Resolution of Inflammation in Murine Autoimmune Arthritis Is Disrupted by Cyclooxygenase-2 Inhibition and Restored by Prostaglandin E2-Mediated Lipoxin A4 Production

Marion Man-Ying Chan and Andrea Rossi Moore

*J Immunol* 2010; 184:6418-6426; Prepublished online 30 April 2010; doi: 10.4049/jimmunol.0903816

http://www.jimmunol.org/content/184/11/6418

---

**References**

This article cites 46 articles, 16 of which you can access for free at: http://www.jimmunol.org/content/184/11/6418.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Resolution of Inflammation in Murine Autoimmune Arthritis Is Disrupted by Cyclooxygenase-2 Inhibition and Restored by Prostaglandin E2-Mediated Lipoxin A4 Production

Marion Man-Ying Chan and Andrea Rossi Moore

Acute inflammation follows defined phases of induction, inflammation and resolution, and resolution occurs by an active process that requires cyclooxygenase-2 (COX-2) activity. This study aims to address whether this paradigm extends to recognized model of chronic inflammation. We demonstrated that murine collagen-induced arthritis follows a similar sequential course. Interestingly, COX-2 and its metabolite, the presumably proinflammatory PGE2, are present in the joints during resolution, and blocking COX-2 activity and PGE2 production within this period perpetuated, instead of attenuated, inflammation. Repletion with PGE2 analogs restored homeostasis, and this function is mediated by the proresolving lipoxigenase metabolite, lipoxin A4, a potent stop signal. Thus, the study provided in vivo evidence for a natural, endogenous link between the cyclooxygenase–lipoxygenase pathways and COX activity, whether with the selective COX-2 inhibitor subsiding. For example, Gilroy et al. (1) observed that blocking inflammatory response does not dissipate but requires an active process that is mediated by products of cyclooxygenase (COX) for subsiding. For example, Gilroy et al. (1) observed that blocking COX activity, whether with the selective COX-2 inhibitor N-[2-(cyclohexyloxy)-4-nitrophenyl]-methanesulfonamide (NS-398) or the dual COX-1/COX-2 inhibitor indomethacin, inhibited carrageenin-induced acute pleurisy in the inflammation phase, but then it significantly perpetuated inflammation by interfering with resolution. Similarly, Fukunaga et al. (2) have shown that pharmacologic inhibition or gene disruption of COX-2 blocked resolution of acid-induced lung injury. COX-2 has also been shown to mediate resolution of acute inflammation in the brain (3), liver (4), small bowel, and colon (5) as well.

Another major impact came from the discovery that a class of arachidonic acid metabolites facilitates resolution of inflammation. These eicosanoids, named lipoxins, resolvins, and protectins, are generated from lipoxygenases or aspirin-acetylated COX and serve as stop signals in the evolution of acute inflammation responses. They act by stimulating the uptake of apoptotic polymorphonuclear leukocytes at sites of inflammation to promote a programmed return to homeostasis (6–8). Analogs and precursors (ω-3-fatty acids) of lipoxins have been shown to relieve inflammatory-related pathology responses, especially in asthma and various airway injuries (9). Currently, it is known that lipoxins are formed in vivo in the course of inflammation and animals with a mutation in their synthetic enzyme, ALOX12/15, suffer exacerbated inflammation (10). However, our knowledge on the endogenous pathway that regulates their biogenic synthesis is still very limited.

Autoimmune diseases are cyclical in nature. It is generally assumed that tissue destruction occurs because the inflammation is chronic (11). The first objective of this study is to determine the relevance of the eicosanoid-directed, proresolving process in an autoimmune condition so as to evaluate whether it can be harnessed for therapeutic intervention; and then to determine whether the mechanism is vulnerable to COX-2 inhibitors for these are blockbuster drugs that are widely prescribed for curbing inflammation (12). We used the murine collagen-induced arthritis (CIA) model, for it has been generally used for evaluating immunological and pharmacological treatments, including COX-2 inhibitors, for rheumatoid arthritis (13). We discovered that PGE2, generally viewed as a culprit for exacerbating arthritis, functions as a critical endogenous signal sufficient for mediating resolution. It coordinates resolution of inflammation by mediating biogenesis of lipoxin A4 (LXA4) for curbing inflammation. This link between PG and lipoxin may explain why clinical treatments with COX inhibitors relieve symptoms but would not halt progression of the disease in humans.

