Dendritic cell subsets in human bronchoalveolar lavage fluid after segmental allergen challenge

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Figure A and table A are available at <http://thorax.bmjournals.com/supplemental>

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Background: Dendritic cells control pulmonary immune reactions. Characteristics of dendritic cells in human bronchoalveolar lavage fluid (BALF) after allergen challenge are unknown.

Methods: 7 patients with allergic asthma (median 23 years, range 19–25 years) underwent segmental challenge and were lavaged 10 min and 24 h after challenge. Dendritic cell subsets and surface markers in BALF and in peripheral blood were analysed using four-colour flow cytometry.

Results: Plasmacytoid dendritic cells (pDCs, median 0.06%, range 0.01–0.08%) and myeloid dendritic cells (mDCs, median 0.47%, range 0.27–0.87%) were detectable in BALF from control segments. CD1a-positive dendritic cells in BALF were identified as a subpopulation of mDCs. Both pDCs (median 0.56%, range 0.09–1.83%) and mDCs (median 1.82%, range 0.95–2.29%) increased significantly in BALF 24 h ($p=0.018$ compared with the control segments for pDCs and mDCs), but not 10 min, after allergen challenge. The percentage increase in pDCs was higher than that of mDCs after allergen challenge, as reflected by an enhanced pDC:mDC ratio after allergen challenge. In peripheral blood, there was a significant decrease in mDCs ($p=0.038$) and a trend to a decrease in pDCs ($p=0.068$) 24 h after allergen challenge. Analysis of dendritic cell surface molecules showed that after allergen challenge, BALF dendritic cells have a less mature phenotype compared with BALF dendritic cells from control segments.

Conclusion: Using a comprehensive strategy to analyse dendritic cell subsets in human BALF, we have shown for the first time that both myeloid and plasmacytoid dendritic cells accumulate in the airway lumen after allergen challenge in patients with asthma.

Dendritic cells form a highly sensitive sentinel network in the lung. By continuously reporting antigenic information from the airways to pulmonary lymph nodes, they are capable of upregulating or downregulating specific immune responses in the lung.^{1,2} Animal models suggest that dendritic cells have a crucial role in asthma.³ Allergen challenge results in a recruitment of dendritic cells into the airways, which is essential for the establishment of allergic airway inflammation.^{4–5} Instillation of allergen-loaded dendritic cells into the airways alone can induce allergic airway inflammation in healthy animals.⁶ Selective elimination of dendritic cells during allergic airway inflammation abolishes the characteristic features of asthma, including eosinophilic inflammation, goblet cell hyperplasia and bronchial hyperresponsiveness.⁷ These data indicate that dendritic cells are vital for both the initiation and the maintenance of allergic airway inflammation in asthma.⁸

Human dendritic cells are identified by the abundant expression of major histocompatibility complex class II (HLA-DR) and the absence of lymphocyte, natural killer cell, monocyte and granulocyte lineage markers.² CD11c⁺ myeloid dendritic cells (mDCs) and CD123⁺ plasmacytoid dendritic cells (pDCs) represent two main dendritic cell subtypes. There is ongoing research to further characterise these two dendritic cell subtypes in human lung parenchyma^{9–11} and in human bronchoalveolar lavage fluid (BALF).^{12–13} Patients with asthma display increased numbers of intraepithelial dendritic cells in the airways compared with healthy individuals.^{14–15} Allergen challenge causes an accumulation of CD11c⁺ mDCs within the bronchial mucosa,¹⁶ and a parallel decline of these cells in

circulating blood.¹⁷ In contrast, CD123⁺ pDC were postulated to be absent from the bronchial mucosa, both before and after allergen challenge,¹⁶ and have not yet been studied in peripheral blood after allergen challenge.¹⁷ However, local increases in pDCs have been reported in patients with atopic rhinitis and dermatitis after allergen challenge,^{18–19} as well as elevated pDC counts in the peripheral blood of patients with allergic asthma.²⁰ The relative contribution of mDCs and pDCs to the pathology of human asthma is, therefore, still unresolved. In addition, no information is available on the accumulation and the characteristics of pDCs and mDCs in BALF fluid after allergen challenge in human asthma. This study investigated dendritic cell subsets in human BALF after segmental allergen challenge using a comprehensive flow cytometric method.

