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Synovial Fibroblasts Directly Induce Th17 Pathogenicity via the Cyclooxygenase/Prostaglandin E2 Pathway, Independent of IL-23

Sandra M. J. Paulissen,*† Jan Piet van Hamburg,*† Nadine Davelaar,*† Patrick S. Asmawidjaja,*† Johanna M. W. Hazes,* and Erik Lubberts*†

Th17 cells are critically involved in autoimmune disease induction and severity. Recently, we showed that Th17 cells from patients with rheumatoid arthritis (RA) directly induced a proinflammatory loop upon interaction with RA synovial fibroblasts (RASF), including increased autocrine IL-17A production. To unravel the mechanism driving this IL-17A production, we obtained primary CD4+CD45RO+CCR6+ (Th17) cells and CD4+CD45RO+CCR6+ (CCR6+) T cells from RA patients or healthy individuals and cocultured these with RASF. IL-1β, IL-6, IL-23p19, and cyclooxygenase (COX)-2 expression and PGE2 production in Th17–RASF cultures were higher than in CCR6+ T cell–RASF cultures. Cytokine neutralization showed that IL-1β and IL-6, but not IL-23, contributed to autocrine IL-17A induction. Importantly, treatment with celecoxib, a COX-2 inhibitor, resulted in significantly lower PGE2 and IL-17A, but not IFN-γ, production. Combined celecoxib and TNF-α blockade more effectively suppressed the proinflammatory loop than did single treatment, as shown by lower IL-6, IL-8, matrix metalloproteinase-1 and matrix metalloproteinase-3 production. These findings show a critical role for the COX-2/PGE2 pathway in driving Th17-mediated synovial inflammation in an IL-23– and monocyte-independent manner. Therefore, it would be important to control PGE2 in chronic inflammation in RA and potentially other Th17-mediated autoimmune disorders. The Journal of Immunology, 2013, 191: 1364–1372.

Although Th17 cells help protect a host against infection, especially infection with extracellular bacteria and fungi (1–3), they also have a pathogenic role in autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis, psoriasis, and inflammatory bowel disease (4–9). The Th17 subset belongs to the class of CD4+ Th cells. Th17 cells produce cytokines such as IL-17A, IL-17F, and IL-22 (10–14). Furthermore, they can induce expression of their master transcription factor retinoic acid–related orphan receptor (ROR)γt, as well as CCR6 cell-surface expression (15–17).

Recently we have shown that in the early stage of RA, Th17 cells, but not Th1 cells, are potent activators of synovial fibroblasts derived from patients with RA (RASF). This activation results in autocrine IL-17A production, which in turn creates a proinflammatory loop characterized by upregulation of the proinflammatory cytokines IL-6 and IL-8, as well as the cartilage-degrading enzymes matrix metalloproteinase (MMP)-1 and MMP-3.

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Abbreviations used in this article: CCR6+, CD4+CD45RO+CCR6+; COX, cyclooxygenase; HPRT, hypoxanthine phosphoribosyltransferase; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; RASF, rheumatoid arthritis synovial fibroblast; ROR, retinoic acid–related orphan receptor; sIL-17R, soluble IL-17R.

Copyright © 2013 by The American Association of Immunologists, Inc. 0022-1767/13/$16.00 (18). This loop may be an important pathway in the progression of arthritis and possibly in other Th17 diseases as well. The autocrine IL-17A production by Th17 cells is critical to sustain the proinflammatory loop (18, 19), but the mechanism underlying the autocrine IL-17A induction is still unknown.

IL-1β, IL-6, and IL-23 promote Th17 cell differentiation. Adding IL-1β to naive T cells induces expression of RORγt and production of IL-17A and IFN-γ, whereas IL-6 addition sustains RORγt expression and induces production of IL-17A, but not IFN-γ. IL-23 promotes the pathological behavior of Th17 cells (10, 20–25).

