

Selective Increase in Blood Dendritic Cell Antigen-3-Positive Dendritic Cells in Bronchoalveolar Lavage Fluid in Allergic Patients

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Abstract

Dendritic cells (DCs) are specific antigen-presenting cells that play critical roles in the initiation and polarization of immune responses. DCs residing in the lungs might be detected in the bronchoalveolar lavage fluid (BALF). We analysed DC compartment in the peripheral blood and BALF of patients with allergy and in controls. Plasmacytoid and four distinct subsets of myeloid DCs [characterized by the expression of blood dendritic cell antigen (BDCA)-1⁺ and -3⁺ and CD16 positivity or negativity] were detected in both tested compartments. We further evaluated the expression of C-type lectins [mannose receptor (MR), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and dendritic and epithelial cells (DEC)-205] relevant to the pathogenesis of asthma. Interestingly, we found a selective increase in the frequency of myeloid DC-expressing BDCA-3 and MR particularly in BALF from allergic patients. Specific and highly statistically significant increase in BDCA-3⁺ and/or MR⁺ DCs brings a novel characteristic to BAL analysis in allergic patients.

Introduction

Dendritic cells (DCs) are bone marrow-derived specific antigen-presenting cells that have critical role in the initiation and polarization of effector immune responses [1–3]. DCs reside specifically in the mucosal compartments at the interface between the environment and the organism, compartments where they constitutively sample antigens [4]. Depending on the nature of the antigenic content, DCs orchestrate the immune response, maintaining tolerance to self and harmless antigens and eliciting adequate immune response against pathogen and/or danger signals. Initiation of an adaptive immune response represents the hallmark of DC function. To fulfil their destiny, DCs constantly migrate between body compartments, particularly between mucosal surfaces and regional lymph nodes [5]. DCs are, therefore, detectable both in peripheral blood, representing the systemic response of organism, and in the bronchoalveolar lavage fluid (BALF), representing a mucosal surface sample. In both of these compartments, DCs form a small, but

well-defined population, representing approximately 0.5% of leucocytes (CD45⁺) [6]. The lung is the largest epithelial surface in the body and is exposed to an enormous challenge of microbes and other environmental antigens [7]. Owing to the obvious importance of lung DCs, there have been several attempts to characterize DC subsets in the lungs [7–10]. Recent work by Ten Berge [9] improved the methodology for working with the BALF DCs isolated from patients' samples. There are, however, still only a few reports on lung DCs, particularly from human studies [11]. There is also not a consensus on the subsets of DCs that reside in the lungs [9, 12, 13], especially in pathology.

Therefore, we initiated the study on lung DCs in respiratory allergy. We present the development of a feasible flow cytometry-based method for the parallel investigation of DC subsets in the peripheral blood and BALF of asthma patients. In the peripheral blood as well as in the BALF, plasmacytoid DCs (pDCs) and myeloid DCs (CD16⁺ mDCs) do not account for the total DC population. Another subpopulation of CD16⁺ mDCs was

Table 1 Characteristics of patients and control group.

Number (n)	Total number	Children Male/female	Adults Male/female
Blood			
Asthma/allergic rhinitis	11	1/1	2/7
Healthy donors	10	0/1	3/6
Bronchoalveolar lavage (BAL)			
Asthma	7	3/4	0
Controls	4	1/3	0

Dendritic cell subsets were investigated in peripheral blood in allergic patients with bronchial asthma or allergic rhinitis ($n = 11$) and healthy controls ($n = 10$). BAL was acquired from seven asthmatic children. Because of the limited diagnostic reasons for bronchoscopy in childhood, we chose four controls for BAL analysis with non-atopic diagnosis.

already described in peripheral blood [8, 14, 15]. In addition to measuring the individual subsets of DCs, we also analysed the expression of lectins dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), dendritic and epithelial cells (DEC)-205 and mannose receptor (MR). These molecules with pattern recognition receptor (PRR) function have crucial roles in respiratory tract diseases [10, 13, 16].

