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Genetic deletion of mPGES-1 abolishes PGE₂ production in murine dendritic cells and alters the cytokine profile, but does not affect maturation or migration

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SUMMARY

We undertook this study to determine the role of microsomal PGE synthase-1 (mPGES-1) and mPGES-1-generated prostaglandin (PG) E₂ on dendritic cell (DC) phenotype and function. Using mPGES-1 knockout (KO) mice, we generated bone marrow derived DCs and determined their eicosanoid production profile, cell surface marker expression, and cytokine production. We also assessed DC migratory and functional capacity *in vivo*. Compared to wild-type, mPGES-1 deficient DCs exhibited a markedly attenuated increase in PGE₂ production upon LPS stimulation, and displayed preferential shunting towards PGD₂ production. mPGES-1 KO DCs did not display deficiencies in maturation, migration or ability to sensitize T cells. However, mPGES-1 deficient DCs generated reduced amounts of the Th1 cytokine IL-12, which may in part be due to increased PGD₂ rather than decreased PGE₂. These findings provide useful information on the effects of inducible PGE₂ on the innate immune system, and have important implications regarding potential consequences of pharmacologic mPGES-1 inhibition.

INTRODUCTION

Prostaglandins (PGs) are lipid molecules that mediate numerous homeostatic and pathologic processes in numerous organ systems [1]. PGE₂, the most abundant and well-studied prostanoid, is generated by many cell types, both under basal conditions and in response to a diverse array of inflammatory stimuli, via immediate and delayed biosynthetic pathways [2,3]. In response to inflammation, much higher levels of PGE₂ are synthesized by the

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CONFLICT OF INTEREST STATEMENT

Dr. Crofford has had grant funding from Pfizer. She is not a consultant and not a participant in any Speaker's Bureau and does not own stock.

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coordinated induction of COX-2 and microsomal PGE synthase-1 (mPGES-1). mPGES-1 is upregulated in a number of human diseases and animal models of inflammation [4,5]

Research has classically focused on the inflammatory properties of PGE₂, especially given the widespread use of prostaglandin-inhibiting non-steroidal anti-inflammatory drugs (NSAIDs). However, there has been increasing recognition of its immunomodulatory properties. There has been interest in the role of PGE₂ as a component of the innate immune response that can significantly shape the acquired immune response [8,9]. In particular, the effects of PGE₂ on dendritic cell (DC) phenotype and function have been the subject of investigation, but the conclusions have been difficult to interpret. As the most potent antigen presenting cells, DCs provide a link between the innate and acquired arms of the immune system [10]. DCs reside in tissues in an immature state, and undergo activation and maturation in response to antigens encountered in the context of a variety of danger signals, many of which are known to induce PGE₂ production. DCs then migrate via afferent lymphatics to lymphoid organs, where they interact with naïve and memory CD4⁺ T cells, promoting maturation towards a Th1, Th2, Th17 or Treg phenotype that influences the final nature of the immune response. This process is determined in large part by the milieu that DCs are exposed to at various points during their maturation, as well as by intrinsic characteristics of different subsets of DCs [11].

DCs both produce PGE₂ and express all four receptors [12]. Research investigating the relationship between PGE₂ and DCs has generated highly variable data, largely dependent on the type and species of precursor cell being studied, the specific culture conditions and stimulatory molecules used, and the timing of exposure to PGE₂. In general, PGE₂ is felt to have a potential role in DC migration; this has been most strongly supported by studies with EP4 knockout (KO) mice, who exhibit decreased Langerhans cell accumulation in regional lymph nodes after cutaneous sensitization [13]. Additionally, *in vitro* studies have shown that DCs matured/stimulated with PGE₂-containing cocktails gain migratory capacity towards chemokines [14,15]. What is less clear is the effect of PGE₂ on the cytokine production profile of DCs, and their subsequent downstream effects on T cells. Early studies suggested that PGE₂ shifted DC cytokine production towards a Th2 promoting profile [9]. More recently, there has been evidence for the role of PGE₂ in supporting Th17 promoting DC responses [16,17]. However, it is critical to differentiate between studies exposing DCs to exogenous PGE₂, which approximate paracrine/endocrine effects, with inhibitor studies, which attempt to define the autocrine role of PGE₂ upon DCs. Additionally, inhibitor studies do not specifically evaluate PGE₂ alone, as the non-specific or COX-2-specific NSAIDs used in these studies also block synthesis of other PGs. Since PGE₂ production is often stimulated in situations where an immune response is needed, it is not unreasonable to suspect that the high levels of inducible PGE₂ exert a differential immunomodulatory effect than basal PGE₂. Thus, as the primary and specific upstream synthase of inducible PGE₂, determining the role of mPGES-1 in DC immunobiology is an important addition to the literature.