Interestingly, PGE2, a lipid mediator that induces inflammation and fever, participates in Th17 expansion and differentiation (26, 27). PGE2 is produced when arachidonic acid is converted by cyclooxygenase (COX), which has two isozymes. COX-1 is expressed constitutively; COX-2 is inducible and is upregulated at inflammation and infection sites (28–30). Upregulation of COX-2 induction and PGE2 production occurs in response to proinflammatory stimuli, including IL-1β, IL-17A, and/or TNF-α (31–37). PGE2, via receptor binding (EP1, EP2, EP3, and EP4), induces production of proinflammatory cytokines (28, 29). PGE2 has a pathogenic role in murine inflammatory disease models, possibly via the IL-23/IL-17 axis (38–40).

PGE2 may be involved in pathogenesis in autoimmunity via Th17 cells, which express EP2 and EP4 receptors. PGE2 binding to these receptors promotes Th1 differentiation (26, 28, 29) and IL-1β/IL-23–mediated Th17 expansion (27–29, 41, 42). Simultaneously, RORγt expression increases (26, 41), and there is evidence for a more pathogenic Th17 phenotype from the increased expression of cytokines, chemokines and chemokine receptors, including IL-17A, IL-17F, CCL20, CCR6, and CCR4 (26, 42). Furthermore, PGE2 indirectly promotes Th17 expansion, because it stimulates IL-23 production by dendritic cells (27) and it alters the Th1/Th17 balance in favor of Th17 by decreasing Th1 differentiation and IFN-γ production (26, 28, 41).
In this study, we show that the autocrine IL-17A production induced by the interaction of Th17 cells and RASF is critically dependent on the cyclooxygenase/PGE2 pathway.

Materials and Methods

Subjects

Blood samples from six treatment-naive early RA patients (five women and one man, mean age $\pm$ SD, 57.5 $\pm$ 9.5 y; mean disease activity score based on 28 defined joints $\pm$ SD, 4.39 $\pm$ 0.82) were studied. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA. None of the patients had been taking disease-modifying anti-rheumatic drugs. Buffy coats from healthy individual blood donors were obtained from the Sanquin Blood Bank (Rotterdam, the Netherlands). This study was embedded in the Rotterdam Early Arthritis Cohort Study and approved by the Medical Ethics Review Board of Erasmus Medical Center Rotterdam.

Flow cytometry, Abs, and cell sorting

mAb preparations, intracellular cytokine detection, and flow cytometry were performed as described previously (43). For intracellular cytokine detection by flow cytometry, cells were stimulated for 4 h with 50 ng/ml PMA, 500 ng/ml ionomycin (Sigma-Aldrich, St. Louis, MO), and GolgiStop (BD Biosciences, San Diego, CA). The following mAbs were purchased from BD Biosciences: CD45RO, CCR6, and CD4. IL-17A mAb was purchased from eBioscience (San Diego, CA). Samples were acquired on a FACSCanto II flow cytometer and analyzed using FlowJo version 7.6 research software (Tree Star, Ashland, OR). Cells were gated on the lymphocyte fraction. T cell populations were sorted from PBMCs isolated from buffy coats from healthy individual blood donors and from peripheral blood from the RA subjects using a FACSaria cell sorter (BD Biosciences). Purity of the obtained T cell populations was $\geq 98\%$.

