Dendritic cell subsets in human bronchoalveolar lavage fluid

after segmental allergen challenge

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ABSTRACT

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

Methods: Seven patients with allergic asthma (median: 23 years, range: 19 - 25 years old) underwent segmental challenge and were lavaged 10 minutes and 24 hours after challenge. DC subsets and surface markers in BALF and in peripheral blood were analysed using four-color flow cytometry.

Results: Plasmacytoid DCs (pDCs, median: 0.06 %, range: 0.01 - 0.08 %) and myeloid DCs (mDCs, median: 0.47 %, range: 0.27 - 0.87 %) were detectable in BALF from control segments. CD1a-positive DCs 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 hours (p = 0.018 to the control segments for pDCs and mDCs), but not 10 minutes after allergen challenge. The percentage increase of pDCs was higher than the percentage increase of mDCs after allergen challenge, as reflected by an enhanced pDC/mDC ratio after allergen challenge. In peripheral blood, there was a significant decrease of mDCs (p = 0.038) and a trend to a decrease of pDCs (p = 0.068) 24 hours after allergen challenge. Analysis of DC surface molecules revealed that BALF DCs after allergen challenge have a less mature phenotype compared to BALF DCs from control segments.

Conclusion: Using a comprehensive strategy to analyse dendritic cell subsets in human bronchoalveolar lavage fluid we demonstrate for the first time that both myeloid and plasmacytoid dendritic cells accumulate in the airway lumen after allergen challenge in patients with asthma.
INTRODUCTION

Dendritic cells (DCs) 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. Animal models suggest that DCs play a crucial role in asthma. Allergen challenge results in a recruitment of DCs into the airways, which is essential for the establishment of allergic airways inflammation. Instillation of allergen-loaded DCs into the airways alone can induce allergic airway inflammation in healthy animals. Selective elimination of DCs during allergic airway inflammation abolishes characteristic features of asthma, including eosinophilic inflammation, goblet cell hyperplasia, and bronchial hyperresponsiveness. These data indicate that DCs are vital for both the initiation and the maintenance of allergic airways inflammation in asthma.

Human DCs 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. CD11c+ myeloid DCs (mDCs) and CD123+ plasmacytoid DCs (pDCs) represent two main DC subtypes. There is an ongoing research to further characterise these two DC subtypes in human lung parenchyma and in human bronchoalveolar lavage fluid (BALF). Patients with asthma display increased numbers of intraepithelial DCs in the airways, as compared with healthy individuals. Allergen challenge causes an accumulation of CD11c+ mDCs within the bronchial mucosa, and a parallel decline of these cells in circulating blood. In contrast, CD123+ pDC were postulated to be absent from the bronchial mucosa, both before and after allergen challenge, and have not yet been studied in peripheral blood after allergen challenge. However, local increases of pDCs have been reported in patients with atopic rhinitis and dermatitis after allergen challenge, as well as elevated pDC counts in peripheral blood of patients with allergic asthma. 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 bronchoalveolar lavage fluid after allergen challenge in human asthma. This study investigates DC subsets in human BALF after segmental allergen challenge using a comprehensive flow cytometric method.
METHODS

Subjects
Seven patients with mild allergic asthma (median: 23 years, range: 19 - 25 years old), the majority 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, 4. a dual reaction following allergen inhalation 21. The calculation of the individual provocation dose was performed 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 21. Briefly, 2.5 ml of saline was instilled into the left S8 (Control 1, C1) and S5 (Control 2, C2) segment, and allergen (diluted in 2.5 ml of saline) was instilled into the right S8 (Allergen 1, A1) and S5 (Allergen 2, A2) segment. The left and right S8 (C1 and A1) segments were lavaged using 100 ml of prewarmed saline after 10 minutes, the left and right S5 segment (C2 and A2) after 24 hours. 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), A2: 60 ml (22 - 64 ml). Prior to each bronchoscopy, venous blood samples were obtained.