Studies using mPGES-1 KO mice have demonstrated a role for mPGES-1 in inflammatory arthritis, wound healing, nociception, and central fever regulation [18], but few have explored the underlying immunologic mechanisms [19]. We now utilize this model to evaluate the immunoregulatory effects associated with the genetic deletion of mPGES-1, specifically evaluating the phenotype and function of DCs.

MATERIALS AND METHODS

Mice

mPGES-1 heterozygote mice were generated as previously described on an inbred DBA/11acJ background [20]. Breeding colonies were maintained in microisolator cages in specific pathogen free barrier facilities at the University of Michigan and the University of Kentucky. Wild-type (WT) and KO mice were generated by matings between heterozygous breeding pairs and used between 8–12 weeks of age. All experiments were approved by the relevant institutional committees for animal use at the University of Michigan, the University of Kentucky, and Mount Sinai School of Medicine.

Reagents

The following monoclonal antibodies (mAbs) and their appropriate controls (BD Pharmingen, San Diego, CA) were used for FACS analysis: CD16/32 (rat), FITC- and PE-CD11c (hamster), PE-Cy5- CD3 (rat), PE-Cy5- CD45R/B220 (rat), PE- CD80 (hamster), PE I-A/I-E (rat), PE CD86 (rat), PE CD40 (rat). Langerin staining was carried out using goat polyclonal anti-Langerin antibody (Santa Cruz), followed by PE-conjugated donkey anti-goat IgG (Jackson ImmunoResearch). Recombinant murine IL-4 was purchased from RDI (Flanders, NJ); conditioned media from a GM-CSF expressing B16 cell line [21] was a kind gift from Dr. Jerold G. Woodward (Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky). LPS (O55:B5), FITC and Optiprep density gradient was purchased from Sigma (St. Louis, MO); collagenase D from Roche (Indianapolis, IN); EDTA from Cambrex (Walkersville, MD); and non essential amino acids, sodium pyruvate, RPMI 1640, HBSS, fetal calf serum, L-glutamine, penicillin/streptomycin, and 2-mercaptoethanol from Gibco/Invitrogen (Carlsbad, California). Rabbit anti-human mPGES-1 polyclonal antibody (pAb), rabbit anti-mouse COX-2 pAb, rabbit anti-human mPGES-2 pAb, rabbit anti-human cPGES pAb, rabbit anti-mouse PGI synthase (PGIS) pAb, rabbit anti-mouse hematopoietic PGD synthase (H-PGDS) pAb, rabbit anti-human TX synthase (TXS) pAb, rabbit anti-mouse COX-1 pAb, PGE₂ and PGD₂ were purchased from Cayman Chemicals, Ann Arbor, MI; horseradish peroxidase-linked donkey anti-rabbit Ig was purchased from Amersham Biosciences, Little Chalfont, England. Recombinant mPGES-1 positive control was a kind gift from Dr. Per Jakobsson (Karolinska Institutet, Stockholm, Sweden).

DC generation and stimulation

Bone marrow-derived DCs (BMDCs) were generated *in vitro* as described by Lutz et al. [22] with a few modifications. Briefly, bone marrow cells from mPGES-1 deficient and WT mice were cultured in complete media plus GM-CSF conditioned media and IL-4 (10 ng/ml). On day 10, nonadherent cells were collected and utilized as immature DCs. There was no difference in DC yield between wild type and mPGES-1 deficient mice (data not shown). DCs were stimulated for 6 or 18 hours with LPS (1 µg/ml), and then cells and supernatant were collected for further analysis.

