

**ORIGINAL ARTICLE** Basic Mechanisms in Allergic Disease

# Iloprost has potent anti-inflammatory properties on human monocyte-derived dendritic cells

T. Müller<sup>1</sup>, T. Dürk<sup>1</sup>, B. Blumenthal<sup>1</sup>, Y. Herouy<sup>2</sup>, S. Sorichter<sup>1</sup>, M. Grimm<sup>1</sup>, E. Panther<sup>3</sup>, S. Cicko<sup>1</sup>, J. Norgauer<sup>4</sup> and M. Idzko<sup>1</sup>Departments of <sup>1</sup>Pneumology, <sup>2</sup>Dermatology, <sup>3</sup>Gastroenterology, University of Freiburg, Freiburg, Germany and <sup>4</sup>Department of Dermatology, University of Jena, Jena, Germany

## Clinical & Experimental Allergy

### Summary

**Background** The stable prostaglandin I<sub>2</sub> analogue (iloprost) iloprost has been shown to inhibit allergic airway inflammation in mice by modulating the function of myeloid dendritic cells (DCs).

**Objective** The aim of the current study was to investigate the biological activity of iloprost on human monocyte-derived DCs.

**Methods** I prostanoide (IP) receptor expression was analysed by RT-PCR. Cytokine secretion by DCs and CD4<sup>+</sup> T cells was measured by ELISA. The expression of the transcription factor FoxP3 after co-culture of DCs with CD4<sup>+</sup> CD45RA<sup>+</sup> T cells was analysed by flow cytometry.

**Results** Human monocyte-derived DCs were found to express mRNA specific for the PGI<sub>2</sub> receptor IP, and stimulation with iloprost resulted in increased cyclic AMP levels in both immature DCs (iDCs) and mature DCs (mDCs). Moreover, iloprost dose dependently inhibited the secretion of TNF- $\alpha$ , IL-6, IL-8 and IL-12p70 in mDCs, while it enhanced IL-10 production. Changes in cytokine secretion were paralleled by an altered T-cell priming capacity of DCs: in co-culture experiments of iloprost-treated mDC and naïve CD45RA<sup>+</sup> T cells, an induction of regulatory T cells could be observed, as demonstrated by increased intracellular FoxP3 expression and IL-10 production. Additionally, iloprost inhibited the MIP-3 $\beta$ -induced migration of mDCs.

**Conclusion** In summary, our results provide evidence that iloprost profoundly affects the function of human myeloid DCs. Therefore, iloprost might also be a new therapeutical option for the treatment of asthma in humans.

**Keywords** dendritic cells, FoxP3, IL-10, iloprost, Tregs

*Submitted 15 October 2009; revised 30 March 2010; accepted 10 May 2010*

### Correspondence:

Dr Marco Idzko, Department of Pneumology, University of Freiburg, Killianstrasse 5, D-79104 Freiburg, Germany.  
E-mail: marco.idzko@uniklinik-freiburg.de

Cite this as: T. Müller, T. Dürk, B. Blumenthal, Y. Herouy, S. Sorichter, M. Grimm, E. Panther, S. Cicko, J. Norgauer and M. Idzko, *Clinical & Experimental Allergy*, 2010 (40) 1214–1221.

### Introduction

Dendritic cells (DCs) are antigen-presenting cells, specialized in the initiation and amplification of immune responses by directing T-cell proliferation and differentiation [1–4]. Immature DCs (iDCs) that reside in peripheral tissues such as the skin, gastrointestinal tract and lungs are specialized in taking up and processing antigens. After interaction with danger signals, e.g. microorganisms, inflammatory cytokines or molecules released during cell damage DCs undergo a maturation process that involves the acquisition of high levels of major histocompatibility complex (MHC) and co-stimulatory molecules such as CD40, CD80, CD83 and CD86 [5]. During maturation, DCs lose their phagocytotic capacity and migrate to secondary

lymphoid organs [6]. In addition, mature DCs (mDCs) produce a broad panel of cytokines (e.g. IL-12, IL-10, TNF- $\alpha$ ) and are capable of presenting processed antigens to T cells [1, 5]. Depending on the interaction with microenvironmental factors during maturation, DCs can stimulate the polarized outgrowth of distinct T-cell subsets [2, 6, 7]. DC-derived IL-12p70 is the most important factor driving the differentiation of T-helper type 1 (Th1) cells, while the production of IL-10 by DCs has been linked to the induction of so-called regulatory T cells (Tregs) [6–8].

Prostaglandins (PGs) are locally acting autacoids generated by a stepwise conversion of arachidonic acid into two short-lived intermediates, PGG and PGH [9–11], through activation of the enzymes COX-1 and COX-2.

The latter can be further metabolized to prostacyclin (PGI<sub>2</sub>) and thromboxane A<sub>2</sub> by the activity of prostacyclin synthase and thromboxane synthase, respectively [12]. PGs have pleiotropic roles in physiological and pathophysiological processes, including inflammation and allergic disorders [9, 11]. In terms of asthma pathophysiology, PGs have generally been regarded as pro-inflammatory mediators in the past. However, non-steroidal anti-inflammatory drugs, e.g. aspirin, which are COX inhibitors and therefore lead to a reduced production of PGs, can be harmful in asthma. This has mainly been attributed to the increased generation of leukotrienes [13]. Additionally, animals lacking the COX1 and COX2 pathway show increased allergic airway inflammation [14, 15]. Furthermore, the administration of PGE<sub>2</sub> and PGD<sub>2</sub> can actually suppress asthmatic airway inflammation, the latter one via the modulation of myeloid DC function and the induction of Tregs [16–18].