Cell cultures

RASF isolation and subsequent culture were performed as described previously (18). In brief, 1.0 $\times$ 10^6 or 2.0 $\times$ 10^5 RASFs were seeded in a flat-bottom plate. After 24 h, RASFs were cultured with or without 2.5 $\times$ 10^5, 1.0 $\times$ 10^5, or 2.0 $\times$ 10^5 allogeneic Th17 cells obtained from healthy individuals or the six subjects. Cells were stimulated with soluble anti-CD3 and anti-CD28 (0.3 and 0.4 $\mu$g/ml, respectively; Sanquin, Amsterdam, The Netherlands) and cultured for 2 or 3 d in a final volume of 200 $\mu$L IMDM medium (Lonza, Basel, Switzerland), supplemented with 10% FCS, 100 U/ml penicillin/streptomycin, 2 mM l-glutamine, and 50 $\mu$M 2-ME (Merck, Darmstadt, Germany). Cultures were grown in the absence or presence of 5–25 $\mu$L meclozib (Sigma-Aldrich), 10 $\mu$L/ml etanercept (Wyeth Pharmaceuticals, Collegville, PA), 25 $\mu$L/ml tocilizumab (Roche Pharmaceuticals, Basel, Switzerland), 2.5 $\mu$L anti-IL-1$\beta$, 2.5 $\mu$L anti-IL-6, 10 $\mu$L/ml anti-IL-21, 2.5 $\mu$L/ml soluble IL-17R (sIL-17R), 10 $\mu$L/ml anti-IL-15, and 2.5 $\mu$L/ml anti-IL-23p19 (all from R&D Systems, Minneapolis, MN). Additionally, RASF mononuclear cultures were grown in the absence or presence of 10 ng/ml IL-17A (R&D Systems) and 10 ng/ml TNF-$\alpha$ (Invitrogen, Carlsbad, CA). To measure DNA synthesis, cells were pulsed with [3H]thymidine for 16–20 h, harvested, and counted using standard methods. For analysis of cell cycle status of Th17 cells, DNA content was determined by fixing in ice-cold ethanol and subsequent staining in PBS containing 0.02 mg/ml propidium iodide, 0.1% (v/v) Triton X-100, and 0.2 mg/ml RNase. CFSE labeling of cells and annexin V and 7-aminocoumarin D staining to determine proliferation and apoptosis of Th17 cells were performed as described previously (44).

PGE2, MMPs, and cytokine measurements

PGE2 expression was determined using a PGE2 parameter ELISA (R&D Systems). IL-1$\beta$, IL-6, IL-8, IL-10, and IFN-$\gamma$ expression was determined using ELISA (Invitrogen). IL-17A, IL-22, TNF-$\alpha$, MMP-1, and MMP-3 expression was measured using DuoSet ELISA (R&D Systems). ELISA was performed according to the manufacturers’ instructions. IL-23 expression was detected by ELISA using the combination of biotin-conjugated anti-human IL-12/23p40 (eBioscience) and anti-human IL-23p19 (R&D Systems).

Quantitative real-time PCR analysis

RNA extraction and cDNA synthesis were performed as described previously (43). Primers were designed with ProbeFinder software and probes were chosen from the universal probe library (Roche Applied Science, Indianapolis, IN). Quantitative real-time PCR was performed using the ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA) and analyzed using SDS version 2.3 software (Applied Biosystems). The transcription values were relative to hypoxanthine phosphoribosyltransferase (HPRT) transcription. Primers used in this study were: Cox1, forward, 5'-AGGTTTGGGATGAAACCTTA-3', reverse, 5'-CTTCGTCGCTATCCTCTTCA-3'; probe no. 12; Cox2, forward, 5'-CTTCCAGACATCATTGGT-3', reverse, 5'-TCACCGTAAATGATTGAAGTTC-CCAC-3'; probe no. 23; HPRT, forward, 5'-TGACCTTGATTITTTTGGCACPCC-3', reverse, 5'-CGAGCAGAGGCTTGTTAGCTGTTG-3'; probe no. 73; IL-1$\beta$, forward, 5'-AGGTTTGGGATGAAACCTTA-3', reverse, 5'-TGGGATTTGAGGCCTAACAAGA-3'; probe no. 5; IL-6, forward, 5'-TAGATGACAAAGTCGTCGTTACA-3'; reverse, 5'-AGGTCTTTGCTGGAGAACTCTA-3'; probe no. 37; IL-23p19, forward, 5'-GGTCGTTGAGTTTCCGAGTGTTCTG-3', reverse, 5'-GCTTTTGCAGAAGACAATTGCTA-3'; probe no. 76; RORC, forward, 5'-CAGGCTTCCAATCTTCCTC-3', reverse, 5'-CCACATCTCCC-ACATGGACT-3'; probe no. 69; and IL-15, forward, 5'-CAAAACACAGTTTGTCTCTAATGG-3', reverse, 5'-GACAAATGTFACAAAAACTCTGCAAATAAA-3'; probe no. 65.

Statistical analysis

Differences between experimental groups were tested with a two-sided paired t test using Prism software version 5.04 (GraphPad Software, La Jolla, CA). A p value $< 0.05$ was considered statistically significant.

