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Differential development of plasmacytoid dendritic cells in Th1- and Th2-like cytokine milieus

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Abstract

Background: Plasmacytoid dendritic cells (pDCs) infiltrate sites of Th1- and Th2-dominant inflammation and many studies have been performed to analyse their role in these immune responses. In contrast, much less is known about the effects of a Th1 or Th2 cytokine milieu on pDC function. Therefore, we investigated the impact of Th1- and Th2-like conditions during the development of pDCs on their antigen expression and function.
Methods: PDCs were matured *in vitro* by the addition of IL-3 under Th1- or Th2-like conditions. Antigen expression and TLR7-ligand-induced cytokine secretion was analysed by flow cytometry and ELISA. Furthermore, the $CD4^+$ T-cell polarizing capacity of pDCs was determined as well as their potential to induce $CD4^+$ T-cell proliferation.
Results: PDCs matured under Th1-like conditions showed a higher expression of antigens involved in T-cell co-stimulation and antigen presentation like CD40, CD80, CD83 and HLA-DR as well as a higher secretion of IL-6 and IFN-α in response to TLR7-ligation compared to Th2-pDCs. Furthermore, Th1-pDCs induced a significantly higher $CD4^+$ T-cell proliferation and primed a higher percentage of $CD4^+$ T cells to express IFN-γ and IL-2 after TLR7-ligation compared to Th2-pDCs. In contrast, Th2-pDCs were characterized by a significant upregulation of BDCA-3 and IL-4 expression following TLR7-ligation.
Conclusion: This study is the first to demonstrate the crucial impact of a surrounding cytokine environment on the development of pDC function including antigen expression. Based on these findings, it can be speculated that antiviral/bacterial pDC functions could be impaired during acute allergic conditions.

Plasmacytoid dendritic cells (pDCs) represent a small cell population in peripheral blood (about 0.2% of leucocytes) and are characterized by high expression levels of CD123 and HLA-DR in the absence of 'lineage' markers (CD3, CD14, CD16, CD19, CD20 and CD56) (1).

They infiltrate into sites of acute Th1- and Th2-dominant inflammation and can be found in high numbers in the airways of patients suffering from bacterial pneumonia and allergen-induced bronchial asthma or nasal allergy (2–4). In contrast, pDCs could rarely be detected at sites of chronic allergic conditions (5).

After recognition of viral and/or bacterial antigens through toll-like receptors (TLR) 7 and 9, pDCs initiate Th1 immune responses in an IFN-α-dependent manner (6–9). In contrast, their role in Th2 immune responses is not clear. PDCs have been described to induce Th2 differentiation from naive $CD4^+$ T cells (10, 11). In addition, in murine models, it has been described that pDCs prevent the production of Th2-cytokines and allergic inflammation to harmless inhaled antigens (12, 13). Furthermore, they have been shown to play a role in immune tolerance by inducing regulatory $CD4^+$ T-cell subsets (14, 15).

Most studies that focused on pDCs have analysed factors which are required to prime pDCs to trigger Th1- or Th2-dominant immune responses, but less is known about how Th1- or Th2-derived mediators can influence pDC function. One study described a reduced expression of TLR9 and a reduced release of IFN-α after CpG-oligonucleotide stimulation by pDCs following FcεRI crosslinking. In addition, there was a downregulation of FcεRI after stimulation with

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CpG-oligonucleotides (16). Furthermore, circulating pDCs stimulated with CpG-oligonucleotides secrete lower amounts of IFN-α following segmental allergen challenge (17). Histamine that is secreted by basophils and mast cells in allergy also has been shown to inhibit IFN-α release by pDCs (18).

The present study was designed to analyse the impact of Th1- and Th2-like cytokine milieus on the development and $CD4^+$ T-cell stimulatory capacity of pDCs. Therefore, pDCs were matured *in vitro* with IL-3 under Th1- or Th2-like conditions, and the expression of maturation-dependent antigens as well as functional antigens was analysed by flow cytometry. In addition, the TLR7-ligand-induced cytokine release by pDCs was measured as well as their potential to induce $CD4^+$ T-cell proliferation and cytokine expression.

Material and methods

Reagents

IL-3, IL-4, IL-12 and IFN-γ were from ImmunoTools (Friesoythe, Germany) and Imiquimod from InvivoGen (San Diego, CA, USA). Anti-IL-4 (clone 3007), anti-IL-12 (clone 24910), anti-IFN-γ (clone 25718), anti-CD3 (clone UCHT1) and anti-CD28 (clone 37407) were from R&D Systems (Wiesbaden, Germany). 5- (and 6-) carboxyfluorescein diacetate succinimydyl ester (CFSE) was from Invitrogen (Karlsruhe, Germany), and Brefeldin A, saponin, as well as propidium iodide were from Sigma (Seelze, Germany). For antibodies used in flow cytometry, see Table 1.

Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly collected EDTA-anticoagulated blood obtained from healthy volunteers using a Biocoll gradient with a density of 1.077 g/ml (Biochrom, Berlin, Germany) according to standard procedures. All subjects gave their written informed consent, and the study protocol was approved by the local ethics committee. PDCs were isolated using the Plasmacytoid Dendritic Cell Isolation Kit and $CD4^+$ T cells with the $CD4^+$ T cell Isolation Kit II (both from Miltenyi Biotec, Bergisch Gladbach, Germany) with a purity >90%.