Western blotting of COXs and PG synthases

DCs were lysed in Tris-buffered saline (TBS) containing 0.1% sodium dodecyl sulfate (SDS). Murine abdominal skin was homogenized in lysis buffer (1x PBS (pH 7.4) with 5mM EDTA, 1% NP40, 0.1% SDS, 0.5% deoxycholic acid, 1 µg/ml leupeptin, 10 mcg/ml aprotinin, 1mM phenylmethylsulfonyl fluoride); lysis debris was spun down (2000g × 15 minutes at room temperature) and supernatants collected and frozen at –80°C until use. Western blotting was then performed as previously described [23].

Phenotypic analysis

5×10^5 cells were first incubated for 10 minutes at room temperature with anti-CD16/CD32 to block Fc γ II/III receptor-mediated nonspecific antibody binding. Then cells were stained with fluorochrome-conjugated mAbs or appropriate isotype matched control antibodies for 30 minutes at 4°C, washed, and fixed in 4% w/v paraformaldehyde. Analysis was performed on a FACScalibur (BD Biosciences).

Eicosanoid and cytokine measurements

Eicosanoids were measured in cell-free supernatant and from homogenized skin samples using commercial ELISA kits (Cayman Chemicals, Ann Arbor, MI) as per manufacturer's protocol. As TXA₂ and PGI₂ are rapidly broken down, we measured supernatant levels of their stable metabolites (TXB₂ and 6-keto-PGF_{1 α} , respectively). The detection limits for PGE₂, TXB₂, PGD₂ and 6-keto-PGF_{1 α} are 15, 11, 1.95 and 11 pg/ml, respectively. Cytokine ELISAs were performed using OptEIA kits (TNF α ; BD Pharmingen, San Diego, CA) and ELISA Ready-SET-Go! kits (IL-12p70, IL-23, and IL-10; eBioscience, San Diego, CA) per manufacturer's protocols. Detection limits for TNF α , IL12p70, IL-23 and IL-10 are 15.6, 15, 30, and 30 pg/ml, respectively.

In vivo skin DC migration assay

Skin sensitization with FITC and DC migration were assessed as previously described [24]. Briefly, FITC dissolved in an acetone:dibutyl phthalate vehicle was applied to the shaved dorsal skin of WT and mPGES-1 deficient mice (5 per group); 18 hours later, draining lymph nodes were harvested, processed, and stained for CD11c, CD86 and Langerin.

Cutaneous PGE₂ determination

Ventral abdominal skin was shaved and either vehicle or FITC solution (15 mg/ml) was applied; 18 hours later the skin was dissected out. Tissue was processed and PGE₂ levels measured using an ELISA kit according to the manufacturer's protocol (Cayman Chemicals, Ann Arbor, MI).

Contact hypersensitivity assay

Shaved abdominal skin of WT and mPGES-1 deficient mice was sensitized with 400 μ l of FITC solution. Five days later, baseline bilateral ear thicknesses were measured using a micrometer, and then 20 μ l of FITC solution was applied to either side of each mouse's right ear; vehicle was applied to the left. 24 hours later ear thickness was re-measured. Ears were fixed in 10% formalin, paraffin embedded, sectioned, and H&E stained.

Statistical Analysis

The data are expressed as mean $\pm$ SEM. Student's *t*-tests were used to calculate all *p* values.

RESULTS

Protein expression of COXs and terminal PG synthases in mPGES-1 WT and KO BMDCs

We performed Western blotting of WT and mPGES-1 deficient BMDCs, both with and without LPS stimulation (Fig. 1). WT DCs did not basally express mPGES-1, but upregulated expression in response to LPS. As expected, KO DCs did not express mPGES-1, either with or without stimulation. COX-2 was also not expressed basally, but increased to a similar degree in both WT and KO DCs after stimulation. All other synthases were present basally and did not increase expression after stimulation with LPS.