METHODS

Participants

Seven patients with mild allergic asthma (median 23 years, range 19–25 years), most of them being medical students at the University of Rostock, volunteered for the study (table 1). Patients were recruited for the study on the basis of previously described criteria: (1) airway hyperresponsiveness; (2) positive allergen skin prick tests; (3) elevated total or specific IgE concentrations; and (4) a dual reaction after allergen

Abbreviations: BALF, bronchoalveolar lavage fluid; BDCA, blood dendritic cell antigen; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell

Table 1 Patient characteristics

No	Sex	Age	FEV ₁ (% predicted)	Drugs	Total IgE (kU/l)	Specific IgE (kU/l)	Allergen	Dose (AU)
1*	M	23	77	BA	548	26.1	Birch	76
2	F	23	92	BA	564	77.1	DP	250
3	F	25	94	BA, IC	96	4.0	Birch	30
4	F	25	105	CR, BA	64	16.9	DP	18
5	F	23	105	BA	545	12.6	Rye	76
6	F	24	102	BA	155	5.2	Rye	4
7	M	19	114	BA	141	18.7	Rye	600

BA, inhaled β_2 agonist; CR, cromoglycate; DP, dermatophagoides pteronyssinus; F, female; FEV₁, forced expiratory volume in the first second; IC, inhaled corticosteroid; IgE, immunoglobulin E; M, male.

Table 1 displays the sex (male/female) and age (in years) of the patients, the prebronchodilator forced expiratory volume in the first second (FEV₁ in % predicted), the drugs taken before the study, serum levels of total (normal range <100 kU/l) and allergen-specific (normal range <0.7 kU/l) immunoglobulin E (IgE) in kilo units (kU/l), and the allergen and the dose (in allergen units, AU) used for segmental allergen challenge. *Dendritic cell counts only from saline and allergen-challenged segments after 24 h.

inhalation.²¹ The individual provocation dose was calculated as described.²² Inhaled and segmental allergen challenges were separated by at least 4 weeks. Corticosteroids were withdrawn at least 7 days before challenge. Patients gave their written informed consent. The study was approved by the local ethics committee.

Segmental allergen challenge

Segmental allergen challenge was performed as described previously.²¹ Briefly, 2.5 ml of saline were instilled into the left S8 (control 1, C1) and S5 (control 2, C2) segments, and allergen (diluted in 2.5 ml of saline) was instilled into the right S8 (allergen 1, A1) and S5 (allergen 2, A2) segments. The left and right S8 (C1 and A1) segments were lavaged using 100 ml of pre-warmed saline after 10 min, and the left and right S5 segments (C2 and A2) after 24 h. Median BALF recoveries were as follows: C1 57 ml (range 40–68 ml), A1 47 ml (40–59 ml), C2 62 ml (50–75 ml) and A2 60 ml (22–64 ml). Before each bronchoscopy, venous blood samples were obtained.

Analysis of cell counts and leucocyte subsets in BALF

BALF samples were filtered through a two-layer sterile gauze into sterile plastic vials, centrifuged at 4°C and 500 *g* for 10 min.

Cells were resuspended in phosphate-buffered saline. A fraction of the suspension was used for cell counts (using a Neubauer chamber; Brand, Wertheim, Germany) and for cytopspins. Cytopspins were stained with May/Grünwald/Giemsa solution (Merck, Darmstadt, Germany) and differential cell counts determined using standard morphological criteria. Results were expressed as the total number of cells per ml of recovered fluid.

Flow cytometry

Freshly collected EDTA blood and isolated BALF cells were incubated with the respective antibodies (table 2) for 20 min. Afterwards, FACS lysing solution (BD Biosciences, Heidelberg, Germany) was added for another 10 min. Cells were centrifuged for 5 min (400 *g*) and washed with phosphate-buffered saline containing 2% fetal calf serum and 0.1% NaN₃. Finally, cells were resuspended in phosphate-buffered saline and analysed on a FACS Calibur using Cell Quest Pro Software (BD Biosciences). To identify dendritic cell subsets in BALF, we used an approach described previously to detect mDCs and pDCs in peripheral blood.²⁰ CD3, CD14, CD16, CD19, CD20 and CD56 negative/dim cells (lin^{neg/dim}) were gated using a commercial lineage cocktail with antibodies against all of these antigens (fig 1A). Among these lin^{neg/dim} cells, distinct populations of CD123⁺HLA-DR⁺ cells