Results

Limited contribution of IL-6 and IL-1$\beta$ to the induction of IL-6, IL-8, MMPs, and autocrine IL-17A production upon Th17–RASF interaction

To investigate the mechanism driving autocrine IL-17A production by Th17 cells upon interaction with RASF, we first focused on the proinflammatory cytokines IL-1$\beta$, IL-6, and IL-23, which are required for Th17 cell differentiation (10, 20–25). Therefore, primary CD4+CD45ROCCR6$^+$ (Th17) and CD4+CD45ROCCR6$^+$CCR6$^+$ (CCR6$^+$ T cells) cells were sorted from peripheral blood of healthy individuals and cocultured with RASFs. Gene transcription and protein production were assessed. Results showed that the Th17–RASF cultures were highly enriched for the Th17-specific transcription factor RORC and IL-17A production (Fig. 1A). Moreover, IL-1$\beta$ and IL-6 gene transcription and IL-6 protein expression in Th17–RASF cultures were significantly higher than those in CCR6$^-$RASF cultures (Fig. 1A).

IL-1$\beta$ protein production was not detectable in the Th17–RASF cultures (detection limit 10 pg/ml, data not shown). To confirm the expression of IL-1$\beta$ in our system, we used higher numbers of Th17 cells and RASF. When Th17 cell numbers were increased at least 4-fold and RASFs 2-fold, IL-1$\beta$ expression was detected (Supplemental Fig. 1).

To investigate the contributions of IL-1$\beta$ and IL-6 in autocrine IL-17A production, neutralizing Abs against these cytokines were added to Th17–RASF cultures. Blockade of IL-1$\beta$ and IL-6 activity significantly suppressed IL-17A production ($\sim$21 and $\sim$26% compared with no blockade, respectively). This blockade had no measurable effects on IFN-$\gamma$ and TNF-$\alpha$ production. Additionally, blockade of IL-1$\beta$, but not IL-6, had limited suppressive effects on IL-8, MMP-1, and MMP-3 expression in Th17–RASF cultures. Combined blockade of IL-1$\beta$ and IL-6 had an additional effect on the suppression of IL-6 expression (Fig. 1B).

IL-23p19 transcription was higher in Th17–RASF cultures than in CCR6$^-$RASF cultures. However, IL-12/23p40 transcription was undetectable (Fig. 1C). Subsequently, no IL-23 protein was detected in supernatant of Th17–RASF. Moreover, blocking of IL-23 in Th17–RASF cultures had no effect on the expression of proinflammatory cytokines and MMPs induced by the proinflammatory loop upon Th17–RASF interaction (Supplemental Fig. 2).

These data show that the proinflammatory cytokines IL-1$\beta$ and IL-6, which are involved in Th17 differentiation, are indeed induced in Th17–RASF cultures. However, these findings do not
fully explain how IL-17A production and the proinflammatory loop are driven in the Th17–RASF cultures, suggesting that additional mechanisms are involved.

**Celecoxib treatment reduces PGE2 and autocrine IL-17A production in Th17–RASF cultures**

Gene expression profiles from RASF cultured with primary Th17 cells or CCR6+ T cells, obtained from peripheral blood of treatment-naive patients with early RA (18), revealed that COX-2 transcription was highly upregulated in Th17–RASF cultures. This is particularly interesting because COX-2 is involved in PGE2 synthesis, which has been shown to induce Th17 expansion (26, 27, 42). To validate these findings, primary peripheral blood Th17 and CCR6+ T cells obtained from healthy volunteers were cultured with RASFs. The gene encoding for COX-2, but not the gene encoding for COX-1, was highly upregulated (∼10 fold) in Th17–RASF cultures compared with CCR6+ T cell–RASF cultures. Additionally, PGE2 production was significantly increased in Th17–RASF cultures (Fig. 2A).

