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# 5-Hydroxytryptamine Modulates Migration, Cytokine and Chemokine Release and T-Cell Priming Capacity of Dendritic Cells In Vitro and In Vivo

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## Abstract

Beside its well described role in the central and peripheral nervous system 5-hydroxytryptamine (5-HT), commonly known as serotonin, is also a potent immuno-modulator. Serotoninergic receptors (5-HTR) are expressed by a broad range of inflammatory cell types, including dendritic cells (DCs). In this study, we aimed to further characterize the immuno-biological properties of serotoninergic receptors on human monocyte-derived DCs. 5-HT was able to induce oriented migration in immature but not in LPS-matured DCs via activation of 5-HTR₁ and 5-HTR₂ receptor subtypes. Accordingly, 5-HT also increased migration of pulmonary DCs to draining lymph nodes in vivo. By binding to 5-HTR₃, 5-HTR₄ and 5-HTR₇ receptors, 5-HT up-regulated production of the pro-inflammatory cytokine IL-6. Additionally, 5-HT influenced chemokine release by human monocyte-derived DCs: production of the potent Th1 chemoattractant IP-10/CXCL10 was inhibited in mature DCs, whereas CCL22/MDC secretion was up-regulated in both immature and mature DCs. Furthermore, DCs matured in the presence of 5-HT switched to a high IL-10 and low IL-12p70 secreting phenotype. Consistently, 5-HT favoured the outcome of a Th2 immune response both in vitro and in vivo. In summary, our study shows that 5-HT is a potent regulator of human dendritic cell function, and that targeting serotoninergic receptors might be a promising approach for the treatment of inflammatory disorders.

## Introduction

Outside the central nervous systems 5-hydroxytryptamine (5-HT), commonly known as serotonin, is found mainly in platelets and can be released during platelet aggregation. Additionally, it has been shown that mast cells are also able to store and release 5-HT, e.g. after cross-linking of membrane bound IgEs by allergens [1–3].

The wide variety of biological activities and the complexity of pharmacologic activities mediated by 5-HT, are due to the existence of different classes of serotoninergic receptors (5-HTR) [4]: the 5-HTR₁ subgroup consists of at least five subtypes namely 5-HTR₁A, 5-HTR₁B, 5-HTR₁D, 5-HTR₁E, and 5-HTR₁F. 5-HTR₁A interacts with several G proteins, eliciting different responses [5]. 5-HTR₁B and 5-HTR₁D are coupled to formation of inositol phosphates through interaction with pertussis toxin–sensitive Gᵢ/ₒ and pertussis toxin–insensitive Gq proteins [6]. The G protein–coupled 5-HTR₂ includes three different subtypes: 5-HTR₂A, 5-HTR₂B, and 5-HTR₂C [7]. The 5-HTR₃ is a ligand-gated cation channel triggering depolarization of the plasma membrane through activation of Na⁺ and K⁺ fluxes [8]. The 5-

HTR₄ receptor has two splice variants (5-HTR₄a and 5-HTR₄b) [9]. The heptahelical 5-HTR₅ subtype is less characterized [10]. 5-HTR₆ and 5-HTR₇ are linked to Gs protein–mediated stimulation of adenylyl cyclase [11,12].

Apart from its well-characterized function as a neurotransmitter, 5-HT has been reported to be a potent immunoregulator: Stimulation of serotoninergic receptors in human monocytes or lung epithelial cells is associated with secretion of pro-inflammatory cytokines [13,14]. Additionally, 5-HT plays a prominent role in T-cell activation and in the interaction between T-cells and dendritic cells [15,16]. Furthermore, 5-HT has chemotactic activity for eosinophils and mast cells [17,18]. Consistently, elevated serotonin levels have been detected in inflammatory diseases such as bronchial asthma where a strong correlation between disease severity and free plasma serotonin has been found [19]. Methysergid, an antagonist at 5-HTR₂ receptors, was able to inhibit airway inflammation and remodelling in an animal model of asthma [20].

Dendritic cells (DCs) are the most potent antigen presenting cells, originating from hematopoietic stem cells [21,22]. Immature DCs

are specialized in capturing and taking up antigens. DC maturation can be induced by danger signals such as bacterial endotoxin [23,24], or pro-inflammatory cytokines, e.g. TNF-α. During maturation MHCII and co-stimulatory molecules on the cell surface are up-regulated and different cytokines and chemokines are produced. Mature DCs migrate to secondary lymphoid organs where they interact with naive T-cells, resulting in either Th1- or Th2-dominated immune responses [21]. DCs also produce several proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8 profoundly affecting the outcome of inflammatory reactions [16,25]. Furthermore DCs have been shown to be essential in the pathogenesis of inflammatory processes such as bronchial asthma [26–28]. Recently, we were able to demonstrate the functional expression of 5-HTR on human DCs, linked to intracellular $Ca^{2+}$-signalling and cytokine secretion [25]. However, the influence of 5-HT on migration, production of chemokines such as CXCL10/IP-10 or CCL22/MDC, and T-cell polarization capacity has not been investigated yet.

In this study, we demonstrated that 5-HT is a chemoattractant for immature but not LPS-matured DC in vitro and in vivo, via activation of $5\text{-HTR}_{1B}$ and $5\text{HTR}_2$ subtypes, while it modulate the secretion of IL-6, CXCL10, CCL22 and the T-cell polarization capacity of mature DC, via activation of $5\text{-HTR}_4$ and $5\text{-HTR}_7$ subtypes.

## Materials and Methods

### Reagents

5-Hydroxytryptamine (5-HT), N-Methyl-5-Hydroxytryptamine (2Me5HT, $5\text{-HTR}_3$ agonist), R-(-)-DOI-hydrochloride (DOI, $5\text{-HTR}_2$ agonist), Ketanserin ($5\text{-HT}_2$ antagonist), and LPS were obtained from Sigma-Aldrich (Deisenhofen, Germany). 5-Carbox-amidotryptamine maleate (5-CT, $5\text{-HTR}_1$ agonist), BRL-54443 ($5\text{-HT}_{1E/F}$ agonist), 8-Hydroxy-DPAT-hydrobromide (8-HDPAT, $5\text{-HTR}_{7/1A}$ agonist), GR 55562 ($5\text{-HT}_{1B}$ antagonist), Anpirtoline hydrochloride (AnHCL, $5\text{-HTR}_{1B}$ agonist), RS67333 hydrochloride ($5\text{-HT}_4$ agonist), RS39604 hydrochloride ($5\text{-HT}_4$ antagonist) and SB-269970 ($5\text{-HT}_7$ antagonist) hydrochloride were purchased from Tocris (Bristol, UK).