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<th>Medication</th>
<th>Total IgE [kU/l]</th>
<th>Specific IgE [kU/l]</th>
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Table 1. Patient characteristics
The table 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 medication prior to the study (BA: Inhaled Beta-2-Agonist, IC: Inhaled corticosteroid, CR: Cromoglycate), 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 (DP: Dermatophagoides pteronyssinus) and the dose (in allergen units, AU) used for segmental allergen challenge. * DC counts only from saline and allergen challenged segments after 24 hours.
Analysis of cell counts and leukocyte subsets in BALF

BALF samples were filtered through a two layer sterile gauze into sterile plastic vials, centrifuged at 4°C and 500 x 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) and for cytospins. Cytospins were stained with May/Grünwald/Giemsa-solution, and differential cell counts determined using standard morphological criteria. Results were expressed as 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 minutes. Afterwards, FACS Lysing solution (BD Biosciences, Heidelberg, Germany) was added for another 10 minutes. Cells were centrifuged for 5 minutes (400 x g) and washed with 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 (BD Biosciences). To identify DC subsets in BALF, we used an approach previously described to detect mDCs and pDCs in peripheral blood 20. CD3, CD14, CD16, CD19, CD20, and CD56 negative/dim cells (lin<sup>neg/dim</sup>) were gated using a commercial lineage cocktail with antibodies against all of these antigens (Fig. 1A). Among these lin<sup>neg/dim</sup> cells, distinct populations of CD123<sup>+</sup>HLA-DR<sup>+</sup> cells (“pDC”) and CD11c<sup>+</sup>HLA-DR<sup>+</sup> cells (“mDC”) were identified, both in peripheral blood and in BALF (Fig. 1A). A population of CD1a<sup>+</sup>HLA-DR<sup>+</sup>lin<sup>neg/dim</sup> cells identified in BALF could hardly be detected in peripheral blood (Fig. 1A). These CD1a<sup>+</sup> DCs co-expressed CD11c in all BALF samples, suggesting that these DCs are a subpopulation of mDCs (Fig. 1B). Backgating revealed that pDCs, mDCs and CD1a<sup>+</sup> DCs 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 (Chicago, IL, USA). Most parameters were non-normally distributed. Therefore, correlation analysis was performed using the 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 the Wilcoxon signed rank test. The comparison of DCs in peripheral blood and BALF was performed using the Mann Whitney U test. Probability values of p < 0.05 were regarded as significant.
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*Table 2. Antibodies used for four-colour flow cytometry*

Abbreviations denote: Blood Dendritic Cell Antigen (BDCA), Fluorescein isothiocyanate (FITC), Phycoerythrin (PE), Allophycocyanin (APC), Peridinin chlorophyll protein (PerCP).
RESULTS

DC subsets in BALF and peripheral blood after segmental allergen challenge

In the BALF collected 24 hours after saline challenge, low amounts of pDCs (median: 0.06 % or 0.06 x 10^3 cells/ml BALF; range: 0.01 - 0.08 % or 0.01 - 0.34 x 10^3 cells/ml BALF), mDCs (median: 0.47 % or 0.63 x 10^3 cells/ml BALF; range: 0.27 - 0.87 % or 0.25 - 3.2 x 10^3 cells/ml BALF) and CD1^a+ mDCs (median: 0.34 % or 0.56 x 10^3 cells/ml BALF; range: 0.24 - 0.56 % or 0.25 - 1.63 x 10^3 cells/ml BALF) were detected. There were no significant differences to DC counts in BALF 10 minutes after saline challenge (Fig. 2). In all patients, the percentages as well as the total numbers of pDCs (median: 0.56 % or 2.71 x 10^3/ml BALF; range: 0.09 - 1.83 % or 0.06 - 16.29 x 10^3/ml BALF; p = 0.018 to the corresponding control), mDCs (median: 1.82 % or 7.45 x 10^3/ml BALF; range: 0.95 - 2.29 % or 0.60 - 20.38 x 10^3/ml BALF; p = 0.018), and CD1^a+ mDCs (median: 1.44 % or 5.22 x 10^3/ml BALF; range: 0.63 - 2.00 % or 0.40 - 17.80 x 10^3/ml BALF; p = 0.018) increased significantly in BALF 24 hours, but not 10 minutes after allergen challenge (Fig. 2). The percentage increase of pDCs 24 hours after allergen challenge was higher than the percentage increase of mDCs 24 hours 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 to control segments (median: 0.11, range: 0.04 - 0.23) after 24 hours (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 hours 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 4 out of 6 patients and mDCs in 5 out of 6 patients in peripheral blood 24 hours after challenge (Fig. 2). This decrease in peripheral blood DC counts was statistically significant in case of mDCs (p = 0.038), but not in case of pDCs (p = 0.068).