To investigate the roles of COX-2 and PGE2 in the production of IL-17A, Th17–RASF cultures were treated with celecoxib, a specific inhibitor of COX-2 activity. This resulted in a dose-dependent decrease of IL-17A and PGE2 production in supernatant. Compared to the control situation, the decrease in IL-17A production as a result of celecoxib treatment (25 μM) was ∼73% (Fig. 2B). Moreover, celecoxib treatment resulted in a significant reduction of the fraction of IL-17A– but not IFN-γ–producing cells (Fig. 2C). Celecoxib treatment did not affect IFN-γ and IL-22 production, whereas TNF-α and IL-10 production were induced (Fig. 2D). The reduction of IL-17A expression by celecoxib was not caused by altered cell proliferation, apoptosis, and cell cycle status (Fig. 2E–H).

In addition to PGE2, IL-15 has also been shown to induce IL-17A expression in PBMCs and T cells obtained from patients with RA (45, 46). We detected IL-15 expression in both CCR6+ T cell–RASF cocultures and Th17–RASF cocultures, although the expression was highest in CCR6+ T cell–RASF cocultures. To analyze the contribution of IL-15 to the induction of IL-17A, we added anti-IL-15 to the cocultures. Although IL-15 blockade in Th17–RASF cocultures reduced the production of IL-17A, the effects were limited compared with celecoxib treatment. Combining IL-15 blockade and celecoxib treatment had no additional effect compared with celecoxib treatment only (Supplemental Fig. 3).

These findings clearly show that COX-2 expression and PGE2 production are induced in Th17–RASF cultures. Moreover, PGE2 suppression by the COX-2 inhibitor celecoxib resulted in a significant reduction of IL-17A production in Th17–RASF cultures.

**Inhibition of COX-2 activity suppressed expression of proinflammatory cytokines and MMPs in Th17–RASF cultures**

Upon interaction with Th17 cells, RASFs are activated and produce the proinflammatory cytokines IL-6, IL-8, MMP-1, and MMP-3 (18). Celecoxib treatment significantly inhibited these processes with regard to IL-6, IL-8, and MMP-3 production (Fig. 3A). Celecoxib had limited effects on RASF directly, because treatment of RASFs with celecoxib resulted in a significant reduction of IL-17A production in Th17–RASF cultures. This is consistent with previous studies (26–28, 42), which have shown that PGE2 is involved in the induction of IL-17A production in Th17–RASF cultures. In addition, celecoxib treatment resulted in a significant reduction of IL-17A production in Th17–RASF cultures. The inhibitory effect of celecoxib treatment on PGE2 production was accompanied by reduced IL-17A production and a reduced fraction of IL-17A–producing Th17 cells (Fig. 2). In earlier studies, we showed that the proinflammatory loop induced upon interaction of Th17 cells and RASF is dependent on IL-17A (18, 19). To
investigate whether the inhibitory effect of celecoxib was dependent on the inhibition of IL-17A production. Th17–RASF cultures were treated with celecoxib and/or sIL-17R to neutralize IL-17A activity. Compared to sIL-17R only treatment, combining celecoxib and sIL-17R treatment results in increased TNF-α, but not of IFN-γ production. Moreover, the combination of celecoxib and sIL-17R treatment had an additional effect on IL-6 and MMP-3 suppression, compared with sIL-17R only treatment (Fig. 4).

Taken together, these findings show that celecoxib treatment of Th17–RASF cultures involves IL-17A suppression. However, in comparison with sIL-17R treatment, celecoxib has additional stimulatory effects on TNF-α expression and inhibitory effects on IL-6 and MMP-3 in Th17–RASF cultures.

Combining celecoxib and TNF-α blockade in Th17–RASF cultures has additional value on the suppression of cytokines and MMPs

Recently, we showed that besides IL-17A, TNF-α was involved in the induction of the proinflammatory loop in Th17–RASF cultures (18, 19). Furthermore, the combination of TNF-α and IL-17A blockade more strongly suppressed IL-6, IL-8, MMP-1, and MMP-3 production in these Th17–RASF cultures than blocking only IL-17A or only TNF-α (18). In this study celecoxib treatment suppressed IL-17A production, and therefore we investigated a possible additional value of celecoxib compared with TNF-α blockade. Th17–RASF cultures were treated with etanercept, a soluble TNF-α receptor, in the presence or absence of celecoxib.