### Preparation of human DC

Peripheral mononuclear cells were separated from buffy coats using a Ficoll gradient. After separation, the leukocyte-containing pellet was resuspended in 2 ml PBS containing 0.15% EDTA and 0.5% BSA. Cells were separated with anti-CD14 mAb-coated MicroBeads using Macs single use separation columns from Miltenyi Biotec (Bergisch Gladbach, Germany). The $CD14^+$ cells were cultured for 5 days in RPMI 1640 medium containing 10% FCS, 1% glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin, 1,000 U/ml IL-4, and 10,000 U/ml GM-CSF (Promocell, Heidelberg, Germany) at 37°C in an humidified atmosphere with 5% $CO_2$. These cells were $CD14^{neg}$, $CD1a^{pos}$, $CD80^{low}$, $CD83^{low}$, $CD86^{low}$, and >95% $CD115^{high}$ and are also referred as immature DC. Maturation of DC was induced by incubation for 24 h in the presence of 3 μg/ml lipopolysaccharide (LPS; Sigma-Aldrich). Mature DC were >95% $CD80^{high}$, $CD86^{high}$ $CD83^{high}$ and $CD115^{low}$. Monoclonal Abs and their respective isotype controls were from Coulter-Immunotech, Krefeld, Germany.

### Migration assay

Experiments were performed in triplicate using 48-well Transwell chambers (Nucleopore, Tübingen, Germany). Buffer or stimuli were added into the lower compartment wells. Thereafter a 10 μm polycarbonate membrane with a pore size of 5 μm (Nucleopore) was placed over the wells. DC ($10^5$ 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 hematoxylin. Migrated DC were counted in 5 randomly chosen high-power (x400) 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.

### Cytokine assays

IL-10 and IL-12p70 were measured in DC supernatants by ELISA using matched pair of mAbs from BD PharMingen (San Diego, CA). Quantikine human TNF-α ELISA was from R&D Systems (Minneapolis, USA). CXCL10 and CCL17 were measured in DC supernatants by ELISA using matched pair of mAbs and ELISA kit from BD PharMingen and R&D Systems, respectively. Release of IFN-γ and IL-5 from T cells was detected using matched pairs of mAbs (BD PharMingen). Samples were assayed in triplicate for each condition.

### T cell differentiation assay

$CD4^+$ T lymphocytes were separated (>95% $CD4^+$ cells) from the heavy density fraction (50–60%) of Ficoll gradients (Amersham-Pharmacia Biotech) performed on PBMC, followed by immuno-magnetic depletion using a mixture of anti-HLA-DR, anti-CD19 and anti-CD8 mAb conjugated beads (Dynal, Oslo, Norway). Allogeneic naive T cells were purified (>95% $CD45RA^+$) by incubation of $CD4^+$ cells with anti-CD45RO mAb followed by a goat anti-mouse Ig coupled to immunomagnetic beads, and then co-cultured ($10^6$ cells/well) with DC ($5\times10^4$ cells/well) in a 24-well plate in 1640 RPMI medium supplemented with 5% human serum. After 5d IFN-γ, IL-5 and IL-13 release were determined by ELISA.

### Animal studies

**Mice.** BALB/c mice (6–8 weeks old) were purchased from Harlan (Zeist, The Netherlands). OVA-TCR transgenic mice (DO11.10) on a BALB/c background were bred at the animal facilities at the University Hospital Freiburg.

### Effect of 5-HT on the T-cell priming capacity of DCs

Ova-specific naive T cells ($1\times10^7$) purified from DO11.10 TCR transgenic mice were injected intravenously in the lateral tail vein of BALB/c mice (day 0). On day 2, the mice received an i.t. injection of $1\times10^6$ OVA-pulsed DCs, 5-HT/OVA-pulsed DCs, or control unpulsed DCs. On day 6, MLNs were collected and homogenized. For cytokine measurement, MLN cells were plated in round-bottom 96-well plates ($1\times10^6$ cells/ml in RPMI1640/5% FCS) and re-stimulated with OVA (10 mg/ml) for 4 days. IL-4, IL-5, IL-13, and IFN-γ were assayed in supernatants by ELISA (R&D Systems, Minneapolis, USA; eBioscience Inc., San Diego, CA).

### Effect of 5-HT on DC migration

To address migration of lung DCs 80 μl of FITC-OVA (10 mg/ml), with or without 5-HT (was administered intratracheally under direct vision through the opening vocal cords using a 18-gauge polyurethane catheter connected to the outlet of a micropipette as previously described (4). Control mice received 80 μl of PBS/DMSO. At 24–36 h after injection, migrating DCs were enumerated in the mediastinal LN as CD11c+MHCII+ cells carrying FITC+ material.