Patients and methods

Patients. We analysed DC subsets in peripheral blood and BALF in allergic patients and healthy controls, and characteristics of the groups are shown in Table 1.

Peripheral blood samples: Peripheral venous blood was collected from 11 patients with asthma and/or allergic rhinitis (pollinosis) and 10 healthy donors into EDTA tubes. Total serum IgE and specific IgE antibodies (against inhaled allergens – birch, grass and tree pollen) were elevated in all allergic patients (Table 2). All healthy controls had a total serum IgE in normal range and did not have any allergic history.

Bronchoalveolar lavage fluid samples: BALF was obtained during fibre-optic bronchoscopy from seven patients with asthma. Four children with non-allergic diseases (interstitial lung fibrosis, Kartagener's syndrome, lung vasculitis and atelectasis of right middle lobe) served as a control group.

Informed consent was obtained from all patients and controls prior to blood collection and bronchoscopy.

Bronchoalveolar lavage fluid. BALF was performed using a flexible fibre-optic bronchoscope (Olympus, Hamburg, Germany) placed in the right middle lobe. BAL fluid was collected in siliconized tubes to prevent cell adherence and was kept at 4 °C. BAL fluid was filtered through sterile gauze and centrifuged for 5 min at 266 g at 4 °C. The supernatant was removed, and the cells were subsequently resuspended in 300 μ l phosphate-buffered saline (PBS) supplemented with 1% foetal bovine serum at 4 °C and stained with monoclonal antibodies.

Staining of dendritic cells. One hundred microlitres of whole peripheral blood and 100 μ l of BALF cell suspension were stained with monoclonal antibodies for 30 min at 4 °C. Subsequently, red blood cells were lysed, cells washed in 3 ml PBS with 2 mmol EDTA, and analysed. We set up an eight-colour flow cytometry panel for simultaneous detection of all DC subsets in one sample tube [17]. We used following monoclonal antibodies: CD3-FITC, CD19-FITC, CD20-FITC, CD56-FITC, CD14-Dyomics590, CD16-Alexa700 (all from Exbio, Prague, Czech Republic), CD45-Pacific Blue (Dako, Glostrup, Denmark), human leucocyte antigen (HLA)-DR-PerCP [Becton Dickinson (BD), Heidelberg, Germany], CD123-PECy7 (eBioscience, San Diego, CA, USA), CD11c-APC (Invitrogen, Paisley, UK), blood dendritic cell antigen (BDCA)-1-PE (Miltenyi Biotec, Bergisch Gladbach, Germany), BDCA-3-PE (Miltenyi Biotec), BDCA-1-APC (Miltenyi Biotec), BDCA-3-APC (Miltenyi Biotec), MR-PE (BD), DC-SIGN-PE (R&D Systems, Minneapolis, MN, USA) and DEC-205-PE (BD). All antibodies were titrated to determine the optimal concentrations. A lineage cocktail (CD3, CD19, CD20 and CD56) was used to exclude other leucocytes subpopulations (T and B lymphocytes, NK cells). Antibody-capture beads (BD) were used for single-colour compensation controls for each reagent used in this study. Gating controls were determined using fluorescence minus one (FMO) controls for CD14, CD16 and lectins.

At least 300 000 events were acquired on FACSaria™ (BD) and subsequently analysed using FLOWJO software (Treestar, Ashland, OR, USA). All samples were processed and analysed immediately after blood sampling.

Statistics. The Mann–Whitney test was used for statistical analysis, and $P < 0.05$ was considered to be significant. Data are expressed as median with range.

Table 2 Laboratory markers in allergic patients and healthy controls measured in peripheral blood and bronchoalveolar fluid.

