

Plasmacytoid dendritic cells in allergic asthma and the role of inhaled corticosteroid treatment

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Summary

Background Plasmacytoid dendritic cells (pDCs) infiltrate sites of acute Th2-dominant inflammation, but their role in allergic asthma remains unclear.

Objective To characterize circulating pDCs from patients with allergic asthma outside their respective allergen season.

Methods Adhesion molecules, co-stimulatory molecules, immunoglobulin receptors and chemokine receptors were quantified on blood pDCs from 20 patients with allergic asthma and 18 healthy controls using flow cytometry. In addition, IL-6-, TNF- α - and IFN- α -secretion were analysed after stimulating isolated pDCs with TLR7- and TLR9-ligands.

Results Plasmacytoid dendritic cells from patients with allergic asthma showed an increased expression of chemokine receptors involved in inflamed tissue homing such as CCR2, CCR4, CCR9, CCR10, CXCR2, CXCR5 and CXCR6, while the expression of the lymph node homing receptor CXCR3 was down-regulated. In addition, these pDCs exhibited a higher expression of activation markers and Th2-associated molecules such as CD40, CD62L, CD64 and Fc ϵ RI α . In contrast, TLR7-mediated IL-6-, TNF- α - and IFN- α -secretion was significantly reduced in pDCs from patients with asthma. The TLR9-mediated cytokine response was only suppressed in those patients who were treated with inhaled corticosteroids (ICS) during previous allergen seasons. The same effect was observed for CD54 and OX40L expression.

Conclusions We report an increased expression of activation markers, and Th2-associated molecules, and an increased migratory potential of circulating pDCs in allergic asthma. These changes are accompanied by a reduced TLR7-mediated cytokine response. In addition, our results suggest a longterm impact of ICS treatment on the characteristics of circulating pDCs.

Keywords asthma, chemokine receptor, inhaled corticosteroids, plasmacytoid dendritic cells, toll-like receptor

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Introduction

Plasmacytoid dendritic cells (pDCs) are essential players in innate immunity and are increasingly recognized for their capacity to modify antigen-driven T cell responses. They represent a small population in peripheral blood (about 0.2% of total leukocytes) and are characterized by high expression of the IL-3 receptor CD123 and HLA-DR in the absence of lineage markers (CD3, CD14, CD16, CD19, CD20, and CD56) [1].

The most prominent role described for pDCs is the initiation of Th1 immune responses in an IFN- α -dependent manner after recognition of viral or bacterial anti-

gens through toll-like receptors (TLR) 7 and 9 respectively [2–5]. In contrast, their role in Th2 immune responses is still in dispute. They infiltrate sites of acute Th2-dominant inflammation such as the airways of patients with allergen-induced bronchial asthma or nasal allergy but they are rarely being detected at sites of chronic allergic conditions [6–8]. Murine models suggest that pDCs prevent Th2-cytokine production and allergic inflammation [9, 10], whereas human pDCs have been reported to induce Th2 differentiation of naïve CD4⁺ T cells [11, 12]. In addition, human pDCs which have been differentiated under Th2-like conditions *in vitro* showed an increased IL-4 expression after

TLR7-ligation, suggesting a more pro-inflammatory role for pDCs in Th2-dominant immune responses [13].

Concentrations of blood pDCs are increased in patients suffering from bronchial asthma and atopic diseases [1, 14, 15]. After segmental allergen challenge pDC numbers in peripheral blood decrease while an increase was observed in the challenged lung segment [16, 17] suggesting a migration from blood to inflamed tissue. In addition, there is evidence for functional differences between blood pDCs of atopic patients and healthy controls: while IL-4 suppresses the IL-3 induced survival of pDCs from healthy subjects, IL-4 enhances the survival of pDCs from atopic patients [14].

This study was designed to systematically analyse the pre-activation state of circulating pDCs from patients with allergic asthma and healthy controls, using a quantification of function-associated molecules and of TLR-mediated cytokine responses.

Methods

Subjects

A total of 20 asymptomatic patients with a physician's diagnosis of mild persistent allergic asthma as well as 18 non-atopic controls participated in this study (asthmatics: 9 women, 11 men, 26 ± 6 years; controls: 12 women, 6 men, 26 ± 3 years). All patients and controls were non-smokers. Atopy was confirmed by the UniCAP sx1-test (Pharmacia Diagnostics, Freiburg, Germany) against common inhaled allergens, while all non-atopic controls showed a negative signal in this test. Median total serum IgE levels were 11.6 kU/l (range < 2 –62 kU/l) for controls and 213 kU/l (range 14–2467 kU/l) in patients with asthma. Eight of 20 patients with allergic asthma have been treated with inhaled corticosteroids (ICS) during previous allergen seasons. The median duration of ICS treatment was 7 years (range 2–31 years), but only during the patients specific allergen seasons. ICS had been withdrawn by these eight patients for 2–4 months (median 3 months) prior to participation in this study because their specific allergen season was over and there were no allergen-induced symptoms during the time of the study. Patient characteristics are shown in Table 1. All patients gave their written informed consent and the study protocol was approved by the local ethics committee (HV 02/2007). All patients were examined outside of their respective allergen season. Accordingly, all anti-asthmatic medication had been stopped by the patients for 2–4 months due to the absence of allergen-induced symptoms.