In all experiments, dead cells were excluded form analysis using propidium iodide. Analysis was performed on a FacsCalibur flow cytometer, using Cellquest and FlowJo software.
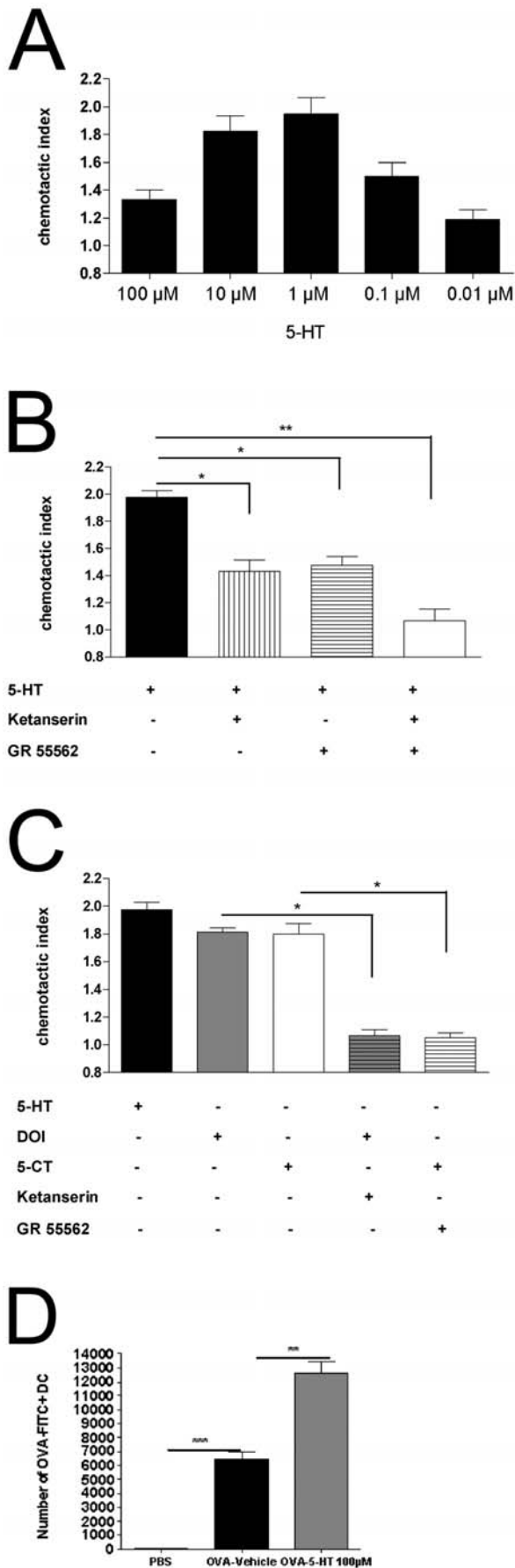
**Figure 1. 5-HT induces migration of immature DCs.** (A) DCs were exposed to the indicated concentrations of 5-HT for 90 min at 37°C in a Boyden chamber as described in material and methods. The chemotactic index was calculated. Data are means±SEM ($n = 5$). (B) Cells were pre-treated with the 5-HTR$_{1B}$ antagonist G55562, 5-HTR$_2$ antagonist ketanserin (both 100 nM) or vehicle. Migration in response to 5-HT (1 μM) was determined as described above and the chemotactic index was calculated as described above ([Fig. 1B]). Data are means±SEM ($n = 5$). (C) Cells were pre-treated with the 5-HTR$_{1B}$ antagonist G 55562, the 5-HTR$_2$ antagonist ketanserin (both 100 nM) or vehicle. Migration in response to 5-HT, the 5-HTR$_2$ agonist DOI (10 μM), and the 5-HTR$_1$ agonist 5-CT (10 μM) was determined and the chemotactic index was calculated as described above ([Fig. 1C]). Data are means±SEM ($n = 5$) *p<0.05, **p<0.01, ***p<0.001. (D) Mice were injected intratracheally with OVA-FITC (10 mg/ml) together with 5-HT (100 μM) or vehicle. One day later, the presence of OVA-FITC carrying CD11c$^+$MHCII$^+$ DCs in mediastinal lymph nodes was analyzed by flow cytometry.
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## Ethics statement

Experiments with human cells were reviewed and approved by the ethics committee of the University hospital Freiburg. Animal experiments were approved by the local animal ethics committee (Regierungspräsidium Freiburg) and performed according to the respective guidelines.

## Statistical analysis

For all experiments, the statistical significance of differences between samples was calculated using the ANOVA, Bonferroni comparison test. Differences were considered significant if p<0.05.

## Results

### 5-HT induces migration of immature but not mature DCs via activation of 5-HTR$_1$ and 5HTR$_2$ receptor subtypes

It has recently been reported that 5-HT is a potent chemoattractant for human mast cells and eosinophils, via activation of 5-HTR$_1$ or 5HTR$_2$ receptor subtypes respectively [17,18]. To investigate the ability of 5-HT to induce migration of DCs, immature and LPS-matured DCs were stimulated with increasing concentrations of 5-HT. As shown in Figure 1A 5-HT elicited a typical dose-dependent bell-shaped chemotactic response for immature DCs, while it failed to induce migration in LPS-matured DCs.

To determine the 5-HTR-subtypes involved in the 5-HT induced migration, immature DC where stimulated with 5-HT (1 μM) in the presence of the 5-HTR$_{1B}$ antagonist GR55562 (100 nM), 5-HTR$_2$ antagonist ketanserin or vehicle. Both antagonists alone were able to partially inhibit 5-HT mediated migration, while when applied in combination they completely abolished this response (Fig. 1B), suggesting the involvement of 5HTR$_{1B}$ and 5-HTR$_{2A}$ subtypes.

Stimulation with the 5-HTR$_1$ agonist 5-CT or the 5-HTR$_2$ agonist DOI also resulted in migration of immature DCs. This response was blocked by pre-incubation with GR55562 or ketanserin (Fig. 1C).

### 5-HT induces migration of DCs to mediastinal lymph nodes

To test whether 5-HT is also a chemotaxin for DCs in vivo, fluorescently labelled OVA was injected intratracheally together with 5-HT or vehicle. The transport of the large molecule FITC-OVA is an exclusive function of lung-derived DCs, being able to transport this complex across the epithelial tight junction barrier. The number of MHCII$^+$CD11c$^+$ DCs carrying fluorescent FITC-OVA cargo was enumerated in the MLNs 1 day after instillation,

dependent inhibition of CXCL10 production was observed (Fig. 2A), while the production of CCL22 was increased. In addition 5-HT also enhanced the secretion of CCL22 in immature DCs (Fig. 2B).

The proinflammatory cytokine IL-6 is involved in both acute inflammation and chronic tissue remodelling. As shown in Figure 3, 5-HT, the 5-HTR$_3$-agonist 2Me5-HT, the 5-HTR$_4$ agonist RS 67333 and the 5-HTR$_{7/1A}$ agonist 8-HDPAT increased IL-6 release of in maturing DCs.

IL-10 and IL-12p70 are cytokines playing a prominent role in T-cell differentiation [29]. Added together with LPS, 5-HT dose dependently inhibited the production of IL-12p70 (Fig. 4A), whereas IL-10 release was increased (Fig. 5A). This effect was mediated by 5-HTR$_4$ and 5-HTR$_7$ receptor subtypes, as preincubation of mDC with a combination of the 5-HTR$_4$ antagonist RS39604 and the 5-HTR$_7$ antagonist SB269970 completely abolished 5-HT induced modulation of IL-10 and IL-12p70 release (Fig. 4/5B). No significant effect of 5-HT on basal IL-10 and IL-12p70 secretion in iDCs could be observed (Fig. 4/5A).