Differentiation of pDCs

PBMCs (2×10^6 cells/ml) or isolated pDCs (1×10^6 cells/ml) were matured in the presence of IL-3 (10 ng/ml) for 16 h in RPMI-1640 (PAA Laboratories, Pasching, Austria) supplemented with 10% foetal calf serum (FCS) (Invitrogen) (9). To obtain a Th1-like cytokine milieu, the key Th1-cytokines IL-12 (10 ng/ml), IFN-γ (10 ng/ml) and a neutralizing antibody to the key Th2-cytokine IL-4 (10 μg/ml) were added. IL-4 (10 ng/ml), anti-IL-12 (10 μg/ml) and anti-IFN-γ (10 μg/ml) were added to obtain Th2-like conditions. Before co-incubation of differentiated pDCs and $CD4^+$ T cells, pDCs were washed three times with RPMI-1640 medium to prevent a spillover of polarizing cytokines.

Table 1 Antibodies used for flow cytometry

Antigen	Label	Clone	Company
Lineage Cocktail 1	FITC		BD Bioscience
CD3	APC	SK7	BD Bioscience
CD14	APC	MφP9	BD Bioscience
CD16	APC	3G8	Invitrogen
CD19	APC	SJ25C1	BD Bioscience
CD20	APC	L27	BD Bioscience
CD40	APC	HB14	Invitrogen
CD56	APC	NCAM16.2	BD Bioscience
CD80	APC	MEM-233	ImmunoTools
CD83	APC	HB15e	Invitrogen
CD86	APC	BU63	Invitrogen
OX40L	PE	IK-1	BD Bioscience
FcεRIα	APC	AER-37	eBioscience, San Diego, CA, USA
HLA-DR	PerCP	L243	BD Bioscience
BDCA-2	PE, APC	AC144	Miltenyi Biotec
BDCA-3	APC	AD5-14H12	Miltenyi Biotec
TLR9	PE	eB72-1665	eBioscience
IL-2	PE	MQ1-17H12	Invitrogen
IL-4	PE	8D4-8	BD Bioscience
IL-5	PE	TRFK5	BD Bioscience
IL-6	PE	MQ2-13A5	BD Bioscience
IL-10	PE	JES3-9D7	BD Bioscience
IL-12	PE	C11.5	BD Bioscience
IL-13	PE	JES10-5A2	BD Bioscience
TNF-α	PE	Mab11	BD Bioscience
IFN-γ	FITC	B27	BD Bioscience
IFN-α	FITC	MMHA-11	PBL Biomedical Laboratories

APC, allophycocyanin; BDCA, blood dendritic cell antigen; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; TLR, toll-like receptor.

Flow cytometric analysis of antigen expression by pDCs

PBMCs were harvested, washed with PBS (PAA Laboratories) and stained with antibodies against surface antigens for 20 min. Then, cells were washed with PBS + FCS (2%), resuspended in PBS and analysed on a FACS Calibur using Cell Quest Pro software (both BD Biosciences, Heidelberg, Germany). For intracellular staining of TLR9, cells were fixed with paraform (4% in PBS) for 10 min after staining of surface antigens and then permeabilized using 0.1% saponin (in PBS + 2% FCS). After staining with antibodies against intracellular antigens for 20 min, cells were washed with permeabilization buffer, resuspended in PBS and analysed by flow cytometry. Median fluorescence intensity (MFI) values were calculated for the whole pDC population by subtraction of the isotype control MFI from the marker MFI. An exception was made for HLA-DR values because HLA-DR is essential for the gating of pDCs in which no appropriate isotype control could be performed.

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Flow cytometric analysis of cytokine expression by pDCs

Differentiated PBMC cultures were stimulated with Imiquimod (5 μg/ml). After 1 h, Brefeldin A (10 μg/ml) was added and cells were cultured for another 2 h in the respective cytokine milieu. Then, cells were washed with PBS, and intracellular cytokine staining was performed as described (19).

Enzyme-linked immunosorbent assay (ELISA)

Isolated and differentiated pDCs were stimulated with Imiquimod (5 μg/ml) and incubated for an additional 8 h in the respective cytokine milieu. Then, cells were harvested and centrifuged for 5 min at 300 ***g***. Culture supernatants were analysed for IL-6 (ImmunoTools), TNF-α (ImmunoTools) and IFN-α (PBL Biomedical Laboratories, Piscataway NJ, USA) using specific ELISAs as described by the manufacturer.

Cell proliferation assay

Isolated $CD4^+$ T cells were resuspended in PBS containing 0.1% bovine serum albumin (Roth, Karlsruhe, Germany) at 1×10^6 cells/ml and then labelled at 37°C for 10 min with CFSE (1 μM). Labelling reactions were stopped with five volumes of ice-cold RPMI-1640 following 5-min incubation on ice and three washing steps with medium. CFSE-labelled $CD4^+$ T cells were cultured with differentiated pDCs in indicated pDC/$CD4^+$ T-cell ratios in the presence of Imiquimod (5 μg/ml) in RPMI-1640 containing 10% FCS for 7 days. Then, cells were harvested, washed with PBS + 2% FCS and stained with anti-human CD3 APC for 20 min. After washing, cells were resuspended in PBS containing propidium iodide (2 μg/ml) and analysed on a FACS Calibur using CELL QUEST PRO software (BD Biosciences, Heidelberg, Germany).