	Peripheral blood		Bronchoalveolar lavage			
	IgE total	C-reactive protein	Macrophages	Lymphocytes	Neutrophils	Eosinophils
	Range (UI/ml)	Range(mg/l)	Range (%)	Range (%)	Range (%)	Range (%)
Allergic patients	30.6–491	<8.0	14.4–97.6	0.4–58	0.2–84.4	0.4–0.8
Controls	0–10.1	<8.0	19.4–73.6	4.4–80	0.3–17.2	0

Results

Identification of DC subsets in the peripheral blood and BALF by eight-colour flow cytometry

The plasmacytoid DCs (pDCs) were characterized as CD45⁺, lineage-negative (CD3⁻, CD19⁻, CD20⁻, CD56⁻), CD14⁻, CD16⁻, HLA-DR⁺ and CD123⁺CD11c⁻. mDCs were characterized as CD45⁺, lineage-negative (CD3⁻, CD19⁻, CD20⁻, CD56⁻), CD14⁻, HLA-DR⁺ and CD11c⁺CD123⁻. These cells were then divided into CD16⁺ and CD16⁻ subgroups. In both subgroups, the expression of lectins (BDCA-1, BDCA-3, MR, DC-SIGN and DEC-205) was analysed.

First, we identified 'classical' myeloid DCs (CD16⁻ mDC) and pDCs in the peripheral blood and in the bronchoalveolar lavage using flow cytometry. Gating strategy is shown on a representative flow cytometry analysis [18, 19] (Fig. 1). Allergic patients had significantly lower frequency of pDCs in the BALF when compared to the blood. On the other hand, we found

a significant increase in CD16⁻ mDCs in the BALF of allergic patients compared to the blood ($P < 0.05$) (Fig. 2A, B).

We detected CD16⁺ mDCs in samples from both PB and BALF in patients and controls (Fig. 1). We did not find any significant differences in CD16⁺ mDCs between patients and controls, or between blood and BALF, but the relative number of CD16⁺ mDCs seemed to be decreased in the BALF compared to the peripheral blood in both groups (Fig. 2C). CD16⁺ mDCs are more frequent than CD16⁻ mDCs in the peripheral blood from patients and controls.

Differential expression of BDCA-1 and BDCA-3 in allergic patients in BALF

Myeloid DCs can be further subclassified according to the expression of BDCA-1 and BDCA-3. In humans, CD16⁻BDCA-1⁺ cells are termed mDC1 and CD16⁻BDCA-3⁺ represents mDC2 [4, 20]. We analysed the expression of BDCA molecules on both CD16⁻ and