Association of DCs with the inflammatory response and allergen dose

Compared to the control segments, there were significantly increased neutrophil (p = 0.043) and eosinophil counts (p = 0.028) in BALF 24 hours, but not 10 minutes after challenge (see Table S1 in the online supplement for details). The amount of infiltrating DCs was correlated with the severity of the local inflammatory response, as reflected by a significant correlation between the total numbers of mDCs, CD1^a+ mDCs and pDCs with eosinophil counts (r > 0.9 and p < 0.001 for all DC subsets) and lymphocyte counts (r = 0.82 - 0.96, p < 0.05 for all DC subsets) in BALF 24 hours after challenge. There was also a correlation between the total numbers of mDCs, CD1^a+ mDCs and pDCs and the allergen dose used for challenge (r > 0.86 and p < 0.01 for all DC subsets).

Surface molecules on DCs in BALF and peripheral blood

Both in peripheral blood and in BALF, the expression of BDCA-2 was restricted to pDCs, while BDCA-1 expression was restricted to a subset of mDCs (Fig. 3, Table 3). In contrast to peripheral blood, BDCA-3 was not only expressed 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 two-fold 24 hours after allergen challenge compared to the control segments (Table 3). Of note, BDCA-3 and BDCA-4 were strongly expressed by other BALF cells which were lin^bright and HLA-DR^+. Since 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 Figure S1 in the online supplement for BDCA expression on BALF cells). Compared to 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 hours after allergen challenge were characterised by a significantly lower expression of CD83 compared to corresponding control segments (p = 0.046) (Fig. 3, Table 3). Due 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 hours after challenge. There was a non-significant trend to a decreased expression of CD40 on BALF pDCs compared to blood pDCs 24 hours after challenge (p = 0.09) (Fig. 3, Table 3).
Table 3. Expression of surface molecules on DC subsets

The table 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 minutes after allergen challenge (A1) is not displayed, because cell counts in BALF were too low to measure surface molecules on DCs. In BALF from saline challenged control segments (C1 and C2), numbers of pDCs were too low to quantify the expression of surface molecules (n.a. denotes not analysable). B1: peripheral blood before allergen challenge; B2: peripheral blood 24 hours after allergen challenge; C1: BALF 10 minutes after saline challenge; C2: BALF 24 hours after saline challenge; A2: BALF 24 hours after allergen challenge. B.d. denotes below detection limit.

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<th>B 2 (%)</th>
<th>C 1 (%)</th>
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<td>62.8 (42.8 – 80.8)</td>
<td>63.5 (38.4 – 82.6)</td>
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<td>85.9 (73.3 – 96.8)</td>
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<td>0.2 (0.0-10.1)</td>
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<tr>
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<td>19.6 (15.6 – 32.6)</td>
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<tr>
<td>BDCA-4</td>
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<td>0.0 (0.0 – 4.6)</td>
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DISCUSSION

In this study, we report a comprehensive strategy for the identification, quantification and characterisation of plasmacytoid DCs (CD123+HLA-DR+linneg/dim) and myeloid DCs (CD11c+HLA-DR+linneg/dim) in human bronchoalveolar lavage fluid using four-color 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. Furthermore, it provides a detailed analysis of the distribution of DC antigens on these infiltrating DCs. Although our study included a comparably small number of subjects, the uniformity of the data suggests that our results are representative for a larger population and that a larger number of subjects would not have changed the findings considerably.

