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mPGES1-Dependent Prostaglandin E2 (PGE2) Controls Antigen-Specific Th17 and Th1 Responses by Regulating T Autocrine and Paracrine PGE2 Production

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The integration of inflammatory signals is paramount in controlling the intensity and duration of immune responses. Eicosanoids, particularly PGE2, are critical molecules in the initiation and resolution of inflammation and in the transition from innate to acquired immune responses. Microsomal PGE synthase 1 (mPGES1) is an integral membrane enzyme whose regulated expression controls PGE2 levels and is highly expressed at sites of inflammation. PGE2 is also associated with modulation of autoimmunity through altering the IL-23/IL-17 axis and regulatory T cell (Treg) development. During a type II collagen–CFA immunization with that of Ag-experienced CD4+ T cells, with mPGES1 competence in the APC compartment enhancing CD4+ IL-17A and their cognate Ag compared with their wild-type counterparts. Additionally, production of PGE2 by cocultured APCs synergized Ag-experienced mPGES1 g and g production under Th17 polarization of naive T cells in vitro. We conclude that mPGES1 is necessary in vivo to mount optimal Treg and Th17 responses during an Ag-driven primary immune response. Furthermore, we uncover a coordination of autocrine and paracrine mPGES1-driven PGE2 production that impacts effector T cell IL-17A and IFN-γ responses. The Journal of Immunology, 2018, 200: 725–736.

Prostaglandin E2 is a ubiquitous eicosanoid that modulates diverse physiologic and pathologic functions. The biosynthesis of PGE2 is controlled by several constitutive (cyclooxygenase-1 [COX-1], phospholipase A2 [PLA2]) and inducible (COX-2, mPGES1) anabolic and catabolic (15-hydroxyprostaglandin dehydrogenase [15-PGDH]) enzymes (1, 2). These enzymes act in concert to tightly regulate the localization and level of PGE2 concentrations during inflammation. PGE2 is the most prominent PG in many chronic inflammatory and neoplastic disorders including rheumatoid arthritis (3, 4) and many forms of cancer (5), including intestinal cancer (6). However, PGE2 can also exert immunosuppressive properties that contribute to the resolution of inflammatory events and help restore tissue homeostasis (1, 7). PGE2 has four known receptors (8) with varying expression levels in different cell types that can trigger negative feedback mechanisms to limit PGE2 concentrations (9, 10).

mPGES1 is a membrane-bound biosynthetic enzyme for PGE2 that acts downstream of COX enzymes (11). mPGES1 can become a highly rate-limiting enzyme that controls PGE2 levels because of its differential expression pattern and inducible nature during inflammation. mPGES1-deficient mice have demonstrated the relevance of mPGES1-driven PGE2 in altering several inflammatory diseases (6, 12–14). Furthermore, absence of mPGES1 can cause shunting of PGs and change the characteristics of the inflammatory response (15), cause deficiencies in Ag-specific humoral responses that are dependent on the T cell (12), mediate collagen-induced arthritis (3), and control carcinogenesis in several cancer models (1, 16). PGE2 exacerbates arthritis development in the collagen-induced arthritis mouse model through the inflammatory IL-23/IL-17 axis (17), and mPGES1 is required to generate inflammatory responses that result in arthritis development in this same model.

PGE2 has pleiotropic effects on many cells of the immune system, influencing both the innate and acquired immune responses. In general, PGE2 suppresses neutrophil and macrophage functions, whereas it stimulates stromal and vascular endothelial cells (2). Cells belonging to the innate immune response arm rapidly react in different ways to PGE2 exposure: PGE2 can promote influx and activation of neutrophils, macrophages, and mast cells (1, 18, 19), but it can also suppress NK cytolysis and granulocyte functions (20). The effects of PGE2 in dendritic cells (DCs) are more complex, acting...
mostly on IL-12 and IL-23 production and promoting CCR7 expression, but being able to promote both proinflammatory and immunosuppressive functions (1, 15, 21–23). PGE2 can therefore serve as a regulator of APC function at many levels. Lymphocytes are also targeted by PGE2, which modulates their function not only depending on its local concentration but additional microenvironment characteristics, especially the composition of the cytokine/chemokine milieu (24, 25).

T cells can also display a multiplicity of responses to PGE2. PGE2 exerts its effect in T cells exclusively via the EP2 and EP4 receptors (9, 25, 26). PGE2 can alter the T cell subset composition in lymphoid organs and several aspects of T cell responses, with marked consequences on T cell commitment (27–29). This latter effect seems particularly relevant in the case of proinflammatory Th1 and Th17 responses, with PGE2 facilitating the expansion of Th17 cells via EP2 and EP4 differential expression when in the presence of IL-1β and IL-23 (25, 30, 31). PGE2 is also capable of increasing IL-17 and reducing IFN-γ production in human memory T cells (32). EP2 expression is almost absent in human Th17 cells due to binding of RORγt to ptg2 with suppressive effects, and Th17 cells from MS patients exhibit a more proinflammatory profile because of enhanced IFN-γ and GM-CSF production compared with healthy individuals (33). Th1 responses can be inhibited by PGE2 (27, 34), but PGE2 can also paradoxically promote Ag-specific Th1 cells (35) and expand Th1 cells in the experimental autoimmune encephalomyelitis (EAE) model in an EP4-dependent fashion (31). Many of the PGE2-Th-promoting effects are triggered by increasing production of polarizing cytokines by surrounding APCs or innate cells, like IL-12 or IL-23, produced by activated DCs (17, 36). PGE2 can also induce Foxp3 expression in CD4+CD25 T cells, and induced regulatory T cells (Tregs) themselves can express COX-2 (37). It is therefore still unclear how PGE2 precisely alters T cell commitment and T cell cytokine profiles, and how the PGE2 signals are integrated in different contexts and inflammatory conditions. Moreover, the relative contribution of T cells themselves to the local PGE2 pools has been barely investigated.