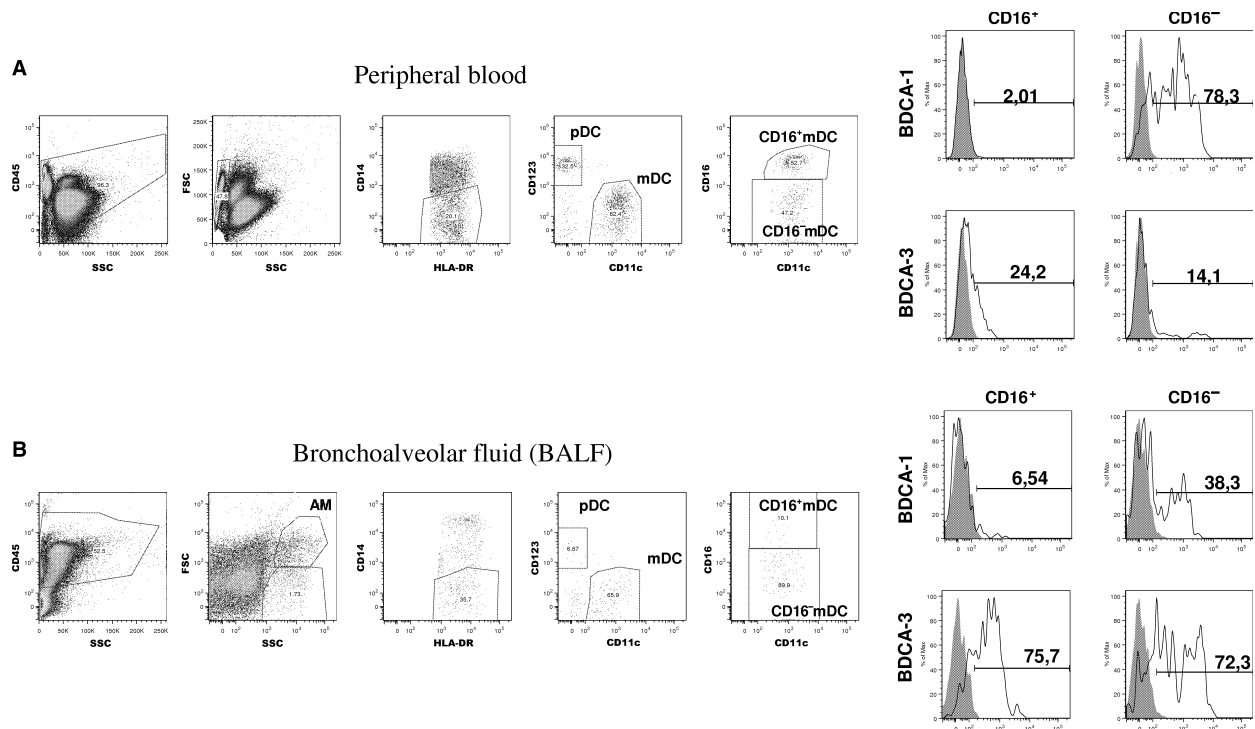


Figure 1 Flow cytometry analysis of dendritic cell (DC) population from whole blood and bronchoalveolar lavage. First, white blood cells were gated based on side scatter (SSC) and CD45 expression, and this was followed by exclusion of T and B lymphocytes, NK cells and granulocytes (CD3⁻, CD19⁻, CD20⁻, CD56⁻) and the inclusion of human leucocyte antigen-DR⁺ cells. In case of peripheral blood, lympho-monocytes were gated based on forward scatter and SSC. In case of bronchoalveolar lavage fluid (BALF) samples, the alveolar macrophages (AM) and CD14⁺ monocytes were excluded. Plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) were distinguished based on the expression of CD123 and CD11c, respectively. Subsequently, gating of mDCs (CD11⁺ cells) was based on the expression of CD16. mDCs were divided into 'classical' mDC (CD16⁻) and CD16⁺ mDCs. Both groups were investigated for the expression of C-type lectins [blood dendritic cell antigen (BDCA)-1, BDCA-3] using histograms. Grey histograms indicate the unstained controls, and white histograms indicate the expression of BDCA-1 or BDCA-3. The results presented here are from one allergic patient and are representative of 20 blood and 10 BALF samples.