Antibodies

Lineage Cocktail 1 fluorescein isothiocyanate, HLA-DR peridinin chlorophyll protein (PerCP) (clone L243),

Table 1. Patient characteristics

Gender	Age(years)	Total IgE (kU/l)	FEV ₁ baseline (l)	FEV ₁ (% pred)	Medication
m	32	203	6.2	124	-
w	25	793	4.6	119	BA
m	40	834	4.8	122	BA, CR
w	31	173	2.6	93	BA, CR
w	23	2467	3.8	108	BA
m	22	1133	4.4	91	BA, CR
w	24	98	4.4	129	-
w	21	179	3.6	104	OAH
w	19	498	2.9	78	BA, OAH
m	21	420	3.8	95	BA, ICS
w	28	164	2.8	87	BA
m	26	205	3.7	79	BA, ICS
m	27	687	5.2	114	-
w	29	217	3.0	95	BA
m	38	123	4.7	106	BA, ICS
m	24	434	4.9	107	BA, ICS
m	21	209	5.3	106	BA, ICS
w	21	14	3.2	100	BA, ICS
m	26	247	4.1	87	BA, ICS
m	27	105	3.2	71	BA, ICS
Mean	26	213*	4.0	102	
SD	6		1.0	15	

BA, beta-agonist; CR cromoglycate; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; OAH, oral antihistamines; SD, standard deviation. All anti-asthmatic medication was at least withdrawn for 2 months.

*Median value.

CD11a allophycocyanin (APC) (clone HI111), CD18 APC (clone 6.7), CD23 APC (clone EBVCS-5), CD32 APC (clone FL18.26), CD54 APC (clone HA58), OX40L phycoerythrin (PE) (clone Ik-1), CCR5 APC (clone 2D7), CXCR1 APC (clone 5A12), CXCR3 APC (clone 1C6), CXCR4 APC (clone 12G5) all from BD Biosciences (Heidelberg, Germany). CD40 APC (clone HB14), CD64 APC (clone 10.1), CD83 APC (clone HB15e), CD86 APC (clone BU63), CD62L PE (clone Dreg-56) all from Invitrogen (Karlsruhe, Germany). CD11b PE (clone M1/70.15.11.5), CD123 PE, APC (clone AC145), BDCA-3 APC (clone AD5-14H12) all from Miltenyi Biotec (Bergisch-Gladbach, Germany). CCR1 PE (clone 53504), CCR2 APC (clone 48607), CCR3 APC (clone 61828), CCR4 APC (clone 205410), CCR6 APC (clone 53103), CCR7 APC (clone 150503), CCR8 APC (clone 191704), CCR9 APC (clone 112509), CCR10 APC (clone 314305), CXCR2 APC (clone 48311), CXCR5 APC (clone 51505), CXCR6 APC (clone 56811), CXCR7 APC (clone 11G8), TRAIL PE (clone 75402) all from R&D Systems (Wiesbaden, Germany). CD80 APC (clone MEM-233), Granzyme B PE (clone GB11) all from ImmunoTools (Friesoythe, Germany). FcεRIα APC (clone AER-37), TLR9 PE (clone eB72-1665) all from eBioscience (San Diego, CA, USA).

Flow cytometry

Flow cytometric detection of surface antigens on pDCs was performed as described [6]. Briefly, freshly collected ethylenediaminetetraacetic acid blood was incubated with the respective antibodies for 20 min. Afterwards, FACS lysing solution (BD Biosciences) was added for another 10 min. Cells were centrifuged for 5 min (400 g) and washed with phosphate-buffered saline (PBS) containing 2% fetal calf serum and 0.1% NaN₃. Finally, cells were resuspended in PBS and analysed on a FACS Calibur using Cell Quest Pro software (both BD Biosciences).

Intracellular staining of Granzyme B and TLR9 was performed as follows. Following incubation with antibodies against surface molecules, cells were fixed with paraformaldehyde (4% in PBS) for 10 min and then permeabilized using 0.1% saponin (in PBS + 2% fetal calf serum). After staining with antibodies against intracellular antigens for 20 min, cells were washed with permeabilization buffer, resuspended in PBS and analysed by flow cytometry using a FACS Calibur and Cell Quest Pro software.

At least 100 PDCs were identified as lineage-negative HLA-DR⁺ CD123⁺ cells among total blood leucocytes. For antigens which are expressed only on a subpopulation of pDCs the percentage of positive pDCs is denoted. For antigens which are expressed on the whole pDC population the net median fluorescence intensity (MFI) was calculated (MFI antigen – MFI control) to show differences in the expression intensity between asthmatics and controls (Fig. 1).

