast, SBFI26 did not affect PGE₂ production, confirming that it does not inhibit enzymes that mediate PGE₂ biosynthesis. These data are consistent with a model wherein FABP5 may...
transport arachidonic acid toward PGE$_2$ biosynthesis, but this is unlikely to fully account for the robust reduction in PGE$_2$ levels observed in paws after inflammation.

**FABP5 regulates mPGES-1 induction**—As noted above, paw inflammation induces COX-2 and mPGES-1 expression, raising the possibility that FABP5 may regulate PGE$_2$ by modulating the induction of these enzymes. We first examined the effect of FABP5 inhibition upon the induction of COX-2. Carrageenan administration enhanced COX-2 expression in paws, which was unaffected by carrageenan deletion (Fig. 5A). In contrast, carrageenan-induced upregulation of mPGES-1 was highly suppressed in FABP5 KO mice (Fig. 5B). The induction of mPGES-1 in FABP4 KO mice was comparable to that observed in WT mice (Fig. 5B), indicating that the regulation of mPGES-1 expression during inflammation is a unique feature of FABP5. A similar profile was observed in WT mice treated with SBF126, confirming that acute pharmacological and genetic FABP5 inhibition produces similar effects upon mPGES-1 induction (Fig. 5C). To confirm this further, we examined the expression and localization of mPGES-1 in carrageenan-injected paws. At baseline, very low levels of mPGES-1 immunoreactivity were observed in paws (Fig. 5D and 5E). After carrageenan injection, elevated mPGES-1 expression was observed in paws of WT mice, which co-localized to a large extent with the macrophage marker F4/80 (Fig. 5D). Weaker staining was also observed in the epidermis and eccrine sweat glands. Carrageenan administration failed to induce mPGES-1 expression in paws of FABP5 KO mice (Fig. 5D), confirming the western blot results.

We previously demonstrated that FABP5 inhibition produces analgesic effects by enhancing activation of cannabinoid receptors and peroxisome proliferator-activated receptor alpha (PPAR$_\alpha$) by their endogenous ligands (15-18). To determine whether FABP5 inhibition suppresses mPGES-1 induction through activation of these receptors, we treated FABP5 KO mice with the cannabinoid receptor 1 and 2 antagonists AM251 and AM630 (3 mg/kg, i.p.), respectively, or with the PPAR$_\alpha$ antagonist GW6471 (4 mg/kg, i.p.) prior to carrageenan injection. Cannabinoid and PPAR$_\alpha$ receptor antagonism did not alter mPGES-1 induction in FABP5 KO mice (Fig. 6A and B). Previous work has demonstrated that cells of the innate immune system express the related receptor PPAR$_\gamma$, whose activation can reduce mPGES-1 induction (40). Treatment of FABP5 KO mice with the PPAR$_\gamma$ antagonist GW9662 (2 mg/kg, i.p.) likewise did not affect mPGES-1 induction (Fig. 6B and C). We corroborated the western blot results by quantifying tissue PGE$_2$, which demonstrated similarly suppressed PGE$_2$ levels in FABP5 KO mice treated with vehicle or the receptor antagonists (Fig. 6D).

**FABP5 induces mPGES-1 via NF-kB**—IL-1$\beta$ is elevated during inflammation and our data demonstrate that FABP5 inhibition reduces IL-1$\beta$ levels in carrageenan-injected paws (Fig. 2E). IL-1$\beta$ is also a major inducer of mPGES-1 expression (34-36), indicating that FABP5 inhibition may curtail mPGES-1 induction indirectly by suppressing IL-1$\beta$ release. It is also possible that FABP5 may directly regulate mPGES-1 induction in cells activated by IL-1$\beta$. To test this, we employed A549 cells, which upregulate mPGES-1 in response to IL-1$\beta$ stimulation (34). Importantly, A549 cells express FABP5 but do not express other FABP isoforms, thus providing an ideal system to examine the contribution of FABP5 toward mPGES-1 induction (38). We confirmed that treatment of A549 cells with IL-1$\beta$ (1 ng/ml) results in the upregulation of mPGES-1 (Fig. 7A). A549 cells bearing a knockdown of FABP5 were generated and we confirmed that cells expressing FABP5 shRNA exhibited attenuated mPGES-1 induction in response to IL-1$\beta$ (Figs. 7A and B).