The following studies were conducted to identify new roles of PGE2 on T cell function by enzymatic fine-tuning of PGE2 production using mPGES1-deficient mice. We also reconcile some of the paradoxical effects that PGE2 has been reported to have on T cells by dissecting its role in naive and Ag-experienced/mature CD4+ populations.

Materials and Methods

Mice and immunization with type II collagen

Wild-type (WT) and mPGES1−/− mice in a BL/6 or DBA background were bred in-house and maintained under specific pathogen-free conditions (3). PGE2 can alter the T cell subset composition in lymphoid organs and several aspects of T cell responses, with marked consequences on T cell commitment (27–29). This latter effect seems particularly relevant in the case of proinflammatory Th1 and Th17 responses, with PGE2 facilitating the expansion of Th17 cells via EP2 and EP4 differential expression when in the presence of IL-1β and IL-23 (25, 30, 31). PGE2 is also capable of increasing IL-17 and reducing IFN-γ production in human memory T cells (32). EP2 expression is almost absent in human Th17 cells due to binding of RORγt to ptg2 with suppressive effects, and Th17 cells from MS patients exhibit a more proinflammatory profile because of enhanced IFN-γ and GM-CSF production compared with healthy individuals (33). Th1 responses can be inhibited by PGE2 (27, 34), but PGE2 can also paradoxically promote Ag-specific Th1 cells (35) and expand Th1 cells in the experimental autoimmune encephalomyelitis (EAE) model in an EP4-dependent fashion (31). Many of the PGE2-Th-promoting effects are triggered by increasing production of polarizing cytokines by surrounding APCs or innate cells, like IL-12 or IL-23, produced by activated DCs (17, 36). PGE2 can also induce Foxp3 expression in CD4+CD25 T cells, and induced regulatory T cells (Tregs) themselves can express COX-2 (37). It is therefore still unclear how PGE2 precisely alters T cell commitment and T cell cytokine profiles, and how the PGE2 signals are integrated in different contexts and inflammatory conditions. Moreover, the relative contribution of T cells themselves to the local PGE2 pools has been barely investigated.

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Cell preparation and flow cytometry

Single-cell suspensions were prepared from the spleen, inguinal, and/or popliteal lymph nodes and stained on ice using predetermined optimal concentrations of each Ab for 20–30 min, washed, and fixed using 1.5% PFA. Cells with the light scatter properties of singlet lymphocytes were analyzed by multicolor immunofluorescence staining and a BD FACS Fortessa II flow cytometer (Becton Dickinson, San Jose, CA). Gates were always positioned to exclude ≥98% of unreactive cells or unstained cells.
these media were supplemented to achieve the following final concentrations: 1 μg/ml soluble anti-CD28 (clone 37.51; BD Pharmingen), 10 μg/ml anti–IFN-γ (clone XMG1.2; BioLegend) and anti–IL-4 (clone 11B11; BioLegend), 1 ng/ml human TGF-β (Miltenyi), and 20 ng/ml rmIL-6 (Miltenyi). For Th1 polarization, the mixture contained instead 1 μg/ml soluble anti-CD28 (clone 37.51; BD Pharmingen), 10 μg/ml anti–IL-4 (clone 11B11; BioLegend), 1 ng/ml rmIL-2 and 10 ng/ml rmIL-12 (Miltenyi Biotech). PGE2 was obtained from Cayman Chemical and stored and diluted according to the manufacturer’s instructions.

Quantitation of eicosanoids by liquid chromatography–mass spectrometry

Analysis was also performed by liquid chromatography–mass spectrometry (LC/MS) as follows: fresh cell culture media or cell culture supernatants were incubated for 30 min in the presence of 50 mM arachidonic acid, and this mixture was added to 5 ml of ice-cold methanol containing 1.0 ng each of the following internal standards: [2H4]-15-F2t-isoprostane ([2H4]-8-iso-PGF2a), [2H4]-PGD2, [2H4]-PGE2, [2H3]-11-dehydro-TXB2 (11-dehydro-TXB2), and [2H4]-6-keto-PGF1α (all purchased from Cayman Chemicals). The lipids were extracted and separated from the solid particulates by centrifugation. The liquid layer was transferred to another tube, and the methanol was dried under a stream of nitrogen. The residue was reconstituted in 10 ml 0.01 M hydrochloric acid and applied to a C-18 Sep-Pak column (Waters) that was prewashed with 5 ml of methanol and 5 ml of H2O (pH 3). For each assay, the precision rate was 65% and the accuracy rate was 95%.

Real-time PCR

Analysis of expression levels of mRNA was performed with TaqMan assays. RNA was obtained from cell culture lysates with QIAGEN mini columns, and DNA digestion was performed during the process. cDNA was generated from the extracted RNA with the VILO SuperScript kit from Invitrogen. cDNA samples were then subjected to RT-PCR TaqMan amplification using the following probes: ptger2 (Mm0042105_m1), ptger4 (Mm00478374_m1), row (Mm01261022_m1), tbx21 (Mm0042105_m1), tgfbr1 (Mm01261022_m1), tgfbr2 (Mm00436064_m1), ptges (Mm01261022_m1), ptger2 (Mm00436064_m1), ptger4 (Mm00436064_m1), d2r3 (Mm00519943_m1), and gapdh (Mm99999915_g1).