Profile of prostanoid expression in mPGES-1 deficient BMDCs

We first determined the role of mPGES-1 in BMDC PGE₂ production under basal and stimulated conditions. The level of PGE₂ under unstimulated conditions was similarly low, but not absent, when comparing WT and mPGES-1 KO mice (Fig. 2). LPS stimulation resulted in a significantly greater increase in PGE₂ production by WT BMDCs compared with mPGES-1 KO BMDCs ($P<0.05$). To determine if there was compensatory shunting of AA into other eicosanoid pathways, we measured levels of PGD₂ and the stable metabolites of thromboxane and prostacyclin (TXB₂ and 6-keto-PGF_{1 α}), the other major eicosanoid products of murine DCs. WT and mPGES-1 deficient BMDCs displayed similar baseline levels of TXB₂ and 6-keto-PGF_{1 α} , that increased significantly upon LPS stimulation, but with no significant differences between genotypes. However, LPS stimulation resulted in significantly higher PGD₂ levels in KO ($P<0.01$) but not WT BMDCs.

Cell surface marker expression of mPGES-1 deficient BMDCs

Next, we analyzed the cell surface expression after LPS stimulation of MHC Class 2, CD40, CD80, and CD86, antigen presentation molecules which DCs upregulate upon maturation. While LPS treatment did increase all examined markers, there were no significant differences in maturation marker expression between BMDCs from WT and mPGES-1 deficient mice, both before and after LPS stimulation (Table 1).

Cytokine expression by mPGES-1 deficient BMDCs

DCs secrete a number of cytokines that mediate some of their effects on other immune cells. In particular, DCs secrete IL-12, which is considered to be the critical Th1 polarizing cytokine [25], and TNF α ; they also secrete IL-10, a Th2 associated cytokine, and IL-23, important for Th17 responses. We measured cytokine release from WT and mPGES-1 deficient LPS-stimulated BMDCs. There was a significant reduction of IL-12 production by mPGES-1 deficient BMDCs (Fig. 3; $p<0.001$). TNF α and IL-23 were similar between WT and KO mice at both 6h and 18h after stimulation. IL-10 levels were 30% higher in KO at 6h, but this difference did not reach statistical significance. There was no difference between WT and KO mice at the 18h time point.

Effect of exogenous PGE₂ and PGD₂ on IL-12 production in LPS-stimulated DCs

We wished to determine whether the observed diminishment in IL-12 production in the absence of mPGES-1 was a function of the altered eicosanoid profile displayed by the KO DCs, as well as further explore the difference between autocrine vs. exogenously supplied PGE₂. To this end, WT and KO BMDCs were stimulated with LPS in the presence or absence of PGE₂ or PGD₂, and IL-12 production was determined (Fig. 4). We discovered that adding PGE₂ to KO DCs did not increase IL-12 levels. However, adding PGD₂ to WT DCs resulted in decreased IL-12 production ($p<0.001$), similar to levels exhibited by mPGES-1 deficient BMDCs. Also, adding PGE₂ to WT DCs resulted in decreased IL-12 production ($p<0.001$). These data suggest that increased PGD₂, not decreased PGE₂, may contribute to the diminished IL-12 production in stimulated mPGES-1 deficient DCs. Additionally, inducible and exogenous PGE₂ appear to exert differential effects on IL-12 production from WT DCs.

DC migration in mPGES-1 deficient mice

To evaluate the paracrine effects resulting from the absence of inducible PGE₂ upon the migration of DCs *in vivo*, we employed a FITC sensitization model; upon application of a FITC solution to the skin of mice, cutaneous DCs phagocytose the FITC and migrate to regional lymph nodes. First, we verified that this model results in upregulated mPGES-1 and inducible PGE₂ production at the site of sensitization, to ensure that WT cutaneous DCs are