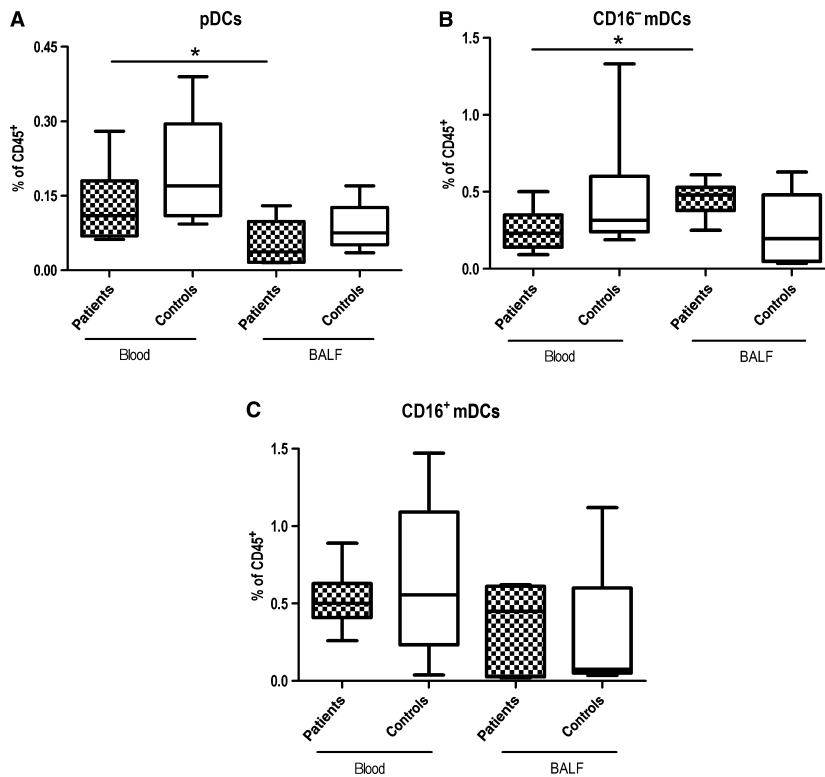


Figure 2 Dendritic cell (DC) subsets in the peripheral blood and bronchoalveolar lavage fluid (BALF). DCs form a small, but well-defined population, representing approximately 0.5% of total leucocytes (CD45⁺) in both the peripheral blood and BALF. The frequency of plasmacytoid DCs (pDCs) (A), 'classical myeloid DCs (mDCs)' CD16⁻mDCs (B) and CD16⁺mDCs (C) was investigated. Significant differences between blood and BALF are indicated by horizontal bars: * $P < 0.05$.

CD16⁺ compartments. Four separate populations of mDCs can thus be identified: CD16⁻BDCA-1⁺ (mDC1), CD16⁻BDCA-3⁺ (mDC2), CD16⁺BDCA-1⁺ and CD16⁺BDCA-3⁺. All of the DC subsets in patients and controls were present in the blood and BALF. There were no significant differences in the distribution of DC subsets between allergic patients and controls in the peripheral blood. The most prevalent population of DCs in the blood in both groups was CD16⁻BDCA-1⁺ followed by both BDCA-3⁺ subsets (CD16⁺BDCA-3⁺ and CD16⁻BDCA-3⁺) and finally the CD16⁺BDCA-1⁺ (Fig. 3A). The distribution of myeloid DC subsets in the BALF from healthy controls resembled their frequency in the peripheral blood, with CD16⁻BDCA-1⁺ DCs being the most frequent subset (Fig. 3B). However, there was a striking difference in the proportion of BDCA-3-expressing mDCs in the BALF from allergic patients. While CD16⁻BDCA-3⁺ DCs only represented $0.035 \pm 0.024\%$ (mean $\pm$ SD) of CD45⁺ cells in the peripheral blood, they accounted for $0.271 \pm 0.076\%$ of CD45⁺ leucocytes in the BALF ($P < 0.001$) and represented the most dominant DC population together with CD16⁻BDCA-1⁺. Similarly, the frequency of CD16⁺BDCA-3⁺ DCs was four times higher in allergic patients $0.06 \pm 0.052\%$ to $0.244 \pm 0.23\%$ ($P = 0.07$) (Fig. 4A). Moreover, the expression of BDCA-3⁺ on DCs in the BALF of allergic patients was significantly higher than on BDCA-3⁺ DCs in the peripheral blood ($P < 0.05$) (Fig. 4B).

In the BALF from asthma patients, BDCA-3⁺ DCs, both CD16⁺ and CD16⁻, formed strikingly dominant population significantly increased in comparison with controls.