Flow cytometric analysis of pDC-induced cytokine expression by $CD4^+$ T cells

Isolated and differentiated pDCs were cultured with autologous $CD4^+$ T cells at a ratio of 1 : 2 in the presence of Imiquimod (5 μg/ml) in RPMI-1640 containing 10% FCS for 7 days. Then, cells were transferred to a 48-well culture plate precoated with anti-CD3 (10 μg/ml) and anti-CD28 (5 μg/ml). After 6 h, Brefeldin A (10 μg/ml) was added and cells were incubated for another 4 h. Cells were harvested, washed with PBS + 2% FCS and stained with anti-human CD3 APC for 20 min. Intracellular cytokine staining was performed as previously described (19).

Statistical analysis

Data were analysed using SPSS 15.0 (SPSS GmbH, München, Germany). Most parameters were asymmetrically distributed. Therefore, differences between groups were analysed using the Wilcoxon signed-rank test. An exception is made in Figure 4 because of the low number of three experiments. Cell proliferation data are presented as mean + standard deviation and were analysed using the two-sided *t*-test for paired samples. *P*-values < 0.05 were regarded as significant.

Results

Differential IL-3-induced antigen expression by pDCs in Th1- and Th2-like cytokine milieus

To analyse the impact of Th1- and Th2-like conditions on IL-3-driven antigen expression by pDCs, we isolated PBMCs (which contain pDCs) from peripheral blood and induced pDC differentiation by adding IL-3. Anti-IL-4, IFN-γ and IL-12 were used to induce a Th1-like milieu, while anti-IFN-γ, anti-IL-12 and IL-4 were added to induce a Th2-like milieu. PDCs were identified as lineage-negative $HLA\text{-}DR^+$ $BDCA\text{-}2^+$ cells, and antigen expression was quantified using flow cytometry (Figure 1).

CD40, CD83, CD86 and HLA-DR expression on pDCs was upregulated after culture in the presence of IL-3 compared to freshly isolated pDCs. Th1-like conditions further enhanced the expression of these antigens, which was statistically significant in the case of CD40, CD83 and HLA-DR. Th2-like conditions, on the other hand, significantly suppressed the IL-3-induced upregulation of CD40, CD83 and HLA-DR (Figure 2). CD80 was upregulated following the addition of IL-3 compared to freshly isolated pDCs and significantly suppressed in Th1- and Th2-like milieus but suppression was significantly more pronounced under Th2-like conditions (Figure 2). OX40L, which has been described to drive Th2-differentiation of T cells (10), was significantly suppressed by culture conditions compared to freshly isolated cells, but this suppression was significantly less pronounced in the Th2-like milieu (Figure 2).

BDCA-3 expression on dendritic cell subsets, which has been associated with Th2-dominant immune responses (20, 21), was significantly upregulated in the presence of IL-3. Th2-like conditions further enhanced BDCA-3 expression by pDCs, while Th1-like conditions significantly suppressed BDCA-3 expression (Figure 2). FcεRIα was significantly downregulated by culture conditions, and no differences were observed in different cytokine milieus (Figure 2). In contrast, toll-like receptor 9 (TLR9) was upregulated by culture conditions but this failed to reach statistical significance (Figure 2).

Differential TLR7-induced cytokine response by pDCs in Th1- and Th2-like cytokine milieus

To analyse the impact of Th1- and Th2-like conditions on TLR-ligand-induced cytokine responses by pDCs, we used the TLR7-ligand Imiquimod because stimulation with CpG-oligonucleotides interferes with the flow cytometric identification of pDCs among PBMCs.

After stimulation of differently matured pDCs (IL-3 only, IL-3 + Th1-like milieu, IL-3 + Th2-like milieu) with Imiquimod, cytokine-expressing pDCs were quantified using flow cytometry. Low amounts of IL-4 expressing pDCs could be detected in the presence of IL-3 (median 2.7%). Th2-like