Isolation and stimulation of Plasmacytoid dendritic cells

Plasmacytoid dendritic cells were isolated using the Plasmacytoid Dendritic Cell Isolation Kit (Miltenyi, Bergisch-Gladbach, Germany). Only pDC-isolates with a purity > 90% were used for further experiments. PDCs could be isolated from 13 healthy controls and 15 patients with asthma. PDC isolation from the other participants failed due to low pDC numbers in peripheral blood or unsatisfactory purity. In three patients with asthma, the numbers of isolated pDCs were too low for both, TLR7 and TLR9 stimulation experiments. Therefore, pDCs from these three patients were only incubated with imiquimod (TLR7-agonist). The pDCs were cultured at 10⁶ cells/ml in RPMI-1640 medium (PAA Laboratories, Pasching, Austria) containing 10% fetal calf serum (Invitrogen, Karlsruhe, Germany) and 1 µM ODN 2216 (InvivoGen, San Diego, CA, USA) or 5 µg/ml Imiquimod (InvivoGen), respectively, for 16 hours. IL-6, TNF-α (ImmunoTools, Friesoythe, Germany) and IFN-α (PBL Biomedical Laboratories, Piscataway, NJ, USA)

were measured in culture supernatants using specific ELISAs as described by the manufacturer.

Statistical analysis

Data were analysed using SPSS 15.0 software (Chicago, IL, USA). The Mann–Whitney *U*-test was performed for the comparison of groups. Probability values of *P* < 0.05 were regarded as significant. Box plots show the median (line within the box), interquartile range (edges of the box), and range of values less distant than 1.5 interquartile ranges from the upper or lower quartile (vertical lines).

Results

Expression of adhesion molecules

No differences were observed between the percentages of peripheral blood pDCs between patients with allergic asthma (A; median 0.19%, range 0.07–0.33%) and healthy controls (HC; median 0.17%, range 0.08–0.30%). There was no detectable expression of CD11b and CD106 (data not shown) and no differences were observed for the expression of CD11a and CD18 by pDCs from allergic asthmatics and healthy controls (Fig. 2a). In contrast, CD62L was significantly increased on pDCs from patients with asthma (A: median MFI 1689, HC: median MFI 1322).

Expression of T cell costimulatory molecules

CD40 expression was significantly increased on pDCs from patients with asthma (A: median 68.2%, HC: median 50.0%), although no differences were observed for CD83 expression. For CD86, there was only a non-significant trend for an increased expression by pDCs from patients with asthma (A: median 9.0%, HC: median 5.3%) (Fig. 2b). CD80 expression could not be detected at significant levels on pDCs from both groups.

Expression of Fc receptors

There was only a trend to an increased expression of the IgG receptor CD32 on pDCs from asthmatics (A: median 3.5%, HC: median 0.5%), while the expression of the IgG receptor CD64 was significantly increased on pDCs from asthmatics (A: median 12.7%, HC: median 5.5%) (Fig. 2c). The low-affinity IgE receptor CD23 could not be detected on pDCs in both groups, whereas the α-chain of the high-affinity IgE receptor (FcεRIα) was significantly increased on pDCs from asthmatics (A: median 93.6%, HC: median 58.5%) (Fig. 2c).