Different expression of lectins on the surface of DCs in peripheral blood and BALF

In addition to the DC-specific lectins BDCA-1 and BDCA-3, we also analysed the expression of other lectin molecules implicated in the binding of an allergen to DCs. DC-SIGN, DEC-205 and MR were all found on the CD16⁺ and CD16⁻ DC subsets in the blood and mainly in the BALF (Fig. 5A). Similar to BDCA-3, there was a dramatic increase in MR⁺ CD16⁺ and CD16⁻mDCs in the BALF compared to the peripheral blood in both groups ($P < 0.01$ and $P < 0.01$, respectively). Moreover, the MR expression on CD16⁻ mDC was significantly increased in the BALF of allergic patients compared to healthy controls ($P < 0.05$) (Fig. 5B). Similar results were found for DC-SIGN and DEC 205 (Fig. 5A).

Discussion

Dendritic cells play the central role in the development of immune reaction. Their unique capacity to translate the activation of the innate immune system to the adaptive immune response puts DCs in a crucial position

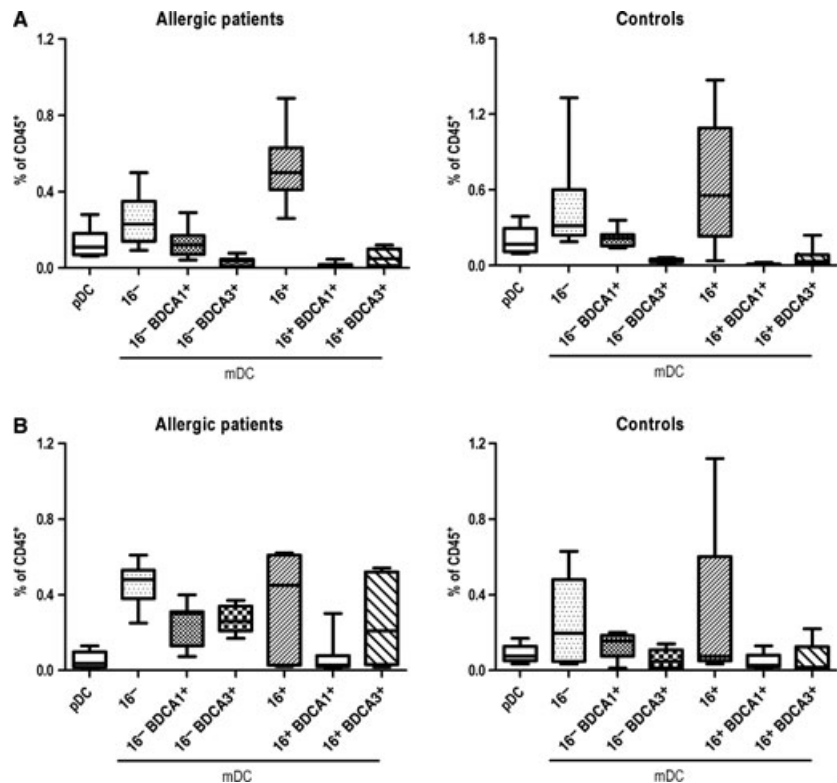


Figure 3 Distribution of dendritic cell (DC) subpopulations in the peripheral blood and bronchoalveolar lavage. Plasmacytoid DCs (pDCs) and four populations of myeloid DCs (mDCs), based on the previously described characteristics of these cells [CD45⁺, human leucocyte antigen (HLA)-DR⁺, CD11c⁺, CD14⁻], blood dendritic cell antigen (BDCA)-1 or BDCA-3 expression and presence of CD16, are shown in the peripheral blood (A) and bronchoalveolar lavage fluid (BALF) (B).