It was previously reported that IL-1$\beta$ stimulation activates NF-kB, which enhances mPGES-1 expression in multiple cell-types including A549 cells (34,39). A549 cells treated with IL-1$\beta$ exhibited robust NF-kB activation as evidenced by enhanced NF-kB phosphorylation, which was reduced in FABP5 knockdown cells (Fig. 7C). Upon activation, NF-kB rapidly translocates into the nucleus to stimulate gene transcription, including that of mPGES-1 (34,39). To determine whether FABP5 inhibition modulates the nuclear entry of NF-kB, A549 cells were stimulated with IL-1$\beta$ and the nuclear and cytoplasmic fractions isolated. The purity of the
fractions was confirmed using GAPDH and histone H3 as cytoplasmic and nuclear markers, respectively (Fig. 7D). Prior to the addition of IL-1β, NF-kB was not detected in the nucleus (Fig. 7D). Upon stimulation with IL-1β, robust NF-kB immunoreactivity was detected in the nuclei of A549 cells (Fig. 7D). In contrast, nuclear NK-kB levels were significantly lower in IL-1β treated A549 cells bearing a knockdown of FABP5 (Fig. 7D). To confirm reduced nuclear NF-kB activity, we examined NF-kB activity using a luciferase reporter containing NF-kB binding sites upstream of the luciferase open reading frame. Binding of NF-kB to its binding sites stimulates luciferase expression. NF-kB reporter activity was elevated after IL-1β stimulation of A549 cells and this was significantly lower in FABP5 shRNA cells, confirming reduced nuclear NF-kB activity upon FABP5 knockdown (Fig. 7E). In addition to mPGES-1, NF-kB mediates the induction of COX-2 in response to IL-1β (34,35). We confirmed that IL-1β induced COX-2 expression in A549 cells, which was reduced upon FABP5 knockdown (Fig. 7A).

To determine whether FABP5 inhibition reduces the activity of the mPGES-1 promoter, we examined mPGES-1 promoter activation in A549 cells expressing a reporter wherein luciferase expression is driven by the human mPGES-1 promoter (Fig. 8A) (41). Treatment of cells with IL-1β produced a significant increase in promoter activity (Fig. 8B). In FABP5 knockdown cells, baseline promoter activity was lower than in the corresponding control cells. Treatment of FABP5 knockdown cells with IL-1β produced a small increase in promoter activity (Fig. 8B), which was significantly lower than observed in IL-1β treated control cells. To determine whether FABP5 regulates mPGES-1 promoter activity via NF-kB mediated activation of the mPGES-1 promoter, we deleted the NF-kB binding sites within the mPGES-1 promoter as described (Fig. 8A) (41). This promoter variant (termed ΔNF-kB) retains binding sites for the Egr-1 transcription factor that can induce mPGES-1 expression (41,42). Compared to the WT promoter, the baseline activity of the ΔNF-kB promoter was significantly lower in both control and FABP5 shRNA cells and showed similar activity levels to the WT promoter when expressed in FABP5 shRNA cells (Fig. 8C). In control and FABP5 knockdown cells, addition of IL-1β produced small but significant increases in promoter activity that were comparable in magnitude (Fig. 8D), indicating that FABP5 knockdown does not affect the activity of the mPGES-1 promoter lacking the NF-kB binding sites.