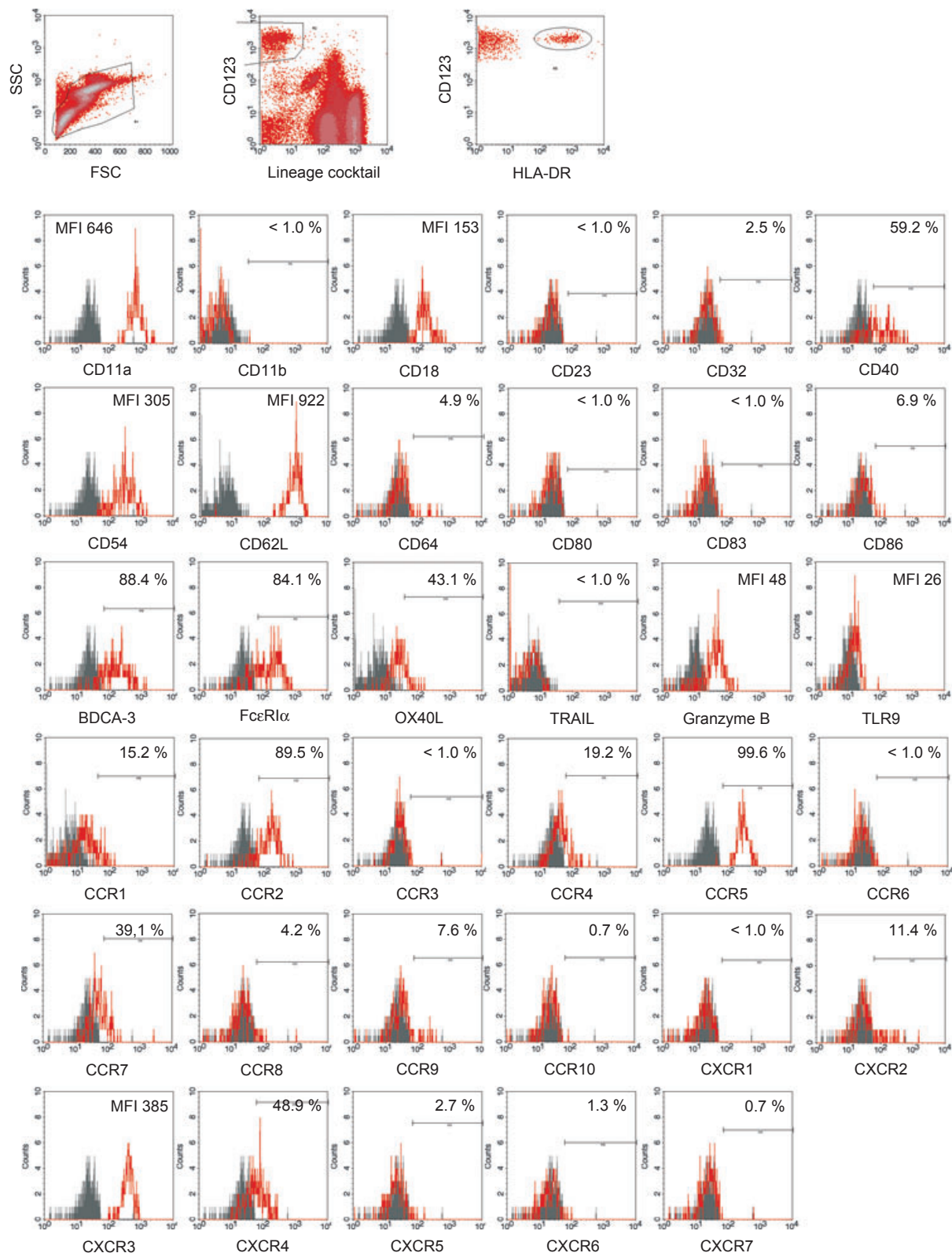


Fig. 1. Density plots show the identification strategy of plasmacytoid dendritic cells (pDCs). Total cells were selected followed by gating of lineage-negative CD123⁺ cells. Among the latter, pDCs were identified as HLA-DR⁺ cells. Histogram plots show specific antigen staining on pDCs (red line) compared with a respective isotype control (grey). Percentages of positive cells or median fluorescence intensity values are presented from one healthy volunteer. The following fluorochromes were used: fluorescein isothiocyanate for the lineage cocktail, PerCP for HLA-DR, phycoerythrin (PE) or allophycocyanin (APC) for CD123, and PE or APC for the respective marker antibody.