## 5-HT influences the T-cell polarizing capacity of mDCs

Starting from our observation that activation of 5-HTR$_4$ and 5-HTR$_7$ subtypes inhibited the production of IL-12p70, which is an important factor influencing the differentiation of Th1 cells, we next analyzed the quality of primary T cell response induced by DC matured in the presence of different concentration of 5-HT. Naive CD4$^+$CD45RA$^+$ allogeneic T cells primed with mDC exposed to various concentration of 5-HT during maturation displayed an impaired Th1 and enhanced Th2 polarization, as determined by the increased production of IL-13 and IL-5 as well as down regulation of IFN-γ (Fig. 6). T cells stimulated with 5-HT pre-treated iDC showed an also higher IL-5 and IL-13 secretion (Fig. 6A/B).

## 5-HT induces Th2-priming in vivo

In vivo relevance of Th2-priming induced by 5-HT was investigated in an animal model: female BALB/c mice received a cohort of naive OVA-TCR Tg (DO11.10) T cells intravenously. Two
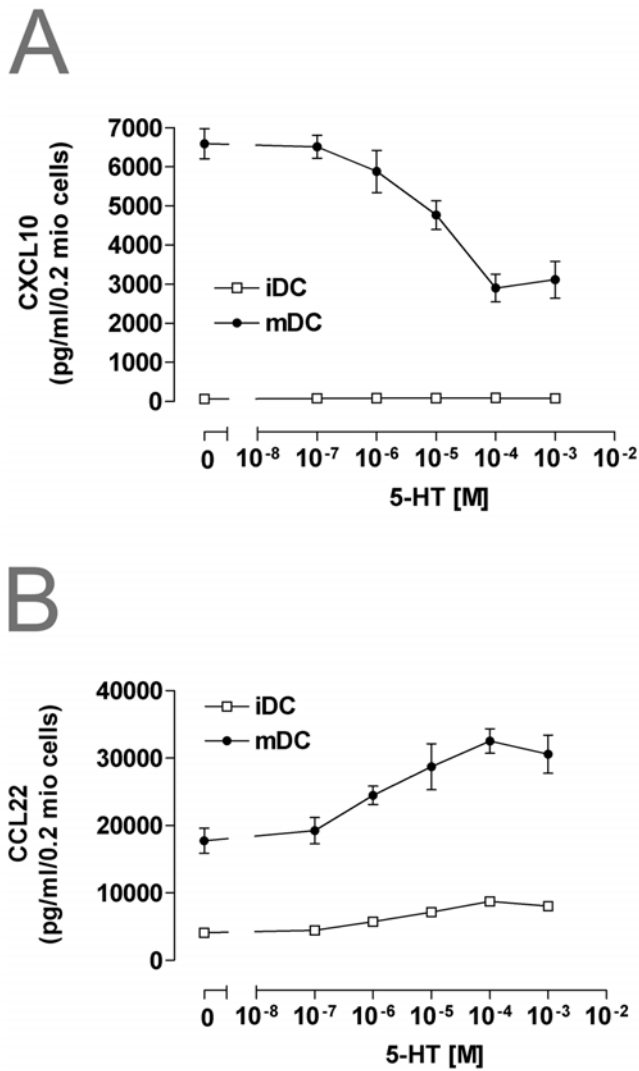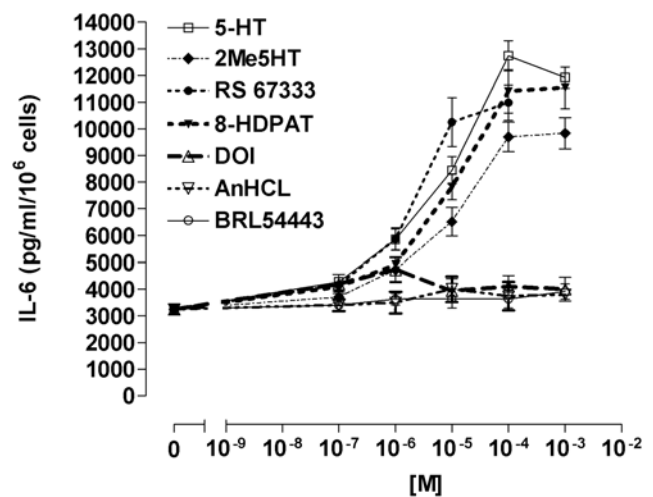
**Figure 2. 5-HT inhibits the release of IP 10/CXCL10 and stimulates MDC/CCL22 secretion in DCs.** Immature and mature DCs were left untreated or were stimulated with the indicated concentration of 5-HT for 24 h (A–B). CXCL10 and CCL22 release was measured by ELISA. The results are expressed as mean pg/ml±SEM (n = 4).
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and, as shown in Figure 1D, 5-HT markedly increased the number of DCs in mediastinal lymph nodes.

## 5-HT modulates cytokine and chemokine production in mDCs

We previously reported that 5-HT can enhance the production of the pro-inflammatory cytokines IL-8 and IL-1β via activation of 5-HTR$_3$, 5-HTR$_4$ and 5-HTR$_7$ subtypes in human monocyte-derived dendritic cells [25]. We therefore were interested if 5-HT is also able to influence secretion of IL-6, IP-10, MDC IL-10 or IL-12p70 in immature and mature DCs. Cells were stimulated for 24 h and cytokine content in the supernatants was analyzed by ELISA.

Immature DCs produce high levels of CCL22/MDC and very low levels of IP-10/CXCL10, whereas in LPS-matured DCs the secretion of both CCL22 and CXCL10 is up-regulated (Fig. 2). When DCs were treated with LPS in the presence of 5-HT, a dose