Materials and Methods

Induction of disease

Experimental protocol used in this study was approved by the Temple University Institutional Animal Care and Use Committee. Arthritis was induced in 6- to 8-wk-old, male DBA/1 mice (The Jackson Laboratory, Bar Harbor, ME) with intradermal injection of chicken collagen II (Chondrex, Redmond, WA) in CFA according to the procedure of Terato et al. (14). Each mouse received a 0.05 ml volume 50 μg collagen emulsified in CFA intradermally in the tail. Immunity was boosted with another injection of 50 μg collagen emulsified in IFA at day 21. Pathogenesis was assessed in a double-blinded manner by measuring the thickness of the hind footpads twice a week with a constant-tension caliper.
Defining resolution

Ability to resolve inflammation was determined by the percent of footpads that has decreased, increased, or remains unchanged in their thickness. A numerical index for each footpad was deduced from the slope of a secant line. In the vehicle control group, the line was drawn from the peak of inflammation to the point of sacrifice or total resolution (<10% swollen) in the resolution phase. For therapeutically treated groups, the line was drawn from the beginning of NS-398 administration. A value of “0” represents no change in thickness. Negative values correspond to decrease in swelling and positive values indicate that swelling increased.

Radiology assessment

Mice were anesthetized with ketamine/xylazine then radiographs were taken using a Faxitron for small animals (model 43855, Faxitron X-Ray, Lincolnshire, IL).

Histological assessment

Knee joints were fixed in 10% neutral buffered formalin for 24h, decalcified in EDTA, embedded in paraffin, and cut in serial sections for histopathological analysis. The sections were then stained with H&E and safranin O to examine the integrity of the cartilage. Formalin-fixed tissue sections were stained with rabbit anti-murine COX-2 Ab (H-62, Santa Cruz Biotechnology, Santa Cruz, CA), then biotinylated anti-rabbit Ab, followed by streptavidin-HRP and DAB solution (all from Dako Scientific, Carpinteria, CA) to assess COX-2 expression. Rabbit normal Ig was used for isotype controls.

PG assays

Lipid was extracted before assaying for the PGs. Randomly selected footpads were harvested, snap frozen, and stored in liquid nitrogen until usage, and then weighed before lipid extraction. The frozen joint tissues were pulverized using a mortar and pestle to obtain a fine powder, which was homogenized (Polytron PRO2000 homogenizer; PRO Scientific, Oxford, CT) and then sonicated in methanol with 0.01 M butyrylcholine and 0.85% formic acid. The lipid extracts were then evaporated to dryness under nitrogen and reconstituted in ELISA buffer. The ELISA kits used for PGE2 and LXA4 were from Cayman Chemicals, Assay Design, and Neogen, respectively. Each sample was assayed in triplicate and at two to three dilutions to ascertain that the reactions occurred within the standard curve and did not reflect interference from cross-reactive substances. The amount of lipid produced was calculated as pg/mg of tissue. A minimum of three repeats were performed for each experiment and they were merged by normalization to the corresponding basal value within each experiment for statistical comparison.

RT-PCR and PCR

Limb joints harvested from euthanized mice were weighed, snap frozen, and stored at −80°C for assessing gene expression. The joints were crushed in liquid nitrogen and homogenized with a micro ultrasonic cell disrupter for RNA extraction in Trizol reagent (Invitrogen, San Diego, CA). Extracts from limbs of randomly selected mice from each group were pooled for reverse transcription and real-time PCR analysis as described in Adapala and Chan (15). Commercial primers and SYBR Green I PCR master mix were used (Superarray). The housekeeping gene 18S RNA was reverse transcribed with random hexamers, and its cDNA solution was diluted to attain an amplification efficiency that was comparable to that of the experimental gene, which cloning threshold values were normalized to those encoding 18S rRNA by the Ct method. A minimum of three repeats were performed for each experiment and, in each, relative units were deduced so that the repeats can be normalized and merged for statistical comparison.