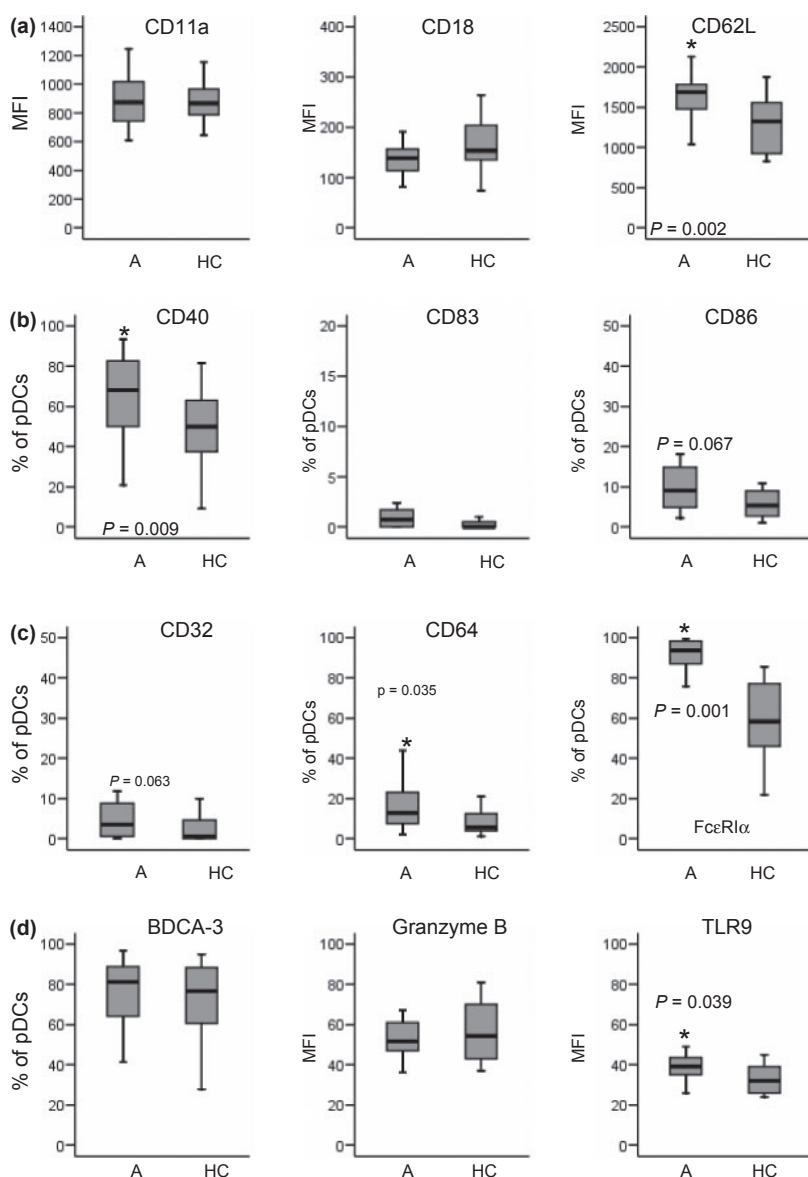


Fig. 2. Antigen expression by circulating plasmacytoid dendritic cells (pDCs) from 20 allergic asthmatics and 18 healthy controls. PDCs were identified as lineage-negative HLA-DR⁺ CD123⁺ cells and antigen expression was quantified in histogram plots as described in fig. 1. (a) adhesion molecules. (b) co-stimulatory molecules. (c) immunoglobulin receptors. (d) BDCA-3 and intracellular antigens. *P*-values < 0.05 were regarded as significant.

Expression of BDCA-3, TLR9 and cytotoxic molecules

There was no significant difference regarding the expression of BDCA-3 and granzyme B by pDCs from both groups, while the expression of TLR9 was significantly up-regulated by pDCs from patients with asthma (A: median MFI 39, HC: median MFI 32) (Fig. 2d). TRAIL expression was not detected on pDCs from both groups.

Expression of chemokine receptors

There was no significant expression of the chemokine receptors CCR3, CCR6 and CXCR1 on peripheral blood pDCs. No differences were found among CCR1, CCR5 and CXCR7 expression. A significant decrease of CXCR3, however, was observed on pDCs from allergic

asthmatics compared to healthy controls (A: median MFI 223, HC: median MFI 298). In addition, there was a trend for a lower expression of CCR7 on pDCs from asthmatic patients (A: median 21.1%, HC: median 40.1%). In contrast, the following chemokine receptors were significantly elevated on pDCs from asthmatics: CCR2 (A: median 97.4%, HC: median 91.0%), CCR4 (A: median 77.2%, HC: median 59.0%), CCR9 (A: median 41.7%, HC: median 12.9%), CCR10 (A: median 13.3%, HC: median 1.7%), CXCR2 (A: median 51.3%, HC: median 27.3%), CXCR5 (A: median 25.0%, HC: median 11.0%) and CXCR6 (A: median 15.5%, HC: median 6.4%). In addition, there was a trend to an increased expression of CCR8 (A: median 14.4%, HC: median 4.6%) as well as CXCR4 (A: median 77.2%, HC: median 62.6%) on pDCs from asthmatics (Fig. 3).