ELISA and multiplex analysis of cytokines

Mouse IL-17A and IFN-γ were measured using the corresponding ELISA Max Deluxe Sets from BioLegend. In some cases, supernatants were also evaluated for IL-17A, IFN-γ, IL-6, IL-22, and TNF-α using the MILLPLEX system from EMD Millipore in a Luminex100 system at the Hormone Analysis Core at Vanderbilt University.

Results

mPGES1 modulates T cell phenotype, but not proliferation, following immunization with CII

Our initial experiments aimed to understand the in vivo role for mPGES1-derived PGE2 during response to a defined Ag. To determine whether mPGES1 was necessary for T cell proliferation during immunization, we transferred CFSE-labeled splenocytes from either WT or mPGES1−/− CII-TCR Tg mice (with T cells that express CII-specific Vβ8.3+) into WT or mPGES1−/− naive animals. The recipient mice were then injected with either IFA alone or CII-IFA, and their draining lymph nodes (dLNs) were recovered and analyzed 3 d later to evaluate their proliferation by CFSE dilution of the transferred cells. Once the dLNs were recovered and processed for flow cytometry analysis, we gated on CD4+Vβ8.3+ cells in the recipient mice (Fig. 1A) and evaluated the frequencies of proliferating cells within that population. Ag-elicited CD4+ proliferation did not significantly differ between the transferred WT or mPGES1−/− donor T cells. Furthermore, the presence or absence of mPGES1 in the recipient mice did not significantly alter proliferation rates of CD4+ cells. These experiments indicate that the physiologic production of PGE2 that depends on mPGES1 does significantly impair the proliferative T cell response during a recall Ag challenge in vivo. It is known that activated CD4+ T cells release picomolar concentrations of PGE2, which are beneficial for proliferation in vitro (9), but how this autocrine PGE2 contributes to different T cell phenotypes in vivo and Ag responses is still largely unknown.

Despite the lack of altered T cell proliferation upon Ag challenge, the question of whether a qualitative and quantitative response regarding the cytokine production identity is different in the context of a proinflammatory response was still unresolved. To address this question, we immunized WT or mPGES1−/− animals with CII-IFA and analyzed the specific response of CD4+ cells in different lymphoid organs. Intracellular production of IL-17A and IFN-γ was evaluated in the spleen and the dLNs on day 10 after immunization. dLNs of WT mice showed significantly higher proportions and numbers of IL-17A+ cells compared with mPGES1−/− mice despite the lack of differences in splenic T cells, as might be expected given the time point evaluated (Fig. 1B). This same effect was also observed on the CD4+Foxp3+ cells in the dLNs, but not in the spleen (Fig. 1C). We hence conclude that mPGES1-driven PGE2 production during a proinflammatory immune response alters both Th17 and regulatory T cell responses in vivo. No significant changes were detected in CD4+IFN-γ+ cells (Supplemental Fig. 1).

Next, WT or mPGES1-deficient mice were immunized with CII-IFA. After 10 d, total splenocytes were isolated and cultured for 4 d in the presence of CII. When restimulated, splenocytes from WT mice released significantly more IL-17A than splenocytes of mPGES1−/− mice into the supernatant (Fig. 1D). Taken together, these results indicate the relevance of mPGES1-derived PGE2 in shaping the phenotype of the developing immune response, with absence of PGE2 reducing the numbers of T cells polarizing toward an IL-17 phenotype. The inverse of these observations suggests that the presence of PGE2 may facilitate polarization toward the IL-17 phenotype.

mPGES1-dependent PGE2 regulates EP2 and EP4 expression in T cells

To better understand our observation of reduced IL-17A production by T cells from mPGES1-deficient animals following immunization, we analyzed the expression levels of genes that control Th17 commitment and integrate PGE2 sensing. Mice deficient in PGE2 receptors 2 or 4 (EP2 or EP4) show impairment in IFN-γ and IL-17A production during inflammatory responses like contact hypersensitivity (31) or during EAE (26). However, whether enzymatic control of PGE2 production alters T cell EP receptor expression levels and susceptibility for cytokine signals is unknown. To identify such differences in an unbiased approach in unmanipulated mice, we sorted freshly isolated splenic populations of T cells from WT and mPGES1−/− mice according to their canonical naive, memory, and Treg markers CD62L, CD44, and CD25. In WT mice, naive T cells showed lower ptger2 but higher ptger4 expression when compared with memory or regulatory T cells (Fig. 2A). mPGES1-competent peripheral Tregs expressed a ~5-fold higher level of ptger2 than naive T cells, whereas their ptger4 expression level was 3-fold lower. Interestingly, absence of mPGES1 reduced ptger2, ptger4, and tgfbr1 expression levels only in naive T cells, with mPGES1−/− naive T cells expressing 5- to 10-fold lower levels of these transcripts. IL-23 is also known to regulate the maintenance and expansion of Th17 cells; however, we did not observe any difference in WT and mPGES1−/− CD4+ cells IL-23R levels. These results suggest a mechanism by which absence of mPGES1 may influence the phenotype of naive T cells due to resistance to TGF-β signaling and also suggest why these cells may be resistant to negative effects of PGE2 on T cell proliferation in vivo.
The initial differences in these receptor levels prompted us to evaluate their differences during in vitro polarization. We cultured naive CD4+ T cells from WT and mPGES1−/− mice under Th17 polarizing conditions for 4 d. To further understand the role that autocrine PGE2 might play in Th17 responses, we examined the expression levels of the genes encoding the key enzymes controlling PGE2 metabolism—mPGES1 (ptges) and COX-2 (ptgs2), and the PGE2 receptors EP2 (ptger2) and EP4 (ptger4)—in WT compared with mPGES1 null cells (Fig. 2B). We found that ptges was very rapidly downregulated under Th17 polarizing conditions during the first 2 d, recovering to initial levels on day 4. This observation may explain the loss of differential IL-17A expression during in vitro polarization compared with in vivo circumstances. Similar to what has been observed in other systems (15), ptgs2 was upregulated in WT cells compared with mPGES1−/− cells, suggesting a positive feedback of PGE2 on COX-2 expression that may rely on ptger2, because this gene was gradually upregulated over the course of Th17 polarization. Expression of ptger4 decreased over time as Th17 polarization progressed, and stayed silenced over the course of 4 d. The rapid and pronounced decline in both ptges and ptger4 suggests the possibility that autocrine PGE2 signaling should be initially robust, but then silenced to complete the commitment to a Th17 phenotype.