Data analyses

For statistical comparison, the data on gene expression and footpad thickness were tested for normality using the Kolmogorov-Smirnov test. Then, the normally distributed populations were compared using unpaired, one-way ANOVA, followed by Bonferroni test. Otherwise, the nonparametric Kruskal-Wallis test was used instead. A p value of 0.05 was chosen as the threshold for statistical significance throughout.

Results

Kinetic studies revealed a resolution phase in the pathogenesis of chronic inflammatory autoimmune arthritis

The study began with examining whether the pathogenesis of autoimmune arthritis in the CIA model would resemble acute injury in having discrete phases. Hind foot thickness showed that, similar to acute inflammation, swelling followed three phases: induction, inflammation, and resolution (Fig. 1A). From days 0–30, arthritis had not developed; the mice were asymptomatic and their footpads were not swollen. Footpad swelling became increasingly prevalent beginning at day 30 and continued to do so until around day 45 when >95% of the hind feet were swollen by 20% or more in thickness, and the incidence of arthritis in the group of mice was 98%. Subsequently, the footpad swelling began to progressively subside with 40% resolved at day 55 and 90% at days 69–70. The progression from induction through inflammation to resolution is illustrated in footpad swelling by a representative footpad (Fig. 1B).

IL-17 and TNF-α are two of the several cytokines that drive the progression of inflammation (16, 17). Therefore, groups of mice were sacrificed at weekly intervals and the joints were collected for reverse transcriptase real-time PCR analysis of these transcriptionally regulated cytokines to verify the state of inflammation by their levels. Their mRNA expression increased by 4- to 6-fold within the inflammatory phase (days 25–55), then it subsided as inflammation resolved at day 70 (Fig. 1C). The state of inflammation was further documented by histology and radiography (Fig. 1D, 1E). The H&E sections show that the synovium of a knee joint at the peak of inflammation is fully infiltrated with neutrophils, whereas that of a resolved one, which footpad thickness had subsided to 18% from 91%, is cleared of infiltrates. The radiographs show that the metatarsophalangeal joints of the footpad regained alignment as inflammation resolved.

COX-2 was expressed in the resolution phase

 Whereas the gene expression of IL-17 and TNF-α subsided as resolution occurred, COX-2 mRNA remained escalated (Fig. 2A). Kinetics studies demonstrated that COX-2 was upregulated by 2- to 3.5-fold during the inflammatory phase between days 35 and 45, declined slightly around day 55, then resurfaced during resolution through day 70. Comparison by unpaired, one-way ANOVA showed that the level of expression during resolution (day 70) and at the peak of inflammation, day 35 or 45, were both significantly higher than baseline (p < 0.005) but they did not differ between each other (p > 0.05). This bimodal pattern was observed in more than three experimental repeats and was analogous to the nature of COX-2 expression in acute inflammation: Gilroy et al. (3) have shown that when carrageenin is used to induce acute pleurisy, COX-2 expression in the rat model shows a bimodal pattern and the enzyme is expressed 3.5-fold higher in the resolution phase (48 h after induction) than in the inflammation phase (2 h after induction). Blaho et al. (18) have also found a similar pattern in an infectious disease model, Lyme disease. Furthermore, Kapoor et al. (19) have shown that COX-2 expression is sustained for repair in the process of wound healing. The presence of COX-2 was confirmed at the protein level by immunohistochemical staining of the knee joints (Fig. 2B). The enzyme was detected in the synovium as well as the infiltrating cells of the resolving knees.

Contradictory duality of COX-2: resolution was COX-2 dependent

Because COX-2 is expressed, it is important to determine the function of COX-2 in the resolution phase. In this experiment, collagen-induced mice were divided into groups of positive control, which received vehicle only; negative control, which were age-
matched normal mice; and the experimental, which received 10 μg NS-398, a COX-2–specific inhibitor, dissolved in saline with 10% ethanol. The dose of NS-398 given was 0.5 mg/kg, and it was administered by gavage every other day in two manners: 1) treatment began at the induction phase to imitate prophylactic use of COX inhibitor (Fig. 3A), or 2) after inflammation has established to mimic patients seeking treatment after symptoms have appeared (Fig. 3B).

