Prostaglandin E2 inhibits IL-23 and IL-12 production by human monocytes through down-regulation of their common p40 subunit[☆]

Khalid W. Kalim^{a,b}, Marcus Groettrup^{a,b,c,*}

^a Division of Immunology, Department of Biology, University of Konstanz, D-78457 Konstanz, Germany

^b Konstanz Research School Chemical Biology, University of Konstanz, D-78457 Konstanz, Germany

^c Biotechnology Institute Thurgau at the University of Konstanz, CH-8280 Kreuzlingen, Switzerland

A B S T R A C T

The heterodimeric cytokine IL-23 is important for the maintenance of Th17 cells, which are pivotal mediators of autoimmune diseases like rheumatoid arthritis, colitis, and multiple sclerosis. Prostaglandin E2 (PGE2) is a soluble regulator of inflammation that has both pro- and anti-inflammatory properties. PGE2 has been shown to elevate the IL-23 production by dendritic cells (DC). Monocytes are also producers of IL-23 but the effect of PGE2 on IL-23 production by human monocytes has hardly been investigated. We show here that PGE2 blocks the production of IL-23 by LPS-stimulated monocytes in an IL-10 and IL-1 β independent manner. This effect was due to the down-regulation of the p40 subunit of IL-23 on mRNA and protein level. The p40 subunit is shared by IL-12 and, consistently, PGE2 also lowered the IL-12 production by monocytes. These effects of PGE2 were cAMP-dependent since the cAMP enhancer forskolin strongly reduced IL-23 and IL-12 production by monocytes. Taken together, PGE2 acts in an anti-inflammatory manner by lowering IL-23 production by monocytes while it has the opposite effect in DC. Our data may help to reconcile controversial point of views on the pro- and anti-inflammatory nature of PGE2 by making a strong case for a cell type-dependent function.

1. Introduction

Prostaglandins are small lipid molecules derived from arachidonic acid (AA) and are produced by the action of cyclooxygenases (COX-1 and COX-2) and prostaglandin synthases (Phipps et al., 1991). PGE2 is the most well studied prostaglandin and is involved in regulating many different fundamental biological functions both in normal physiology and pathophysiology. PGE2 acts via four EP receptors (EP1–EP4), which are present on multiple cell types reflecting the diverse functions of PGE2 (Hata and Breyer, 2004). The functions of PGE2 are discussed very controversially as it can have pro- and anti-inflammatory properties. It is an important mediator of active inflammation with local vasodilatory function along with activation and recruitment of macrophages and mast cells (Nakayama et al., 2006; Weller et al., 2007; Yu and Chadee, 1998). But it also has the ability to suppress multiple

pro-inflammatory cytokines by inducing anti-inflammatory IL-10 and thus promoting immune suppression (Phipps et al., 1991; Wang et al., 2007). PGE2 modulates the activities of professional APCs such as dendritic cells and macrophages by regulating the production of cytokines by these cells. It plays an important role in the migration of mature DCs by enhancing the signal transduction of the chemokine receptor CCR7 (Scandella et al., 2002). Moreover, PGE2 induces the production of IL-23 and inhibits the release of IL-12 from bone marrow derived dendritic cells (Sheibanie et al., 2004). PGE2 also modulates the proliferation of CD4⁺ T-cells and their ability to produce cytokines (Choudhry et al., 1999a,b).

CD4⁺ T-cells undergo distinct differentiation pathways depending on the kind of cytokine present in the microenvironment. Initially, two effector T-helper subsets were distinguished: Th1 and Th2 cells (Mosmann and Coffman, 1989). Th1 differentiation is promoted by IL-12 with IFN- γ as an important effector cytokine while Th2 differentiation is promoted by IL-4 and IL-10 (Fort et al., 2001; Mosmann and Coffman, 1989). Recently, a distinct T helper cell subset designated Th17 was characterized in mice (Aggarwal et al., 2003; Cua et al., 2003; Harrington et al., 2005; Langrish et al., 2005; Park et al., 2005) and humans (Acosta-Rodriguez et al., 2007; Annunziato et al., 2007; Chen and O'Shea, 2008; van Beelen et al., 2007). Th17 cells produce high amounts of IL-17A and IL-17F and are driven by the master transcription factor ROR γ t (Ivanov et al., 2009). Th17 cells play a role in

Abbreviations: PGE2, prostaglandin E2; AA, arachidonic acid.