In contrast to celecoxib treatment, TNF-α blockade had no effects on PGE_2 and IL-17A production (Fig. 5A, 5B). In line with our previous findings (18), TNF-α blockade inhibited IL-6, IL-8, MMP-1, and MMP-3 production (Fig. 5C). Importantly, combining celecoxib and TNF-α blockade had an additional value compared with TNF-α blockade only in suppressing IL-6 and IL-8 expression (Fig. 5C).

The decrease in proinflammatory cytokine and MMP expression after celecoxib treatment was accompanied by an increase in IL-10 concentration (Fig. 2D). However, IL-10 blockade did not reverse the effects of celecoxib treatment on IL-17A, IFN-γ, IL-6, IL-8, MMP-1, or MMP-3 production in Th17–RASF cultures (Supplemental Fig. 4). This indicates that the effects of celecoxib treatment in these cultures were independent of IL-10 induction.

These data show that in contrast to celecoxib, TNF-α blockade has no suppressive effects on PGE_2 and IL-17A production in Th17–RASF cultures. Moreover, combining celecoxib treatment and TNF-α blockade has an additional value in suppression of IL-6 and IL-8 production in these cultures.
Celecoxib, but not IL-1β and IL-6 blockade, inhibits autocrine IL-17A production in cocultures of RASFs and Th17 cells from treatment-naive patients with early RA

To investigate the contribution of IL-1β and IL-6 to Th17 polarization under pathological conditions, RASFs were cultured with primary Th17 cells isolated from peripheral blood of treatment-naive patients with early RA. These Th17–RASF cultures were treated with anti-IL-1β and/or tocilizumab, a humanized monoclonal against the IL-6R. Single or combined treatment of Th17–RASF cultures with anti-IL-1β and tocilizumab had no effects on the production of IL-17A. However, both anti-IL-1β and tocilizumab inhibited the expression of IL-6 and IL-8 production. Additionally, treatment of Th17–RASF cultures with anti-IL-1β suppressed the production of both MMP-1 and MMP-3 (Fig. 6A).

In contrast to anti-IL-1β and tocilizumab, treatment of these Th17–RASF cultures with celecoxib resulted in significantly lower (~4-fold, *p < 0.05) IL-17A production compared with the control conditions. Celecoxib treatment induced the production of TNF-α production but had no effects on IFN-γ production. Furthermore, celecoxib treatment resulted in significantly reduced IL-6, IL-8, MMP-1, and MMP-3 production in the Th17–RASF cultures (Fig. 6B).

Collectively, these data show that PGE2 production via COX-2 activation is the main contributing factor in driving IL-17A production when Th17 cells interact with RASF. Consequently, treatment with the COX-2 inhibitor celecoxib results in inhibition of the proinflammatory loop induced upon Th17–RASF interaction.

Discussion

Recently we showed that when Th17 cells interact with RASF, a proinflammatory loop is induced, resulting in increased autocrine IL-17A production (18). This IL-17A/TNF-α-dependent loop may be an important mechanism in the role of Th17 cells in the progression of chronic persistent inflammation in arthritis, and possibly in other Th17-mediated diseases. In the present study we showed that PGE2 production via COX-2 activation is the main contributing factor in driving IL-17A production when Th17 cells interact with RASF (Fig. 7). Consequently, treatment with the
COX-2 inhibitor celecoxib resulted in a specific inhibition of IL-17A production, which led to reduced synovial inflammation, as was shown by reduced IL-6, IL-8, MMP-1, and MMP-3 expression (Fig. 7).

The proinflammatory cytokines IL-1β, IL-6, and IL-23 are required for Th17 cell differentiation (25). Therefore, their effect on the autocrine IL-17A induction upon Th17–RASF interaction was investigated. Although IL-1β and IL-6, but not IL-23, were increased in the Th17–RASF cultures compared with CCR6− RASF cultures, neutralization experiments showed a limited role of these cytokines in the total production of IL-17A in Th17–RASF cultures. In concordance with this, neutralization of IL-6 and IL-1β in a coculture of mesenchymal cells and PBMCs only marginally reduced IL-17A production (47).