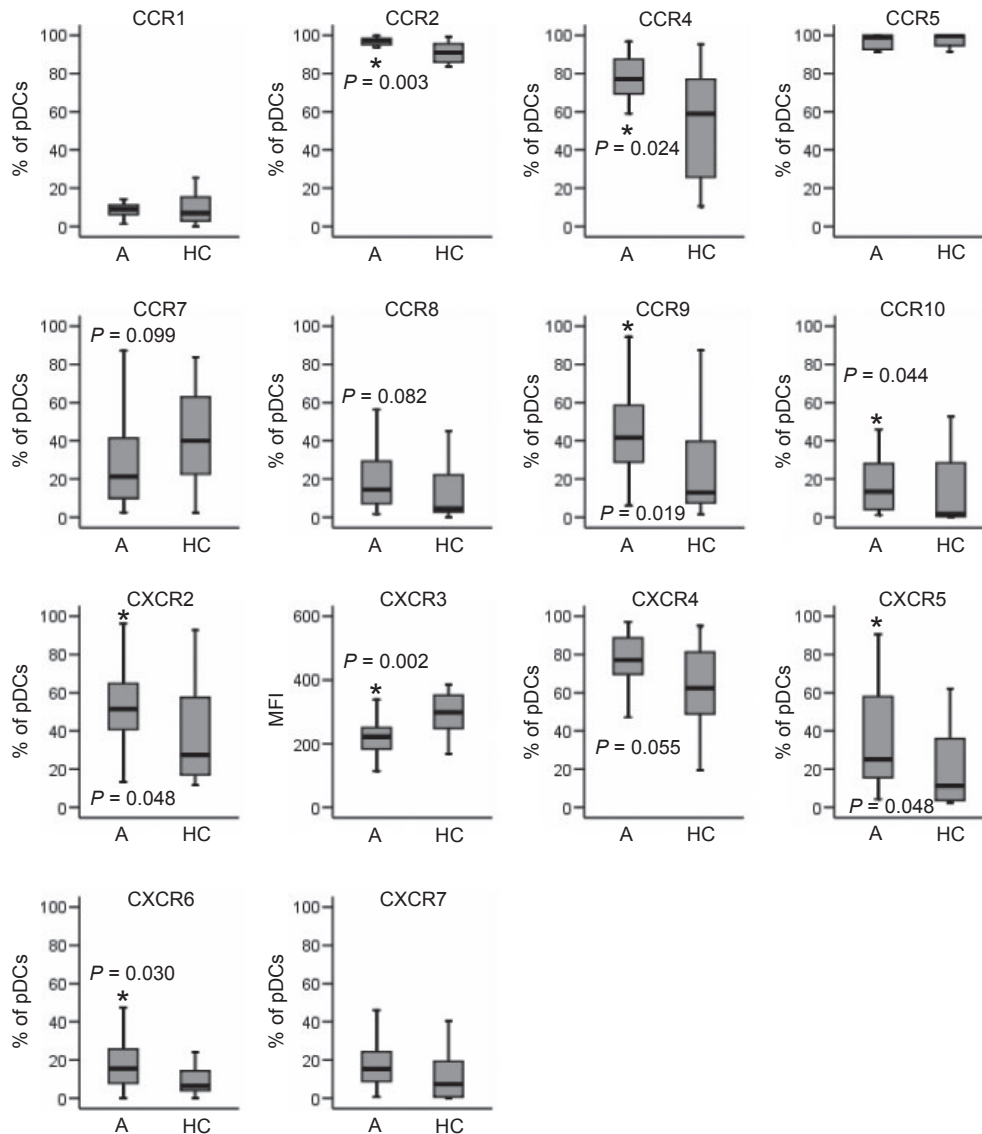


Fig. 3. Chemokine receptor expression by circulating plasmacytoid dendritic cells (pDCs) from 20 allergic asthmatics (A) and 18 healthy controls (HC). pDCs were identified as Lineage-negative HLA-DR⁺ CD123⁺ cells and antigen expression was quantified in histogram plots as described in fig. 1. *P*-values < 0.05 were regarded as significant.

The TLR7-mediated cytokine response is impaired in pDCs from allergic asthmatic patients

After ligation of TLR7 with imiquimod, pDCs from asthmatics showed a significant decrease in IL-6 (A: median 0.7 ng/ml, HC: median 1.2 ng/ml), TNF- α (A: median 3.2 ng/ml, HC: median 5.7 ng/ml), and IFN- α (A: median 43.7 ng/ml, HC: median 82.6 ng/ml) secretion compared to pDCs from healthy controls (Fig. 4).

Impact of treatment with inhaled corticosteroids

The medication status of the patients showed only a significant impact on the expression of the molecules

CD54 and OX40L. CD54 was significantly decreased on pDCs from patients who had been using ICS (median MFI 189) in the past compared with those who had not been exposed to ICS (median MFI 254) during allergen exposure in their respective allergen season and healthy controls (median MFI 328). The same phenomenon was observed for the T cell co-stimulatory molecule OX40L (A with ICS: median 15.5%, A without ICS: median 30.7%, HC: median 26.1%) (Fig. 5a).

TLR9-mediated IL-6 secretion by pDCs of ICS-treated patients (median 0.5 ng/ml) was significantly reduced compared with ICS naive patients (median 1.1 ng/ml) and healthy controls (median 1.5 ng/ml). Similar observations were made for IFN- α (A with ICS: median 0.2 μ g/ml, A without ICS: median 1.2 μ g/ml, HC: med-

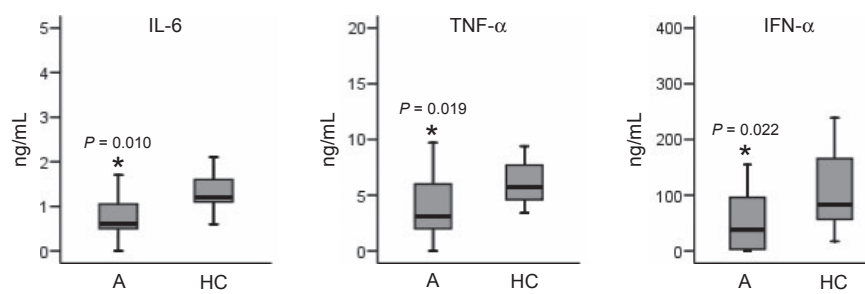


Fig. 4. The TLR7-mediated cytokine response by circulating pDCs is decreased in allergic asthma. pDCs from 15 patients with allergic asthma (A) and 13 healthy controls (HC) were isolated and stimulated with imiquimod for 16 hours. Concentrations of IL-6, TNF- α , and IFN- α were determined using specific ELISAs. *P*-values < 0.05 were regarded as significant.