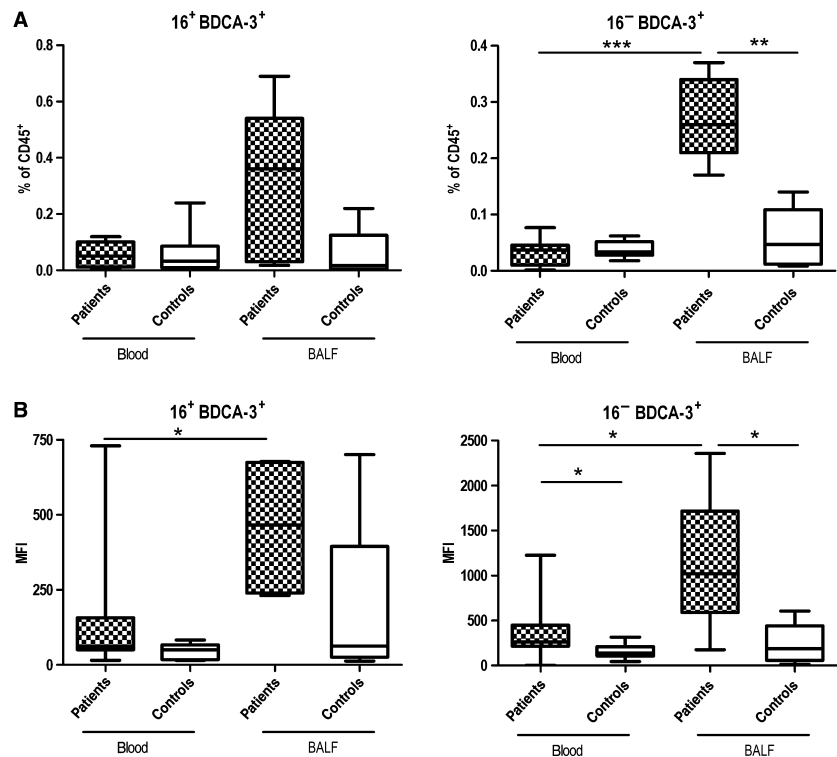
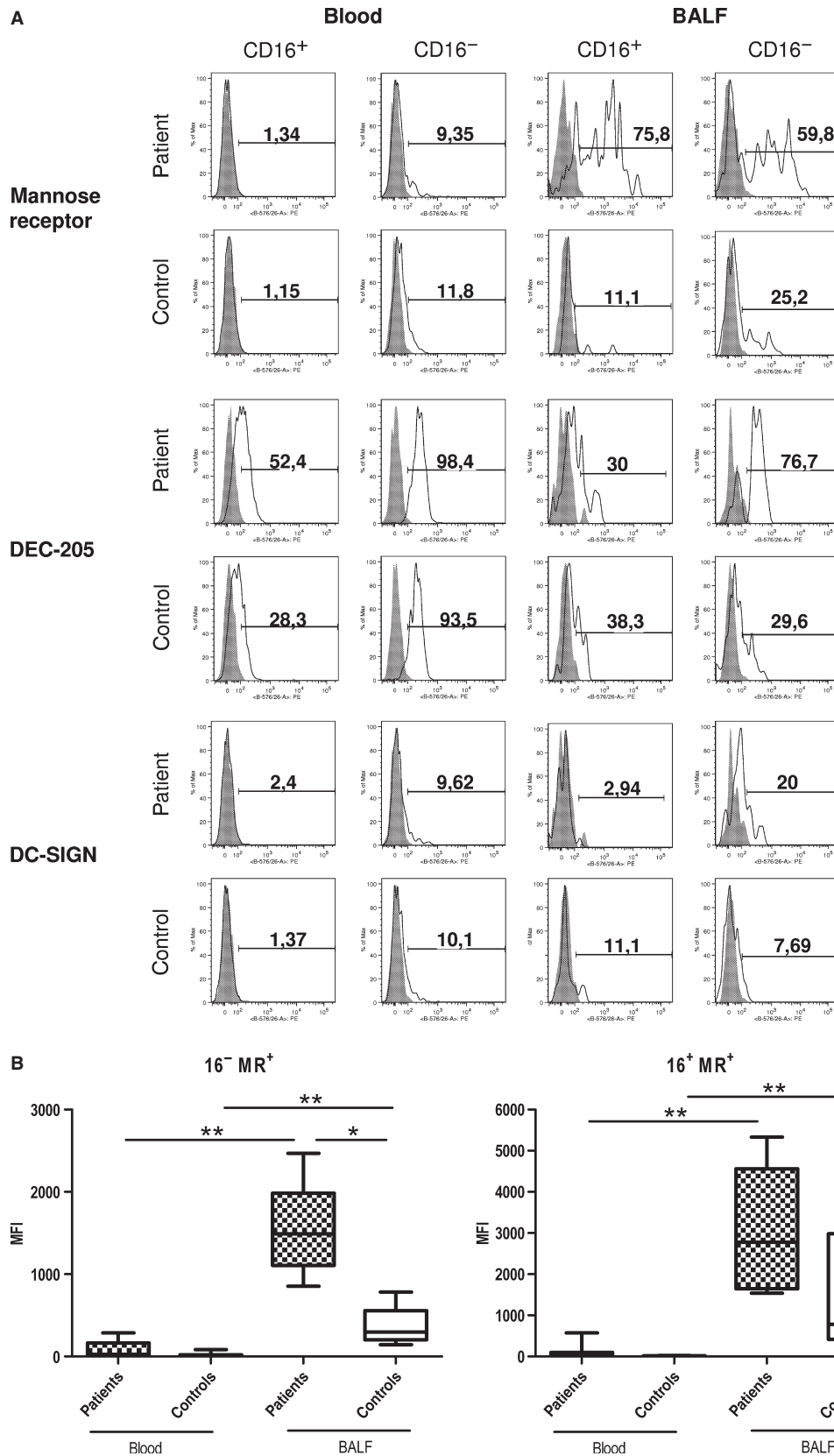