While others could demonstrate that deficiency of the PGI<sub>2</sub>-exclusive I prostanoid (IP) receptor was associated with exaggerated features of asthmatic airway inflammation [19, 20], we showed previously that intrapulmonary administration of the stable PGI<sub>2</sub> analogue iloprost to ovalbumin-sensitized animals suppresses the cardinal features of asthma [21]. Some of these effects can be linked to the PGI<sub>2</sub>-induced modulation of DC function, such as inhibition of lung DC migration, altered cytokine production and T-cell priming capacity [21, 22]. In addition, very recently, it has been reported that the stable PGI<sub>2</sub> analogue iloprost suppressed CpG oligodeoxynucleotide-(or imiquimod-) induced TNF- $\alpha$  and IFN- $\alpha$  production, while it induced the production of IL-10 in human plasmacytoid DCs (pDCs) [23]. However, the effect of PGI<sub>2</sub> on the function of human myeloid DCs has not been studied so far.

## Material and methods

### Materials

Iloprost-trometamol (iloprost) was from Cayman Chemical and Ficoll-Paque from Amersham-Pharmacia Biotech AB (Uppsala, Sweden).

### Preparation of human dendritic cell

CD14<sup>+</sup> monocytes were isolated from 200 mL of peripheral blood, obtained from healthy human volunteers, using Ficoll-Paque gradients and anti-CD14 monoclonal antibody (mAb)-coated MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) [24]. Isolated cells were resuspended in a serum-free X-VIVO-15 medium (Bio Whittaker Walkersville, MD, USA) containing 1% glutamine, 50 IU/mL penicillin, 50  $\mu$ g/mL streptomycin (Life Technologies GmbH, Karlsruhe, Germany), 1000 U/mL IL-

4, 200 ng/mL GM-CSF (Natutec, Frankfurt, Germany) and 1% human plasma. After 6 days of culture at 37 °C in a humidified CO<sub>2</sub> (5%) atmosphere, monocyte-derived iDCs were >95% CD1a<sup>+</sup>, CD80<sup>low</sup>, CD86<sup>low</sup>, CD83<sup>low</sup> and CD115<sup>high</sup>. Further differentiation into mDC was induced by treatment with 3  $\mu$ g/mL lipopolysaccharide (LPS) (*Escherichia coli*, serotype 0111:B4, Sigma, Deisenhofen, Germany) for 48 h. mDC were >95% CD80<sup>high</sup>, CD86<sup>high</sup>, CD83<sup>high</sup> and CD115<sup>low</sup>. Analysis of cell surface molecules was performed by flow cytometry using FACScan (Becton Dickinson, Heidelberg, Germany). mAbs and their respective isotype controls were from Coulter-Immunotech (Krefeld, Germany).

### Detection of messenger RNA by reverse transcriptase polymerase chain reaction analysis

mRNA was isolated using QIAshredder and RNeasy kits (Qiagen, Hilden, Germany). mRNA, M-MLV reverse transcriptase and pd(N)<sub>6</sub> primers (Gibco BRL, Gaithersburg, MD, USA) were used to obtain cDNA. All oligonucleotides used as primers in PCR were designed to recognize sequences specific for each target cDNA. The primer sequences were as follows: IP: sense: 5'-TTTTTCAGGCTCTGCAAGGA-3', anti-sense: 5'-GGC TGC AAG ATG AGA ACT GG-3';  $\beta_2$ -microglobulin: sense: 5'-CCTTGAGGCTATCCAGCGTA-3', anti-sense: 5'-GTTACACGGCAGGCATACT-3'. Thirty-five PCR cycles were run at 94 °C (denaturation, 1 min), 62 °C (annealing, 1 min) and 72 °C (extension, 1 min). PCR products were subjected to electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. The identity of the PCR products was confirmed by sequencing after cloning in pCRII vectors. Control run without reverse transcriptase yielded no PCR products.

### Measurement of intracellular cyclic AMP

Intracellular cyclic AMP (cAMP) was measured by cAMP EIA from Amersham-Pharmacia Biotech AB. Forskolin (Sigma-Aldrich, Taufkirchen, Germany) was used as a positive control. Briefly, DCs (1  $\times$  10<sup>5</sup>/mL) were stimulated with different concentrations of iloprost or forskolin for 10 min at 37 °C, and then cells were lysed and cAMP levels were measured according to the manufacturer's instruction. The cAMP levels are expressed as an index representing the ratio between values obtained in stimulated cells and cells incubated in the control medium.

### Cytokine assays

IL-12p70 was analysed using the OptiEIA kit (BD Pharmingen, Heidelberg, Germany). Quantikine human TNF- $\alpha$  ELISA was from R&D Systems (Minneapolis, MN, USA). CXCL8, IL-6 and IL-10 were measured in DC supernatants

using ELISA from R&D Systems. The release of IFN- $\gamma$  and IL-5 from T cells was detected using matched pair of mAbs (R&D Systems). Samples were assayed in triplicate for each condition.

#### *T-cell differentiation assay*

CD4<sup>+</sup> T lymphocytes were separated (>95% CD4<sup>+</sup> cells) by Ficoll gradients (Amersham-Pharmacia Biotech, Freiburg, Germany) and immunomagnetic depletion using a mixture of anti-HLA-DR, anti-CD19 and anti-CD8 mAb-conjugated beads (Dyna, Oslo, Norway) [24]. Allogeneic naïve T cells were purified (>95% CD45RA<sup>+</sup>) by incubation of CD4<sup>+</sup> cells with anti-CD45RO mAb and goat anti-mouse Ig coupled to immunomagnetic beads. Recovered CD4<sup>+</sup> CD45RA<sup>+</sup> cells were more than 95% pure, as indicated by flow cytometry. Then purified CD45RA<sup>+</sup> (10<sup>6</sup> cells/well) cells were co-cultured with allogeneic LPS-DCs or iloprost-LPS-DCs (10<sup>5</sup> cells/well) in a 24-well plate in 1640 RPMI medium supplemented with 5% human serum for 6 days. The T cells generated were stimulated with anti-CD3/ anti-CD28 Dynabeads for 24 h and then Dynabeads were removed, the supernatants were harvested and cells were stained for intracellular FOXP3.