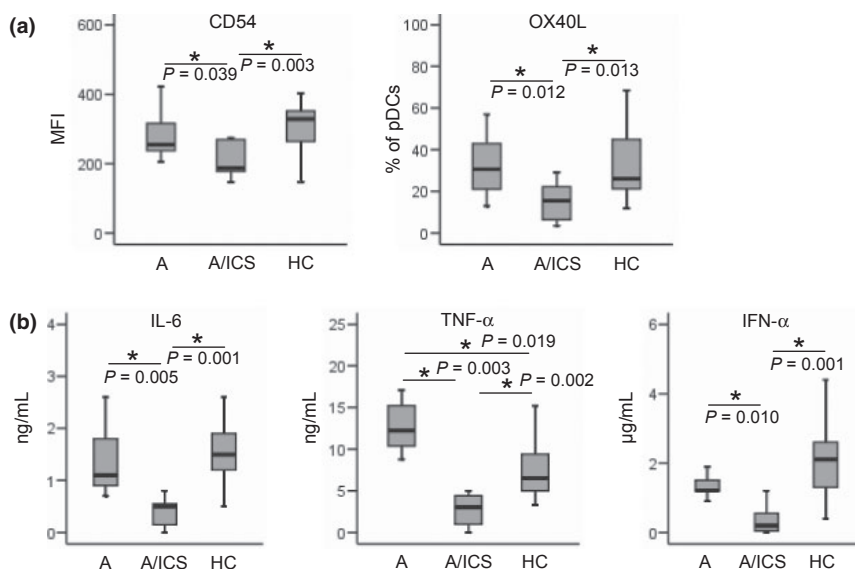


Fig. 5. Impact of inhaled corticosteroid (ICS) treatment on antigen expression and TLR9-mediated cytokine response by plasmacytoid dendritic cells (pDCs). (a) CD54 and OX40L expression by pDCs from 20 allergic asthmatics (A/ICS: treated with ICS *n* = 8, A: not treated with ICS *n* = 12) and 18 healthy controls. (b) Cytokine secretion by pDCs from 12 patients with allergic asthma (A/ICS: treated with ICS *n* = 7, A: not treated with ICS *n* = 5) and 13 healthy controls after stimulation with ODN2216. *P*-values < 0.05 were regarded as significant.

ian 2.1 $\mu\text{g}/\text{mL}$). Interestingly, TNF- α secretion was significantly elevated in ICS naïve asthmatics compared with healthy controls (A without ICS: median 12.2 ng/mL, HC: median 6.5 ng/mL) while patients treated with ICS in the past showed a decreased TNF- α secretion (median 3.0 ng/mL) (Fig. 5b). In contrast, TLR7-mediated cytokine release was not influenced by the use of inhaled corticosteroids. *P*-values for the comparison of ICS-treated asthmatics and ICS-naïve asthmatics were 0.536 (IL-6 release), 0.281 (TNF- α release) and 0.336 (IFN- α release).

Discussion

In this study, circulating pDCs from asymptomatic patients with allergic asthma (who were examined outside of their respective allergen season) were analysed to elucidate potential differences in the pre-activation status of naïve pDCs compared to healthy controls.

One of the main findings of this study is an up-regulation of tissue homing chemokine receptors on circulating pDCs from patients with allergic asthma. There was a significant increase in the expression of CCR4 and CCR10, which are involved in the homing of

immune cells into the skin and inflamed epithelia [18, 19]. CCR9, which has been described as a homing receptor for pDCs to the small intestine was also up-regulated on pDCs from allergic asthmatics [20]. In addition, the receptors for pro-inflammatory chemokines CCL2 (CCR2), CXCL5 (CXCR2), CXCL13 (CXCR5), and CXCL16 (CXCR6), which are released into the lungs of asthmatic patients are up-regulated on pDCs from asthmatic patients [21–24]. In contrast, CXCR3, which has been suggested as a lymph node homing receptor for pDCs is down-regulated in allergic asthma [25]. Furthermore, the well-known lymph node homing receptor CCR7 shows a tendency for down-regulation in allergic asthma. Together, the expression profile of chemokine receptors suggests that circulating pDCs in allergic asthma have an elevated potential to migrate to inflamed tissues rather than to lymph nodes. Interestingly, the skin and gut homing receptors CCR4 and CCR9, respectively, are also elevated on pDCs from allergic asthmatics. This may reflect the systemic course of allergic disease because many patients with allergic asthma also suffer from allergic symptoms in the skin and intestine. In addition, CCR4 has been shown to play a key role in Th2 cell recruitment into the human air-

ways [26]. So the increased expression of CCR4 on pDCs from asthmatic patients might also reflect a higher lung migratory potential of these pDCs. CCR9 has been shown to define tolerogenic pDCs [27], which suggests that the pDC compartment from asthmatic patients exhibits a higher tolerogenic potential.

Circulating pDCs from patients with allergic asthma showed an increased expression of the α -chain of the high-affinity IgE receptor, which is in line with the results of previous studies [28, 29] and which reflects an elevated Th2-pro-inflammatory potential of circulating pDCs in allergic asthma. This is underlined by the increased expression of CD62L on pDCs from asthmatic patients as L-selectin has been reported to be essential for Th2-dominant inflammation in a murine model of contact hypersensitivity [30].