Production of PGE2 by Ag-stimulated T cells is mPGES-1 dependent and acts in synergy with APCs

The intrinsic capacity of T cells to produce PGs has been scarcely investigated. T cells are known to produce PGE2 upon strong TCR-driven stimuli (33, 40), but the enzymatic control of such PGE2 production and whether this differs within different T cell subsets upon inflammation is barely known. To explore the role of PGE2 in an Ag-specific manner, we isolated CD4+CD25− and CD25+ T cells from WT mice following a CII-CFA–specific immunization response (day 10 after immunization). We observed different levels of PGE2 production when T cells were rechallenged ex vivo with their cognate Ag in the presence of splenic DCs acting as APCs (Fig. 3A), with CD4+CD25− WT mPGES1-competent cells producing nearly 3-fold more PGE2 than CD4+CD25+ cells. To corroborate the capacity to elicit Ag-specific T cell activation and PGE2 production ex vivo, we also isolated CD4+CD25− and CD25+ cells from unimmunized CII-TCR Tg animals and incubated them for 4 d in the presence of CII (Fig. 3A). CD4+ cells from unimmunized CII-TCR Tg CD4+ and from day 10 CII-CFA-immunized mice responded similarly, with CD4+CD25− CII-TCR Tg cells showing increased PGE2 production compared with CD4+CD25+ cells. These data demonstrate that PGE2 production is an integral component of an Ag-specific T cell response.

Because mPGES1 is one of the terminal enzymes controlling PGE2 production, and in other systems absence of mPGES1 can lead to shunting from PGE2 to other PGs, we investigated a variety of PGs during T cell activation. Purified CD4+CD25− cells from naive WT and mPGES1−/− unmanipulated mice were stimulated with anti-CD3/CD28 for 4 d, and the supernatants were analyzed for different PGs. Comparing WT and mPGES1−/− T cells, PGE2, PGF2α, and PGD2 were either not detected or expressed at low levels, but did not show differences between WT and mPGES1−/− mice (Fig. 3B). However, when WT CD4+CD25− cells from day 10 CII-immunized mice were restimulated with anti-CD3/CD28, we observed higher levels of PGF2α and PGE2 (Fig. 3B, 3C), and revealed a significant difference in PGE2 concentrations between WT and mPGES1−/− T cells. We did not see shunting from PGE2 to an alternate terminal PG in these conditions. These data demonstrate that CD4+CD25+ T cells acquire the capacity to produce different PGs during the course of a proinflammatory immune response.
response following immunization with Ag, and that mPGES1 controls the magnitude of the corresponding increase in PGE2.

Because PGE2 is the predominant PG in T cells during an inflammatory immunization, we next inquired to what extent an APC would collaborate, and whether this response would be substantially altered by antigenic restimulation. To answer this question, purified CD4+CD25− T cells from naive mice were sorted into the indicated naive, memory, and regulatory subsets (CD25+CD4+CD62L−, CD25+CD4+CD62L+, and CD25−) and analyzed for their expression levels of ptger2, ptger4, tgfbr1, and il23r. All values are relative to the WT CD4+CD25+ T cell population. (B) Expression levels kinetics of the indicated mRNAs were evaluated at the indicated time points (freshly isolated, days 1, 2, 3, and 4) under Th17-polarizing conditions. Results are compiled from three different experiments with three to four pooled mice cells and four replicates. Statistically significant differences from WT cells for each data point in a one-way ANOVA test are indicated. *p < 0.05, **p < 0.01.

T cell autocrine and paracrine PGE2 sources coordinate to control Ag-specific T cell cytokine responses

Our previous data demonstrate that PGE2 production is enhanced during immunization in T cells in vivo, and that presence of mPGES1 increased CD4+ cell IL-17 production in vivo. However, there remained uncertainty as to how PGE2 generated by T cells or by APCs or other surrounding cells are integrated to control T cell cytokine phenotype during a recall response. To better delineate what is the relative contribution of both T cell autocrine and paracrine mPGES1-driven PGE2, we isolated CD4+CD25− T cells from the spleens and dLNs of day 10 CIA-CFA–immunized WT and mPGES1−/− mice, and cocultured them with either WT or mPGES1-deficient BMDCs in the presence of their cognate Ag (CII) for 4 additional days in vitro. Ag rechallenge with CII induced a much larger IL-17A production in WT CD4+CD25− cells than in the mPGES1-deficient counterparts (Fig. 4A). Absence of mPGES1 in both T cells and APCs resulted in almost absent IL-17A concentrations in the supernatant. mPGES1-competent BMDCs induced a 7-fold higher production of IL-17A from WT T cells, whereas mPGES1−/− T cells IL-17A production could be only partially rescued by the presence of mPGES1 in the BMDCs. Production of IFN-γ paralleled what was observed with IL-17A, but showed independence of mPGES1 in BMDCs. These results suggest autocrine and paracrine PGE2 may cooperate to coordinate T cell IL-17A and IFN-γ when both the T cell and the APCs are mPGES1 competent during a cognate Ag interaction. To enhance the strength of the T cell–APC interaction and the T cell response and PG production by BMDCs, we also stimulated the cocultured BMDCs for 2 h with LPS prior to activation of T cells in the same coculture conditions. As anticipated, LPS stimulation of the APCs increased cytokine production by T cells (Fig. 4A, 4B). The results recapitulated what was observed without LPS stimulation, but these conditions unveiled significant differences in IL-17A production between WT and mPGES1−/− CD4+CD25− cells when the BMDCs were mPGES1 competent (Fig. 4B). These results demonstrate that IL-17A production relies more on PGE2 production by cognate APCs than IFN-γ production, and suggest that APC stimulatory capacities enhance cytokine production together with PGE2.