exposed to a higher PGE₂ environment than KO mice (Fig. 5). Skin from mPGES-1 KO mice, as expected, displayed absent mPGES-1 protein expression and low PGE₂ levels, both with and without FITC sensitization. In contrast, WT skin displayed mPGES-1 expression both basally and after sensitization; cutaneous PGE₂ levels were higher in unsensitized ($p<0.05$) and sensitized ($p<0.002$) WT skin compared with KO. Next, migration of cutaneous DC was determined by flow cytometry. The frequency of CD11c⁺ FITC⁺ (migratory) DCs in regional draining lymph nodes was not significantly different between WT and mPGES-1 KO mice (Fig. 6B). To specifically look at Langerhans cells (a specialized population of migratory DCs), CD11c⁺ cells were gated for Langerin. As demonstrated in Fig. 6C, this subset of migratory DCs was also not differentially present in WT and KO lymph nodes. Of note, the density of Langerhans was similar prior to FITC staining between the two genotypes; epicutaneous application of FITC reduced the density of Langerhans cells in the epidermis by about 20% at 18 h for each genotype (data not shown). Lastly, we determined if the maturation status of the migratory DCs in the lymph node was altered in the absence of mPGES-1. Levels of CD86 were not different between WT and mPGES-1 KO DCs in the lymph node (Fig. 6D), suggesting that maturation of migratory DCs *in vivo* is not dependent upon inducible PGE₂.

Contact hypersensitivity in mPGES-1 KO mice

Finally, we determined whether mPGES-1 plays a role in the immunomodulatory properties of DCs by performing a contact hypersensitivity assay. Contact hypersensitivity requires functional DCs that not only uptake antigen and migrate correctly, but interact with T cells that can subsequently generate rapid inflammatory responses upon re-exposure to the antigen. We sensitized and subsequently challenged WT and KO murine skin with FITC applied to the ears. We saw no difference in the degree of contact hypersensitivity elicited between the two genotypes, as ascertained by measuring changes in ear swelling pre-and post FITC challenge (Fig. 7a). Histologically, there were no differences in the amount or type of inflammatory infiltrate by H&E staining (Fig. 7b).

DISCUSSION

In this study we report several novel findings. First, mPGES-1 deficient BMDCs do not markedly increase PGE₂ production in response to LPS stimulation, and display shunting of prostanoid synthesis towards PGD₂. Second, mPGES-1 deficient BMDCs produce less IL-12 than WT DCs upon stimulation; this appears not to be a function of decreased PGE₂, but due to increased PGD₂ levels. Finally, the absence of mPGES-1 does not impair migration of cutaneous DCs or their ability to sensitize T cells *in vivo*.

We have demonstrated that absence of mPGES-1 does not change the expression of other PG synthases, including the constitutive PGE₂ synthases cPGES and mPGES-2. Also, mPGES-1 deficiency does not alter COX-2 expression; this implies that neither mPGES-1 nor its associated PGE₂ regulate their upstream synthase in a feedback loop. Thus, the synthetic machinery for other PGs remains intact. We have also shown that mPGES-1 is required for the markedly increased production of PGE₂ in response to LPS by BMDCs. This correlates with similar findings in other tissues and cells such as macrophages and embryo fibroblasts [23,26,27]. There is a much smaller increase in PGE₂ production upon LPS stimulation of mPGES-1-deficient DCs. Potentially, this may arise through the actions of other PGES isozymes, which can couple with COX-2 in certain situations [28].

Interestingly, we saw evidence for increased production of PGD₂ by BMDCs from mPGES-1 KO mice; this appears to be due to shunting of PG precursors down the PGD₂ synthetic pathway in the absence of mPGES-1. Shunting of prostanoid synthesis towards PGD₂ could potentially be one mechanism of immunomodulation in the absence of

mPGES-1. Dendritic cells express the DP1 receptor [29], thereby allowing for both autocrine and paracrine effects. Studies have demonstrated a potential role for PGD₂ in DC cytokine production and migration [30,31]. However, the autocrine effects of PGD₂ are largely unexplored.

Our results for eicosanoid shunting in BMDCs are unlike what has been reported in other knockout cells. These other studies differed from each other, however, in terms of which prostanoids were found to be preferentially upregulated. Our data support the growing body of evidence that there exists considerable variability and cell specificity to prostanoid diversion in the absence of mPGES-1 [32]. Additionally, we saw no evidence for diminished basal PGE₂ production in the absence of mPGES-1 in unstimulated BMDCs, as has been previously described [23,27]. This may be the result of mPGES-1 functionally coupling to upstream synthases in a cell-specific manner, thus differentially contributing to basal PGE₂ production.