Blockade of IL-1β and/or IL-6 signaling was able to suppress IL-17A production by peripheral blood Th17 cells obtained from healthy volunteers, but not from patients with early RA (Figs. 1B, 6A). In an earlier study we found that RA Th17 cells are more polarized to the IL-17A–producing phenotype than are healthy Th17 cells (18). It may be possible that because of this polarization, RA Th17 cells are less sensitive for IL-1β and IL-6 signaling, which are especially important during differentiation and polarization, compared with healthy Th17 cells. This polarization status may be reflected in higher activity of RA Th17 cells, explaining the higher levels of IL-6 induced by RA Th17 cells (∼500 ng/ml, Fig. 6A) compared with healthy Th17 cells (∼250 ng/ml, Fig. 1B).

Next to IL-1β, IL-6, and IL-23, PGE2 has been reported to induce IL-17A expression by Th17 cells (26, 27, 41, 42). Treatment of Th17–RASF cultures with celecoxib reduced the production of PGE2 and IL-17A. The effects of celecoxib on IL-6, IL-8, and MMP production were explained in large part by IL-17A blockade, as celecoxib inhibited IL-8 and MMP-1 production in a similar extent as did sIL-17R. However, in comparison with IL-17A blockade, celecoxib had additional inhibitory effects on IL-6 and MMP-3 expression. This indicates that besides IL-17A, celecoxib may affect additional factors as well. These may include mechanisms involved in proinflammatory cytokine and MMP production by activated RASFs (Fig. 3B).

The observed decrease in proinflammatory cytokine and MMP expression after celecoxib treatment was accompanied by increased IL-10 production. Boniface et al. (26) found that PGE2 through EP4 receptor signaling inhibits IL-10 expression. Blocking PGE2 by celecoxib in our cocultures may lead to an inverse process leading to increased IL-10 production. The induction in IL-10 production may explain the inhibitory effects of celecoxib on the proinflammatory loop. However, IL-10 blockade did not reverse the effects of celecoxib treatment in Th17–RASF cultures.
This shows that the inhibitory action of celecoxib on the proinflammatory loop is independent of IL-10.

Additionally, we observed increased TNF-α levels after celecoxib treatment. It was shown that celecoxib increases TNF-α production in LPS-stimulated human monocytes, in human RA synovial membranes cultures, and in whole blood of human subjects following celecoxib treatment in vivo (48), which is in concordance with our results. In the present study, TNF-α blockade has no suppressive effects on PGE2 and IL-17A production in RA synovial inflammation. In the Th17–RASF cultures, however, combining celecoxib treatment and TNF-α blockade has an additional value in suppression of IL-6 and IL-8 production in these cultures. These data show that celecoxib and TNF-α blockade both downregulate inflammation, but they exert their effects via different routes.

Many reports on the effects of PGE2 on Th17 differentiation and expansion show that these effects are IL-23 dependent (26, 27, 37, 42). A proposed model is that IL-23 and PGE2 act synergistically to achieve these Th17 effects, possibly via PGE2 mediated upregulation of IL-23R (26), but our results show an IL-23–independent manner. Moreover, inhibiting COX-2 expression, which results in PGE2 synthesis and secretion. Next, PGE2 induces autocrine IL-17A expression by Th17 cells enabling the development of a proinflammatory loop. This loop may explain the progression of an early synovial inflammation toward chronic destructive arthritis. The induction of autocrine IL-17A expression and thereby the proinflammatory loop are blocked by treatment of the COX-2 inhibitor celecoxib.

FIGURE 7. Conceptual model of Th17 cell–mediated synovial inflammation. Activated Th17 cells secrete the proinflammatory cytokine IL-17A. Upon interaction of activated Th17 cells with RASF, IL-17A induces production of the proinflammatory cytokines IL-1β, IL-6, and IL-8 and the tissue-degrading enzymes MMP-1 and MMP-3 by RASFs. Simultaneously, IL-17A induces COX-2 expression, which results in PGE2 synthesis and secretion. Next, PGE2 induces autocrine IL-17A expression by Th17 cells enabling the development of a proinflammatory loop. This loop may explain the progression of an early synovial inflammation toward chronic destructive arthritis. The induction of autocrine IL-17A expression and thereby the proinflammatory loop are blocked by treatment of the COX-2 inhibitor celecoxib.
Disclosures
The authors have no financial conflicts of interest.

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