The effect of NS-398 in the inflammation and the resolution phases is opposite. Blocking COX-2 prior to manifestation of symptoms (initiating NS-398 feeding at day 14) attenuated footpad swelling during the nominal inflammation phase. This was apparent when the prevalence of arthritis was enumerated by percent or by the degree of swelling in the hind footpads. Among mice in the vehicle control group, >75% of the hind feet had swollen, whereas NS-398 feeding reduced the incidence to ~40%. The thickness of their footpads had increased by 40.4% on average, whereas those of the NS-398–fed group had swollen by only 21.4% (Fig. 3A). Correspondingly, the mRNA levels of proinflammatory cytokines, TNF-α and IL-17, in the footpads that were harvested between days 40 and 45 were also suppressed by NS-398 (Fig. 3C). Henceforth, considering that both groups of mice are comparable in weight, averaging ~20 g throughout the experiment, our results demonstrate what would be expected; NS-398 reduces inflammation when given in the induction phase.

In contrast, blocking COX-2 during the resolution phase perpetuates inflammation (Fig. 3B). This phenomenon is best illustrated by the percent of mice that have qualitatively been defined by a zero, a positive, or a negative slope. Among the footpads that have developed arthritis (82%), 87% of those in the vehicle control group had experienced resolution, as indicated by the rate of change in footpad thickness (negative slope, downward), and only 14% of them remained unchanged or continued to swell. In contrast, inflammation persisted or progressed (zero or positive slope) in 54% of the footpads in the group that was fed NS-398 after inflammation has established (therapeutically). When secant lines were drawn to deduce numbers, the slopes of the footpads in the vehicle control group average to −2.35, indicating inflammation was resolving naturally, whereas those of NS-398 groups that were fed therapeutically was +0.38, showing that resolution has failed to occur.

**FIGURE 1.** Three phases of pathogenesis in arthritis. In A, open bars indicate the percent of footpads that have swollen by 20%. Filled bars indicate the percent of swollen limbs that have decreased by >20% in thickness, n = 50–200. The number of footpads decreases as mice were sacrificed for cytokine analyses. Mean and SDs are plotted. The degree of swelling for each footpad was calculated as 100 × (thickness − thickness on day 0)/ (thickness on day 0, range from 2.0–2.3 mm). B, The progression of inflammation in a typical arthritic limb is shown. In C, IL-17 and TNF-α mRNA were normalized against 18S RNA and then the relative level of expression was deduced. Three independent experimental repeats were performed. Within each experiment, 4–6 limbs from randomly selected mice were pooled, each data point is averaged from the three repeats, so total n = 12–18. The data were tested for normality, and unpaired one-way ANOVA, followed by Bonferroni test was used for statistical comparison. Their levels at peak of inflammation (day 0) were significantly different from when the joints were normal (day −40) or resolved (day +30), p < 0.05, but the levels in normal and resolved joints were not different, p > 0.05. D, H&E shows a highly cellular synovium in the inflamed (70% swollen at day 40) but not in the resolved (subsided from 91% to 12% swelling at day 70) knee joints (original magnification ×40). E, Sequential x-ray of representative metatarsal joints of a footpad is shown; note the subsiding of soft tissue swelling and the return of joint integrity and bone density in the fifth phalange.
The degree of inflammation corresponded to the levels of proinflammatory cytokines in the harvested limbs. Real-time PCR analysis (Fig. 3D) confirmed that the levels of TNF-α and IL-17 mRNA were significantly higher in the NS-398–treated groups, and the differences were statistically significant. Fig. 3E illustrates how NS-398 affected the course of resolution kinetically using a representative limb from each group. The footpads in the vehicle control swelled and then subsided; however, resolution was impaired in the group that was fed NS-398. Even in mice that were fed NS-398 preventively (initiated in the induction phase), the suppression of inflammation was temporary in 67% of the footpads; the onset of swelling eventually occurred.

The medical consequence of loss of resolution was increased destruction of the joints (Fig. 4). Radiography showed that the degree of soft tissue swelling, digital misalignment, ankylosis, and loss of bone density were more severe in the limbs that had delayed onset (preventive treatment) or perpetuated inflammation (therapeutic treatment) than in those that were able to resolve (vehicle control). The degree of deterioration was further delineated by histological staining. The pannus in the knee joints of the NS-398–fed mice were more proliferative and the cartilage and bone were more severely damaged than those of the vehicle controls.