#### *Mixed leucocyte reaction assay*

CD4<sup>+</sup> T lymphocytes were separated (>95% CD4<sup>+</sup> cells) by Ficoll gradients (Amersham-Pharmacia Biotech) and immunomagnetic depletion using a mixture of anti-HLA-DR-, anti-CD19- and anti-CD8 mAb-conjugated beads (Dyna) [24]. Allogeneic naïve T cells were purified (>95% CD45RA<sup>+</sup>) by incubation of CD4<sup>+</sup> cells with anti-CD45RO mAb and goat anti-mouse Ig coupled to immunomagnetic beads. CD45RA<sup>+</sup> (10<sup>4</sup> cells/well) were co-cultured with DC (10<sup>3</sup> cells/well), which had been matured in the presence or absence of iloprost, in 96-well plates in 1640 RPMI medium supplemented with 5% human serum. Co-cultures were pulsed after 5 days with 1  $\mu$ Ci/well (0.037 MBq/well) [<sup>3</sup>H]-thymidine (Amersham, Little Chalfont, UK) for 16 h at 37 °C, and then harvested onto fibre-coated 96-well plates (Packard Instruments, Groningen, the Netherlands). Radioactivity was measured in a  $\beta$ -counter (Topcount; Packard Instruments, Meriden, CT, USA).

#### *Analysis of FoxP3 expression*

For the detection of forkhead box p3 (FoxP3)-positive cells, a commercially available kit (Human Regulatory T cell Staining Kit #2, e-Bioscience, San Diego, CA, USA) was used, according to the manufacturer's description. All samples were analysed using a BD FACScan flow cytometer and FACSDiva software (BD Bioscience, San Diego, CA, USA).

#### *Migration assay*

Experiments were performed in triplicate using 48-well Transwell chambers (Nucleopore, Tübingen, Germany). Buffer or stimuli were added to the lower compartment wells. Thereafter, a 10  $\mu$ m polycarbonate membrane with a pore size of 5  $\mu$ m (Nucleopore) was placed over the wells. DC (10<sup>5</sup> cells/well) were added to the upper compartment and incubated at 37 °C for 90 min in a humidified atmosphere. After removing the cells from the upper side of the membrane by wiping over a profiled rubber, migrated DC on the lower side of the membrane were fixed in methanol and stained with haematoxylin. Migrated DCs were counted in five randomly chosen high-power ( $\times$ 400) fields and a mean value for each sample was calculated. The chemotactic index was calculated as the ratio between DC migrated in the presence and in the absence of stimuli.

#### *Statistical analysis*

Unless stated otherwise, values are expressed as mean SEM. ANOVA was used to compare experimental groups with the control values. When the global test of differences was significant at the 5% level, pairwise tests of differences between groups were applied (Bonferroni comparison test).

## **Results**

#### *Expression of the messenger RNA for I prostanoid and the influence of iloprost on cyclic AMP production*

The expression of mRNA for the PGI<sub>2</sub> receptor IP was analysed in iDCs and mDCs by RT-PCR. Figure 1 shows that iDCs and mDCs express similar amounts of IP receptor-specific mRNA. No products were obtained after omitting reverse transcription in the reaction (data not shown).

PGI<sub>2</sub> signals through G protein-coupled IP receptors, which, in most cells, couple to G<sub>s</sub> proteins and activate adenylyl cyclase [25]. To analyse the functional expression of this ligand-dependent signalling pathway in DCs, we measured cAMP levels. Figure 2 shows that iloprost enhances the intracellular cAMP levels in both iDCs and mDCs in a concentration-dependent manner.

#### *Modulation of cytokine and chemokine secretion by iloprost*

Others and we could show recently that iloprost can modulate the cytokine production of bone marrow-derived myeloid DCs *in vitro* [21, 22]. Therefore, we characterized the influence of iloprost on IL-6, IL-12p70, TNF- $\alpha$  and IL-10 secretion. No significant effect of different iloprost concentrations could be observed on the release

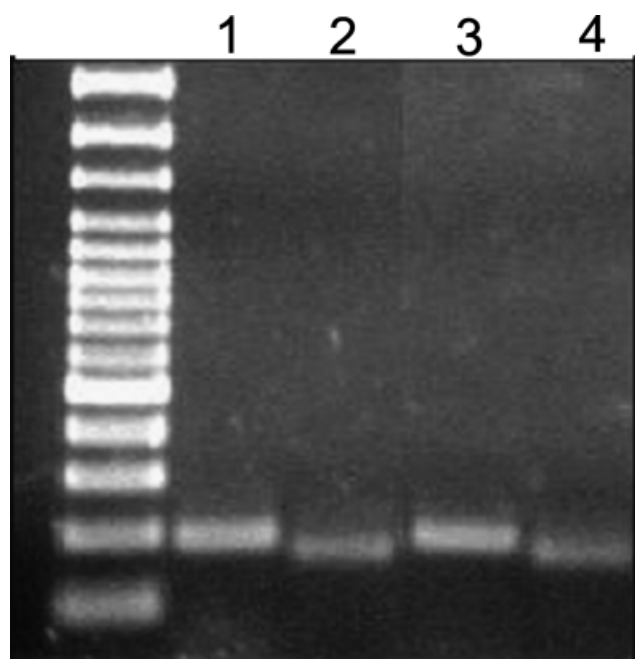


Fig. 1. Expression of I prostanoid (IP) receptor mRNA transcripts by immature dendritic cells (iDCs) and mature dendritic cells (mDCs). RNA from iDCs and mDCs was isolated and reverse transcription was performed. PCR reaction was carried out with primer pairs specific for the IP-receptor and  $\beta_2$ -microglobulin as a positive control. Lane 1, iDCs  $\beta_2$ -microglobulin; 2, iDCs IP-receptor; 3, mDCs  $\beta_2$ -microglobulin; 4, mDCs IP-receptor. One representative experiment out of three is shown ( $n = 3$ ).

of these cytokines in iDCs (Fig. 3a–e). However, iloprost added, together with LPS, inhibited the production of IL-12p70, TNF- $\alpha$ , IL-8 and IL-6 in a dose-dependent manner, whereas IL-10 production was dose dependently increased (Fig. 3a–e).