To confirm that the cytokine profile was being regulated by PGE2 during T cell activation, we stimulated WT or mPGES1-deficient purified CD4+CD25− T cells from mice immunized in the same manner as before with anti-CD3/anti-CD28 rather than cognate Ag for 3 d (Fig. 4C). The capacity of in vivo–primed T cells to produce IL-17 was 5-fold larger in T cells from WT mice compared with mPGES1−/− mice, and the increase was fully abrogated by specifically inhibiting COX-2 activity with NS-398, confirming the effect is related to PGE2 biosynthesis. Furthermore, when measured by multiplex, IL-6, IL-17A, TNF-α, and IFN-γ were also significantly higher in supernatants of WT compared with mPGES1-knockout (KO) T cells (Fig. 4C). When PG production was blocked by COX-2 inhibition, levels of IL-6, IL-17A, and TNF-α from WT T cells were reduced to the level of mPGES1−/− T cells in all cases. These data confirm that the changes in production of these cytokines are also due to changes in PGE2 synthesis on T cells. It is of interest to note that magnitude of reduction in IFN-γ in KO T cells was not as dramatic as IL-17A, and thus may not be as dependent on PGE2.

Altogether, our results demonstrate that T cell mPGES1-driven autocrine PGE2 controls T cell cytokine profiles and significantly enhances IL-17A, IL-6, and IFN-γ production during Ag-specific and nonspecific T cell activation. In addition, IL-17A and IFN-γ were differentially regulated during Ag-specific versus nonspecific T cell activation, with IL-17A more strongly affected by paracrine PGE2 levels derived from BMDCs.
PGE$_2$ regulates ROR$\gamma$ expression and promotes IFN-$\gamma$ production of naive CD4$^+$ T cells under Th1 and Th17 conditions

Previous reports have demonstrated that PGE$_2$ can expand T cells committed to the Th17 lineage both in mice and in humans, but PGE$_2$ can also promote the proliferation of murine Th1 cells. We tested whether mPGES1 deficiency would impact either the generation or proliferation of naive T cells undergoing Th1 or Th17 polarization in vitro. During Th17 differentiation, WT cells show a relative increase in both $rorc$ and $tbx21$ expression (Fig. 5A), although they were not significant except for $rorc$ at day 4. We then evaluated the intracellular expression of either Tbet or ROR$\gamma$ at day 4 of differentiation but found no significant differences between the WT and mPGES1$^{-/-}$ proportions of CD4$^+$Tbet$^+$ cells under Th1 differentiation or CD4$^+$ROR$\gamma^+$ under Th17 polarizing conditions (Fig. 5B). Under these conditions, mPGES1 also did not significantly alter the proportions of IL-17A$^+$ cells under Th17 polarization or IFN-$\gamma^+$ in Th1 conditions (Fig. 5C). We hence concluded that T cell autocrine PGE$_2$ does not have the capacity to alter Th1 or Th17 commitment of naive T cells in the absence of a cognate APC interaction and/or during a non-antigen–driven T cell response. This lack of significantly lower IL-17A during in vitro polarization of naive CD4$^+$ T cells contrasts with our observation that mPGES1-deficient cells differentiated in vivo demonstrated significantly lower levels of IL-17A. To address this issue, we examined the effect of exogenously provided PGE$_2$ during polarization. We polarized purified naive CD4$^+$ T cells under Th1 or Th17 conditions for 4 d in the presence of increasing concentrations of PGE$_2$ and analyzed the intracellular production of IL-17A and IFN-$\gamma$. Surprisingly, under Th17 polarization, gradual increase in PGE$_2$ concentrations caused a drop in the percentage of cells capable of producing IL-17A, whereas at the same time promoting the intracellular accumulation of IFN-$\gamma$ (Fig. 5D, 5E), although concentrations $>$10 nM had a negative
impact on overall T cell proliferation and survival (data not shown). Interestingly, intermediate concentrations of PGE2 did not alter T cell proliferation of IL-17A+ cells during the first days of in vitro Th17 polarization (Fig. 5F), in contrast with what seems to be the case during the expansion phase of such cells (31) or human memory T cells (32). PGE2 did, however, inhibit proliferation of all other IL-17A+ cells, and hence might provide a competitive advantage during inflammatory conditions. In the case of Th1 cells, PGE2 also favored proliferation of IFN-γ+ cells, consistent with reports in mice that show a synergistic amplification of IL-12 signaling (36), but contrary to what seems to happen during restimulation of expanded human naive T cells (25). PGE2 also downregulated CD25 expression selectively on IL-17A+ cells under Th17 conditions (Fig. 5G).