Dendritic cells in BALF have initially been identified using immunocytochemistry, and were postulated to yield approximately 0.4 % of all cells in BALF of healthy subjects 23. In subsequent studies, the same group identified DCs by their low autofluorescent properties in flow cytometric analyses, and divided these low autofluorescent cells in subgroups of CD1a+ and CD1a- cells 24,25. Using flow cytometry with a different gating strategy compared to our study, Donnenberg and Donnenberg recently described CD123+ plasmacytoid DC (0.02 %) and CD11c+ myeloid DC (0.06 %) in BALF from healthy volunteers 12. Our study is the first to use all three markers (CD1a, CD11c, CD123) to characterise DCs in BALF. With this approach, we identified CD1a+DCs 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+DCs were previously described as intraepithelial DCs, with a yet undefined relationship to 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 DCs 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 linbrightHLA-DR+ cells within the lymphocyte/DC gate. BDCA-4 was also expressed on mDCs and a linbrightHLA-DR+ cell population in BALF. These data suggest that BDCA-3 and BDCA-4 are not specific for mDCs or pDCs in BALF of patients with allergic asthma. In addition, the previously reported BDCA-3+HLA-DR+ DC 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-negative 10 and that the T-cell proliferation induced by mDC2 ranges between the T-cell proliferation 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 play 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 pro-allergic role of mDCs and anti-allergic properties of
pDCs. However, there is uncertainty whether this concept is applicable to human asthma. Plasmacytoid DCs are increased in peripheral blood of patients with allergic asthma and other atopic diseases, and are positively correlated with IgE-levels and eosinophil counts. In patients with atopic rhinitis and dermatitis, there is a strong local increase of pDCs after allergen challenge. 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. A recent report suggests that the decision whether pDCs will trigger either a Th1-type or Th2-type immune response may be dependent on the local microenvironment and a pre-stimulation with Th1-type or Th2-type mediators. Therefore, the precise role of pDCs in human asthma is still unclear.

We demonstrate 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 the percentage increase of mDCs in BALF, as reflected by an enhanced pDC/mDC ratio after allergen challenge. In keeping with previous data, there was a decrease of mDCs in peripheral blood following allergen challenge. In addition, there was a trend to a decrease of peripheral blood pDCs in this condition. These data suggest that both mDCs and pDCs are recruited from peripheral blood to the human respiratory tract following allergen challenge. A migration of immature peripheral blood DCs 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 of mature CD83⁺ mDCs in BALF might also be due to a 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 DCs after allergen challenge. This finding suggests that the amount of DCs recruited into the airways might be linked to or triggered by airways inflammation. Thus, according to the concept that DCs play a role in maintaining an established airways inflammation in asthma, an inflammation-triggered recruitment of DCs 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 describe a strategy to comprehensively analyse DC subsets and DC surface molecules in human bronchoalveolar lavage fluid. Using this strategy, we demonstrate a marked local increase of not only mDCs but also pDCs after allergen challenge in patients with asthma which suggests that both DC subsets are involved in the pathogenesis of asthma.
FIGURE LEGENDS

Figure 1. Identification of DC subsets in BALF
A: Total cells were identified in a FSC/SSC-Plot (first row). Lineage negative/dim (linneg/dim) cells were further gated (second row) to identify dendritic cells. Among linneg/dim cells, plasmacytoid dendritic cells were identified by CD123 and HLA-DR expression (third row), myeloid dendritic cells by CD11c and HLA-DR expression (fourth row) and CD1a+ dendritic cells by co-expression 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.

Figure 2. DC subsets in BALF and peripheral blood after allergen challenge
Shown are percentages of DCs in BALF and total DC counts in BALF and peripheral blood. A1: BALF 10 minutes after allergen challenge; A2: BALF 24 hours after allergen challenge; B1: peripheral blood before SAP; B2: peripheral blood 24 hours after SAP; C1: BALF 10 minutes after saline challenge; C2: BALF 24 hours after saline challenge. Significant differences between allergen challenged and corresponding control segments, and between peripheral blood before and 24 hours after allergen challenge, are marked with an asterisk (*). Median values are displayed as bars for A2, B1 and B2.

Figure 3. Surface molecules on DCs in BALF and peripheral blood
Plasmacytoid dendritic cells (CD123+HLA-DR+linneg/dim) and myeloid dendritic cells (CD11c+HLA-DR+linneg/dim) were gated as described in Fig. 1. Histograms display the expression of surface molecules (red) compared with corresponding isotype controls (grey).
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COMPETING INTERESTS
The authors have no competing interests to declare.

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ETHICS APPROVAL
The study was approved by the local ethics committee of Rostock, Germany.