Table 2 Antibodies used for four-colour flow cytometry

	Antigen	Label	Clone	Company
Lineage cocktail	CD3	FITC	SK7	BD Biosciences
	CD14	FITC	MφP9	BD Biosciences
	CD16	FITC	3G8	BD Biosciences
	CD19	FITC	SJ25C1	BD Biosciences
	CD20	FITC	L27	BD Biosciences
	CD56	FITC	NCAM16.2	BD Biosciences
Other antibodies	CD11c	PE	S-HCL-3	BD Biosciences
	CD11c	APC	S-HCL-3	BD Biosciences
	CD123	PE	9F5	BD Biosciences
	HLA-DR	PerCP	L243	BD Biosciences
	CD40	APC	HB14	Caltag/Invitrogen
	CD83	APC	HB15e	Caltag/Invitrogen
	CD86	APC	BU63	Caltag/Invitrogen
	CD80	APC	MEM-233	Immunotools
	CD1α	PE	NA1/34	Dako
	BDCA-1	APC	AD5-8E7	Miltenyi Biotec
	BDCA-2	APC	AC144	Miltenyi Biotec
	BDCA-3	APC	AD5-14H12	Miltenyi Biotec
	BDCA-4	APC	AD5-17F6	Miltenyi Biotec

APC, allophycocyanin; BDCA, blood dendritic cell antigen; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

Caltag/Invitrogen, Karlsruhe, Germany; Dako, Glostrup, Denmark; Miltenyi Biotec, Bergisch Gladbach, Germany; Immunotools, Friesoythe, Germany.

Figure 1 Identification of dendritic cell subsets in bronchoalveolar lavage fluid (BALF). (A) Total cells were identified in forward scatter/side scatter (FSC/SSC) plots (first row). Lineage negative/dim ($\text{lin}^{\text{neg/dim}}$) cells were further gated (second row) to identify dendritic cells. Among $\text{lin}^{\text{neg/dim}}$ cells, plasmacytoid dendritic cells were identified by CD123 and HLA-DR expression (third row), myeloid dendritic cells by CD11c and HLA-DR coexpression (fourth row) and CD1a⁺ dendritic cells by coexpression of CD1a and HLA-DR (fifth row). (B) BALF-derived CD1a⁺ dendritic cells were gated and analysed for CD11c expression (black) compared with an isotype control antibody (grey). (C) Backgating of dendritic cell subsets showed distinct cell populations (blue) in the FSC/SSC plot.

(pDC) and CD11c⁺HLA-DR⁺ cells (mDC) were identified, both in peripheral blood and in BALF (fig 1A). A population of CD1a⁺HLA-DR⁺ $\text{lin}^{\text{neg/dim}}$ cells identified in BALF could hardly be detected in peripheral blood (fig 1A). These CD1a⁺ dendritic cells coexpressed CD11c in all BALF samples, suggesting that these dendritic cells are a subpopulation of mDCs (fig 1B). Backgating showed that pDCs, mDCs and CD1a⁺ dendritic cells from BALF form distinct populations in the forward scatter/side scatter plot, without an overlap with lymphocyte, granulocyte and alveolar macrophage regions (fig 1C).

Statistical analysis

Data were analysed using SPSS v 11.0. Most parameters were non-normally distributed. Therefore, correlation analysis was

performed using Spearman's correlation coefficient, and the comparison of BALF parameters between allergen-challenged and saline-challenged control segments, and the comparison of blood parameters before and after allergen challenge was performed using Wilcoxon's signed rank test. The comparison of dendritic cells in peripheral blood and BALF was performed using the Mann-Whitney U test. Probability values of $p < 0.05$ were regarded as significant.