**Discussion**

This study identifies FABP5 as a novel modulator of inflammatory mPGES-1 induction and PGE₂ biosynthesis, thus positioning FABP5 inhibitors as potential anti-inflammatory and analgesic drugs (15,16). Although FABP4 and FABP5 are co-expressed in innate immune cells and FABP4 inhibition can reduce the inflammatory output of macrophages (21,23,43), our data demonstrate that the modulation of inflammatory PGE₂ biosynthesis is restricted to FABP5. Inflammation induces the release of cytokines and PGE₂, which sensitize nociceptors and lead to the development of hyperalgesia (24). Consequently, inhibition of COX-1/2 or mPGES-1, and blocking pro-inflammatory cytokines produces analgesic effects (24,27,44). In this study, we demonstrated that FABP5 inhibition reduces tissue PGE₂ and pro-inflammatory cytokine levels, as well as paw edema and hyperalgesia after carrageenan administration. Consistent with the antinociceptive effects observed using behavioral approaches, FABP5 KO mice also exhibited attenuated spinal CGRP release, indicative of reduced nociceptor sensitization. This raises the possibility that the antinociceptive effects observed after FABP5 inhibition may stem at least in part from reduced inflammatory output at the site of inflammation, such as via suppressed PGE₂ release. We recently demonstrated that FABP5 is abundantly expressed in nociceptors (18) and its inhibition within this site may contribute to the observed analgesic effects that are mediated by cannabinoid and PPARα receptors. The identification and functional characterization of the site(s) wherein FABP5 modulates nociception will require animal models bearing selective disruption of FABP5 in each of these cell populations.

Our data demonstrate that FABP5 regulates inflammatory PGE₂ biosynthesis through two independent mechanisms (Fig. 9). FABP5 serves as an intracellular carriers for arachidonic.
acid and we hypothesized that FABP5 may transport arachidonic acid to COX enzymes, which reside within the endoplasmic reticulum of cells (45,46). Indeed, our data demonstrate that FABP5 inhibition in activated THP-1 cells reduced PGE_2 biosynthesis, suggesting that arachidonic acid transport may be partially disrupted after FABP5 inhibition. The second, and likely more important mechanism, stems from our discovery that the induction of mPGES-1 at the site of inflammation is suppressed upon FABP5 deletion. To our surprise, cannabinoid and PPAR receptor activation did not account for the effects of FABP5 inhibition upon mPGES-1 expression. Instead, our data reveal that FABP5 modulates mPGES-1 through at least two complementary mechanisms. Pro-inflammatory cytokines such as IL-1β induce mPGES-1 expression during inflammation (34-36). Pharmacological and genetic FABP5 inhibition reduced tissue IL-1β levels (Fig. 2E), thus likely suppressing its ability to upregulate mPGES-1. FABP5 can also regulate mPGES-1 expression downstream of IL-1β signaling. Specifically, FABP5 knockdown reduced mPGES-1 induction in cells treated with IL-1β, effects that were mediated via suppressed NF-kB activation of the mPGES-1 promoter. Downregulation of NF-kB activity following FABP5 inhibition has also been reported in other settings (47,48), consistent with our data. It is noteworthy that in addition to mPGES-1, NF-kB upregulates COX-2 in cells treated with IL-1β (34,35), and we confirmed that knockdown of FABP5 suppressed COX-2 induction in A549 cells as well. In contrast to these results, FABP5 inhibition did not affect COX-2 expression in carrageenan-injected paws (Fig. 5). This indicates that additional pathways may regulate COX-2 induction and/or distinct cell-types may upregulate its expression in vivo, independently of FABP5.

Carrageenan injection induces an influx of FABP5-expressing macrophages into the site of inflammation (Fig. 3). To our knowledge, the expression of mPGES-1 in paws after an inflammatory challenge has not been previously reported. Our histological results indicate that mPGES-1 is upregulated predominantly in macrophages and to a lesser extent in the epidermis, which may account for the ability of macrophage- and skin-expressed FABP5 to modulate its expression during inflammation. As noted above, mPGES-1 is the principal enzyme that synthesizes PGE_2 (26). Our data indicate that FABP5 deletion reduced paw PGE_2 levels by ~60%, which was accompanied by a near complete suppression of mPGES-1 induction (Figs. 2F and 5). This is consistent with previous reports indicating that selective inhibition of mPGES-1 reduces tissue PGE_2 levels by ~70% (49), indicating that other PGE_2 biosynthetic enzymes may account for the remainder of PGE_2 production.