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* Corresponding author at: University of Konstanz, Department of Biology/Immunology, Universitaetsstr. 10 D-78457 Konstanz, Germany.

Tel.: +49 7531 882130; fax: +49 7531 883102.

E-mail address: Marcus.Groettrup@uni-konstanz.de (M. Groettrup).

protection against extracellular bacteria and fungi, and are important mediators of autoimmune diseases like rheumatoid arthritis, experimental autoimmune encephalomyelitis, and inflammatory bowel disease (Langrish et al., 2005; Murphy et al., 2003; Yen et al., 2006). IL-23 is a pro-inflammatory cytokine that consists of a unique p19 subunit and a p40 subunit, which it shares with IL-12 (Oppmann et al., 2000). IL-23 in combination with IL-1 is sufficient to induce naive human T-cells to produce IL-17A, IL-17F, IL-22 and the transcription factor ROR γ t (Wilson et al., 2007).

PGE2 inhibits the production of IL-12 from monocytes (van der Pouw Kraan et al., 1995) and DCs (Kalinski et al., 1997), thus inhibiting Th1 differentiation and promoting a Th2 response, which is evident by the overproduction of PGE2 in multiple Th2-associated diseases, most prominently in atopic dermatitis and asthma (Kapsenberg et al., 1999). PGE2 has also been shown to promote IL-17 producing Th17 through EP2–EP4 signaling by suppressing IL-12 production while enhancing IL-23 production from DC (Boniface et al., 2009; Esaki et al., 2010; Khayrullina et al., 2008; Sheibanie et al., 2007; Yao et al., 2009). Nevertheless, the role of PGE2 as a paracrine mediator of pro-inflammatory and anti-inflammatory immune responses has remained a matter of very controversial debate (Kalinski, 2012; Rieser et al., 1997).

Monocytes have a possible role in the acute exacerbation of autoimmune diseases like MS and rheumatoid arthritis (Highton et al., 1989; Imamura et al., 1993). Monocytes activated in a pro-inflammatory environment can initiate autoimmune diseases by entering the target organs before T-cells, by traversing normal endothelium without help from any other cells (de Jong et al., 1996; Lidington et al., 1998; Stoy, 2002). While there is a positive effect of PGE2 on IL-23 production by DC, the effect of PGE2 on human monocytes in this respect has not been studied in detail. Here we show that PGE2 blocks the production of IL-23 from LPS-stimulated human monocytes and this effect was due to the down-regulation of the p40 subunit of IL-23 and IL-12 at mRNA and protein level. We conclude that PGE2 has the opposite effects on monocytes and DC with respect to IL-23 production emphasizing the cell type dependence of its function.

2. Material and methods

2.1. PBMCs and monocyte isolation

Human PBMCs were separated from whole blood by standard density gradient centrifugation on Ficoll-PaqueTM Plus (GE Healthcare). For isolation of CD14⁺ monocytes, PBMCs were incubated with anti-CD14 conjugated microbeads (Miltenyi Biotech) and positively selected for monocytes by magnetic separation using MACS columns (Miltenyi Biotech). Blood donations were conducted after informed consent and were approved by the Institutional Review Board of Konstanz University.

2.2. Generation of human monocyte derived DCs (MoDCs) and monocyte-derived macrophages

For generation of MoDCs, isolated monocytes were cultured at 2×10^6 cells/ml in AIM-V medium (Invitrogen) along with 50 ng/ml GM-CSF (Peprotech) and 50 ng/ml IL-4 (Peprotech) for 5 days. For generation of monocyte-derived macrophages, isolated monocytes at 2×10^6 cells/ml were cultured in AIM-V medium along with 50 ng/ml GM-CSF (Peprotech) for 5 days. The same volume of media along with the same amount of cytokines were added on day 2 to the culture. On day 6, cells were harvested and stimulated with 5 μ g/ml LPS (Sigma) in the presence or absence of 1 μ g/ml PGE2 (Sigma). Finally, the supernatant was harvested after 48 h for analysis by ELISA.