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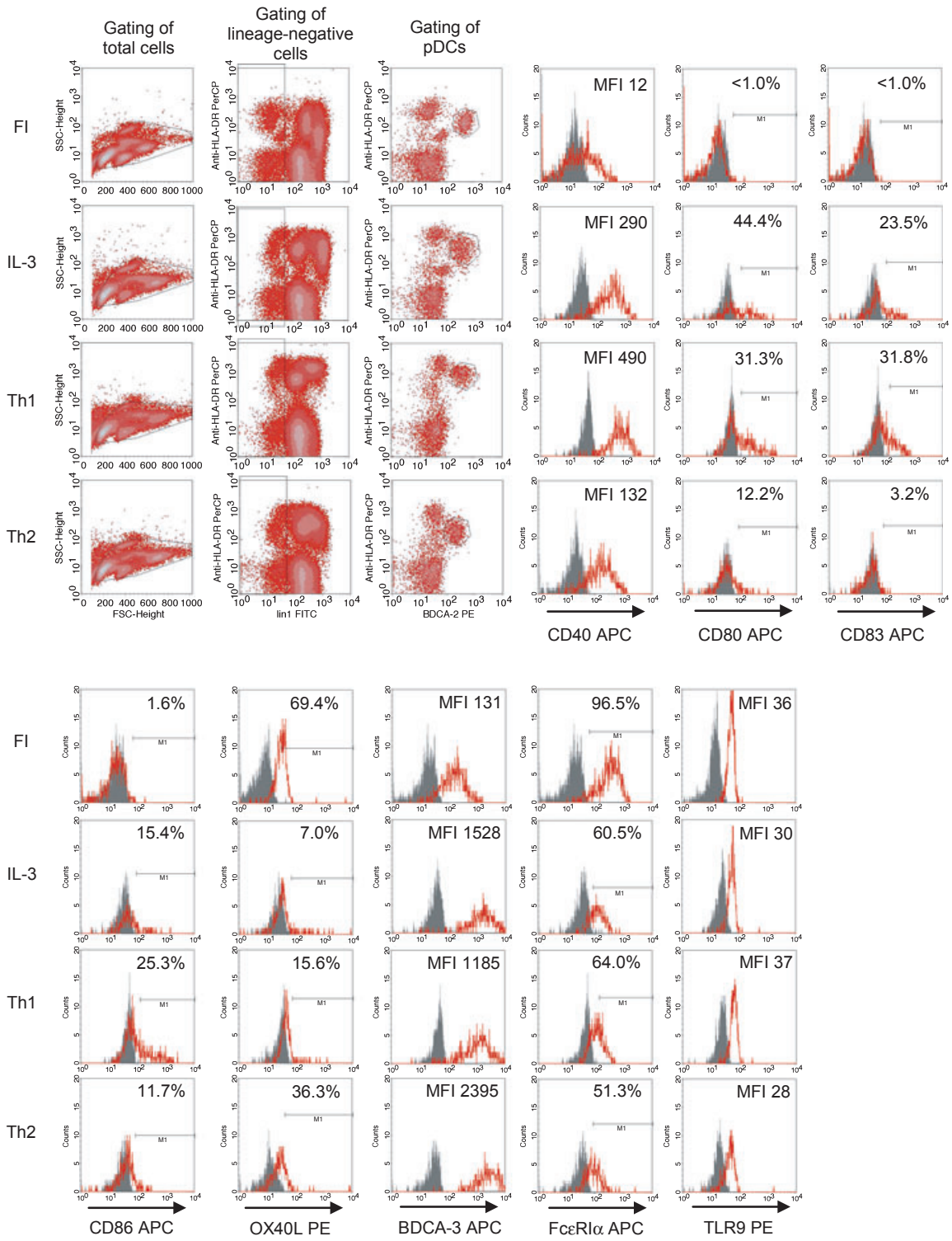

Figure 1 Gating of pDCs and determination of antigen expression. Density plots show the identification strategy of pDCs among PBMCs. Total cells were selected followed by gating of lineage-negative cells. Among the latter, pDCs were identified as HLA-DR$^+$ BDCA-2$^+$ cells. Histogram plots show specific antigen staining on pDCs (red line) compared with a respective isotype control (grey). Percentages of positive cells or median fluorescence intensity (MFI) values are presented. FI, freshly isolated PBMCs; IL-3, PBMCs stimulated with IL-3 only; Th1, PBMCs incubated in a Th1-like cytokine milieu; Th2, PBMCs incubated in a Th2-like cytokine milieu.

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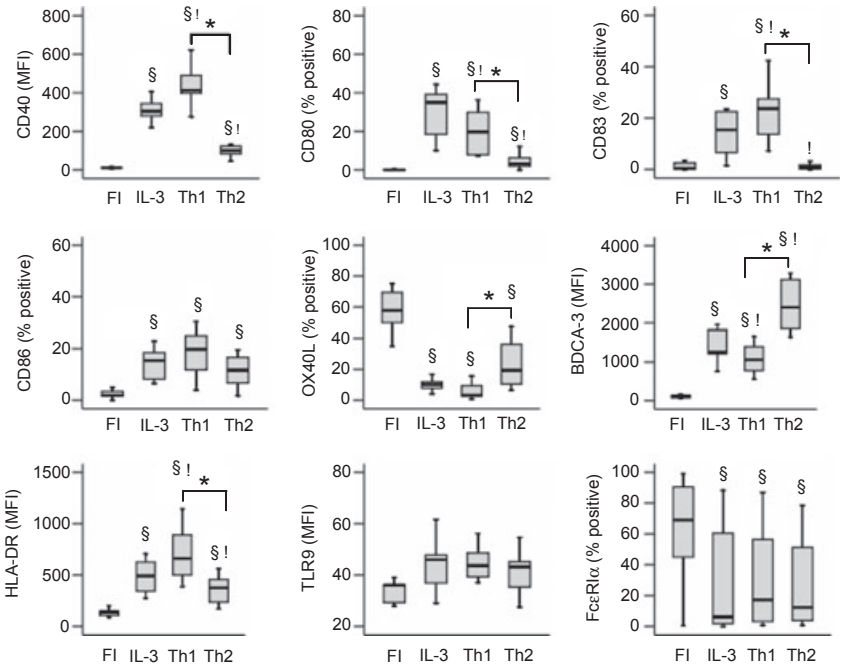

Figure 2 IL-3-induced antigen expression by pDCs is influenced by the surrounding cytokine milieu. PBMCs from nine different subjects were cultured in the presence of IL-3 (IL3), IL-3/anti-IL-4/IL-12/IFN-γ (Th1) or IL-3/IL-4/anti-IL-12/anti-IFN-γ (Th2) for 16 h. PDCs were identified as lineage-negative HLA-DR$^+$ BDCA-2$^+$ cells and analysed for antigen expression by flow cytometry. 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). Differences between groups were analysed using the Wilcoxon signed-rank test, and *P*-values < 0.05 were regarded as significant. §significant to freshly isolated cells (Fl); !significant to IL-3 only stimulated cells (IL3); *significance between Th1- and Th2-like conditions; Fl, freshly isolated cells; MFI, median fluorescence intensity.