In conclusion, the present results identify FABP5 as a novel modulator of inflammation and hyperalgesia. FABP5 is required for mPGES-1 induction and nociceptor sensitization during carrageenan-induced inflammation. Thus, in addition to cannabinoid and PPARα receptors (16,17), suppression of PGE_2 biosynthesis may contribute to the antinociceptive effects observed after FABP5 inhibition. These results, coupled with our previous findings that pharmacological FABP5 inhibition produces anti-inflammatory and antinociceptive effects, positions FABP5 as an attractive target for the development of novel analgesics.

**Experimental Procedures**

**Chemicals**—LPS (026:B6), phorbol myristate acetate, λ-carrageenan were from Sigma, human IL-1β was from R&D Systems, diclofenac, GW9662, GW6471, AM251, AM630, and arachidonic acid were from Cayman Chemical. SBF126 was synthesized as previously described (18).

**Constructs**—The FABP5 shRNA, empty vector, and beta-galactosidase constructs were previously described (10). The mPGES-1 promoter construct contains 631 base pairs of the mPGES-1 promoter inserted upstream of luciferase using XhoI and HindIII as described (41). To generate the ΔNF-kB construct lacking the NF-kB binding sites (Δ-631 to -177), the mPGES-1-Luc construct was digested with SacI and re-ligated as described (41). The NF-kB luciferase reporter construct was kindly provided by Dr. Laurie Krug (Stony Brook University) and was previously described (50). All constructs were validated by sequencing.

**Cells**—THP-1 and A549 cells were obtained from ATCC. THP-1 cells were cultured in RPMI 1640...
containing 10% fetal bovine serum and 20 μM beta-mercaptoethanol. A549 cells were cultured in DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin/streptomycin, 2 mM L-glutamine, and 1 mM sodium pyruvate in a humidified incubator containing 95% air and 5% CO₂.

Lentiviral transduction—The shRNA targeting FABP5 was previously described (10). To obtain stable knockdown cells, shRNA or control plasmids were packaged into lentiviral particles in HEK293T cells and subsequently utilized to transduce A549 cells. The human FABP5 shRNA clone was co-transfected with third-generation lentiviral packaging plasmids pRSV-REV (#12253, Addgene, Cambridge, MA), pCMV-VSV-G (#8454, Addgene), and pGpV (#320024, Cell Biolabs Inc., San Diego, CA) using GenJet Plus transfection reagent (SignaGen, Rockville, MD) according to manufacturer’s instructions. Forty-eight hours later, packaged lentivirus was collected and used to transduce A549 cells. Forty-eight hours after infection, 1 μg/mL puromycin (Fisher Scientific, Hampton, NH) was added to the culture medium to select for puromycin-resistant stably infected cells.

Animals—The experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Stony Brook University Institutional Animal Care and Use Committee (protocol #277150). This study employed male C57Bl/6 mice. FABP4 KO and FABP5 KO mice were kindly provided by Dr. Gokhan Hotamisligil. The mice were maintained on a 12:12 h light:dark cycle with ad libitum access to food and water.

Inflammation and hyperalgesia measurement—Baseline thermal withdrawal latencies were obtained using a Hargreaves plantar apparatus as previously described (15-18). Mice received an intraplantar injection of 1% λ-carrageenan into the hind paw and saline into the contralateral paw and thermal withdrawal latencies were measured 4 h later. Edema was measured using a plethysmometer (Ugo Basile) in which fluid displacement by the hind paw before and after inflammation was measured. Edema is reported as the change in fluid displacement from baseline to the 4 h time point for the carrageenan-injected paw minus the change in fluid displacement for the saline-injected paw.