**FIGURE 3.** NS-398 increased the incidence and severity of swelling in the resolution phase. A, Depiction of the relative increase in thickness of footpad (mm) due to arthritis induced by chicken collagen. The maximum thickness obtained within days 30–55, the inflamed phase, was plotted for each foot. The data are compiled from four independent trials. The black diamonds (n = 112) represent footpads of mice that were induced for arthritis but fed vehicle as control. The gray triangles (n = 58) represent footpads of experimental mice that were fed NS-398 prior to manifestation of symptoms, and they are congregated at the bottom. B, The incidence and severity of swelling in the resolution phase. Each black diamond (n = 38) and gray triangle (n = 48) represents a footpad. The data were compiled from three independent experiments. The open squares, in A and B, both n = 20, represent footpads of normal mice that were not injected. The horizontal bars indicate the average value in each group, and the groups were compared by Kruskal-Wallis test, and p < 0.05 was considered significant. C and D, The difference in levels of mRNA in a subgroup of limbs randomly selected and harvested from the group in A or the group in B, is shown, respectively. The levels of mRNA expression were normalized against 18S RNA and the results shown are the mean ± SEM from 4–6 mice and representative of three experimental repeats. The data were tested for normality and then statistical analysis by ANOVA. The differences between NS-398–fed groups and the vehicle control group was significant (p < 0.05). E, Kinetically plots a representative limb from the vehicle control group (dashed line–open triangles) and a group where NS-398 treatment was initiated after inflammation has established (solid line–filled triangles). E is also used to delineate the three phases (lines) and illustrate treatment regimen. Closed arrow points to when therapeutic NS-398 treatment started.

**FIGURE 4.** Radiography showed the degree of soft tissue swelling, digital misalignment, ankylosis, and loss of bone density were more severe in the limbs that had delayed onset (preventive treatment) or perpetuated inflammation (therapeutic treatment) than in those that were able to resolve (vehicle control). The degree of deterioration was further delineated by histological staining. The pannus in the knee joints of the NS-398–fed mice were more proliferative and the cartilage and bone were more severely damaged than those of the vehicle controls.

**PGE2 was produced in the resolution phase and COX-2 dependent**

Among the COX-2 metabolites, PGE2 is usually considered as a culprit that causes inflammation, whereas 15-deoxy-Δ^{12,14}-PGJ2 (15d-PGJ2) is a ligand for the immunosuppressive transcription factor peroxisome proliferator-activated receptor-γ and an inhibitor of NF-κB (20). A class switching from PGE2 to 15d-PGJ2.
has been observed during transition from inflammation to resolution on acute models (19, 21). However, in murine CIA, the level of PGE$_2$ is sustained.

In three independent experiments, similarly, the amount of PGE$_2$ was elevated in the inflamed and the resolution phases. Compared with the normal control, the average increases in the two phases were $2.40 \pm 0.43$-fold and $2.57 \pm 0.63$-fold, respectively (Fig. 5A). They are statistically different from the normal control but not from each other. Intrigued by the synthesis of PGE$_2$ in the phase of resolution, we proceeded to determine whether COX-2 is synthesizing the PG during the resolution phase. In the mice that were fed NS-398, the relative level of PGE$_2$ decreased to $1.46 \pm 0.13$ from $2.22 \pm 0.22$, by $\sim 60\%$ when compared with the vehicle control (Fig. 5B, see legend for actual concentrations in pg/mg tissue).