Exogenous PGE₂ is a potent maturation agent for human monocyte-derived DCs [33] and is used in cocktails for enhancing the efficacy of DC tumor vaccines [34]. However, the contribution of endogenous PGE₂ to DC maturation is less well understood. Our data suggest that the absence of inducible PGE₂ alone does not affect BMDC maturation in an autocrine fashion. These findings do not eliminate a role for paracrine effects of mPGES-1-induced PGE₂ on BMDC maturation. Higher PGE₂ levels generated by surrounding cells could accelerate or increase maturation beyond what is seen in isolated cultured BMDCs exposed only to their own endogenous PGE₂, and thus systemic mPGES-1 deficiency might result in a “less” mature phenotype than possible in WT BMDCs.

Exogenous PGE₂ has been shown to inhibit production of inflammatory chemokines and cytokines by DC [35,36]. More specifically, *in vitro* exposure of immature DCs to exogenous PGE₂ decreased production of IL-12, the classical Th1 promoting cytokine [37,38]. This suggests that the presence of PGE₂ at the initial peripheral site of damage (where the immature DC sentinels resides) could potentially mature DCs that promote a downstream Th2 response, and that inhibition of PGE₂ might possibly skew towards a subsequent Th1 response (via increased IL-12 production). However, a recent study demonstrated that *in vitro* differentiation of DCs in the presence of exogenous PGE₂ alters the IL-12/IL-23 balance, with resultant promotion of Th17 differentiation [39]. Additionally, prostaglandin inhibitor studies have generated differing data. Some studies have found that inhibition of endogenous prostanoids using selective and non-selective COX-2 inhibitors did not alter cytokine production [41,42]. Other groups have found otherwise [17,36,43]. Some of these varying results may in part be due to technical differences with variable culture conditions and stimulatory molecules, making direct comparison between studies difficult. Murine genetic background plays a role in the amount and effect of PGE₂ production and subsequent Th1/Th2 effects [40] and may cause differences between various mouse models studied.

Our data revealed a significant decrease in IL-12 production after LPS stimulation in mPGES-1 deficient BMDCs compared with WT, a finding apparently in contradiction to published data showing downregulation of IL-12 in the presence of PGE₂. As exogenous PGE₂ has been shown to downregulate IL-12 production via a cAMP dependent mechanism [44], we measured cAMP levels basally and after stimulation in WT and KO BMDCs. We found no differences between the genotypes (data not shown), indicating that the machinery for response to PGE₂ is intact in the absence of mPGES-1. Additionally, we found that adding exogenous PGE₂ does cause our WT BMDCs to decrease IL-12 production. This illustrates one potentially important difference between the effect of exogenous PGE₂ vs. the effect of autocrine PGE₂ and/or its synthase.

However, the diminished IL-12 production by mPGES-1 deficient BMDCs does not appear to be the result of diminished autocrine inducible PGE₂ levels, as adding exogenous PGE₂ to KO DCs did not increase IL-12 and restore the WT phenotype. In fact, the altered IL-12 profile may at least in part be due to increased PGD₂ produced by KO cells, as adding PGD₂ to WT DCs downregulated IL-12. This is consistent with other studies that have shown exogenous PGD₂ to decrease IL-12 production by murine BMDCs [30,45].

Studies have suggested a primary role for PGE₂ in controlling DC migration. Exogenous PGE₂ enhances the function of CCR7, the key migratory chemokine receptor for DCs [14,46], and stimulates migration [15,47]. However, these *in vitro* studies demonstrated that PGE₂ is able to confer a migratory phenotype to DCs; they did not preclude the possibility of other mediators having similar capabilities in the absence of PGE₂. More compellingly, mice deficient in the EP4 receptor for PGE₂ demonstrated impaired migration of sensitized cutaneous DCs to regional lymph nodes [13]. However, certain subsets of human DCs have been shown to not require PGE₂ in order to obtain a migratory capacity [15,48].