#### *Iloprost modulates cytokine production by exclusive activation of the I prostanoid receptor*

To prove the exclusive involvement of the IP receptor in the iloprost-induced modulation of cytokine production by mDCs, cells were pre-treated with the selective IP-receptor antagonist CAY10449 (10  $\mu$ M) 30 min before stimulation with iloprost. As shown in Fig. 3f, blocking of the IP receptor completely reversed the iloprost-induced changes in cytokine production.

#### *Iloprost reduces the capacity of mature dendritic cell to stimulate mixed leucocyte reaction response*

To investigate whether iloprost can affect the capacity of mDCs to stimulate the allogeneic T-cell response, CD4<sup>+</sup> CD45RA<sup>+</sup> T cells were cultured with iloprost-treated mDCs or vehicle-treated mDCs for 5 days. Cell proliferation was measured by [<sup>3</sup>H]-thymidine uptake in a 16-h

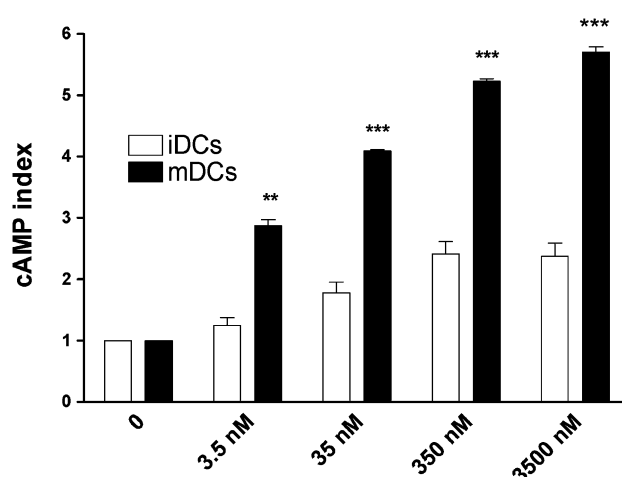


Fig. 2. Stimulation of immature dendritic cells (iDCs) and mature dendritic cells (mDCs) with iloprost increases cyclic AMP (cAMP). iDCs and mDCs were stimulated with the indicated concentrations of iloprost, and cAMP levels were determined using a commercially available kit. Data are means  $\pm$  SEM ( $n = 4$ ). \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared with unstimulated cells.

pulse. As shown in Fig. 4, compared with vehicle-treated DCs, iloprost-treated DCs induced a significantly lower proliferative T-cell response.

#### *Mature dendritic cells matured in the presence of iloprost induce the generation of regulatory T cells*

Finally, we questioned whether iloprost can modulate the T-cell priming capacity of mDCs. For this purpose, CD4<sup>+</sup> CD45RA<sup>+</sup> T cells were cultured with iloprost-mDCs or vehicle-mDCs for 6 days. Then the cells were collected, washed and re-stimulated with anti-CD3/anti-CD28 beads for an additional 24 h. After the removal of beads, the supernatants were collected and cells were stained for intracellular FoxP3 expression. As shown in Fig. 5a, T cells co-cultured with iloprost-treated DCs produced higher amounts of IL-10 and lower amounts of IFN- $\gamma$  and IL-5, suggesting the differentiation of Tregs. Indeed, intracellular staining revealed an increased expression of the transcription factor FoxP3<sup>+</sup> in CD4<sup>+</sup> CD25<sup>+</sup> T cells (Fig. 5b).

#### *Effect of iloprost treatment on the migratory capacity of mature dendritic cell*

We could show recently that iloprost can inhibit the CCR7-mediated migration of myeloid lung DCs to the mediastinal lymph nodes in mice [21]. Therefore, we questioned whether iloprost can also modulate human mDC migration *in vitro*. For this purpose, DCs were matured in the presence or absence of iloprost. Then cells were washed, placed into the upper chamber of a transwell plate and different concentrations of the CCR7 agonist