To gain insight into the mechanisms controlling the shift in IL-17A to IFN-γ, we evaluated how PGE2 alters the levels of mRNA encoding for RORγt, Tbet, mPGES1, and COX-2 at the concentration that exerted the strongest shift in IL-17A to IFN-γ production (10 nM) without majorly impacting proliferation or survival during Th1 and Th17 polarization. Exogenously added PGE2 decreased rorc expression when compared with untreated cells (Fig. 6A), and this downregulation was largely independent of mPGES1. Examination of the intracellular expression of RORγt and IL-17A revealed that in vitro exogenous PGE2 caused an expected decrease in RORγt+ cells, but the most accentuated inhibition took place in RORγt+IL-17A+ cells. Production of IL-17A was strictly segregated to RORγt+ cells (Fig. 6A, middle dot plots), which made us disregard the possibility of a non-Th17 concomitant population or Th cells that could be plastic enough to contribute to the pool of IL-17A+ cells. In search of a further mechanism explaining the role of PGE2 in T cell function, we analyzed the phosphorylation levels of pSTAT family members involved in Th17 commitment. Exogenous addition of PGE2 during Th17 polarization downregulated the phosphorylation of both pSTAT3 and pSTAT5 in CD4+RORγt+ cells (Fig. 6B), although there was no difference due to mPGES1 competence. This suggests that exogenous PGE2 alters the activation of STAT proteins of already committed cells. Contrary to the Th17 conditions, under Th1 polarization extrinsic PGE2 increased tbx21 expression at day 2 (Fig. 6C, left) and significantly increased intracellular IFN-γ+ expression in Tbet+ cells (Fig. 6C, right dot plots and bar graph). Interestingly, Th1 conditions increased ptgs-2 compared with Th17 conditions, which might contribute to reinforce the positive feedback loop that IFN-γ+ exerts on Th1 cells.

FIGURE 4. PGE2 from T cell–intrinsic and –extrinsic sources controls Ag-specific T cell cytokine responses in immunized mice. CD4+CD25+ T cells from WT or mPGES1−/− DBA mice immunized with CII-CFA were isolated on day 10. These T cells were cocultured with WT or mPGES1−/− BMDCs for 4 d in the presence of CII. IL-17A and IFN-γ production was measured in the supernatants of CD4+CD25+ cells cocultured with (A) unstimulated BMDCs or (B) BMDCs previously stimulated with LPS at 1 μg/ml for 2 h. (C) Pooled purified CD4+CD25+ from WT and mPGES1-deficient mice were isolated on day 10 of CII-CFA immunization and stimulated with anti-CD3/CD28 for 4 d in vitro. Cytokines from the supernatants were measured in the presence or absence of the COX-2 inhibitor NS398 at 10 μM. Compiled results of three different experiments with n = 3 replicates each and expressed as fold-difference relative to WT cells. *p < 0.05, **p < 0.01, using a two-tailed heteroscedastic Student t test. All results are relative to WT T cells + WT BMDCs (A and B) or to WT T cells (C).
Expression of \textit{ptgs-2} was not altered due to initial exposure to PGE$_2$ under Th17 differentiation, but it was decreased when PGE$_2$ was provided under Th1 conditions. Together, these data demonstrate that PGE$_2$ concentrations are critical in defining the commitment potential of undifferentiated cells at the exact moment that cells receive a TCR proliferative signal, and that extrinsic PGE$_2$ supplies alter Th1 and Th17 commitment. Moreover, extrinsic PGE$_2$ shifts IL-17A production to IFN-\(\gamma\) production under Th17 polarization, and it increases the overall IFN-\(\gamma\) production of Th1 cells, whereas T cell-mPGES1 does not exert a measurable effect.

**Discussion**

In this report, we demonstrate that mPGES1-driven PGE$_2$ regulates T cell responses during an Ag-specific immune response, with the regulatory and Th17 compartments demonstrating a requirement
for PGE2 to optimally expand. We also show that T cells themselves alter their PG secretion capacities in an Ag-specific manner, with PGE2 becoming a dominant PG that is dependent on mPGES1 during immune responses. Furthermore, we illustrate how mPGES1-dependent PGE2 facilitates T cells to increase their IL-17A, IFN-γ, and IL-6 cytokine production capacity upon restimulation with their cognate Ag. Finally, we show that after in vivo priming, T cell autocrine and paracrine PGE2 act synergistically to achieve maximal IL-17A and IFN-γ cytokine secretion potential. Consistent with this, exposure to intermediate levels of PGE2 during in vitro Th1 polarization of naive CD4+ cells increases their IFN-γ production, but it surprisingly inhibits IL-17A during Th17 polarization, whereas EP2 and EP4 are detected at lower levels only in the naive CD4+ population, and EP4 is downregulated during Th17 polarization, whereas EP2 is upregulated (Fig. 2). The net effect of PGE2 production on T cells upon an autoimmune inflammatory response is highly complex due to the diversity and variability of EP receptors expressed in different cells and tissues together with the different capacities to secrete PGE2 (1, 43). During a CII-CFA immunization, DBA mice deficient in mPGES1 show impaired generation of regulatory and IL-17A+ cells in the dLNs compared with WT mice (Fig. 1), but this is not due to a general lack of proliferative capacity of T cells. We conclude that this effect must be due to both the absence of mPGES1 in the immunized mouse, which alters the production of PGE2 by neighboring cells and APCs, as well as a T cell–intrinsic effect that is at least in part due to their altered EP receptor expression.