RESULTS

Dendritic cell subsets in BALF and peripheral blood after segmental allergen challenge

In the BALF collected 24 h after saline challenge, low amounts of pDCs (median 0.06% or 0.06×10^3 cells/ml BALF; range

Figure 2 Dendritic cell subsets in bronchoalveolar lavage fluid (BALF) and peripheral blood after allergen challenge. Shown are percentages of dendritic cells in BALF and total dendritic cell counts in BALF and peripheral blood. A1, BALF 10 min after allergen challenge; A2, BALF 24 h after allergen challenge; B1, peripheral blood before SAP; B2, peripheral blood 24 h after SAP; C1, BALF 10 min after saline challenge; C2, BALF 24 h after saline challenge. *Significant differences between allergen challenged and corresponding control segments, and between peripheral blood before and 24 h after allergen challenge. Median values are displayed as bars for A2, B1 and B2. mDC, myeloid dendritic cells; pDC, plasmacytoid dendritic cells.

0.01–0.08% or $0.01\text{--}0.34 \times 10^3$ cells/ml BALF), mDCs (median 0.47% or 0.63×10^3 cells/ml BALF; range 0.27–0.87% or $0.25\text{--}3.2 \times 10^3$ cells/ml BALF) and CD1a⁺ mDCs (median 0.34% or 0.56×10^3 cells/ml BALF; range 0.24–0.56% or $0.25\text{--}1.63 \times 10^3$ cells/ml BALF) were detected. There were no significant differences in dendritic cell counts in BALF 10 min after saline challenge (fig 2). In all patients, the percentages as well as the total numbers of pDCs (median 0.56% or 2.71×10^3 cells/ml BALF; range 0.09–1.83% or $0.06\text{--}16.29 \times 10^3$ cells/ml BALF; $p = 0.018$ compared with the corresponding control), mDCs (median 1.82% or 7.45×10^3 cells/ml BALF; range 0.95–2.29% or $0.60\text{--}20.38 \times 10^3$ cells/ml BALF; $p = 0.018$) and CD1a⁺ mDCs (median 1.44% or 5.22×10^3 cells/ml BALF; range 0.63–2.00% or $0.40\text{--}17.80 \times 10^3$ cells/ml BALF; $p = 0.018$) increased significantly in BALF 24 h, but not 10 min, after allergen challenge (fig 2). The percentage increase in pDCs was higher than the percentage increase in mDCs 24 h after allergen challenge in all patients. This was reflected by a

significantly enhanced pDC:mDC ratio in allergen-challenged segments (median 0.36, range 0.09–0.80) as compared with control segments (median 0.11, range 0.04–0.23) after 24 h ($p = 0.018$). In peripheral blood, distinct populations of pDCs and mDCs were detected before (median of pDCs 0.31%, range 0.18–0.36%; median of mDCs 0.26%, range 0.17–0.36%) and 24 h after allergen challenge (median of pDCs 0.14%, range 0.09–0.25%; median of mDCs 0.16%, range 0.06–0.27%). Total numbers of pDCs decreased in four of six patients and mDCs in five of six patients in peripheral blood 24 h after challenge (fig 2). This decrease in dendritic cell counts in peripheral blood was statistically significant in the case of mDCs ($p = 0.038$), but not in the case of pDCs ($p = 0.068$).

Association of dendritic cells with the inflammatory response and allergen dose

Compared with the control segments, there were significantly increased neutrophil ($p = 0.043$) and eosinophil counts

Figure 3 Surface molecules on dendritic cells in bronchoalveolar lavage fluid (BALF) and peripheral blood. Plasmacytoid dendritic cells (CD123⁺HLA-DR⁺lin^{neg/dim}) and myeloid dendritic cells (CD11c⁺HLA-DR⁺lin^{neg/dim}) were gated as described in fig 1. Histograms display the expression of surface molecules (red) compared with corresponding isotype controls (grey). APC, allophycocyanin; BDCA, blood dendritic cell antigen.

($p = 0.028$) in BALF 24 h, but not 10 min, after challenge (see supplementary table A online at <http://www.thorax.bmjournals.com/supplemental>). The amount of infiltrating dendritic cells was correlated with the severity of the local inflammatory

response, as reflected by a significant correlation between the total numbers of mDCs, CD1a⁺ mDCs and pDCs with eosinophil counts ($r > 0.9$ and $p < 0.001$ for all dendritic cell subsets) and lymphocyte counts ($r = 0.82-0.96$, $p < 0.05$ for all dendritic cell