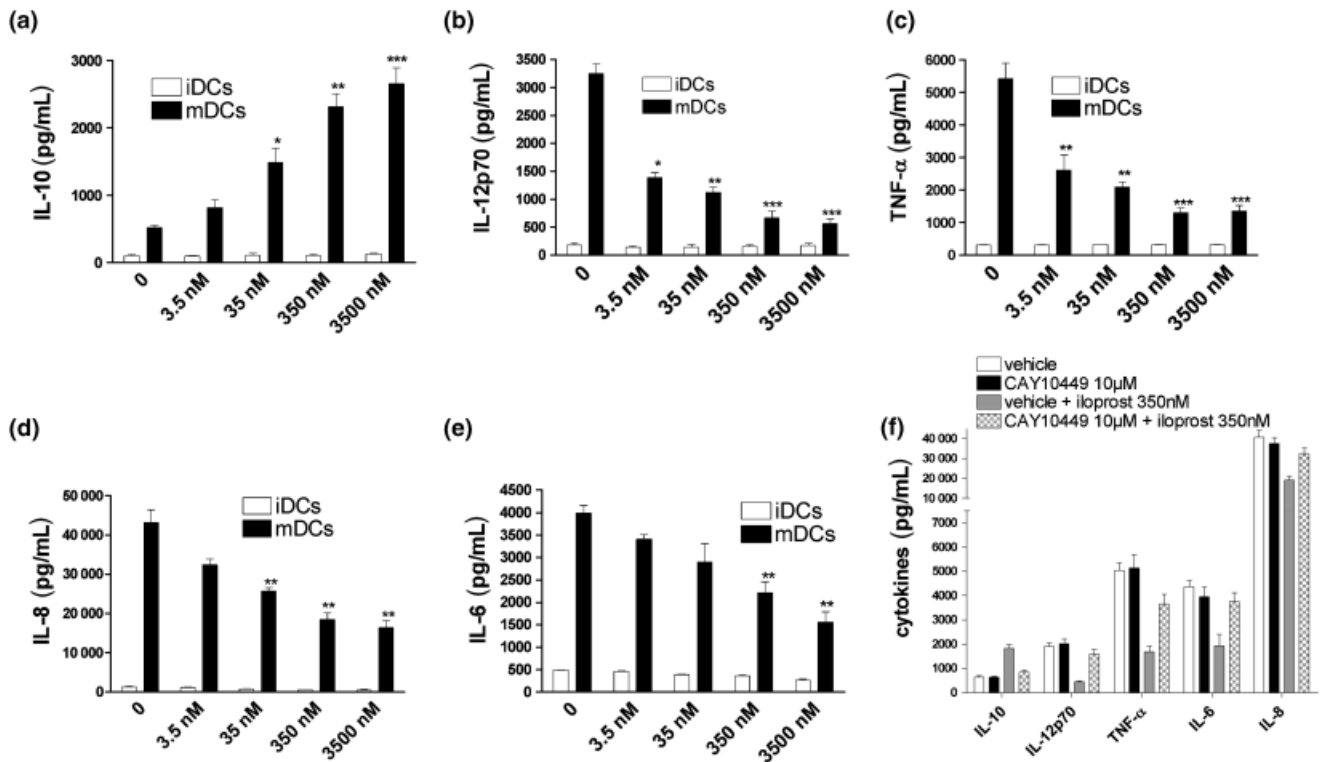


Fig. 3. Iloprost inhibits IL-12p70 and TNF- $\alpha$  secretion from mature dendritic cells (mDCs). DCs were left untreated (white bars) or were stimulated with 3  $\mu$ g/mL lipopolysaccharide (LPS) (black bars) in the presence of the indicated concentration of iloprost for 24 h (a–e). Cytokine release was quantified by ELISA. Results are expressed as mean  $\pm$  SEM ( $n = 3$ –5). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . DCs were pre-treated with the selective IP-receptor antagonist CAY10449 (10  $\mu$ M) 30 min before stimulation with iloprost or vehicle, followed by pulsing with LPS (3  $\mu$ g/mL). Twenty-four hours later, the supernatants were collected and cytokine concentrations were determined by ELISA (f).

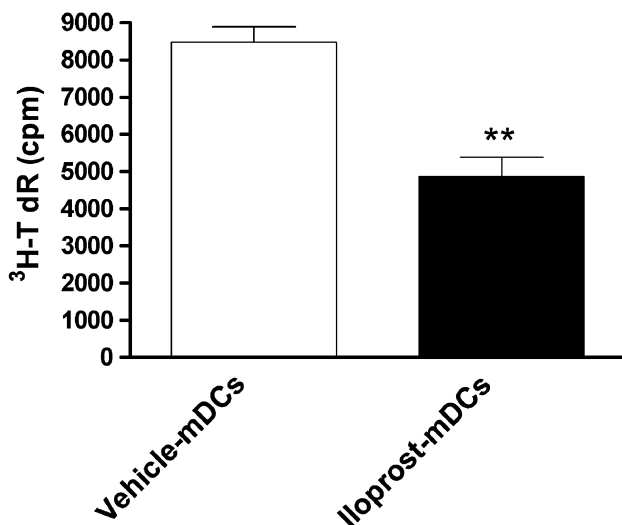


Fig. 4. Dendritic cells (DCs) matured in the presence of iloprost show a reduced ability to stimulate a mixed leucocyte reaction response. CD4 $^{+}$  CD45 $^{+}$  T cells were cultured with allogeneic mDCs, which had been pre-treated with vehicle or iloprost before lipopolysaccharide stimulation, at a ratio of 10:1 in 96-well plates. At day 5, cells were pulsed with 1  $\mu$ Ci/well  $^3$ H-thymidine for 16 h at 37  $^{\circ}$ C. One representative experiment in triplicate out of four is shown. \*\* $P < 0.01$ .

CCL19 (MIP3 $\beta$ ) were added to the lower chamber. Pre-treatment with iloprost significantly decreased the capacity of mDCs to migrate in response to CCL19 (Fig. 6), which was not due to down-regulation of CCR7, as detected by flow cytometry (data not shown).

## Discussion

Recently, it has been reported that iloprost can inhibit allergic airway inflammation in mice by altering the function of myeloid DCs [21]. In this study, we investigated whether iloprost is also capable of modulating the function of human monocyte-derived DCs.

We were able to demonstrate that both iDCs and mDCs express IP receptor-specific mRNA and that IP receptor activation via the PGI $_2$  analogue iloprost results in elevated intracellular cAMP levels. In addition, changes in intracellular cAMP were associated with a decreased production of pro-inflammatory cytokines, while the secretion of the anti-inflammatory cytokine IL-10 was increased. Furthermore, DCs matured in the presence of iloprost showed a reduced ability to induce T-cell proliferation and induced the *in vitro* differentiation of