Histology—The protocol for immunofluorescence has been recently described (18). Briefly, mice were anesthetized and transcardially perfused first with saline then with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), pH 7.4. Paws were immersed in fixative overnight at 4°C then transferred to 30% sucrose in 0.1 M PB. Tissues were embedded and frozen using a liquid nitrogen-chilled isopentane bath. Cryostat sections 20µm thick were thaw-mounted on slides, dried, and stored at -20°C. For immunoprocessing, slides were thawed, immersed in 4% PFA for 5 minutes, and rinsed in PBS three times. Following a 30 minutes block consisting of 10% normal donkey serum (NDS, Jackson ImmunoResearch Labs Cat# 017-000-121) in PBS, slides were incubated 24-48 h at 4°C in a mixture of primary antibodies diluted in PBS containing 0.1% Tween-20 and 1% bovine serum albumin. Primary antisera used were rabbit anti-FABP5 (Biovendor, # RD181060100) diluted 1:600, rabbit anti-mPGES-1 (Cayman, #160140) diluted 1:250, and rat anti-F4/80 (Abcam, #ab6640,) diluted 1:100. Following secondary antibody incubations, the slides were washed with PBS and immediately mounted with ProLong® Gold antifade mounting medium with DAPI (Life Technologies, #P36931). Fluorescent immunoreactivity was observed with a Zeiss Axioplan 2 epifluorescent microscope. Images were captured using Zeiss AxioCam HRm monochrome digital camera, and AxioVision Rel. 4.6 software. Images were only adjusted for brightness and contrast.

Western blotting—Western blots were performed as previously described (18). The following antibodies were used: mPGES-1 (Abcam #ab180589), mPGES-1 (Cayman Chemical #160140), COX-2 (Abcam #ab15191), FABP5 (Biovendor # RD181060100), NF-kB (Cell Signaling #8242), phospho-NF-kB (Cell Signaling #3033), GAPDH (Cell Signaling #5174), Histone H3 (Cell Signaling #4499). The following secondary antibody was used: goat anti-rabbit
HRP (Life Technologies #A16104). The blots were imaged using the C-digit scanner and band intensities were quantified. Data are reported as a ratio of target band intensity / GAPDH or Histone H3 intensities.

CGRP release—Mice received an intraplantar injection of 1% l-carrageenan into the hind paw and saline into the contralateral paw. Four hours later the mice were euthanized and the lumbar spinal column was removed and submerged into cold EC1a solution (10 mM HEPES, 13 mM glucose, 151 mM NaCl, 2.5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, pH 7.4). Each hemisphere of the vertebrae was cut to expose the spinal cord. The lumbar enlargement was then cut out and bisected along the midline. The contralateral and ipsilateral spinal sections were subsequently incubated in EC1a buffer for 30 min at 37°C to obtain baseline CGRP release. The media were collected and the sections were incubated with EC1a buffer containing 1 μM capsaicin for 30 min at 37°C and the media were collected. CGRP levels in the media were quantified using the CGRP ELISA kit (Betrin Pharma) according to the manufacturer’s instructions.

Biochemical assays and cell fractionation—For the THP1 experiments, the cells were differentiated into macrophages by incubation with 100 ng/ml phorbol myristate acetate for 72 h followed by activation with 5 μg/ml LPS for 24 h. The media were subsequently removed and the cells were incubated for 2 h in the presence or absence of SBF26 or diclofenac. The media were subsequently collected and PGE2 levels quantified using the PGE2 monoclonal ELISA kit (Cayman Chemical) according to the manufacturers’ instructions. To quantify PGE2 levels in paws, the paws were homogenized in ice-cold PBS and tissue lysates were generated and used for quantification. COX-1 and COX-2 inhibition by SBF26 or diclofenac was examined using the COX inhibitor screening kits (Cayman Chemical). THP-1 membranes were isolated as we previously described (51). Fractionation of cells and isolation of nuclei and cytoplasmic fractions was performed using the NE-PER Nuclear and Cytoplasmic Extraction Reagent (Pierce) according to the manufacturer’s instructions.