Several studies have demonstrated the relevance of PGE2 in CD4+ T cells during immune responses leading to autoimmunity, setting the path to understand how PGE2 might act in promoting disease progression. Sensing of PGE2 in T cells is paramount, because efficient T cell priming and activation require EP receptors (9). EP4 (ptger4) variants have been described as candidate risk factors in joint damage in RA patients (41) and in inflammatory bowel disease patients (42). CD4+ T cell conditional EP4 KO mice are protected from EAE (26), and EP2 and EP4 antagonists suppress the differentiation of Th1 and Th17 cells in vivo (31). In this report, we show that in mice deficient in mPGES1, a terminal PGE2 biosynthetic enzyme, EP2 and EP4 are detected at lower levels only in the naive CD4+ population, and EP4 is downregulated during Th17 polarization, whereas EP2 is upregulated (Fig. 2). The net effect of PGE2 production on T cells upon an autoimmune inflammatory response is highly complex due to the diversity and variability of EP receptors expressed in different cells and tissues together with the different capacities to secrete PGE2 (1, 43). During a CII-CFA immunization, DBA mice deficient in mPGES1 show impaired generation of regulatory and IL-17A+ cells in the dLNs compared with WT mice (Fig. 1), but this is not due to a general lack of proliferative capacity of T cells. We conclude that this effect must be due to both the absence of mPGES1 in the immunized mouse, which alters the production of PGE2 by neighboring cells and APCs, as well as a T cell–intrinsic effect that is at least in part due to their altered EP receptor expression.

The contribution of IL-17A and Th17 cells to many autoimmune and inflammatory diseases is widely documented, with promising results in treatment of psoriatic arthritis and RA in targeted therapies like the anti–IL-17 mAb secukinumab (44–47). IL-17A
is known to be increased in the synovial fluid of RA patients, being more prevalent in ACPA+ RA (48). PGE2 can promote both human and mouse Th17 proliferation and expansion. Koeller et al. (33) demonstrated that human Th17 cells selectively downregulate EP2, whereas all other T cell–induced fates did not alter it, which contrasts with our results in mouse Th17 cells (Fig. 2). However, it remains unresolved whether this is due to the distinct source and nature of naïve T cell populations (secondary lymphoid organs versus blood) or if it is a species-specific issue. In either case, our data support the notion that sensing of locally available PGE2 upon activation of naïve T cells is critical for Th17 differentiation. In addition, EP2 and EP4 control Th17 cell expansion and activity (25, 31). However, most of these studies show how sensing of PGE2 alters responses in cells that are already committed to a certain phenotype, like Th17 polarized cells, and use EP2 and EP4 germline/conditional T cell KOs or administration of antagonists of EP2/EP4 to investigate how PGE2 functions. We show that T cells undergoing Th17 polarization first suppress expression of ptger2 to recover over time, whereas ptger-2 is upregulated in the absence of mPGES1. We also show how ptger2 is gradually upregulated, whereas ptger4 is starkly and consistently suppressed (Fig. 2B).

Inflammatory signals can induce PGE2 release in many different cell types (1). Activation of T cells also involves autocrine production of PGE2, although this has been previously studied only regarding the involvement of COX-2 (40). We demonstrate that stimulation of CD4+ cells induces PGE2 production in an mPGES1-dependent fashion and is Ag dependent (Fig. 3). More importantly, CD4+CD25+ cells alter their PG profile during immunization, with a large increase in PGE2 production that relies on mPGES1, with no concomitant differences in PGD2 and PGE2 (Fig. 3B, 3C).

PGE2 acts synergistically with IL-23 to favor human Th17 expansion (30), and TCR triggering in the presence of PGE2 increases IL-17 and reduces IFN-γ production by freshly PBMC-isolated human memory T cells or T cell clones (32). In agreement with our results, BMDCs that produce PGE2 can favor ex vivo Th1 and Th17 responses while being detrimental for Th2 responses, in large extent due to an imbalance in their IL-12/IL-23 secretory profile caused by PGE2 (22, 49). All of these results suggest that nonlymphoid paracrine PGE2 governs Th17 expansion. Dissecting the relative contribution of PGE2 by different cell types allowed us to determine that mPGES1 competence is necessary in both cell types during cognate APC–CD4+ T cell interactions. We demonstrate that the presence of mPGES1 is strikingly important to mount IL-17A and IFN-γ Ag-specific responses upon Ag challenge in BMDC/CD4+CD25+ coculture assays (Fig. 4). Most interestingly, requirement for mPGES1 revealed both an autocrine and paracrine role, with mPGES1 presence acting synergistically on APCs and CD4+ cells to achieve optimal generation of IL-17A and IFN-γ. This is consistent with the capacity mPGES1 in BMDCs to specifically enhance IL-12 production (15). Stimulation of WT BMDCs with LPS prior to incubation increased IL-17A production in a synergistic manner with WT T cells (Fig. 4B), an effect that was not observed for IFN-γ or in the absence of mPGES1 in BMDCs. Moreover, autocrine cytokine production in the absence of APCs proved again to be largely dependent on mPGES1, with 5-fold lower IL-17A and IL-6 responses in mPGES1−/− cells (Fig. 4C). IFN-γ responses were, however, different, because they showed less dependency on mPGES1 competence in BMDCs, although it was necessary on T cells (Fig. 4). Importantly, cytokine production in all these instances was fully abolished by specific inhibition of COX-2, demonstrating the requirement of upstream PGH2 generation and the specificity of mPGES1 activity, because blockade of COX-2 generated cytokine values virtually identical to those from mPGES1−/− CD4+CD25+ cells.