Table 3 Expression of surface molecules on dendritic cell subsets

	B1 (%)	B2 (%)	C1 (%)	C2 (%)	A2 (%)
mDC					
BDCA-1	62.8 (42.8 to 80.8)	63.5 (38.4 to 82.6)	81.3 (65.0 to 94.7)	85.9 (73.3 to 96.8)	92.6 (84.3 to 97.5)
BDCA-2	0.0 (0.0 to 6.8)	0.2 (0.0 to 10.1)	0.0 (0.0 to 0.4)	0.0 (0.0 to 0.4)	0.8 (0.0 to 2.3)
BDCA-3	19.6 (15.6 to 32.6)	23.8 (11.4 to 48.8)	73.1 (57.6 to 94.2)	76.1 (61.2 to 87.7)	87.5 (69.4 to 96.4)
BDCA-4	0.0 (0.0 to 2.9)	0.0 (0.0 to 4.6)	20.0 (0.2 to 52.2)	23.3 (15.0 to 43.2)	45.1 (10.8 to 81.5)
CD40	43.4 (13.4 to 54.2)	33.0 (11.2 to 75.8)	98.0 (93.1 to 100.0)	95.1 (92.9 to 99.3)	96.6 (90.1 to 99.1)
CD80	0.0 (0.0 to 0.9)	0.0 (0.0 to 0.5)	70.8 (50.6 to 80.9)	58.6 (39.8 to 75.8)	43.3 (16.9 to 62.0)
CD83	0.0	0.0	26.5 (19.9 to 33.3)	27.6 (12.7 to 38.1)	11.1 (7.3 to 25.1)
CD86	1.0 (0.0 to 7.2)	1.5 (0.0 to 8.2)	84.3 (65.8 to 90.0)	87.0 (67.6 to 93.0)	78.9 (39.4 to 86.8)
CD1a	bd	bd	71.8 (60.0 to 100.0)	69.6 (51.1 to 91.8)	72.9 (59.3 to 87.3)
pDC					
BDCA-1	0.0	0.0	NA	NA	0.0 (0.0 to 3.4)
BDCA-2	99.2 (94.7 to 99.7)	98.9 (96.1 to 100.0)	NA	NA	98.1 (91.1 to 100.0)
BDCA-3	53.2 (17.3 to 73.5)	52.1 (28.4 to 65.5)	NA	NA	97.1 (94.9 to 100.0)
BDCA-4	94.2 (92.3 to 99.7)	96.5 (92.9 to 100.0)	NA	NA	96.4 (77.4 to 99.5)
CD40	88.4 (27.2 to 92.6)	82.6 (23.8 to 89.9)	NA	NA	57.1 (36.8 to 77.7)
CD80	0.0	0.0	NA	NA	9.5 (2.5 to 30.0)
CD83	0.0	0.0	NA	NA	0.0 (0.0 to 2.3)
CD86	0.0 (0.0 to 0.6)	0.0 (0.0 to 3.2)	NA	NA	9.0 (0.6 to 12.5)

A2, bronchoalveolar lavage fluid (BALF) 24 h after allergen challenge; B1, peripheral blood before allergen challenge; B2, peripheral blood 24 h after allergen challenge; bd, below detection limit; BDCA, blood dendritic cell antigen; C1, BALF 10 min after saline challenge; C2, BALF 24 h after saline challenge; mDC, myeloid dendritic cells; NA, not analysable; pDC, plasmacytoid dendritic cells. Values in parentheses denote ranges (minimum to maximum).

subsets) in BALF 24 h after challenge. There was also a correlation between the total numbers of mDCs, CD1a⁺ mDCs and pDCs and the allergen dose used for challenge ($r > 0.86$ and $p < 0.01$ for all dendritic cell subsets).