2.3. Cytokine assays

Isolated monocytes were cultured in RPMI 1640 (Invitrogen) at 2×10^6 cells/ml in the presence or absence of 5 μ g/ml LPS (Sigma) under different conditions as indicated in the figures. Supernatants were collected after different time points as indicated and analyzed by ELISA by means of commercially available kits to detect IL-23 (eBioscience), IL-12p70 (eBioscience), IL-6 (BD Pharmingen), IL-10 (BD Pharmingen), and IL-1 β (BD Pharmingen) according to the manufacturers' protocols.

2.4. Cell staining and flow cytometry

Isolated monocytes were stimulated with 5 μ g/ml LPS (Sigma) in the presence or absence of 1 μ g/ml PGE2 (Sigma) along with brefeldin A (Sigma) for 18 h. Subsequently, cells were harvested and surface stained with FITC-conjugated CD14 antibody (Miltenyi Biotech). Cells were fixed with Fixation-Permeabilisation buffer (eBioscience) according to the manufacturer's instruction. Cells were then stained intracellularly with efluor 660-conjugated IL-23p19 (eBioscience), alexafluor 647-conjugated IL-12p35 (clone SNKY35, eBioscience), and PE-conjugated IL-12/23p40 (clone eBioHP40, eBioscience) in permeabilization buffer overnight at 4 °C. Matured monocyte-derived DCs were surface stained for maturation markers PE-conjugated CD80, PE-conjugated CD83, PE-conjugated CD86, and APC-conjugated CCR7 (all from BD Biosciences). Flow cytometric analyses were performed on a BD FACS AriaTM IIIu (BD Biosciences) and data were analyzed with Flowjo software (Tree Star).

2.5. RNA isolation and cDNA synthesis

Isolated monocytes were stimulated with 5 μ g/ml LPS (Sigma) in the presence or absence of 1 μ g/ml PGE2 for 5 h. Cells were harvested thereafter and processed for total RNA isolation by using the Nucleospin[®] RNA isolation kit (Macherey Nagel) as per the manufacturer's protocol. 1 μ g of RNA was used for synthesizing cDNA using the reverse transcription kit from Promega. The resulting cDNA was used for real time RT-PCR.

2.6. Real time RT-PCR

Real time RT-PCR was performed with the Light Cycler (Roche) and the Light Cycler-DNA Master SYBR Green I kit (Roche) according to the manufacturer's protocol. After initial denaturation for 30 s at 95 °C, thermal cycling was performed for 38 cycles with steps of 95 °C for 10 s, 60 °C for 7 s, and 72 °C for 8 s, with fluorescence being read at the end of each cycle. The following oligonucleotides were used as primers for the PCR: IL-23p19 fwd-5'GCTTCAAAATCCTTCGAG 3'; IL-23p19 rev-5'TATCTGAGTGCCATCCTTGAG 3'; IL-12/23p40 fwd-5'GTCAGAGGGGACAACAAGGA 3'; IL-12/23p40 rev-5'TGATGAAGAAGCTGCTGGTG 3'. The data analysis was performed with Light Cycler software 3. The values were normalized to 18S rRNA which was used as a reference. All PCR products were analyzed by determination of melting profiles as well as by agarose gel electrophoresis.

2.7. Statistical analysis

The statistical significance of the differences was determined using Student's *t*-test. The Graph Pad Prism software (version 4.03) (Graph Pad) was used for all statistical analyses.