Reconstitution with PGE$_2$ was associated with return of resolution

To determine whether PGE$_2$ play a functional role in resolving arthritis, reconstitution studies were performed. Because PGE$_2$ is unstable, we used stable analogs that resist degradation in vivo and would activate the PGE receptors, EP$_1$-4, 16,16 dimethyl-PGE$_2$ (dmPGE$_2$), and misoprostol (22). The latter is a PGE$_1$ analog used clinically for preventing nonsteroidal anti-inflammatory drug (NSAID)-induced stomach ulcers in humans (23). Arthritis was allowed to develop for 45 d and then the mice were divided into groups: 1) vehicle control that would resolve naturally, 2) NS-398 and PBS-treated that would fail to resolve, and 3) NS-398–treated groups: 1) vehicle control that would resolve naturally, 2) NS-398 allowed to develop for 45 d and then the mice were divided into three times a week (Fig. 6). The analogs were effective in nanogram units were derived by normalization to their respective normal control, and $n = 16$, respectively. The values for the three experimental repeats were 36.1, 9.49, and 25.8 pg/mg tissue for normal, 103, 19.89, and 78.6 pg/mg for resolved footpods. B shows PGE$_2$ levels in footpods, harvested during resolution, from mice that were not induced for arthritis ($n = 24$), fed vehicle ($n = 33$), and treated with NS-398 ($n = 46$). For the individual experiments, the values for the normal control were 4.6, 24.9, and 5.86 pg/mg footpad, vehicle control were 9.46, 53.44, and 12.87, and NS-398–treated group 7.59, 35.86, and 7.52, respectively. In both A and B, relative units were derived by normalization to their respective normal control, and then compared by ANOVA, followed by Bonferroni procedure.
MPGES-1 did not synthesize the PGE2 during resolution

PGE2 is synthesized by the enzyme PG E synthase, which has several isoforms. The COX-2-associated inducible isoform, microsomal PGES-1 (mPGES-1), is currently regarded as a potential therapeutic target to replace COX-2, which inhibition has cardiovascular side effects (25, 26). Therefore, it is important to examine whether it is the source of PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

MPGES-1 did not synthesize the PGE2 during resolution

PGE2 is synthesized by the enzyme PG E synthase, which has several isoforms. The COX-2-associated inducible isoform, microsomal PGES-1 (mPGES-1), is currently regarded as a potential therapeutic target to replace COX-2, which inhibition has cardiovascular side effects (25, 26). Therefore, it is important to examine whether it is the source of PGE2 during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.

Return of resolution was associated with restoration of LXA4 production

Having established that PGE2 has a functional role, we were curious as to how it may promote the resolution of arthritis. Clearance of apoptotic neutrophils from the inflammatory tissue by professional phagocytes is a hallmark of LXA4, an anti-inflammatory eicosanoid from the LOX pathway; this prompted us to examine whether COX-2 affects its production (8). First, a kinetic study was conducted to determine whether LXA4 and ALOX12/15, the enzyme that controls production of LXA4, was upregulated during resolution. Gene expression analysis showed that mPGES-1 was expressed only in the inflamed phase; its levels in the resolving footpads were the same as basal (Fig. 7). Thus, mPGES-1 is not likely the downstream terminal synthase for PGE2, even though further investigation is needed to establish that it does not affect the resolution process.
LXA₄ was reduced by 50%, from 3.5 pg/mg in the vehicle control group to 1.5 pg/mg. However, with administration of 600 and 1200 ng of misoprostol, in a dose-dependent manner, the levels of LXA₄ returned to 2 and 2.7 pg/mg tissue, a reconstitution by 25% and 50%, respectively. Reconstitution with 600 ng of dmPGE₂ also restored LXA₄ production to 2.9 pg/mg tissue. Bonnans et al. (27) have shown that PGE₂ induces the expression of ALX, the receptor of LXA₄. DmPGE₂ also reconstituted the expression of ALX, thus supporting the argument that PGE₂ regulates the operation of a lipoxin-mediated resolution mechanism (Fig. 9B).

Discussion

Chronic inflammation has long been known as the cause of many tissue destructive diseases, such as arthritis, asthma, colitis, and periodontitis. In the past decade, investigations have uncovered that the impact of inflammation is much more extensive. It is the underlying culprit in many prevalent diseases that were previously not considered to be inflammatory in nature, such as coronary heart conditions, Alzheimer’s, and cancer. This revelation intensified interests in controlling inflammation for disease prevention and treatment. The momentum has led to a growing body of evidence within acute inflammation models, which shows that turning off the inflammatory response requires an active process mediated at least in part by products of the COX and lipoxygenase pathways. However, acute inflammation is self-limiting and without relapse; some of its models have been criticized as lacking the complexity of autoimmune diseases and incongruous with chronic inflammation, which are cyclic and laden with flares (28). For example, Seibert et al. (28) doubted whether COX-2 inhibitors will apply to rheumatoid arthritis when Gilroy et al. (1) first discovered the anti-inflammatory property of COX-2 in 1995. This study in a murine CIA, a model that, to a large extent, has been regarded as resembling the clinical disease, addresses the challenge. It critically expands the clinical significance of the COX-2/PGE-mediated resolution mechanism by revealing that it is an active and integral process, and hence, potentially can be harnessed for therapeutic intervention of the autoimmune conditions (Fig. 10).