culture conditions significantly enhanced the percentage of IL-4 expressing pDCs (median 5.6%), while they were significantly reduced in Th1-like conditions (median 0.6%) (Figure 3B). Equal percentages of pDCs expressed IL-6 in the presence of IL-3 (median 88.4%) and under Th1-like conditions (median 86.1%), while a significant reduction was observed under Th2-like conditions (median 65.9%) (Figure 3B). Isolated pDCs cultured under Th2-like conditions also secreted significantly lower amounts of IL-6 in response to Imiquimod compared to isolated pDCs cultured under Th1-like conditions (Figure 3C). In PBMC cultures, a median value of 50.5% of pDCs cultured only with IL-3 expressed TNF-α following Imiquimod stimulation. TNF-α expressing pDCs were slightly but significantly upregulated under Th2-like conditions (median 58.9%), while a strong upregulation was observed under Th1-like conditions (median 87.8%) (Figure 3B). In contrast, no difference concerning TNF-α secretion was detected in cultures of isolated pDCs (Figure 3C), suggesting an indirect effect of the added cytokines and antibodies on this finding in PBMC cultures. Following Imiquimod stimulation in PBMC cultures, equal percentages of IL-3-stimulated pDCs (median 28.3%) and Th2-pDCs (median 31.7%) expressed IFN-α, while IFN-α expression was significantly upregulated in pDCs under Th1-like conditions (median 60.3%) (Figure 3B). This finding was confirmed by measuring IFN-α in culture supernatants of isolated pDCs (Figure 3C). In PBMC cultures, no expression of IL-5, IL-10, IL-12 and IL-13 by pDCs following Imiquimod stimulation could be observed (data not shown).

Th2-like conditions suppress the capacity of pDCs to induce CD4$^+$ T-cell proliferation

To analyse the impact of Th1- and Th2-like conditions on the capacity of pDCs to induce CD4$^+$ T-cell proliferation, we used a flow cytometry–based proliferation assay. Differently matured pDCs (IL-3 + Th1-like milieu, IL-3 + Th2-like milieu) were co-cultured with autologous CFSE-labelled CD4$^+$ T cells in different ratios in the presence of the TLR7-ligand Imiquimod. After 7 days, CD4$^+$ T-cell proliferation was analysed.

While CD4$^+$ T cells alone showed no proliferation after 7 days, addition of pDCs induced a significant proliferation. CD4$^+$ T-cell proliferation induced by Th1-pDCs was higher compared to Th2-pDCs and reached statistical significance when a pDC/CD4$^+$ T-cell ratio of 1 : 2 was used (Figure 4).

Differential CD4$^+$ T-cell cytokine-inducing capacity of pDCs matured in TH1- and TH2-like cytokine milieus

Because the cytokine milieu that predominates during the maturation of pDCs significantly influences their ability to induce T-cell proliferation, we next investigated the impact of Th1- and Th2-like conditions on the capacity of pDCs to

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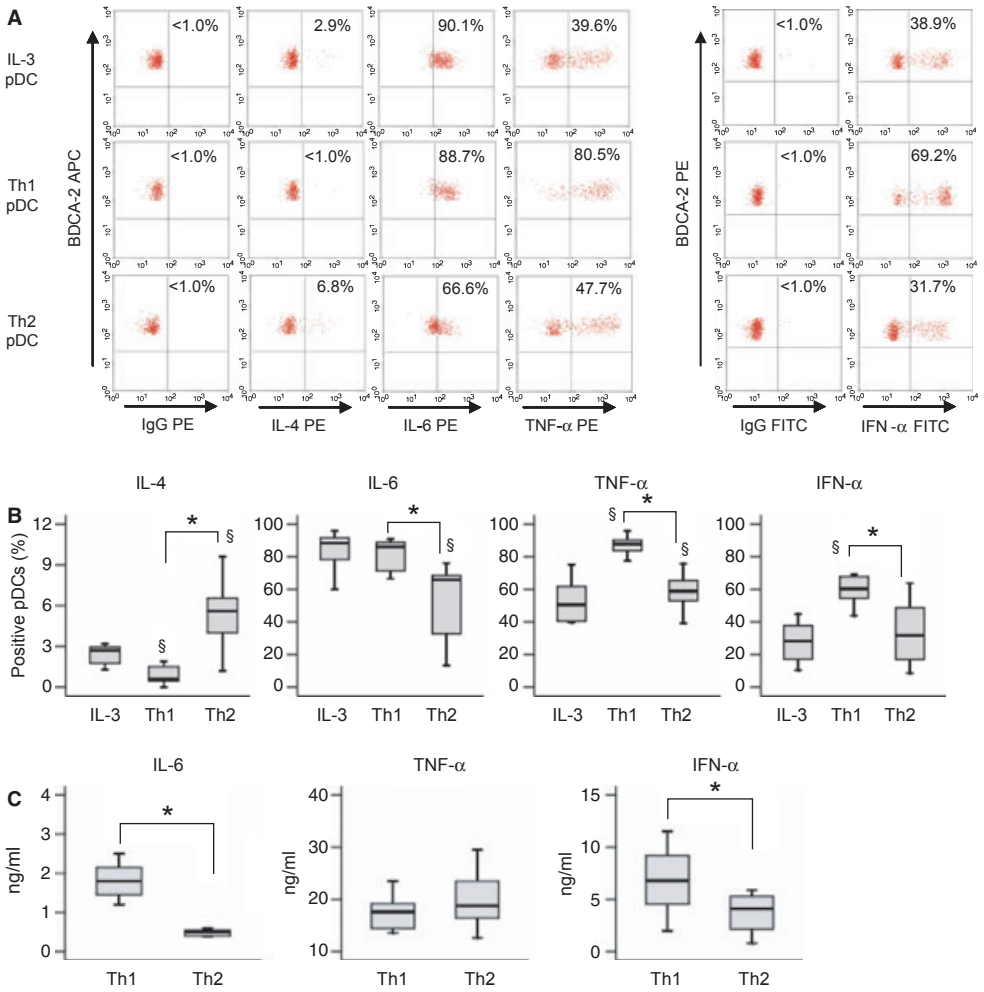