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blood BALF (saline) BALF (allergen)

**A**

- **gating of total cells**
- **gating of lineage neg/dim cells**

- CD123⁻ HLA-DR⁻ linneg/dim (pDC)
- CD11c⁻ HLA-DR⁻ linneg/dim (mDC)
- CD1a⁻ HLA-DR⁻ linneg/dim (mDCsub)

**B**

- **CD1a⁺ DCs**

**C**

- "backgating"
CD123+ HLA-DR+ linneg/dim (pDC)

CD11c+ HLA-DR+ linneg/dim (mDC)

CD1a+ HLA-DR+ linneg/dim (mDCsub)

% of total BALF cells

cells (10^3/ml BALF)

cells (10^3/ml blood)

Bratke et al., Fig. 2
Dendritic cell subsets in human bronchoalveolar lavage fluid
after segmental allergen challenge

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Werner Luttmann1, and J. Christian Virchow1

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<th></th>
<th>Macrophages (10^3/ml BALF)</th>
<th>Lymphocytes (10^3/ml BALF)</th>
<th>Neutrophils (10^3/ml BALF)</th>
<th>Eosinophils (10^3/ml BALF)</th>
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<tr>
<td>C1</td>
<td>133.0 (29.8 – 186.4)</td>
<td>2.9 (0.0 – 5.5)</td>
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<td>0.0 (0.0 – 2.7)</td>
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<td>A1</td>
<td>69.0 (11.2 – 148.2)</td>
<td>1.4 (0.5 – 2.2)</td>
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<td>0.0 (0.0 – 0.6)</td>
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<tr>
<td>C2</td>
<td>131.7 (54.7 – 671.8)</td>
<td>5.2 (1.0 – 14.1)</td>
<td>2.8 (0.0 – 7.3)</td>
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<tr>
<td>A2</td>
<td>125.5 (60.4 – 487.7)</td>
<td>30.5 (0.4 – 105.3)</td>
<td>8.2 * (2.3 – 81.9)</td>
<td>198.6 * (0.0 – 288.4)</td>
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**Table S1. Cell counts in BALF**

Total BALF cell counts are displayed as median values (range) from the 7 included patients with asthma. **A1**: BALF 10 minutes after allergen challenge; **A2**: BALF 24 hours after allergen challenge; **C1**: BALF 10 minutes after saline challenge; **C2**: BALF 24 hours after saline challenge. Significant differences to the corresponding control are marked with an asterisk (*).
Expression of blood dendritic cell antigens (BDCA) on BALF cells

To analyse the specificity of anti-BDCA antibodies for dendritic cell staining in BALF cells from patients with asthma, the lymphocyte and dendritic cell region of a forward scatter/side scatter plot was gated, excluding cells with high autofluorescence properties, e.g. alveolar macrophages and eosinophils. In this gate, BDCA-2 stained only $\text{lin}^{\text{neg/dim}}$ cells. BDCA-1 was mainly observed on $\text{lin}^{\text{neg/dim}}$ cells, but also stained a very low percentage of $\text{lin}^{\text{bright}}$ cells. In contrast, a strong expression of BDCA-3 and BDCA-4 was detected on a $\text{lin}^{\text{bright}}$ cell population which was also strongly positive for HLA-DR (Fig. S1, A). On alveolar macrophages, a strong expression of BDCA-3 and a weaker expression of BDCA-4 were found, while BDCA-1 and BDCA-2 could not be detected (Fig. S1, B). In the granulocyte region of the forward scatter/side scatter plot, low amounts of BDCA-3 and BDCA-4 positive cells were detected (Fig. S1, C).

Figure Legend

Figure S1. Blood dendritic cell antigen (BDCA) expression on BALF cells

A: BALF cells in the lymphocyte and dendritic cell region of a forward scatter/side scatter plot were gated (excluding cells with high autofluorescence such as alveolar macrophages and eosinophils) and $\text{lin}^{\text{neg/dim}}$ as well as $\text{lin}^{\text{bright}}$ cells were analysed for BDCA-1, 2, 3, 4, and HLA-DR expression. B: Alveolar macrophages were gated and analysed for BDCA-1, 2, 3, 4, and HLA-DR expression. C: Granulocytes (eosinophils and neutrophils) were gated and analysed for BDCA-1, 2, 3, 4, and HLA-DR expression.
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