**Figure 3. Stimulation of serotoninergic receptors induces secretion of IL-6 in mature DCs.** Mature DCs were stimulated with the indicated concentrations of 5-HT or isotype receptor agonists. Supernatants were collected 24 h after stimulation and IL-6 concentration was measured by ELISA. Results are given as mean ± SEM (n = 4).
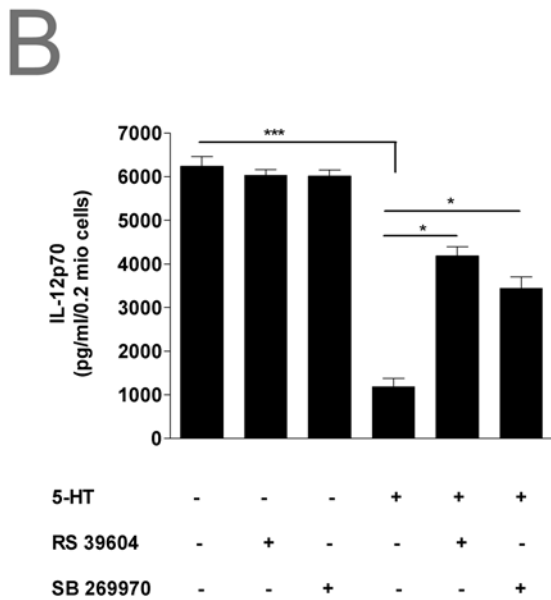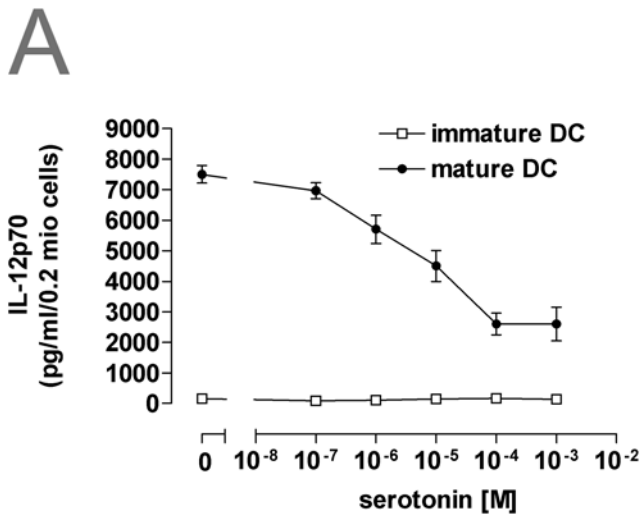doi:10.1371/journal.pone.0006453.g003

**Figure 4. 5-HT modulates IL-12p70 production of human monocyte-derived DCs.** (A) Immature and mature DCs were stimulated with the indicated concentration of 5-HT concentrations. Supernatants were collected 24 h after stimulation and cytokine content was measured by ELISA. Results are given as mean±SEM (n = 4). (B) Mature DCs were preincubated with $10^{-7}$ M of the selective 5-HTR$_4$ antagonist RS39604 or the 5-HTR$_7$ antagonist SB269970 30 min prior to stimulation with 5-HT. 24 hours later production of IL-12p70 was measured by ELISA. Data are means±SE (n = 5) *p<0.05, **p<0.01, ***p<0.001.
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**Figure 5. 5-HT modulates IL-10 production of human monocyte-derived DCs.** (A) Immature and mature DC were stimulated with the indicated concentration of 5-HT concentrations. Supernatants were collected 24 h after stimulation and IL-10 content measured by ELISA. Results are given as mean±SEM (n = 4). (B) Mature DCs were preincubated with $10^{-7}$ M of the selective 5-HTR$_4$ antagonist RS39604 or the 5-HTR$_7$ antagonist SB269970 30 min prior to stimulation with 5-HT. 24 hours later production of IL-10 was measured by ELISA. Data are means±SE (n = 5) *p<0.05, **p<0.01, ***p<0.001.
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days later, they were injected intratracheally with vehicle treated OVA-pulsed DCs, serotonin-treated OVA-pulsed DCs, or control unpulsed DCs. As shown in figure 6D, mediastinal lymph nodes cells of mice receiving serotonin-pulsed DCs produced higher amounts of IL-4, IL-5, and IL-13 whereas production of IFN-γ was inhibited.