Promoter activity assays—For mPGES-1 promoter assays, A549 cells were co-transfected with a plasmid expressing luciferase under the control of the mPGES-1 promoter and a plasmid expressing beta-galactosidase to control for transfection efficiency. To measure NF-kB activity, cells were transfected with the NK-kB luciferase plasmid alongside beta-galactosidase. Twenty-four hours later, the cells were incubated with vehicle or 1 ng/ml IL-1β for 6 h in DMEM containing 0.1% fetal bovine serum. The cells were rinsed with PBS and lysed with Glo Lysis Buffer (E2661, Promega). The cells were subsequently incubated with luciferase substrate (Bright-Glo Luciferase Assay System, E2610, Promega) and luminescence was quantified using a F5 Filtermax Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA, USA). Beta-galactosidase 2X activity buffer was then added to all wells (Beta-Galactosidase Assay System, E2000, Promega). After a 30-min incubation at 37°C, 1 M sodium carbonate was added to the wells and absorbance was measured at 405 nm. Background signals in untransfected cells were substracted from all samples. Luciferase activity is reported as a ratio of luminescence / beta-galactosidase for each sample.

Statistical analysis—Data are presented as mean +/- SE. Student’s t-test, or one-way or two-way ANOVA followed by Dunnett or Tukey post-hoc test, were used to determine significances of differences between means as appropriate.

Acknowledgements: We would like to thank Liqun Wang for help with molecular biology and members of our laboratories for helpful discussions. We would also like to thank Sabine Grosch for providing the mPGES-1 luciferase construct.

Conflict of Interest: The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
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FOOTNOTES
This work was supported by the National Institutes of Health grants R01 DA035949 and DA035923 to M.K.