Figure 3 TLR7-ligand-induced cytokine expression by pDCs is influenced by the surrounding cytokine milieu. (A, B) PBMCs from seven different subjects were cultured in the presence of IL-3 (IL3), IL-3/anti-IL-4/IL-12/IFN-γ (Th1) or IL-3/IL-4/anti-IL-12/anti-IFN-γ (Th2) for 16 h. Cytokine expression was induced by adding the TLR7-ligand Imiquimod for 3 h. PDCs were identified as lineage-negative HLA-DR$^+$ BDCA-2$^+$ cells and analysed for cytokine expression by flow cytometry. Dot plots show percentages of IL-4, IL-6, TNF-α and IFN-α positive pDCs. (C) PDCs from seven different subjects were cultured in the presence of IL-3/anti-IL-4/IL-12/IFN-γ (Th1) or IL-3/IL-4/anti-IL-12/anti-IFN-γ (Th2) for 16 h. Cytokine secretion was induced by adding Imiquimod for another 8 h. Cytokine concentrations were determined in culture supernatants by ELISA. 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). Differences between groups were analysed using the Wilcoxon signed-rank test and *P*-values < 0.05 were regarded as significant. §significant to IL-3 only stimulated cells; *significance between Th1- and Th2-like conditions.

induce cytokine expression in CD4$^+$ T cells. Differently matured pDCs (IL-3+ Th1-like milieu, IL-3+ Th2-like milieu) were co-cultured with autologous CD4$^+$ T cells in a ratio of 1 : 2 in the presence of Imiquimod for 7 days. Then, cytokine expression in CD4$^+$ T cells was induced by stimulation with anti-CD3/anti-CD28 and analysed by flow cytometry.

PDCs matured under Th2-like conditions had no effect on IL-2 expression in CD4$^+$ T cells (without pDCs: median 2.6%, with Th2-pDCs: median 2.9%), while pDCs matured under Th1-like conditions induced IL-2 expression in significantly higher percentages of CD4$^+$ T cells compared to Th2-pDCs (median 12.3%) (Figure 5). A median value of 18.7% CD4$^+$ T cells expressed IL-4 after a 7-day culture period without pDCs. Addition of Th1-pDCs (median 4.5%) or Th2-pDCs (median 6.8%) suppressed the expression of IL-4 by autologous CD4$^+$ T cells, but reached statistical significance only in the case of Th1-pDCs (Figure 5). IFN-γ expression was rarely detectable in CD4$^+$ T cells cultured without pDCs (median 0.5%). Addition of Th2-pDCs slightly increased the percentage of IFN-γ expressing CD4$^+$ T cells (median 0.9%), while a strong increase was observed in the presence of Th1-pDCs (median 22.2%) (Figure 5). No expression of IL-5, IL-10 and IL-13 could be detected in

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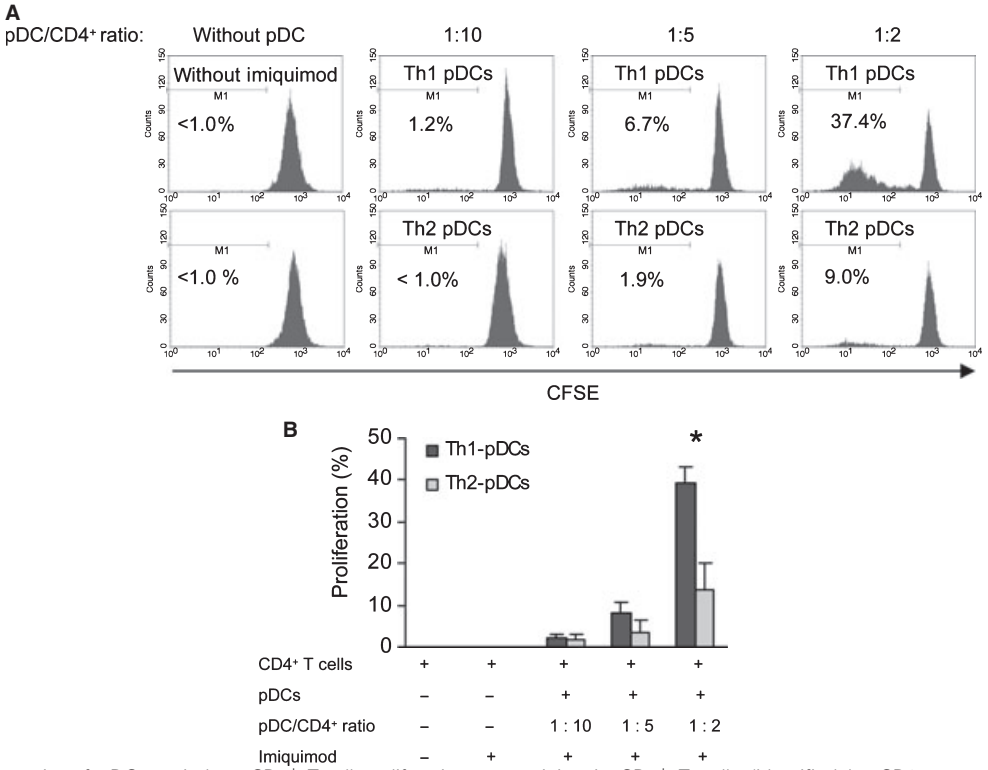