3. Results

3.1. PGE2 inhibits IL-23 production by human PBMCs and isolated monocytes

To assess the effect of the lipid immunomodulator PGE2 on the ability of human monocytes to produce IL-23, human PBMCs were isolated from whole blood and stimulated with LPS in the presence or absence of PGE2 for 24 h. The analysis of the supernatant by ELISA showed that PGE2 strongly inhibited IL-23 production from PBMCs whereas the solvent control did not show any effect (Fig. 1A). In contrast, there was no effect of PGE2 on IL-6 production (Fig. 1A), documenting the specificity of PGE2 in down-regulating IL-23 production. To investigate whether the ability of PGE2 to block IL-23 production was a direct effect on monocytes, CD14⁺ monocytes were magnetically isolated from PBMCs to a purity of more than 95% and stimulated *in vitro* with LPS for 24 h in the presence or absence of PGE2. As evidenced for PBMCs before, PGE2 largely inhibited IL-23 production from isolated monocytes as well (Fig. 1B) thus indicating a direct effect of PGE2 on monocytes in blocking IL-23 production. Again, IL-6 production from monocytes was not affected by PGE2 (Fig. 1B), illustrating the specific effect of PGE2 on the suppression of IL-23 production. We also tested the effect of PGE2 on IL-23 production by isolated monocytes after stimulation with poly I:C and found that also with this TLR ligand PGE2 led to a down regulation of IL-23 production while the IL-6 production remained unaltered (Fig. 1C).

3.2. PGE2 promotes IL-23 production by monocyte derived DCs but not by monocyte derived macrophages

Since PGE2 has been reported to up-regulate the production of IL-23 by monocyte derived DCs (MoDCs) (Sheibanie et al., 2004, 2007), we decided to compare MoDCs and monocyte derived macrophages in this respect. To this aim we differentiated monocytes to immature MoDCs with GM-CSF and IL-4 for 5 days followed by stimulation with LPS in the presence or absence of PGE2 for 48 h to induce maturation. Subsequently, the supernatants were collected and analyzed for IL-23 and IL-6 production by ELISA and the cells were characterized for the surface expression of DC maturation markers. PGE2 led to an up-regulation of surface markers CD80, CD83, CD86, and CCR7 on matured MoDCs (Supplementary Fig. S1). In accordance with previous studies (Sheibanie et al., 2004, 2007), PGE2 up-regulated the production of IL-23 and IL-6 from immature MoDCs upon LPS stimulation (Fig. 2A). We also cultured monocytes with GM-CSF only to differentiate them to monocyte-derived macrophages and then stimulated them with LPS in the presence or absence of PGE2 for 48 h. The supernatants were collected thereafter and analyzed for IL-23 and IL-6 content by ELISA. PGE2 did not affect the production of IL-23 and IL-6 from monocyte derived macrophages upon LPS stimulation (Fig. 2B). These data shows a cell context specific effect of PGE2 with respect to governing the production of IL-23.

3.3. PGE2 inhibits IL-23 and IL-12 production from isolated monocytes through down-regulation of the common p40 subunit both at the mRNA and protein level

IL-23 is a heterodimer of the subunits p19 and p40. Since PGE2 was leading to a down-regulation of IL-23 production by monocytes, we wanted to investigate the effect of PGE2 on the individual p19 and p40 subunits of IL-23. To this aim, we performed intracellular staining for the individual subunits of IL-23, i.e. p19 and p40. Isolated monocytes in the presence of brefeldin A were stimulated with LPS in the presence or absence of PGE2 for 18 h. They were subsequently surface stained for CD14 followed by

intracellular staining for p19 and p40. Sorted CD14 positive cells showed a CD14^{high} and CD14^{low} population (Supplementary Fig. S2). Upon LPS stimulation, there was an up-regulation of CD14 and an increase in the CD14^{high} population. We consistently observed an increase in p19 and p40 producing cells upon LPS stimulation in both the CD14^{high} and CD14^{low} populations (Supplementary Fig. S2). PGE2 led to a significant down-regulation of p40 producing cells in both the CD14^{high} and CD14^{low} population (Supplementary Fig. S2). We also performed a co-staining for p19 and p40, and found that PGE2 led to a significant down-regulation of p19/p40 double positive cells, which could be functional IL-23 producing cells (Fig. 3A, top). Since the p40 subunit is also shared by IL-12, we wanted to investigate whether the suppression of p40 by PGE2 is also affecting IL-12 production by monocytes. Hence, co-stainings for the individual subunits of IL-12 p35 and p40 were performed (Fig. 3A, bottom). PGE2 also led to a significant down-regulation of p35/p40 double positive cells, which could be functional IL-12 producing cells (Fig. 3A). To determine whether the suppression of p40 also occurred on mRNA level, real time RT-PCR analyses were performed. PGE2 led to a significant down-regulation of the mRNA for the p40 subunit while there was no significant effect on the level of mRNA for the p19 subunit (Fig. 3B).