Although the transcriptional control of CD4+ T cell commitment to determined cytokine-biased fates has been vastly studied, much less is known about how PGE2 affects the molecular mechanisms behind it. RORyt binds the ptger2 promoter and represses EP2 expression in mouse and human CD4+ cells. In addition, Th17 cells from patients with MS exhibited reduced RORC binding to the ptger2 promoter region, which promotes IFN-γ and GM-CSF production in such cells (33). EP2 expression can be partially restored by increasing TCR signal strength, as well in RORCx+ cells. Surprisingly, it has been recently reported that Tbet or continued RORyt expression is not strictly required for Th17-associated immunopathology in mouse models of Helicobacter hepaticus–induced intestinal inflammation or EAE (50). We report that in naïve CD4+ cells, exogenous PGE2 downregulates RORyt and IL-17A expression during Th17 polarization and at the same time shifts production of IL-17A to IFN-γ. Expression of IFN-γ does not seem to happen through a reversion of previously IL-17A+ cells, because we did not observe any differences in double IL-17A+ IFN-γ+ cells. In contrast, during Th1 polarization PGE2 did not alter expression of Tbet, but it increased proliferation of IFN-γ+ cells (Fig. 5E, 5F) and IFN-γ production (Fig. 6C), demonstrating that Tbet and RORyt-expressing cells are differentially sensitive to PGE2 concentrations. Our results also implicate that the effects of PGE2 provided exogenously or produced in an autocrine manner by T cells cannot be considered equivalent. Early exposure of naïve CD4+ T cells to high PGE2 concentrations in the 50–100 nM range indiscriminately affect T cell proliferation, as well as function (Supplemental Fig. 2). At intermediate concentrations (10 nM), PGE2 has the capacity to provide competitive proliferative advantage to IL-17A+ or IFN-γ+ cells (Fig. 5F, 5G) and suppression of IL-17A together with enhancement of IFN-γ responses (Figs. 5, 6). In contrast, gradual accumulation of PGE2 during T cell proliferation in vivo enhances Th17, but not Th1 (Figs 1–4). This might be reconciled when considering that EP4, which has a higher affinity for PGE2 than EP2 (51), is strongly downregulated during Th17 polarization (Fig. 2C), and hence will render T cells less responsive to newly generated PGE2. Alternatively, it is possible that the Th17 bias imposed by PGE2 is subordinated to strong T cell activation accompanied by IL-2, so that when PGE2 concentrations inhibit proliferation this effect is subverted, and Th1 cells have a competitive advantage. Our data are therefore consistent with previous reports that PGE2 can favor a Th1 response under certain circumstances (36), because it has been shown to be the case for promoting IFN-γ-producing Th17 cells in MS patients (33).

Differentiation of T cell phenotypes that rely on TGF-β and IL-2 like Tregs and Th17 cells require a TCR signal that is less robust than in other T effector responses (52, 53). Phosphorylation at Tyr705 in STAT3 is induced by IL-6 and IL-23 and controls Th17 commitment and function (54). In contrast, during T cell priming, IL-2–induced STAT5 restricts Th17 commitment, and STAT3 induces ainos to silence the il2 locus (55, 56), whereas it generally promotes regulatory T cell expansion (52, 57). However, STAT5 phosphorylation is also reduced by IL-2 under Th17 polarizing conditions (58). Reconciliation of how STAT3 and STAT5 signals are integrated to result in such a diversity of outcomes in T cell responses is still needed. Inflammatory cytokines like IL-1β, IL-6, and IL-23, together with other environmental signals like retinoic acid (59), also highly modify STAT3- and STAT5-mediated signaling (57). A balance-tipping antagonism between regulatory T cell and Th17 cell commitment because of the integration of IL-1 and retinoic acid signals was recently unveiled by Basu et al. (59), demonstrating that STAT3 and STAT5 antagonize each other by...
binding reciprocally similar sites on the Foxp3 and il-17a/IL-17f regulatory regions. We show that PGE2 can downregulate phosphorylation of both STAT3 and STAT5 under Th17 polarizing conditions (Fig. 5B), which might partially explain its capacity to inhibit IL-17A while promoting IFN-γ. However, the precise molecular mechanisms that explain how these phenomena are achieved remain to be resolved. Our results reveal an added layer of interaction among APCs and T cells during immune responses that is codependent on these cells capacities to regulate PGE2 through mPGES1. In lieu of the evidence for T cell fate and cytokine potential plasticity (60) and our results, it is tempting to suggest that PGE2 might constitute a significant modulator of regulatory-to-effector T cell transitions during later phases of inflammatory responses, which we are currently studying. The concomitant production and sensing of PGE2 by different cell types might contribute to T plasticity and how this attunes with our knowledge of the properties of PGE2 in mounting but also resolving inflammatory responses. The relevance of T cell–derived PGE2 might be highlighted as well in lymphoid organs or infiltrates, where T cell density is high and IL-2 becomes limiting during proliferative responses, and is also able to influence the effector versus regulatory fate.

In conclusion, mPGES1 deficiency results in loss of induced PGE2 expression, but preservation of other PGs. Our in vivo data demonstrating that mPGES1 deficiency inhibits the capacity of CD4+ cells to become Th17 cells are consistent with previous data showing that increased PGE2 promotes Th17 responses in vivo. Our research supports the notion that Th17 cells are more significantly affected by PGE2 generated by cогnate interactions in the in vivo microenvironment than are Th1 cells. Our results also highlight the fact that there is both autocrine (T cell) and paracrine (APCs or stromal cells) mPGES1-dependent PGE2 during CD4+ cell stimulation, of different nature and magnitude but with synergistic enhancing effects on cytokine production. We also demonstrate that the variations are not solely due to sensing of PGE2 (EP2/EP4 genetic deficiency and/or agonist drug studies) or up-stream generalized COX-PG blockade, but derive from responses that are controlled by the inducible enzyme mPGES1 and are hence highly PGE2-specific and physiologically relevant during inflammatory responses. Our research therefore provides a rationale for understanding how different concentrations of PGE2 can fine-tune T cell commitment into regulatory, Th1, or Th17 types, and hence locally reshape the proinflammatory and anti-inflammatory T cell landscape during inflammatory responses.

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Disclosures
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