The abbreviations used are: FABP, fatty acid binding protein; PGE2, prostaglandin E2; mPGES-1, microsomal prostaglandin E synthase 1; mPGES-2, microsomal prostaglandin E synthase 2; cPGES, cytosolic prostaglandin E synthase; COX, cyclooxygenase; TNFα, tumor necrosis factor alpha; IL6, interleukin 6; IL-1β, interleukin 1 beta; IL-1R, interleukin 1 receptor; LPS, lipopolysaccharide; NSAID, Nonsteroidal anti-inflammatory drug
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**Figure 1.** Biosynthetic pathway for PGE\(_2\). Arachidonic acid is converted into prostaglandin H\(_2\) by COX-1 and COX-2 enzymes. Prostaglandin H\(_2\) is subsequently converted into PGE\(_2\) by mPGES-1, mPGES-2, and cPGES enzymes. COX-2 and mPGES-1 are the enzymes that contribute towards the bulk of PGE\(_2\) biosynthesis during inflammation.
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Figure 2. FABP5 inhibition produces anti-inflammatory and antinociceptive effects. (A) WT, FABP4 KO, and FABP5 KO mice received intraplantar injections of carrageenan and paw edema was measured 4 h later. The change in paw edema from baseline is indicated. *, p < 0.05 vs WT mice (n = 6). (B) Thermal withdrawal latencies in WT, FABP4 KO, and FABP5 KO mice before and 4 h after intraplantar injection of carrageenan. *, p < 0.05 vs WT mice after carrageenan injection (n = 9). (C) CGRP release in sections of lumbar spinal cords obtained from carrageenan and saline injected WT mice. Mice received an intraplantar injection of carrageenan or saline and CGRP release was measured 4 h later in spinal sections obtained from the ipsilateral (carrageenan-injected) or contralateral (saline-injected) side. **, p < 0.01 (n = 6). (D) Capsaicin evoked CGRP release in lumbar spinal slices from WT and FABP5 KO. Spinal sections were obtained as in (C). CGRP release was quantified in ipsilateral (carrageenan-injected) slices before and after treatment with capsaicin (1 μM, 30 min). *, p < 0.05; **, p < 0.01; ***, p < 0.001 (n = 6). (E) TNFα, IL-1β, and IL-6 levels in paws at baseline and 4 h after carrageenan injection. Mice were injected with vehicle or SBFI26 (20 mg/kg, i.p.) 30 min before carrageenan. Cytokine levels were normalized to baseline levels in WT mice. *, p < 0.05 vs carrageenan injected WT mice (n = 6). (F) PGE2 levels in paws at baseline and 4 h after carrageenan injection in WT mice treated with vehicle or 20 mg/kg SBFI26 and in FABP5 KO mice. **, p < 0.01 vs carrageenan injected WT mice (n = 6).
Figure 3. FABP5 expression in paws after inflammation. (A) Immunolocalization of FABP5 in sections of paw tissue before and after intraplantar injection of carrageenan. Left panels: FABP5 was predominantly expressed in the epidermis of control paws, with some expression in F4/80+ macrophages. Right panels: Carrageenan injection resulted in the recruitment of FABP5-expressing F4/80+ macrophages to the site of inflammation. (B) Higher magnification merged images of those shown in (A) demonstrating robust expression of FABP5 in F4/80+ macrophages in carrageenan-injected paws.
**Figure 4.** Effect of FABP5 inhibition upon PGE₂ levels in activated THP-1 cells. (A) Western blot of COX-2 induction in THP-1 cells after incubation with 5 μg/ml LPS for 2 or 24 h. (B) PGE₂ levels in THP-1 cells before and after activation with LPS. The cells were incubated with vehicle (PBS, control) or 5 μg/ml LPS for 24 h to induce PGE₂ biosynthesis. The cells were subsequently washed and incubated with SBFI26, diclofenac, or vehicle (0.1% DMSO) for 2 h and PGE₂ levels quantified in the media. *, p < 0.05; **, p < 0.01 vs LPS-activated vehicle-treated cells (n = 6). (C) Effect of SBFI26 upon purified COX-1 and COX-2 activity (n = 4). (D) PGE₂ biosynthesis in membrane fractions of control and LPS activated THP-1 cells. Membranes were isolated 24 h after LPS stimulation and were incubated with 1 μM arachidonic acid in the presence or absence of SBFI26 or diclofenac. **, p < 0.01 vs LPS-activated vehicle-treated membranes (n = 6).
Figure 5. Effect of FABP5 inhibition upon COX-2 and mPGES-1 induction in carrageenan-injected paws. (A) Left: COX-2 expression in hind paws of WT and FABP5 KO mice injected with saline or carrageenan. The paws were harvested 24 h after carrageenan administration. Right: Quantification of COX-2 and GAPDH intensity ratios. COX-2 levels are reported as the ratio of COX-2 / GAPDH signal intensity (n = 6). (B) Left: mPGES-1 induction in hind paws of WT, FABP4, and FABP5 KO mice injected with carrageenan. Right: Quantification of mPGES-1 signal intensities. *, p < 0.05 vs carrageenan injected WT mice (n = 6). (C) Left: Induction of mPGES-1 in carrageenan-injected hind paws of WT mice receiving an intraperitoneal injection of vehicle or 20 mg/kg SBFI26. Right: Quantification of mPGES-1 and GAPDH signal intensities. **, p < 0.01 vs carrageenan injected mice (n = 6). (D) Immunofluorescence of mPGES-1 and F4/80+ macrophage expression in the paws of WT and FABP5 KO mice. Note the low mPGES-1 expression at baseline and its increase primarily in F4/80+ macrophages after carrageenan injection in WT mice. In FABP5 KO mice, carrageenan injection did not lead to an increase in mPGES-1 expression. (E) Higher magnification merged images of those shown in (D).