3.4. PGE2 acts in a concentration dependent manner to inhibit IL-12 and IL-23 production by isolated monocytes after different time points of LPS stimulation

In order to confirm the effect of PGE2 on the production of the heterodimeric IL-12 p70 complex by LPS-stimulated monocytes on the protein level analyses of supernatants by ELISA were performed. For this analysis isolated human monocytes were stimulated with LPS and IFN- γ in the presence or absence of PGE2. The supernatants were collected after 24 and 48 h of stimulation and the concentrations of IL-12p70 were determined with a p70 specific antibody detecting the p35/p40 heterodimer. There was a significant up-regulation of IL-12 production by monocytes after LPS and IFN- γ stimulation (Fig. 4A, bottom). The IL-12 concentration reached its maximum after 24 h of stimulation and was significantly down-regulated by the effect of PGE2 at both 24 and 48 h post stimulation without any effect on IL-6 production (Fig. 4A). In addition to IL12, we also measured the content of the IL-23 p19/p40 heterodimer in the supernatants after 24 and 48 h post LPS stimulation in the presence or absence of PGE2. IL-23 production peaked at 24 h and its concentration started to decline in the supernatant thereafter, which could be due to its consumption by cells in the culture (Fig. 4A, top). PGE2 resulted in a down-regulation of IL-23 at 24 and 48 h post stimulation without any effect on IL-6 production. We also investigated on the effect of PGE2 on IL-12 and IL-23 production by isolated monocytes at different concentrations of PGE2. PGE2 inhibited IL-12 and IL-23 production by isolated monocytes in a dose-dependent manner upon LPS stimulation (Fig. 4B). Even at the lowest concentration of 10 ng/ml PGE2, there was a nearly 50% down-regulation of IL-12 and IL-23 while there was no effect on IL-6 levels in the supernatants at all concentrations of PGE2 tested (Fig. 4B).

3.5. PGE2 inhibits IL-12 and IL-23 production from isolated monocytes in a cAMP dependent manner

Monocytes exclusively express the PGE receptors EP2 and EP4, the stimulation of which leads to the enhancement of intracellular cAMP levels (Panzer and Uguccioni, 2004). We investigated whether the suppressive effect of PGE2 on IL-12 and IL-23 production by monocytes was cAMP mediated by applying the cAMP enhancer forskolin. We quantified IL-12 and IL-23 production from isolated monocytes stimulated with LPS in the presence or absence

Fig. 1. Effect of PGE2 on IL-23 and IL-6 production by human PBMCs and isolated monocytes. (A) Human PBMCs and (B) isolated monocytes (2×10^6 cell/ml) were stimulated with 5 μ g/ml LPS in the presence or absence of 1 μ g/ml of PGE2 and supernatants were collected after 24 h. IL-23 and IL-6 concentrations were measured in the supernatant by ELISA. Error bars represent standard deviations from 3 different donors for PBMCs and 5 different donors for isolated monocytes. (C) Isolated monocytes were stimulated with polyI:C in the presence and absence of PGE2 and supernatants were collected after 24 h. IL-23 and IL-6 concentrations were measured in the supernatant by ELISA. Error bars represent standard deviations from 3 different donors.

Fig. 2. Influence of PGE2 on IL-23 and IL-6 production from human monocyte derived DCs and monocyte derived macrophages. (A) Immature monocyte derived DCs and (B) monocyte derived macrophages were stimulated with LPS in the presence or absence of 1 μ g/ml PGE2 and supernatants were collected after 48 h. IL-23 and IL-6 concentrations were measured by ELISA. Error bars represent standard deviations of triplicate readings from the same donor. Data from two different donors are shown.