## Discussion

5-HT is one of the major mediators secreted during platelet aggregation or following cross-linking of IgE antibodies on mast cells [2,3]. Moreover, increasing evidence suggests that 5-HT is involved in
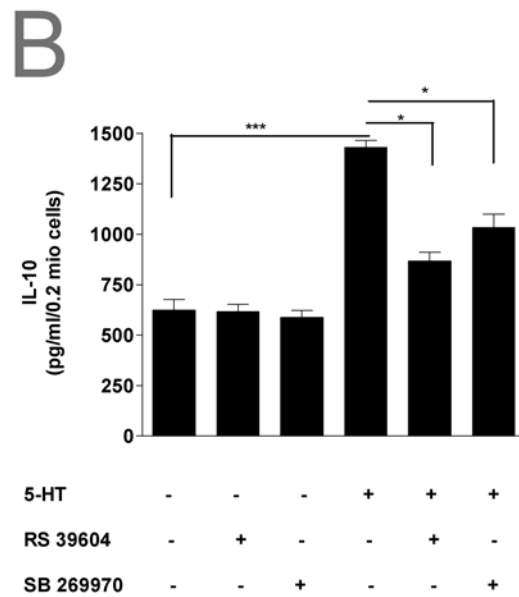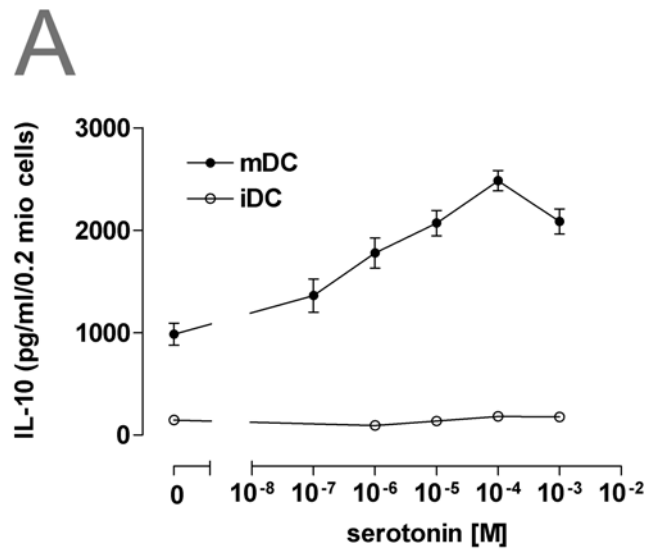
the pathogenesis of allergic asthma, as elevated 5-HT levels have been detected in blood and sputum samples of asthmatic patients compared to healthy individuals [19]. Additionally, a negative correlation between serum 5-HT levels and pulmonary function tests was found in asthmatics [19]. Treatment of asthmatic patients with the 5-HT reuptake accelerator tianeptine has been shown to lead to a clinical improvement [30–32]. Recently, the functional expression of different 5-HTR subtypes on human monocyte-derived dendritic cells has been reported [25]. In this study, we provide additional evidence that 5-HT is a powerful modulator of dendritic cell function thus being able to enhance allergic airway inflammation.
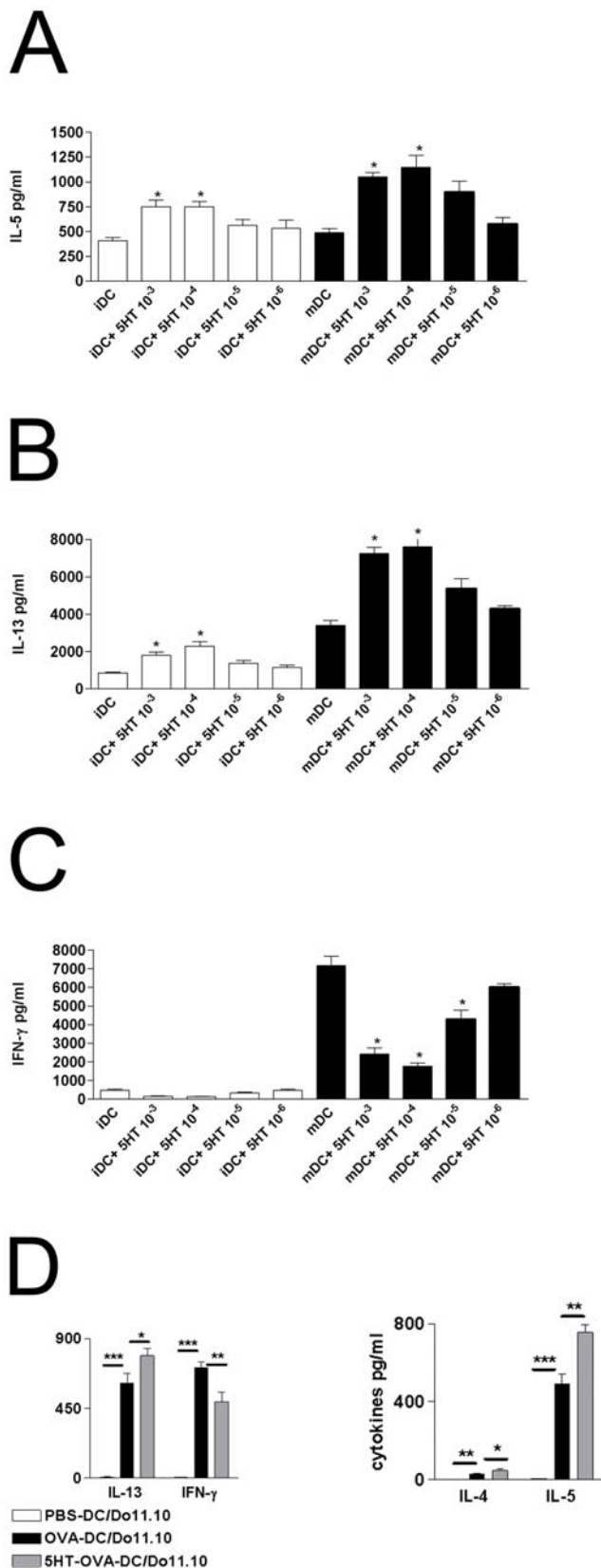
for 24 h. DCs were then used to prime purified allogeneic CD4+CD45RA+ naive T cells. After 5 days, supernatants from T cells were evaluated for the secretion of IL-5 (A), IL-13 (B) and IFN-γ (C). Results are expressed as mean pg/ml±SD (n=3). *p<0.05 between cytokines secreted by T cells stimulated with DC treated or not with 5-HT. (D) Naive mice received $10\times10^6$ DO11.10 CD4$^+$ T-cells intravenously on day-2. On day 0 mice were instilled intratracheally with $10^6$ OVA-pulsed, OVA-pulsed 5-HT treated DCs, or unpulsed DCs. On day4 mediastinal lymph node cells were collected and cultured for 4 days. Four days later, supernatants were harvested and analyzed for the presence of IL-4, IL-5, IL-13, and IFN-γ using commercially available ELISAs .

Thereby, we demonstrated for the first time that 5-HT is a direct chemo-attractant for immature but not mature human DCs. By using the 5-HTR$_{1B}$ antagonist GR 55562 and the 5-HTR$_{2A}$ antagonist ketanserin we could show that both receptor subtypes are involved in the 5-HT-induced migration of iDC. Interestingly, 5-HT has been reported to induce migration of human eosinophils [17] and human aortic smooth muscle cells [33] by selective activation of 5-HTR$_{2A}$-subtypes, while others reported that the receptor subtypes 5HTR$_{1B}$ and 5HTR$_{1A}$ were involved in 5-HT-mediated chemotaxis of human aortic endothelial cells [34] and mast cells [18]. However, during maturation DCs loose the ability to migrate in response to 5-HT. Similar to other well-described mediators of allergic inflammation such as histamine or nucleotides this might be a prerequisite for the departure of DCs to secondary lymphoid organs [35,36]. As shown by the increased number of DCs in mediastinal lymph nodes following administration of 5-HT, these findings are relevant in vivo. Therefore, 5-HT release during allergen challenge might be important for the recruitment of DCs to the site of inflammation.

Previous studies were able to demonstrate that 5-HT is a potent regulator of cytokine secretion in different kinds of cells. Here we show that 5-HT increase production of the pro-inflammatory cytokine IL-6 in mature DCs via 5-HTR$_3$, 5-HTR$_4$ and 5-HTR$_7$ subtypes. These findings are in accordance with previous studies conducted with human monocytes [14] and airway epithelial cells [13]. Additionally, via activation of 5-HTR$_4$ and 5-HTR$_7$ receptors, 5-HT up-regulated production of IL-10 by human LPS-matured DCs. Similar effects of 5-HT on IL-10 release have been observed in human LPS-treated monocytes [14], whereas no effect on IL-10 production has been seen in human peripheral blood mononuclear cells treated with 5-HT [37]. IL-12p70 is known as a cytokine favouring Th1-polarisation [38]. According to previous data of our group, IL-12p70 secretion was dose-dependently inhibited by 5-HT [25].