Surface molecules on dendritic cells in BALF and peripheral blood

Both in peripheral blood and in BALF, the expression of blood dendritic cell antigen (BDCA)-2 was restricted to pDCs, whereas BDCA-1 expression was restricted to a subset of mDCs (fig 3, table 3). In contrast with peripheral blood, BDCA-3 was expressed not only on a subset of mDCs but also on pDCs and most BDCA-1⁺ mDCs in BALF. BDCA-4, which was restricted to pDCs in peripheral blood, was also expressed on a subset of BALF mDCs (fig 3, table 3). This subset of BDCA-4⁺ BALF mDCs increased twofold 24 h after allergen challenge compared with the control segments (table 3). Of note, BDCA-3 and BDCA-4 were strongly expressed by other BALF cells that were lin^{bright} and HLA-DR⁺. As eosinophils, neutrophils and macrophages were excluded by gating, other lineage-positive cells must account for this expression. In addition, some expression of BDCA-3 and BDCA-4 was found on alveolar macrophages and granulocytes (see supplementary fig A online at <http://www.thorax.bmj.com/journal/supplement>). Compared with peripheral blood mDCs, the expression of CD40, CD80 and CD86 was significantly stronger on mDCs from BALF of control segments ($p < 0.05$ in all cases; fig 3, table 3). The dendritic cell maturation marker CD83 was absent on blood mDCs, but detectable on approximately 25% of BALF mDCs from the control segments. The mDCs in BALF 24 h after allergen challenge were characterised by a significantly lower expression of CD83 compared with the corresponding control segments ($p = 0.046$; fig 3, table 3). Owing to the very low number of pDCs in BALF, it was not possible to reliably analyse surface markers on pDCs in the control segments. Only CD40, but not CD80, CD83 or CD86, was expressed on pDCs in peripheral blood. In contrast, a low expression of CD80 and CD86 was found on BALF pDCs 24 h after challenge. There was a non-significant trend to a decreased expression of CD40 on BALF pDCs compared with blood pDCs 24 h after challenge ($p = 0.09$; fig 3, table 3).

Table 3 displays the percentage of marker-positive mDCs and pDCs in blood (B1, B2) or BALF (C1, C2, A2). The median

values (range) of six patients with asthma are presented. The time point 10 min after allergen challenge (A1) is not displayed, because cell counts in BALF were too low to measure surface molecules on dendritic cells. In BALF from saline-challenged control segments (C1 and C2), numbers of pDCs were too low to quantify the expression of surface molecules.

DISCUSSION

In this study, we have reported a comprehensive strategy for the identification, quantification and characterisation of plasmacytoid dendritic cells (CD123⁺HLA-DR⁺lin^{neg/dim}) and myeloid dendritic cells (CD11c⁺HLA-DR⁺lin^{neg/dim}) in human BALF using four-colour flow cytometry. In addition, this is the first study in humans to show that allergen challenge is associated with a marked influx of both pDCs and mDCs into the airway lumen of patients with allergic asthma. Further, it provides a detailed analysis of the distribution of dendritic cell antigens on these infiltrating dendritic cells. Although our study included a comparably small number of patients, the uniformity of the data suggests that our results are representative for a larger population and that a larger number of patients would not have changed the findings considerably.

Dendritic cells in BALF were initially identified using immunocytochemistry, and were postulated to yield approximately 0.4% of all cells in the BALF of healthy patients.²³ In subsequent studies, the same group identified dendritic cells by their low autofluorescent properties in flow cytometric analyses, and divided these low autofluorescent cells into subgroups of CD1a⁺ and CD1a⁻ cells.^{24, 25} Using flow cytometry with a different gating strategy compared with our study, Donnenberg and Donnenberg recently described CD123⁺ plasmacytoid dendritic cells (0.02%) and CD11c⁺ myeloid dendritic cells (0.06%) in BALF from healthy volunteers.¹² Our study is the first to use all three markers (CD1a, CD11c and CD123) to characterise dendritic cells in BALF. With this approach, we identified CD1a⁺ dendritic cells as a subset of mDCs in BALF. This CD1a⁺ subset of mDCs represented the majority of BALF mDCs, but was nearly absent among peripheral blood mDCs, suggesting that CD1a is upregulated during the passage of mDCs into the airways. CD1a⁺ dendritic cells were previously described as intraepithelial dendritic cells,

with a yet undefined relationship with mDCs.² Our data provide evidence that these cells represent a subgroup of mDCs in BALF.