Fig. 3. Effect of PGE2 on IL-23 and IL-12 production from isolated monocytes at the mRNA and protein level. Isolated human monocytes (2×10^6 cell/ml) treated with brefeldin A were stimulated with LPS in the presence or absence of 1 μ g/ml PGE2. (A) Cells were harvested after 15 h and surface stained for CD14 followed by intracellular co-staining for the IL-12 and IL-23 subunits p35, p40 and p19, respectively, and then analyzed by flow cytometry. Recombinant human IFN- γ was added to the culture along with LPS for induction of IL-12. The shown dot plots are representative for three independent experiments from different donors with similar outcomes. (B) Isolated monocytes (2×10^6 cell/ml) were stimulated with LPS in the presence or absence of PGE2 for 4 h and subsequently cells were harvested followed by RNA isolation and real time RT-PCR for IL-23p19 and the common p40 subunit of IL-12 and IL-23. Data from three different donors are shown.

of PGE2 and also in the presence or absence of 50 μ M forskolin. PGE2 was leading to a down-regulation of IL-12 and IL-23 as observed in previous experiments (Fig. 5A and B). Forskolin was able to mimic the effect of PGE2 as the supplementation of the culture medium with 50 μ M forskolin led to a down-regulation of IL-12 and IL-23 in the supernatant of LPS stimulated monocytes which was similar to that of PGE2 (Fig. 5A and B). Again, there was no effect on IL-6 content after forskolin treatment (Fig. 5A and B). Our data strongly suggests that PGE2 acting through EP2 and EP4 receptors leads to an elevation of cAMP level and this in turn appears to be responsible for the down-regulation of IL-12 and IL-23 production by isolated monocytes.

4. Discussion

IL-23 is a heterodimeric cytokine belonging to the IL-12 family of cytokines. IL-23 is constituted from a unique p19 subunit and the p40 subunit, which it shares with the heterodimeric cytokine IL-12. IL-23 plays a crucial role in the differentiation and maintenance of human Th17 cells and therefore has been implicated in several inflammatory autoimmune disorders. The effect of the non-cytokine immunomodulator prostaglandin E2 on IL-23 production by DCs has been studied extensively but its effect on human monocytes, which also play an important role in the progression of autoimmune disorders by secreting pro-inflammatory cytokines (Chofflon et al., 1992; Imamura et al., 1993), has not been investigated in detail.

We have shown in this study that PGE2 is affecting the expression of the common p40 subunit in activated monocytes leading to its suppression both at mRNA and protein level, which was responsible for the down-regulation of IL-12 and IL-23 in monocytes. PGE2 acts through EP2 and EP4 receptors leading to the enhancement of cAMP levels. The effect of PGE2 of blocking IL-12 and IL-23 production from both total PBMCs and isolated monocytes was attributed to the enhancement of cAMP levels since forskolin, which is a pharmacologic enhancer of intracellular cAMP, was able to show the same effect like that of PGE2 in blocking these pro-inflammatory cytokines. LPS signaling in monocytes induces a wide range of signaling pathways and transcription factors, which include MAP kinase and NF- κ B signaling pathways. Both p38 MAP kinase and NF- κ B are important in LPS mediated transcriptional induction of the p40 gene; in particular the NF- κ B component c-Rel plays an important role in the induction of IL-23 (Zhang et al., 2000). However, a detailed experimental promoter analysis of p40 is needed to clarify which regulatory proteins and transcription factors possess binding sites in the promoter region of the p40 gene. It would be interesting to investigate the effect of PGE2 on those regulatory proteins, which might be responsible for the observed down-regulation of p40 mRNA levels. It would also be interesting to look at the effect of PGE2 on other MAP kinase pathways activated by LPS stimulation like the JNK and ERK pathways. It has been shown earlier that cAMP inhibits p38 MAP kinase activity leading to the down-regulation of IL-12p40 (Feng et al., 2002; Zhang et al., 2006), which could be a possible mechanism underlying our finding of the fulminant down-regulation of the p40 mRNA. It would be interesting

Fig. 4. Dose dependent effect of PGE2 on IL-12 and IL-23 production by isolated human monocytes after different time points of LPS stimulation. (A) Isolated monocytes (2×10^6 cell/ml) were stimulated with LPS in the presence or absence of $1 \mu\text{g/ml}$ PGE2. Supernatants were collected after 24 and 48 h. ELISAs were performed for IL-23 (p19/p40 dimer) and IL-12 (p35/p40 dimer). Error bars represent standard deviation of triplicate readings. The data is representative of three independent experiments with different donors. (B) Isolated monocytes (2×10^6 cell/ml) were stimulated with LPS in the presence or absence of different concentrations of PGE2 for 24 h. Supernatants were analyzed for the concentration of IL-23 and IL-12 by ELISA. Error bars represent standard deviations of triplicate readings from the same donor. Data is representative of at least three independent experiments with different donors.