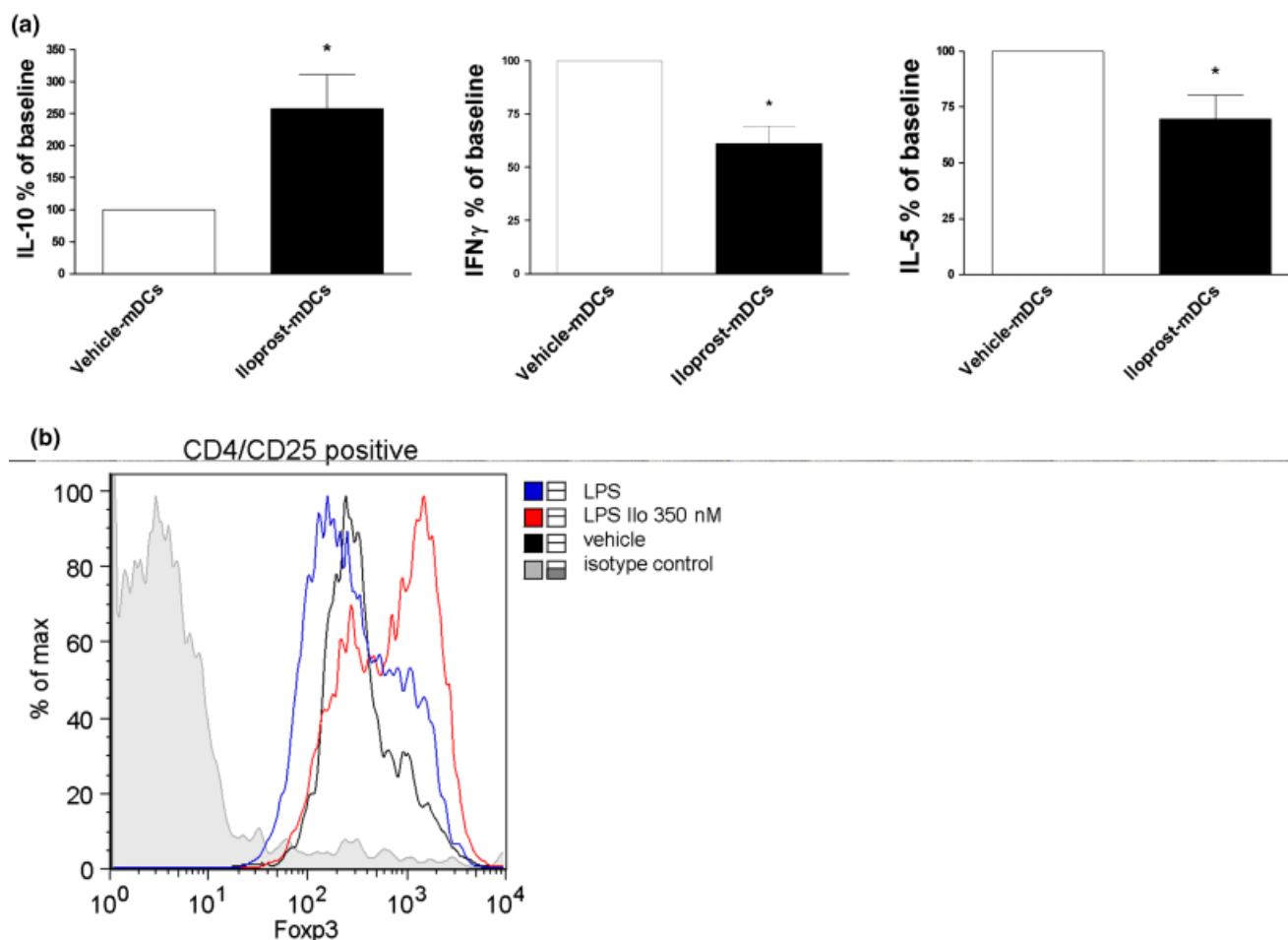


Fig. 5. Iloprost-matured dendritic cells (mDCs) induce the generation of IL-10-producing regulatory T cells.  $CD4^+ CD45^+$  T cells were cultured with allogeneic mDCs, which had been pre-treated with vehicle or iloprost before lipopolysaccharide (LPS) stimulation, at a ratio of 10 : 1 for 6 days. Then cells were stimulated with anti-CD3/CD28 Dynabeads. After 24 h, beads were removed, and the supernatants were harvested. (a) Supernatants from T cells evaluated for the secretion of IL-10 and IL-5 after the initial activation with DCs. Results are expressed as mean  $pg/mL \pm SEM$  ( $n = 3$ ).  $*P < 0.05$ . (b) After the removal of Dynabeads, T cells were permeabilized and stained for intracellular FoxP3 expression using anti-human FoxP3-allophycocyanin mAb. One representative out of three experiments is shown ( $n = 3$ ).

IL-10-producing FoxP3 $^+$  Tregs. Moreover, iloprost affected the ability of mDCs to migrate towards the CCR7-ligand CCL19 (MIP3 $\beta$ ). These findings support recent studies, suggesting that the anti-inflammatory effects of iloprost involve an altered function of DCs [21–23].

Elegant experiments have demonstrated the essential role of myeloid DCs in the induction and maintenance of allergic airway inflammation [26]. By their ability to present antigens via the MHC II complex together with co-stimulatory signals and by releasing different cytokines and chemokines, myeloid DCs can activate local effector Th2 cells in the airway wall, resulting in eosinophilic airway inflammation. Modulating DC function is therefore a promising approach for the treatment of bronchial asthma [21, 27].

Our results, demonstrating that iloprost inhibits the release of IL-12p70, TNF- $\alpha$ , IL-6 and IL-8, while it

increases IL-10 secretion in maturing DCs, are in accordance with previous studies showing a similar pattern of iloprost-induced changes in cytokine release by bone marrow-derived DCs [21, 22, 27]. Additionally, very recently, similar effects of iloprost on TNF- $\alpha$  and IL-10 production in human pDCs could be observed [23]. The direct inhibition of the pro-inflammatory cytokine production and especially the up-regulation of IL-10, which is essential in suppressing inflammatory responses (e.g. by limiting Th1 immune responses or induction of Tregs), further support the overall anti-inflammatory effect of PGI2 analogues on myeloid DC function [28, 29].