Recently, the use of magnetic beads targeting blood dendritic cell antigens (BDCA 1–4) has been proposed as a straightforward strategy to isolate subsets of dendritic cells from lung homogenates^{10–11} and BALF.¹³ In peripheral blood, BDCA-2 and BDCA-4 are confined to pDCs, whereas BDCA-1 and BDCA-3 are confined to mDCs.²⁶ In agreement with this distribution in peripheral blood, BDCA-1 was confined to mDCs and BDCA-2 to pDCs in BALF. However, there was a rather unspecific expression of BDCA-3 and BDCA-4 in BALF. BDCA-3 was also expressed on pDCs, BDCA-1⁺ mDCs and a variety of other BALF cells including a population of lin^{bright}HLA-DR⁺ cells within the lymphocyte/dendritic cell gate. BDCA-4 was expressed on mDCs and a lin^{bright}HLA-DR⁺ cell population in BALF. These data suggest that BDCA-3 and BDCA-4 are not specific for mDCs or pDCs in the BALF of patients with allergic asthma. In addition, the previously reported BDCA-3⁺HLA-DR⁺ dendritic cell subset in lung homogenates termed “mDC2”^{10–11} might also contain pDCs. This hypothesis is supported by the findings that a subpopulation of mDC2 is CD11c[−],¹⁰ and that the T cell proliferation induced by mDC2 ranges between that induced by mDC1 and pDC.¹¹ Therefore, further analysis of the cellular distribution of BDCA molecules in human BALF or lung homogenates will be required before anti-BDCA-3 and anti-BDCA-4 beads can be recommended as a suitable tool to isolate subsets of pulmonary dendritic cells.

There is accumulating evidence from animal models that mDCs have a crucial role in the development and maintenance of allergic asthma.^{3–6} In contrast, pDCs have been reported to inhibit allergic airway inflammation and Th2-type cytokine production in a mouse model of asthma.²⁷ It has therefore been hypothesised that mDCs and pDCs might be in a yin-yang balance in allergic asthma, with a proallergic role of mDCs and antiallergic properties of pDCs. However, there is uncertainty whether this concept is applicable to human asthma. Plasmacytoid dendritic cells are increased in the peripheral blood of patients with allergic asthma and other atopic diseases, and are positively correlated with IgE levels and eosinophil counts.^{20–28–29} In patients with atopic rhinitis and dermatitis, there is a strong local increase in pDCs after allergen challenge.^{18–19} Human pDCs can stimulate allergen-dependent T cell proliferation and Th2-type cytokine production as efficiently as mDCs, but are also capable of Th1-type cytokine production after stimulation with CpG motifs.³⁰ A recent report suggests that the decision as to whether pDCs will trigger either a Th1-type or a Th2-type immune response may be dependent on the local microenvironment and pre-stimulation with Th1-type or Th2-type mediators.³¹ Therefore, the precise role of pDCs in human asthma is still unclear.

We show for the first time that allergen challenge is associated with a marked influx of both mDCs and pDCs into BALF in patients with asthma. Of note, the percentage increase of pDCs in BALF was higher than that of mDCs in BALF, as reflected by an enhanced pDC:mDC ratio after allergen challenge. In keeping with previous data,¹⁷ there was a decrease in mDCs in peripheral blood after allergen challenge. In addition, there was a trend to a decrease in peripheral blood pDCs in this condition. These data suggest that both mDCs and pDCs are recruited from peripheral blood to the human respiratory tract after allergen challenge. Migration of immature peripheral blood dendritic cells into the airways would also explain the observation that the percentage of mature CD83⁺ mDCs was significantly reduced in BALF after allergen challenge. However, the decrease in mature CD83⁺ mDCs in BALF might also be due to migration of mature endobronchial

mDCs to mediastinal lymph nodes after allergen challenge. Notably, the number of local effector cells (such as eosinophils and lymphocytes) was correlated with the number of infiltrating dendritic cells after allergen challenge. This finding suggests that the amount of dendritic cells recruited into the airways might be linked to or triggered by airway inflammation. Thus, according to the concept that dendritic cells have a role in maintaining an established airway inflammation in asthma,⁸ an inflammation-triggered recruitment of dendritic cells into the airways could represent a vicious cycle in allergic asthma. It remains to be elucidated, however, whether the recruited mDCs and pDCs have similar or opposing roles in this condition.

In conclusion, we described a strategy to comprehensively analyse dendritic cell subsets and dendritic cell surface molecules in human bronchoalveolar lavage fluid. Using this strategy, we showed a marked local increase of not only mDCs but also pDCs after allergen challenge in patients with asthma, which suggests that both dendritic cell subsets are involved in the pathogenesis of asthma.

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