COX inhibitors are first-line therapies of arthritis and have been widely adopted for treatment of many chronic inflammatory disorders and autoimmune diseases (29). In the murine CIA, we found that a COX-2 inhibitor, NS-398, at a dose of 0.5 mg/kg, will interfere with resolution when given after the disease is symptomatic, and when given earlier will only delay onset. This may explain why NSAID and COX inhibitors are palliative rather than curative, and why they must be given relatively early to be effective and would not halt the progression of disease in humans (12).

There are examples that dual inhibitors of COX-1 and COX-2 induce clinical relapse in patients with osteoarthritis and inflammatory bowel diseases. Reijman et al. (30) observed that patients who received long-term treatment with diclofenac (180 d)
As early as the 1980s, fish oil has been shown to modulate arthritis in rodent models through products of COX and lipoxinase from the LOX pathway to mediate resolution of inflammation in a feedback manner.

had a 2.4-fold and a 3.2-fold higher risk of osteoarthritic damage in their hips and knee, respectively, than subjects who received the NSAID for a short term (1–30 d). In a small-scaled retrospective study, Matuk et al. (31) found that 40% of patients on coxib and 70% of those on celecoxib had a relapse within 6 wk. In a study that used 109 subjects, Takeuchi et al. (32) found that 17–28% of patients with quiescent Crohn’s disease or ulcerative colitis had a relapse after 4 wk on conventional NSAIDs (naproxen, diclofenac, or indomethacin), whereas no patients taking acetaminophen did. It has also been reported that indomethacin exacerbates psoriasis and rofecoxib prolongs the time needed to resolve inflammation caused by tooth extraction (33–35).

In several acute models, such as heart inflammation and skin wound healing, production switches from pro- to anti-inflammatory metabolites, PGE$_2$ to 15d-PGJ$_2$, as the inflammation evolves into resolution (19, 21). However, in chronic murine autoimmune arthritis, PGE$_2$ production is sustained throughout both phases. Since the mid-1980s, there have been reports on the pro- as well as anti-inflammatory effects of PGE$_2$; however, the view that PGE$_2$ promotes inflammation has dominated. More recently, the pendulum has begun to swing toward its anti-inflammatory nature. In cancer, for example, PGE$_2$ was thought to promote tumor growth by activating cell proliferation, migration, apoptosis, and/or angiogenesis. However, it is now considered that COX-2 expression promotes carcinogenesis because PGE spares the transformed cells from immune elimination (36). The enzyme that degrades PGE, 15-PG dehydrogenase, is now classified as a tumor suppressor. PGE has been shown to suppress many immune functions, for example, it activates regulatory T lymphocytes (37) and inhibits NF-κB activation to reduce the production of proinflammatory mediators (38). It has also been shown to activate the Th-2 subset of T cells, while suppressing the Th-1 subset (39). The PG signals cellular events by binding to its receptors, EP1, EP2, EP3, and EP4, which activate nonidentical functions. The EP1 receptor activates G protein, Gq, and enhances intracellular Ca$^{2+}$ levels. The EP2 receptor inhibits adenylate cyclase via Gi to reduce cAMP levels, whereas, contrarily, the EP3 and EP4 receptors activate G proteins that stimulate cAMP production via adenylate cyclase activation (40). It has been reported that PGE$_2$-signaling through EP3 suppresses skin inflammation in murine contact hypersensitivity (41). In contrast, it has also been shown that PGE$_2$ can exert immunosuppressive effects by binding to EP2 and EP4 (42). To date, a consensus on which EP is pro- or anti-inflammatory has not been reached.

ACKNOWLEDGMENTS

We thank Dr. Dunne Fong and Dr. Samuel Spadone for valuable discussion and statistic analysis, Dr. Robert Christman for help in analyzing the x-ray, and Dr. Suresh Adapala, Dr. Xinnin Zhang, Rodger Brown, and Kyle Evans for assistance throughout the project.

DISCLOSURES

The authors have no financial conflicts of interest.

REFERENCES