Figure 4 The capacity of pDCs to induce $CD4^+$ T-cell proliferation is suppressed under Th2-like conditions. Isolated pDCs were cultured in the presence of IL-3/anti-IL-4/IL-12/IFN-γ (Th1) or IL-3/IL-4/anti-IL-12/anti-IFN-γ (Th2) for 16 h. Then, pDCs and autologous $CD4^+$ T cells were co-cultured at indicated ratios in the presence of the TLR7-ligand Imiquimod for 7 days. (A) Histograms show CFSE staining in $CD4^+$ T cells (identified by CD3 expression). (B) Bars show the percentage of proliferating cells ($CFSE^{low}$ cells). Mean values + standard deviation from three different experiments are shown. Differences between groups were analysed using the two-sided *t*-test for paired samples. **P*-values < 0.05 were regarded as significant.

$CD4^+$ T cells cultured in the absence or presence of pDCs (data not shown).

Discussion

The present study describes the differential development of pDCs maturing in Th1- or Th2-like milieus using an *in vitro* culture system. PDCs matured under Th1-like conditions showed high expression of surface molecules involved in antigen presentation and T-cell co-stimulation, such as HLA-DR, CD40, CD80, CD83 and CD86, while most of these molecules were efficiently suppressed in pDCs matured under Th2-like conditions. This corresponds to *in vivo* data where none to low expression of CD80, CD83 and CD86 was found on endobronchial pDCs after segmental allergen challenge (3).

In addition, Th1-pDCs secreted significantly higher amounts of IL-6 and IFN-α in response to TLR7-ligation compared with Th2-pDCs. An inhibitory impact on IFN-α secretion by pDCs was previously reported for histamine and crosslinking of the high affinity IgE-receptor (16, 18). This is consistent with data from several groups who reported an impaired IFN-α release from blood cultures from atopic patients following virus or CpG-oligonucleotide stimulation (22–24). In addition, circulating pDCs secrete significantly lower amounts of IFN-α in response to CpG-oligonucleotide stimulation after segmental allergen challenge (17) suggesting a role for Th2-mediators in the downregulation of TLR7- and TLR9-mediated immune responses not only *in vitro* but also *in vivo*.

Virus- and CD40L-stimulated pDCs have a high Th1-polarizing capacity for $CD4^+$ T cells, which depends on IFN-α (6, 25). Th1-pDCs in our study, which secrete higher amounts of IFN-α compared to Th2-pDCs, primed significantly more $CD4^+$ T cells to express IFN-γ and IL-2 compared to Th2-pDCs. In addition, Th1-pDCs significantly suppressed the ability of $CD4^+$ T cells to express IL-4. Th1-pDCs also induced a significantly higher $CD4^+$ T-cell proliferation compared to Th2-pDCs. This is in line with the higher IL-2 expression in $CD4^+$ T cells induced by Th1-pDCs. It was previously described that endobronchial pDCs isolated from an allergen- and LPS-challenged lung segment failed to induce lymphocyte proliferation (26), confirming our finding that Th2-like conditions suppress the T-cell stimulatory capacity of pDCs.

PDCs matured under Th2-like conditions showed a significant increase in IL-4 expression following stimulation of TLR7. To our knowledge, this is the first study that reports IL-4 expression by pDCs suggesting that these cells not only initiate Th1 immune responses but can also contribute to maintain a Th2-dominant immune response by secreting IL-4. In addition, pDCs use an OX40L-dependent mecha-

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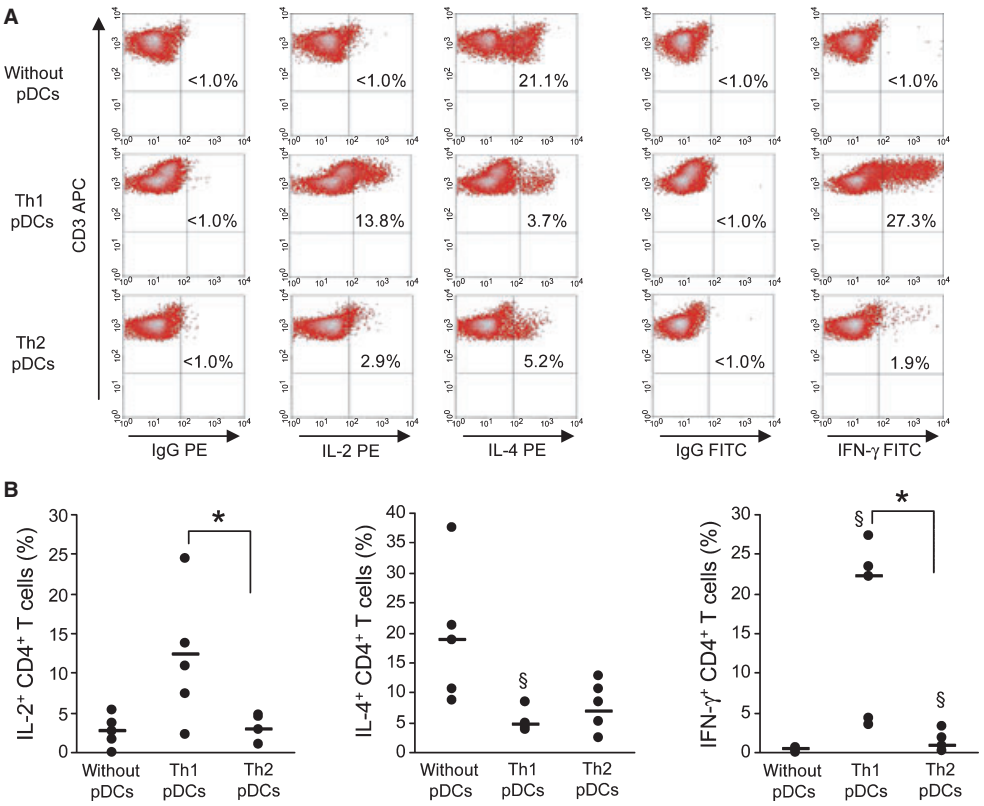