DCs are known to produce different chemokines thereby regulating the traffic of Th1 and Th2 cells into inflamed tissue [39]. Besides its influence on cytokine secretion, stimulation with 5-HT up-regulated production of MDC/CCL22 in both immature and mature DCs, while secretion of CXCL10 was inhibited in mature DCs. 5-HT is known to increase intracellular cAMP levels via activation of 5-HTR$_4$ and 5-HTR$_7$ receptors [25]. In accordance, cAMP-elevating agents, such as histamine or prostaglandins have similar effects on CCL22 and CXCL10 secretion [40,41]. CCL22 preferentially attracts Th2 cells, while CXCL10 is a key chemokine for the recruitment of Th1 cells, it can be assumed that DCs exposed to 5-HT (e.g. released during allergen challenge) preferably attract Th2 cells leading to a Th2-dominated microenvironment [42,43].

Recently Katoh et al reported that 5-HT reduced the capacity of immature DCs to activate allogeneic T-cells [44]. However, the effect of 5-HT on mature DCs has not been investigated in this

**Figure 6. 5-HT modulates T-cell priming capacity of human monocyte-derived DCs.** Immature DCs were left untreated or stimulated with 5-HT, or were induced to undergo maturation with LPS in the absence or the presence of indicated concentration of 5-HT

study. By using unselective 5-HTR$_{1/6/7}$ agonists and antagonists the authors suggest the involvement of 5-HTR$_{1E}$ and 5-HTR$_7$ in this cellular response. In contrast, we could show here, that DCs matured in the presence of 5-HT induced a Th2-polarisation in naive CD4$^+$CD45RA$^+$ T-cells, as demonstrated by downregulation of IFN-y and upregulation of IL-5/IL-13 production consistent with the capacity of 5-HT to increase IL-10 and to inhibit IL-12p70 secretion in maturing DCs. This in vitro observation could be confirmed in an animal model, where 5-HT favoured the outcome of a Th2-response in mediastinal lymph nodes.

DCs are essential for the induction and maintenance of allergic diseases such as bronchial asthma [28,45]. Here we provide additional evidence that 5-HT can influence human dendritic cell function in a pro-asthmatic way. 5-HT which is released following allergen challenge has chemotactic activity on immature but not mature DCs leading to accumulation of DCs at the site of inflammation. Under the influence of 5-HT DCs secrete larger amounts of IL-6, a cytokine important for airway-remodelling and mucus hypersecretion [46,47]. By increasing IL-10 and inhibiting IL-12p70 secretion 5-HT influences the T-cell priming capacity of DCs favouring the outcome of a Th2-response [22]. Additionally, up-regulation of CCL22 and down-regulation of CXCL10 release by 5-HT might lead to selective accumulation of Th2-cells into inflamed tissue [43].

In conclusion our data provide further evidence that 5-HT is involved in the pathogenesis of allergic diseases. Therefore, the use of specific 5-HTR antagonists targeting the function of DCs, might be a new therapeutic option in allergy treatment.

## Author Contributions

Conceived and designed the experiments: TM TD MI. Performed the experiments: TM TD MG EP. Analyzed the data: TM TD BB MG SC EP YH FDV DF JN. Contributed reagents/materials/analysis tools: FDV DF. Wrote the paper: TM TD BB SS JN MI.

## References

1. Cloez-Tayarani I, Changeux JP (2007) Nicotine and serotonin in immune regulation and inflammatory processes: a perspective. J Leukoc Biol 81: 599–606.

2. Kushnir-Sukhov NM, Brown JM, Wu Y, Kirshenbaum A, Metcalfe DD (2007) Human mast cells are capable of serotonin synthesis and release. J Allergy Clin Immunol 119: 498–499.

3. Yoshida A, Ohba M, Wu X, Sasano T, Nakamura M, et al. (2002) Accumulation of platelets in the lung and liver and their degranulation following antigen-challenge in sensitized mice. Br J Pharmacol 137: 146–152.

4. Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71: 533–554.

5. Hamblin MW, Guthrie CR, Kohen R, Heidmann DE (1998) Gs protein-coupled serotonin receptors: receptor isoforms and functional differences. Ann N Y Acad Sci 861: 31–37.

6. Wurch T, Pauwels PJ (2000) Coupling of canine serotonin 5-HT(1B) and 5-HT(1D) receptor subtypes to the formation of inositol phosphates by dual interactions with endogenous G(i/o) and recombinant G(alpha15) proteins. J Neurochem 75: 1180–1189.

7. Jerman JC, Brough SJ, Gager T, Wood M, Coldwell MC, et al. (2001) Pharmacological characterisation of human 5-HT2 receptor subtypes. Eur J Pharmacol 414: 23–30.

8. Jackson MB, Yakel JL (1995) The 5-HT3 receptor channel. Annu Rev Physiol 57: 447–468.

9. Pindon A, van Hecke G, van Gompel P, Lesage AS, Leysen JE, et al. (2002) Differences in signal transduction of two 5-HT4 receptor splice variants: compound specificity and dual coupling with Galphas- and Galphai/o-proteins. Mol Pharmacol 61: 85–96.

10. Matthes H, Boschert U, Amlaiky N, Grailhe R, Plassat JL, et al. (1993) Mouse 5-hydroxytryptamine5A and 5-hydroxytryptamine5B receptors define a new family of serotonin receptors: cloning, functional expression, and chromosomal localization. Mol Pharmacol 43: 313–319.

11. Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, et al. (1996) Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. J Neurochem 66: 47–56.

12. Ruat M, Traiffort E, Leurs R, Tardivel-Lacombe J, Diaz J, et al. (1993) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT7) activating cAMP formation. Proc Natl Acad Sci U S A 90: 8547–8551.

13. Bayer H, Muller T, Myrtek D, Sorichter S, Ziegenhagen M, et al. (2007) Serotoninergic receptors on human airway epithelial cells. Am J Respir Cell Mol Biol 36: 85–93.

14. Durk T, Panther E, Muller T, Sorichter S, Ferrari D, et al. (2005) 5-Hydroxytryptamine modulates cytokine and chemokine production in LPS-primed human monocytes via stimulation of different 5-HTR subtypes. Int Immunol 17: 599–606.

15. Leon-Ponte M, Ahern GP, O'Connell PJ (2007) Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. Blood 109: 3139–3146.

16. O'Connell PJ, Wang X, Leon-Ponte M, Griffiths C, Pingle SC, et al. (2006) A novel form of immune signaling revealed by transmission of the inflammatory mediator serotonin between dendritic cells and T cells. Blood 107: 1010–1017.