The migration of myeloid DCs from the lung to the draining lymph nodes, enabling them to interact with naïve or effector T cells, is an important step in asthma pathophysiology that depends on CCR7 signalling [30–32]. We reported recently that iloprost inhibits the

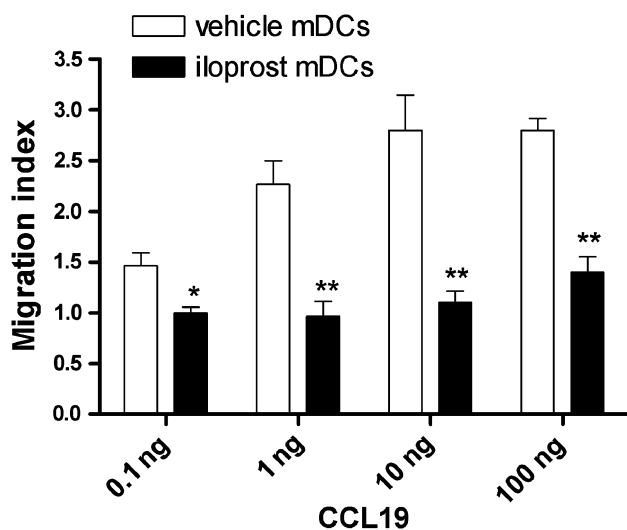


Fig. 6. Iloprost inhibits CCL19-induced migration of human mature dendritic cells (mDCs). Vehicle-treated mDCs or iloprost-treated mDCs were stimulated with increasing concentrations of CCL19. Oriented migration was measured using the modified Boyden chamber as described above. Data are mean  $\pm$  SEM ( $n = 5$ ) \* $P < 0.05$ , \*\* $P < 0.01$ .

migration of murine mDCs *in vivo* and *in vitro* by reducing their chemotactic response to the CCR7-ligand MIP-3 $\beta$  (CCL19). In accordance, human DCs matured in the presence of iloprost have lost their ability to migrate towards CCL19, further supporting the concept of an immunosuppressive effect of iloprost in DC biology.

On reaching the draining lymph nodes, DCs have the ability to induce the proliferation and polarization of naïve T cells into different subsets, including Tregs [2]. It has been reported that cAMP-elevating agents causing a decreased production of IL-12p70 but enhanced secretion of IL-10 by myeloid DCs promote the induction of Tregs [33]. In this work, we demonstrated that DCs matured in the presence of iloprost had a reduced ability to stimulate proliferation of CD4<sup>+</sup> CD45RA<sup>+</sup> T cells, and furthermore were able to promote the induction of Tregs, as seen by increased IL-10 and decreased IFN- $\gamma$ /IL-5 production. However, in contrast to previous studies in which FoxP3<sup>+</sup> IL-10-producing Tr1 cells were induced by DCs, we could detect an increased percentage of FoxP3<sup>+</sup> cells [33]. Nevertheless, there is strong evidence that FoxP3 expression is not restricted to natural Tregs but can also occur in the periphery by conversion of CD25<sup>+</sup> FoxP3<sup>+</sup> cells [34, 35].

In summary, we demonstrate that iloprost suppresses the production of pro-inflammatory cytokines, while it enhances IL-10 secretion by human monocyte-derived DCs. Furthermore, iloprost reduces the capacity of mDCs to migrate in response to CCL19 and the ability to induce T-cell proliferation. Additionally, naïve T cells co-cultured with iloprost-treated mDCs showed a regulatory phenotype. The modulatory effects were associated with

selective activation of the IP receptor pathway and intracellular cAMP elevation.

A clear therapeutic effect of iloprost on experimental asthma has been shown, which was due to an altered function of myeloid DCs [21]. Although, in the majority of patients, asthma can be controlled with inhalative corticosteroids, there is still a subgroup needing oral steroids or suffering from steroid-insensitive asthma. Thus, new therapeutic approaches for the treatment of asthma are still needed [36]. Inhalative iloprost is a well-tolerated treatment for pulmonary hypertension with little side-effects mainly due to its vasodilatory effects. However, systemic hypotension in iloprost-treated patients was not associated with clinical deterioration [37, 38]. Consequently, the findings of this manuscript should lead to investigations concerning the effects of iloprost in humans with asthma.

#### Acknowledgement

This work was supported by a grant from the German Research Foundation (DFG) to M. I. (DFG ID7/4-1).

#### References

- 1 Banachereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; 392:245–52.
- 2 Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev* 2003; 3:984–93.
- 3 Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. *Science* (New York, NY) 2000; 290:92–7.
- 4 Ueno H, Klechevsky E, Morita R *et al*. Dendritic cell subsets in health and disease. *Immunol Rev* 2007; 219:118–42.
- 5 Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr Opin Immunol* 2001; 13:114–9.
- 6 Panther E, Idzko M, Corinti S *et al*. The influence of lysophosphatidic acid on the functions of human dendritic cells. *J Immunol* 2002; 169:4129–35.
- 7 Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 2000; 18:593–620.
- 8 Idzko M, Panther E, Corinti S *et al*. Sphingosine 1-phosphate induces chemotaxis of immature and modulates cytokine-release in mature human dendritic cells for emergence of Th2 immune responses. *FASEB J* 2002; 16:625–7.
- 9 Carey MA, Germolec DR, Langenbach R, Zeldin DC. Cyclooxygenase enzymes in allergic inflammation and asthma. *Prostaglandins Leukotrienes Essent Fatty Acids* 2003; 69:157–62.
- 10 Park GY, Christman JW. Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. *Am J Physiol* 2006; 290:L797–805.
- 11 Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB J* 2004; 18:790–804.