Our results demonstrate that, while WT cutaneous DCs are clearly exposed to higher levels of PGE₂ than KO mice in a well-established FITC sensitization model [49], no impairment of DC migration was demonstrable in mPGES-1 KO mice, despite the absence of inducible PGE₂ in the inflammatory milieu and likely in the DCs themselves. Potentially, basal PGE₂ exposure and production is adequate to maintain DC migration in the presence of other inflammatory signals, possibly via the EP4 receptor, which has been shown to be important utilizing the same FITC sensitization model we used [13]. Alternately, there may be redundancy with regards to which mediators confer migratory capacity; in the absence of inducible PGE₂, other compounds may be sufficient. Differences in inbred mouse strains used may also account for some of the discrepancy between our data and the EP4 knockout findings. Inducible PGE₂ also does not appear to affect the maturation status of migratory DCs, as we saw no difference in CD86 expression between WT and KO DCs. This addresses the issue of the paracrine effects of PGE₂ on DC maturation; WT cutaneous DCs are exposed to much higher PGE₂ levels than mPGES-1 deficient DCs, and yet do not exhibit higher maturation marker expression upon arrival to the lymph nodes. Additionally, migratory DCs from the mPGES-1 deficient animals were capable of engaging T cells in the lymph nodes appropriately and eliciting functional downstream immune responses, as we saw equivalent contact hypersensitivity responses between the two genotypes. This assay also gives us indirect insight into the effect of inducible PGE₂ on T cells, as KO mice mounted appropriate T cell-mediated delayed type hypersensitivity.

Much of the literature regarding the effects of PGE₂ on DC phenotype and function, especially using human derived cells, has been conducted in order to understand and refine DC vaccine use in immunotherapy. To that end, many studies have been performed *ex vivo* with exogenous PGE₂ administration. Advantages of using the mPGES-1 knockout model include better study of *in vivo* physiologic and pathologic effects, as well as evaluation of the autocrine effects of inducible PGE₂ without eliminating alternate PGs. Our study finds that while mPGES-1 is essential for increased PGE₂ production in BMDCs, there is a minimal role for mPGES-1 generated PGE₂ on DC maturation and migration.

In addition to expanding our understanding of the specific role of mPGES-1 and mPGES-1 induced PGE₂ on DC biology, these data provide information on the potential consequences of mPGES-1 inhibition in a therapeutic setting. This is of particular importance since mPGES-1 is an attractive pharmacologic target, given its specificity for inducible, inflammatory PGE₂. However, as has been demonstrated by the adverse effects associated with COX-2-specific NSAIDs [50], inducible prostaglandins also have a number of physiologic functions, and it is critical to understand the full spectrum of biologic effects

associated with absence or inhibition of mPGES-1 in order to determine the ultimate clinical usefulness of this enzyme as a therapeutic target.

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Figure 1. Protein expression of COX and terminal PG synthases in mPGES-1 WT and KO DCs
 mPGES-1 WT and KO DCs were harvested at 18 hrs post LPS stimulation, and protein expression of mPGES-1, COX-2, mPGES-2, cPGES, PGIS, H-PGDS, TXS, COX-1 and β -actin was determined by Western blotting. Representative data are shown from 4 separate experiments.

Figure 2. Eicosanoid profile of BMDCs

BMDCs were generated by culturing bone marrow cells in complete media supplemented with GM-CSF/IL-4, and then stimulated with LPS or vehicle for 18 hours. Supernatants were analyzed by ELISA for PGE₂, PGD₂, 6-keto-PGF_{1α} and TXB₂. Stimulated mPGES-1 deficient DCs fail to strongly upregulate PGE₂; however, they do significantly upregulate PGD₂ upon stimulation with LPS. Results are the mean of 10 separate experiments. * = p < 0.05, ** = p < 0.01 and *** = p < 0.001; by student's *t*-test.

Figure 3. Cytokine production by BMDCs

Cultured mPGES-1 DCs stimulated with LPS (1 $\mu\text{g/ml}$) for 6 and 18 hours display significantly decreased IL-12 (A) production compared to WT DCs, as measured by ELISA ($*=p<0.001$ by student's *t*-test). There is no significant difference in the levels of IL-23 (B), TNF α (C), or IL-10 (D) comparing WT and KO BMDCs. Results are expressed as the percent difference from the corresponding stimulated wild type production (designated as 100%) and are the mean of 3–6 experiments.