17. Boehme SA, Lio FM, Sikora L, Pandit TS, Lavrador K, et al. (2004) Cutting edge: serotonin is a chemotactic factor for eosinophils and functions additively with eotaxin. J Immunol 173: 3599–3603.

18. Kushnir-Sukhov NM, Gilfillan AM, Coleman JW, Brown JM, Bruening S, et al. (2006) 5-hydroxytryptamine induces mast cell adhesion and migration. J Immunol 177: 6422–6432.

19. Lechin F, van der Dijs B, Orozco B, Lechin M, Lechin AE (1996) Increased levels of free serotonin in plasma of symptomatic asthmatic patients. Ann Allergy Asthma Immunol 77: 245–253.

20. Lima C, Souza VM, Soares AL, Macedo MS, Tavares-de-Lima W, et al. (2007) Interference of methysergide, a specific 5-hydroxytryptamine receptor antagonist, with airway chronic allergic inflammation and remodelling in a murine model of asthma. Clin Exp Allergy 37: 723–734.

21. Lanzavecchia A, Sallusto F (2000) Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science 290: 92–97.

22. Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392: 245–252.

23. Rescigno M, Granucci F, Citterio S, Foti M, Ricciardi-Castagnoli P (1999) Coordinated events during bacteria-induced DC maturation. Immunol Today 20: 200–203.

24. Sparwasser T, Koch ES, Vabulas RM, Heeg K, Lipford GB, et al. (1998) Bacterial DNA and immunostimulatory CpG oligonucleotides trigger maturation and activation of murine dendritic cells. Eur J Immunol 28: 2045–2054.

25. Idzko M, Panther E, Stratz C, Muller T, Bayer H, et al. (2004) The serotoninergic receptors of human dendritic cells: identification and coupling to cytokine release. J Immunol 172: 6011–6019.

26. Oz-Arslan D, Ruscher W, Myrtek D, Ziemer M, Jin Y, et al. (2006) IL-6 and IL-8 release is mediated via multiple signaling pathways after stimulating dendritic cells with lysophospholipids. J Leukoc Biol 80: 287–297.

27. Idzko M, Hammad H, van Nimwegen M, Kool M, Vos N, et al. (2007) Inhaled iloprost suppresses the cardinal features of asthma via inhibition of airway dendritic cell function. J Clin Invest 117: 464–472.

28. van Rijt LS, Jung S, Kleinjan A, Vos N, Willart M, et al. (2005) In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. J Exp Med 201: 981–991.

29. Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, et al. (1998) The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu Rev Immunol 16: 495–521.

30. Lechin F, van der Dijs B, Orozco B, Jara H, Rada I, et al. (1998) Neuropharmacologic treatment of bronchial asthma with the antidepressant tianeptine: a double-blind, crossover placebo-controlled study. Clin Pharmacol Ther 64: 223–232.

31. Lechin F, van der Dijs B, Orozco B, Jara H, Rada I, et al. (1998) The serotonin uptake-enhancing drug tianeptine suppresses asthmatic symptoms in children: a double-blind, crossover, placebo-controlled study. J Clin Pharmacol 38: 918–925.

32. Lechin F, van der Dijs B, Lechin AE (2004) Treatment of bronchial asthma with tianeptine. Methods Find Exp Clin Pharmacol 26: 697–701.

33. Matsusaka S, Wakabayashi I (2005) 5-Hydroxytryptamine augments migration of human aortic smooth muscle cells through activation of RhoA and ERK. Biochem Biophys Res Commun 337: 916–921.

34. Matsusaka S, Wakabayashi I (2005) 5-Hydroxytryptamine as a potent migration enhancer of human aortic endothelial cells. FEBS Lett 579: 6721–6725.

35. Idzko M, Dichmann S, Ferrari D, Di Virgilio F, la Sala A, et al. (2002) Nucleotides induce chemotaxis and actin polymerization in immature but not mature human dendritic cells via activation of pertussis toxin-sensitive P2y receptors. Blood 100: 925–932.

36. Idzko M, la Sala A, Ferrari D, Panther E, Herouy Y, et al. (2002) Expression and function of histamine receptors in human monocyte-derived dendritic cells. J Allergy Clin Immunol 109: 839–846.

37. Cloez-Tayarani I, Petit-Bertron AF, Venters HD, Cavaillon JM (2003) Differential effect of serotonin on cytokine production in lipopolysaccharide-

stimulated human peripheral blood mononuclear cells: involvement of 5-hydroxytryptamine2A receptors. Int Immunol 15: 233–240.

38. Theiner G, Gessner A, Lutz MB (2006) The mast cell mediator PGD2 suppresses IL-12 release by dendritic cells leading to Th2 polarized immune responses in vivo. Immunobiology 211: 463–472.

39. Moser B, Loetscher P (2001) Lymphocyte traffic control by chemokines. Nat Immunol 2: 123–128.

40. McIlroy A, Caron G, Blanchard S, Fremaux I, Duluc D, et al. (2006) Histamine and prostaglandin E up-regulate the production of Th2-attracting chemokines (CCL17 and CCL22) and down-regulate IFN-gamma-induced CXCL10 production by immature human dendritic cells. Immunology 117: 507–516.

41. Kuroda E, Sugiura T, Okada K, Zeki K, Yamashita U (2001) Prostaglandin E2 up-regulates macrophage-derived chemokine production but suppresses IFN-inducible protein-10 production by APC. J Immunol 166: 1650–1658.

42. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 187: 875–883.

43. la Sala A, Sebastiani S, Ferrari D, Di Virgilio F, Idzko M, et al. (2002) Dendritic cells exposed to extracellular adenosine triphosphate acquire the migratory properties of mature cells and show a reduced capacity to attract type 1 T lymphocytes. Blood 99: 1715–1722.

44. Katoh N, Soga F, Nara T, Tamagawa-Mineoka R, Nin M, et al. (2006) Effect of serotonin on the differentiation of human monocytes into dendritic cells. Clin Exp Immunol 146: 354–361.

45. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, et al. (2007) Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. Nat Med 13: 913–919.

46. Kuhn C 3rd, Homer RJ, Zhu Z, Ward N, Flavell RA, et al. (2000) Airway hyperresponsiveness and airway obstruction in transgenic mice. Morphologic correlates in mice overexpressing interleukin (IL)-11 and IL-6 in the lung. Am J Respir Cell Mol Biol 22: 289–295.

47. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, et al. (2003) Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. J Biol Chem 278: 17036–17043.