Furthermore, the elevated expression of T cell costimulatory antigens CD40 (significant) and CD86 (trend) as well as IgG receptors CD64 (significant) and CD32 (trend) suggests a more activated state of circulating pDCs in patients with allergic asthma. The elevated expression of CD40 was unexpected because a suppression of CD40 on pDCs has been previously reported in *in vitro* generated Th2 conditions [13]. *In vivo*, about 57% of endobronchial pDCs expressed CD40 after segmental allergen challenge [6] suggesting that certain *in vivo* stimuli override this *in vitro* effect. In the absence of Th1-stimuli, such as viruses, pDCs preferentially secrete Th2 cell-attracting chemokines after stimulation with CD40L [31]. Accordingly, the increased expression of CD40 might also reflect an elevated Th2-pro-inflammatory potential of circulating pDCs in allergic asthma.

Plasmacytoid dendritic cells can recognize RNA-viruses by TLR7 and then initiate a Th1 immune response by secreting pro-inflammatory cytokines such as IFN- α [5]. In line with a previous study, we show a reduced secretion of IFN- α by circulating pDCs from patients with allergic asthma [29]. In addition, we report a decreased secretion of the pro-inflammatory cytokines IL-6 and TNF- α by pDCs from asthmatics. These findings suggest a reduced potential of the circulating pDCs pool of asthmatic patients to initiate anti-viral immune responses by TLR7. This is in line with the findings of Roponen et al. who demonstrated a reduced TLR7 function in adolescents with asthma [32].

There was an increase in TLR9 expression in patients with allergic asthma, which might reflect a stronger response of these pDCs to bacterial or viral DNA. Of note, this TLR9 up-regulation does not appear to be a general phenomenon in patients with allergies. Patients with allergic rhinitis have been reported to have an unchanged TLR9 expression on blood pDCs [28], whereas patients with hymenoptera venom allergy even display a decreased TLR9 expression on blood pDCs

[33]. Thus, the increase in TLR9 expression might rather be related to the presence of asthma than to the presence of allergies.

In contrast to TLR7-mediated cytokine secretion, TLR9-mediated cytokine secretion was significantly suppressed only in patients with allergic asthma who had been treated with ICS during previous allergen seasons. It is currently not clear if this observation is due to previous ICS treatment or a more severe form of asthma. Tversky et al. also reported a suppressed IFN- α release by pDCs after TLR9 activation, but most of their patients were also treated with topical steroids [28]. Prednisolone, which can impact on pDC concentrations in general [34], suppresses TLR9-mediated IFN- α secretion in pDCs *in vitro* [35]. In addition, IFN- α secretion by circulating pDCs is suppressed in patients receiving oral corticosteroids [36]. In the latter, IFN- α reached normal levels within days after withdrawal of oral corticosteroids, while in our study ICS had already been withdrawn for at least 2 months. This discrepancy is not easily explained and is unlikely to be due to the different corticosteroid derivatives used for oral and topical therapy.

In addition, OX40L and CD54 were significantly suppressed in those patients with seasonal ICS treatment. For dexamethasone, mometasone furoate and budesonide, CD54 suppression on dendritic cells, lung fibroblasts and an epithelial cell line has already been demonstrated *in vitro* [37–39]. At present, there is little if any clinical correlate for this finding, but the hypothesis that previous ICS treatment might have the potential for a long-lasting change in the expression of pDC-derived cytokines and surface molecules requires further investigation. Taken together, these findings suggest that previous ICS treatment can have an impact on blood pDC function. The issue whether this observation is due to a prolonged immunomodulatory effect of ICS on systemic pDCs or indirectly reflecting more severe forms of asthma is still open.

In conclusion, we report an increased activation state of circulating pDCs and an increased expression of Th2-associated molecules, accompanied by a reduced TLR7-mediated cytokine response in patients with mild allergic asthma. The chemokine receptor expression profile of circulating pDCs in asthma suggests a higher potential for migration into inflamed tissues and a reduced potential for lymph node homing compared to healthy controls. But the role pDCs play in allergic inflammation remains still unclear. Murine models suggest a tolerogenic function of pDCs [9, 10], which is in line with our finding of an increased percentage of CCR9⁺ pDCs in asthma patients. In addition, virus or CpG-oligonucleotide-stimulated pDCs are able to induce regulatory T cells [40, 41], which underlines their anti-inflammatory properties, but to date it is not clear

if allergen-stimulated pDCs are also capable of inducing regulatory T cells. Other studies using human cells show that pDCs can induce Th2 immune responses [11, 12] and are able to secrete IL-4, especially under Th2 conditions [13], which suggests a more pro-inflammatory role for pDCs in allergic inflammation. This might be reflected by the increased expression of the high-affinity IgE receptor, CD40 and CD62L by pDCs from asthmatic patients. So it might be possible that different pDC populations exist, which exhibit different functions in allergic inflammation.

Furthermore, we report a reduced TLR9-mediated cytokine response as well as lower CD54 and OX40L

expression on circulating pDCs in patients previously treated with ICS. This finding might reflect a long-term immunomodulatory effect of ICS on blood pDCs, and requires further investigation.

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Conflict of interest: The authors have no conflict of interest to declare.

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