**Figure 6.** Involvement of cannabinoid and PPAR receptors in mPGES-1 induction. (A) Induction of mPGES-1 in WT and FABP5 KO mouse hind paws after injection with carrageenan. Mice were administered vehicle, the cannabinoid receptor 1 and 2 antagonists AM251 and AM630 (3 mg/kg, i.p.), or the PPARα antagonist GW6471 (4 mg/kg, i.p.). Paws were harvested 24 h after carrageenan injection. (B) Quantification of mPGES-1/GAPDH band intensities of western blots described in (A) and (C). *, p < 0.05 vs carrageenan injected WT mice (n = 6-8). (C) Induction of mPGES-1 in paws of WT and FABP5 KO mice treated with vehicle or the PPARγ antagonist GW9662 (2 mg/kg, i.p.). (D) PGE2 levels in hind paws of WT and FABP5 KO mice administered AM251/AM630, GW6471, or GW9662. PGE2 levels were quantified at baseline and 24 h after carrageenan injection. *, p < 0.05; **, p < 0.01 vs carrageenan injected WT mice (n = 6).
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**Figure 7.** Effect of FABP5 inhibition upon mPGES-1 induction and NF-kB activity in A549 cells. (A) Left: Induction of mPGES-1 and COX-2 in control- and FABP5 shRNA-expressing A549 cells treated with 1 ng/ml IL-1β or vehicle. Right: Quantification of western blots. *, p < 0.05 vs IL-1β treated control cells at the same time point (n = 6). (B) Left: knockdown of FABP5 by shRNA. Right: Quantification of western blots. p < 0.05 (n = 3). (C) Left: Phosphorylated and total NF-kB in control and FABP5 shRNA-expressing A549 cells treated with 1 ng/ml IL-1β. Right: Quantification of phosphorylated / total NF-kB. *, p < 0.05 vs IL-1β treated control cells at the same time point (n = 6). (D) Left: Western blot of nuclear and cytoplasmic NF-kB in control and FABP5 shRNA-expressing A549 cells incubated with 1 ng/ml IL-1β or vehicle for 30 min. The cells underwent fractionation and nuclear and cytosolic fractions were collected. The purity of the fractions was confirmed using GAPDH and Histone H3 as cytosolic and nuclear markers, respectively. C: cytosolic fraction, N: nuclear fraction. Right: Quantification of nuclear NF-kB / histone H3 levels. *, p < 0.05 vs IL-1β treated control cells (n = 4). (E) NF-kB reporter activity in control and FABP5 shRNA A549 cells expressing the NF-kB luciferase reporter construct. The cells were treated with 1 ng/ml IL-1β or vehicle for 6 h and luciferase and beta-galactosidase signals were quantified. Results are reported as Luciferase / beta-galactosidase signal and normalized to control cells. ***, p < 0.01 vs IL-1β treated control cells (n = 6).
Figure 8. FABP5 modulates mPGES-1 promoter activity in A549 cells. (A) Schematic of the mPGES-1 promoter luciferase constructs, which contain the human mPGES-1 promoter upstream of luciferase. The ΔNF-kB construct lacks base pairs -631 to -177 that contain the NF-kB binding sites. (B) WT mPGES-1 promoter activity in control and FABP5 shRNA A549 cells treated with 1 ng/ml IL-1β or vehicle for 6 h. Promoter activity is represented as luciferase / beta-galactosidase and is normalized to control A549 cells. *, p < 0.05; ***, p < 0.001. ##, p < 0.01 vs control cells (n = 6). (C) Baseline promoter activity in control and FABP5 shRNA-expressing A549 cells transfected with the WT or ΔNF-kB constructs. **, p < 0.01 vs control cells expressing the WT construct (n = 6). (D) Promoter activity in control and FABP5 shRNA A549 cells expressing the ΔNF-kB promoter construct. The cells were treated with 1 ng/ml IL-1β or vehicle for 6 h and luciferase and beta-galactosidase activity was quantified. Data are normalized to control cells. *, p < 0.05 (n = 6).
**Figure 9.** Schematic representation of the pathways through which FABP5 modulates PGE\(_2\) biosynthesis during inflammation. (A) Activation of the interleukin 1 receptor (IL-1R) by IL-1β triggers NF-kB phosphorylation and nuclear translocation, wherein it binds to the mPGES-1 promoter and initiates mPGES-1 transcription. In parallel, FABP5 may transport arachidonic acid to COX-1/2 and mPGES-1, the main biosynthetic enzymes for PGE\(_2\), which reside on the endoplasmic reticulum. (B) Inhibition of FABP5 blunts NF-kB activation and nuclear translocation, resulting in attenuated induction of mPGES-1. FABP5 inhibition may additionally reduce arachidonic acid delivery to COX-1/2, resulting in suppression of PGE\(_2\) biosynthesis.
Fatty acid binding protein 5 controls microsomal prostaglandin E synthase 1 (mPGES-1) induction during inflammation

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J. Biol. Chem. published online February 13, 2018

Access the most updated version of this article at doi: 10.1074/jbc.RA118.001593

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