Figure 5 The cytokine milieu during the maturation of pDCs influences their $CD4^+$ T-cell polarizing capacity. PDCs from five different subjects were cultured in the presence of IL-3/anti-IL-4/IL-12/IFN-γ (Th1) or IL-3/IL-4/anti-IL-12/anti-IFN-γ (Th2) for 16 h. Then, pDCs and autologous $CD4^+$ T cells were co-cultured in the presence of the TLR7-ligand Imiquimod for 7 days. Cytokine expression in $CD4^+$ T cells was induced by stimulation with anti-CD3 and anti-CD28 for 10 h and analysed by flow cytometry. (A) Density plots show IL-2, IL-4 and IFN-γ expression by $CD4^+$ T cells, which were identified by CD3 expression. Percentages of positive cells are indicated. (B) Plots show percentages of cytokine expressing $CD4^+$ T cells. Median values (–) are indicated. Differences between groups were analysed using the Wilcoxon signed-rank test, and *P*-values < 0.05 were regarded as significant. §significant to $CD4^+$ T cells without pDCs; *significance between Th1- and Th2-pDCs.

nism to prime Th2 immune responses (10). Comparable to IL-4 expression, OX40L expression by pDCs is significantly suppressed under Th1 conditions, but almost maintained in a Th2-like cytokine milieu. Together, these data convincingly support the hypothesis that the potential of pDCs to prime Th2-dominant immune responses is maintained in a Th2-cytokine milieu, while Th1 conditions effectively suppress the Th2 potential of pDCs.

PDCs play an important role in the initiation of antiviral and antibacterial immune responses because they secrete IFN-α after recognizing microbial DNA and/or RNA through TLR7 and 9, respectively (6–9). Our finding of a reduced TLR7-mediated IFN-α release and Th1-polarizing potential for $CD4^+$ T cells by pDCs matured under Th2-like conditions could contribute to a delayed clearance of viral and/or bacterial infections during acute allergic attacks. Furthermore, it is tempting to speculate that the increased amounts of IL-4 synthesized by pDCs matured under Th2-like conditions in response to TLR7-ligation might contribute to asthma exacerbations caused by pulmonary infections (27).

Interestingly, BDCA-3 (Thrombomodulin, CD141) expression by pDCs is highly upregulated under Th2-like conditions. Previously, we reported the upregulation of BDCA-3 on endobronchial pDCs following segmental allergen challenge (20). Thus, these *in vitro* findings are compatible with those reported *in vivo* (20). Another study reported an allergen-enhanced BDCA-3 expression on myeloid DCs and showed that these cells have a higher Th2-polarizing capacity compared with BDCA-3-negative myeloid DCs (21). Together, these results suggest a specific but to date unclear role for BDCA-3 expression on DCs in Th2-dominant immune responses. BDCA-3 has anti-inflammatory properties mediated through its *N*-terminal lectin-like domain and/or through the activation of protein C (28, 29). In a mouse model, pDCs have been shown to prevent asthmatic reactions to harmless inhaled antigens (12). It is therefore tempting to speculate that the anti-inflammatory properties of BDCA-3 might play a role in the regulation of Th2 immune responses, but further investigations are needed to elucidate the role of BDCA-3 expression by pDCs under Th2-conditions.

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In conclusion, this study demonstrates a crucial impact of the surrounding cytokine milieu on pDC phenotype and function. PDCs matured under Th1-like conditions showed high expression of antigens involved in T-cell co-stimulation and antigen presentation as well as a potent Th1-polarizing capacity for $CD4^+$ T cells after TLR7-ligation, while their Th2-like potential is strongly suppressed. In contrast, Th2-like conditions during the development of pDCs suppressed the expression of T-cell co-stimulatory antigens and their $CD4^+$ T-cell stimulatory capacity, which would be compatible with an impaired antiviral/bacterial pDC function. Furthermore, Th2-like conditions favour the expression of BDCA-3 by pDCs as well as IL-4 secretion after TLR7-ligation, suggesting a mechanism which may participate in asthma exacerbations during pulmonary infections.

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Author contribution

KB designed the study, performed experiments, analysed the data and wrote the manuscript; CK performed experiments; MK analysed the data; ML wrote the manuscript; JCV designed the study and wrote the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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