- 12 Helliwell RJ, Adams LF, Mitchell MD. Prostaglandin synthases: recent developments and a novel hypothesis. *Prostaglandins Leukotrienes Essent Fatty Acids* 2004; **70**:101–13.
- 13 Hamad AM, Sutcliffe AM, Knox AJ. Aspirin-induced asthma: clinical aspects, pathogenesis and management. *Drugs* 2004; **64**:2417–32.
- 14 Gavett SH, Madison SL, Chulada PC *et al.* Allergic lung responses are increased in prostaglandin H synthase-deficient mice. *J Clin Invest* 1999; **104**:721–32.
- 15 Jaffar Z, Wan KS, Roberts K. A key role for prostaglandin I2 in limiting lung mucosal Th2, but not Th1, responses to inhaled allergen. *J Immunol* 2002; **169**:5997–6004.
- 16 Hammad H, Kool M, Soullie T *et al.* Activation of the D prostanoid 1 receptor suppresses asthma by modulation of lung dendritic cell function and induction of regulatory T cells. *J Exp Med* 2007; **204**:357–67.
- 17 Kunikata T, Yamane H, Segi E *et al.* Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. *Nat Immunol* 2005; **6**:524–31.
- 18 Pavord ID, Wong CS, Williams J, Tattersfield AE. Effect of inhaled prostaglandin E2 on allergen-induced asthma. *Am Rev Respir Dis* 1993; **148**:87–90.
- 19 Nagao K, Tanaka H, Komai M, Masuda T, Narumiya S, Nagai H. Role of prostaglandin I2 in airway remodeling induced by repeated allergen challenge in mice. *Am J Respir Cell Mol Biol* 2003; **29**:314–20.
- 20 Takahashi Y, Tokuoka S, Masuda T *et al.* Augmentation of allergic inflammation in prostanoid IP receptor deficient mice. *Br J Pharmacol* 2002; **137**:315–22.
- 21 Idzko M, Hammad H, van Nimwegen M *et al.* Inhaled iloprost suppresses the cardinal features of asthma via inhibition of airway dendritic cell function. *J Clin Invest* 2007; **117**:464–72.
- 22 Zhou W, Hashimoto K, Goleniewska K *et al.* Prostaglandin I2 analogs inhibit proinflammatory cytokine production and T cell stimulatory function of dendritic cells. *J Immunol* 2007; **178**:702–10.
- 23 Hung CH, Chu YT, Suen JL *et al.* Regulation of cytokine expression in human plasmacytoid dendritic cells by prostaglandin I2 analogues. *Eur Respir J* 2009; **33**:405–10.
- 24 Panther E, Corinti S, Idzko M *et al.* Adenosine affects expression of membrane molecules, cytokine and chemokine release, and the T-cell stimulatory capacity of human dendritic cells. *Blood* 2003; **101**:3985–90.
- 25 Lo RK, Liu AM, Wise H, Wong YH. Prostacyclin receptor-induced STAT3 phosphorylation in human erythroleukemia cells is mediated via Galpha(s) and Galpha(16) hybrid signaling. *Cell Signal* 2008; **20**:2095–106.
- 26 van Rijt LS, Jung S, Kleinjan A *et al.* *In vivo* depletion of lung CD11c<sup>+</sup> dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J Exp Med* 2005; **201**:981–9.
- 27 Idzko M, Hammad H, van Nimwegen M *et al.* Local application of FTY720 to the lung abrogates experimental asthma by altering dendritic cell function. *J Clin Invest* 2006; **116**:2935–44.
- 28 Henry E, Desmet CJ, Garze V *et al.* Dendritic cells genetically engineered to express IL-10 induce long-lasting antigen-specific tolerance in experimental asthma. *J Immunol* 2008; **181**:7230–42.
- 29 Lau AW, Biester S, Cornall RJ, Forrester JV. Lipopolysaccharide-activated IL-10-secreting dendritic cells suppress experimental autoimmune uveoretinitis by MHCII-dependent activation of CD62L-expressing regulatory T cells. *J Immunol* 2008; **180**:3889–99.
- 30 Hammad H, Lambrecht BN, Pochard P *et al.* Monocyte-derived dendritic cells induce a house dust mite-specific Th2 allergic inflammation in the lung of humanized SCID mice: involvement of CCR7. *J Immunol* 2002; **169**:1524–34.
- 31 Sanchez-Sanchez N, Riolo-Blanco L, Rodriguez-Fernandez JL. The multiple personalities of the chemokine receptor CCR7 in dendritic cells. *J Immunol* 2006; **176**:5153–9.
- 32 Yamashita N, Tashimo H, Matsuo Y *et al.* Role of CCL21 and CCL19 in allergic inflammation in the ovalbumin-specific murine asthmatic model. *J Allergy Clin Immunol* 2006; **117**:1040–6.
- 33 D'Ambrosio A, Colucci M, Pugliese O, Quintieri F, Boirivant M. Cholera toxin B subunit promotes the induction of regulatory T cells by preventing human dendritic cell maturation. *J Leukocyte Biol* 2008; **84**:661–8.
- 34 Ganesh BB, Cheatem DM, Sheng JR, Vasu C, Prabhakar BS. GM-CSF-induced CD11c<sup>+</sup> CD8a<sup>+</sup> dendritic cells facilitate Foxp3<sup>+</sup> and IL-10<sup>+</sup> regulatory T cell expansion resulting in suppression of autoimmune thyroiditis. *Int Immunol* 2009; **21**:269–82.
- 35 Lagaraine C, Lemoine R, Baron C, Nivet H, Velge-Roussel F, Lebranchu Y. Induction of human CD4<sup>+</sup> regulatory T cells by mycophenolic acid-treated dendritic cells. *J Leukocyte Biol* 2008; **84**:1057–64.
- 36 Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. *Lancet* 2009; **373**:1905–17.
- 37 Gombert-Maitland M, Olschewski H. Prostacyclin therapies for the treatment of pulmonary arterial hypertension. *Eur Respir J* 2008; **31**:891–901.
- 38 Gessler T, Schmehl T, Hoepfer MM *et al.* Ultrasonic versus jet nebulization of iloprost in severe pulmonary hypertension. *Eur Respir J* 2001; **17**:14–9.