Figure 4. Effect of exogenous PGE₂ and PGD₂ on IL-12 production in LPS-stimulated DCs DCs from mPGES-1 WT and KO were cultured with or without PGE₂ or PGD₂ in the presence of LPS (1 μg/ml) for 18 hours. The levels of IL-12 in supernatant were measured by ELISA. Addition of both exogenous PGD₂ and PGE₂ to WT DCs results in diminished IL-12 production. Addition of exogenous PGE₂ to KO DCs does not alter IL-12 production to resemble that of WT. Results are expressed as the percent difference from the corresponding stimulated WT production (designated as 100%) and are the mean of 4 experiments. *= $p < 0.001$ vs. WT stimulated with LPS alone (student's *t*-test). Raw values for the stimulated WT after LPS stimulation are $922.5 \text{ pg/ml} \pm 265.1$

Figure 5. Effect of FITC sensitization upon cutaneous mPGES-1 and PGE₂ expression
 Shaved WT skin displayed baseline mPGES-1 expression, and basal PGE₂ levels higher than KO. Application of a FITC solution for 18 hours results in upregulation of mPGES-1 protein and PGE₂ production in WT, but not mPGES-1 deficient, murine skin. Panel 5A shows a representative Western blot of mPGES-1 expression in WT and KO skin, with and without FITC sensitization, as well as the ratio of mPGES-1 to β-actin expression, normalized to WT. +control is a recombinant mPGES-1 protein used as a positive control. Panel 5B shows the mean cutaneous PGE₂ production from 3 mice per treatment group; levels are normalized to total protein expression. *= $p<0.05$; **= $p<0.002$ by student's *t*-test.

Figure 6. In vivo DC migration

After epicutaneous application of a FITC solution for 18 hours, draining lymph nodes were harvested, stained and analyzed by flow cytometry. Panel 6A shows the percent of CD11c^{hi}FITC⁺ (migratory) DCs in representative histograms of WT and mPGES-1 KO lymph nodes; 6B shows the number of FITC⁺ DCs in the lymph nodes of 5 mice per genotype. 6C shows the proportion of Langerhans cells in representative histograms of each genotype. 6D shows the expression of CD86 on FITC⁺ migratory DCs. There were no differences between WT and mPGES-1 deficient mice in the frequency of migratory DCs found in lymph nodes (both total DCs and Langerhans cells specifically) or their maturation status (as determined by CD86 expression).

Figure 7. Contact hypersensitivity assay

Mice were sensitized to FITC solution and subsequently challenged with FITC or vehicle application to their ears. 24 hours later the mice were sacrificed and the change in ear swelling measured. mPGES-1 KO mice display the same contact hypersensitivity response as WT mice. There were no significant differences clinically or histologically between WT and mPGES-1 KO mice, with either vehicle or FITC application. Results are the mean of 7–8 mice per group, and are reported as the change in ear thickness pre-and post FITC or vehicle challenge, $\pm$ SEM.

Cell surface marker expression of BMDCs

Cultured BMDCs were stimulated with LPS (1 µg/ml) or vehicle for 18 hours, stained with fluorochrome-conjugated antibodies, and analyzed by flow cytometry. mPGES-1 deficient BMDCs are phenotypically similar to WT DCs. Results are presented as percent positivity (%), mean fluorescent intensity (MFI), and geometric mean of fluorescent intensity (geoMFI), and are the mean of three experiments; there were no statistically significant differences between WT and KO cells.

Table 1

Phenotypic marker	Un-treated		LPS-treated	
	WT	KO	WT	KO
CD11c	%	82.98	77.92	85.80
	MFI	2296.71	2381.21	1582.23
	geoMFI	1364.69	1380.76	1025.19
CD40	%	3.71	4.09	56.65
	MFI	508.40	652.18	768.94
	geoMFI	256.36	275.12	423.73
CD80	%	78.43	79.66	91.07
	MFI	907.41	919.86	3390.57
	geoMFI	511.78	545.06	2122.61
CD86	%	35.78	41.53	78.43
	MFI	1556.90	1384.18	3503.80
	geoMFI	642.36	610.19	1896.69
MHC class2	%	49.13	53.94	70.41
	MFI	3298.21	3201.17	5005.41
	geoMFI	1197.12	1192.18	2249.21