to investigate further downstream of cAMP, the cell context specific effect of PGE2 on monocytes and monocytes derived DCs with respect to IL-23 production. cAMP signals through PKA and Epac, the guanine nucleotide exchange factor for the small GTPase Rap1 (de Rooij et al., 1998) and the crosstalk between cAMP versus PKA and Epac signaling is responsible for DC maturation (Garay et al., 2010). It could be possible that cAMP signaling via PGE2 and its cross talk with PKA and Epac signaling is operating differentially in the two cell types, i.e. monocytes and monocytes derived DCs.

IL-10 is a potent inhibitor of many of the functions of monocytes, including oxidative burst, nitric oxide production, phagocytosis, and the production of pro-inflammatory cytokines like IL-12

(Bogdan et al., 1991; Fiorentino et al., 1991). It has been shown that IL-10 inhibits the transcription of inflammatory cytokines produced by monocytes upon LPS stimulation (Wang et al., 1994), and IL-1 β is involved in the production of IL-12 by human monocytes (van de Wetering et al., 2009). Therefore, we have investigated in this study whether the effect of PGE2 of blocking IL-12 and IL-23 production was mediated by altering the expression of IL-10 and IL-1 β in monocytes, but we could not detect any effect of PGE2 on IL-10 and IL-1 β production by isolated monocytes upon LPS stimulation (Supplementary Fig. S3). Moreover, as concluded from our intracellular staining experiments and real time RT-PCR analyses of isolated monocytes, PGE2 does not seem to act via the induction of any other

Fig. 5. The effect of PGE2 on IL-23 and IL-12 down-regulation in isolated monocytes is cAMP mediated. Isolated human monocytes (2×10^6 cell/ml) were stimulated with LPS and IFN- γ under different conditions in the presence of 1 μ g/ml of PGE2 or 50 μ M forskolin as indicated for 24 h. Supernatants were collected thereafter and ELISAs were performed for quantitating (A) IL-23 and (B) IL-12. Data is representative of at least three independent experiments from different donors.

soluble mediator or cytokine, which might be responsible for the reduced IL-12 and IL-23 production by monocytes. Instead, our data strongly suggest that PGE2 exerts this effect directly by binding to EP2 and/or EP4 receptors on highly purified monocytes leading to the enhancement of intracellular cAMP levels which in turn most likely suppress the transcription of these pro-inflammatory cytokines produced by monocytes upon LPS stimulation. We should point out, however, that we have not ruled out that the reduction in the steady state level of p40 mRNA is due to an accelerated degradation of the mRNA encoding p40 rather than a reduced transcription of the p40 gene.

PGE2 has been known for its unique status of a pro-inflammatory factor with both immunosuppressive as well as immuno-stimulating activity. PGE2 promotes Th17 differentiation by inducing IL-23 production by DCs. It would be interesting to look at the effect of PGE2 on Th1 and Th17 differentiation in the context of monocytes.

5. Conclusion

Our data strongly suggest that PGE2 affects T-helper cell differentiation in a cell context dependent manner. When PGE2 is acting on DCs it promotes Th17 differentiation whereas when it is acting on monocytes it inhibits both Th1 and Th17

differentiation due to its inhibitory effect on IL-12 and IL-23 production by human monocytes, respectively. This dichotomy between monocytes in the peripheral blood and mature DCs in secondary lymphoid organs may be a means to counteract an overshooting pro-inflammatory response and cytokine storm in the circulation whereas DCs licensed by TLR ligands and PGE2 are allowed to promote a pro-inflammatory response in secondary lymphoid organs. This dichotomy and cell type as well as differentiation state specific consequences of PGE2 stimulation, especially when comparing monocytes with DCs, is an important paradigm, which may help to reconcile controversial findings on the role of PGE2 in the regulation of cytokines of the IL12 family.

Authors' contribution

K.W.K. designed and performed all experiments, analyzed the data, and wrote the manuscript.; M.G. provided advice for experimental design, supervised the experiments and refined the manuscript.

Conflict of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molimm.2012.08.014>.

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