Figure 4 Relative numbers (percentage of CD45⁺) of CD16⁺ blood dendritic cell antigen (BDCA)-3⁺ and CD16⁻BDCA-3⁺ myeloid DCs (mDCs) in the peripheral blood and bronchoalveolar lavage fluid (BALF). The frequency (A) and surface expression (B) of BDCA-3⁺ mDCs (CD16⁺ mDCs and CD16⁻ mDCs) were analysed in samples from both the peripheral blood and lungs. Significant differences between blood and BALF or between groups are indicated by horizontal bars: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

in an immune network [1, 2]. This network is of particular importance on mucosal and epithelial surfaces, specifically in the lungs. Lung DCs continuously sample and

present inhaled antigens and induce tolerance to harmless substances and adequate immune response to pathogens and other antigens [10]. DCs residing in the lungs con-



sist of several subsets with specific functions as recently reviewed [12, 21]. There is, however, still no consensus on the lung DC spectrum. The situation is particularly difficult in inflammatory conditions in which the cellularity of the lung tissue and surfaces undergoes striking changes. Here, we present a feasible flow cytometry-based method for DC identification in the BALF.

There is a general consensus that two major subsets of DCs, myeloid (mDCs) and plasmacytoid DCs (pDCs), reside in the lungs (for detailed review see Ref. [7]). While pDCs represent a well-characterized subset, mDCs consist of a heterogeneous population. BDCA-1 and BDCA-3 are considered as specific markers of mDC subsets and were found in samples from both the peripheral blood and lungs [4, 8]. We developed an eight-colour flow cytometry method to distinguish pDCs and mDCs, defined as lineage-negative, CD45⁺, HLA-DR⁺, CD11c⁺, CD16[−] and BDCA1⁺ or BDCA3⁺. However, applying these criteria did not cover the total DC lung population (Fig. 1). Another subpopulation of mDCs, CD16-positive, is clearly present in samples from both the peripheral blood and BALF. In agreement with Piccioli *et al.* [22], we analysed pDCs and all four subpopulations of mDCs, based on BDCA-1 or BDCA-3 expression and CD16 positivity. All investigated DC populations were present both in the peripheral blood and in the BALF.

Concerning pDCs, we observed their lower numbers in allergic lungs. Such finding is consistent with described role of pDCs in the maintenance of tolerance to antigens in lungs [23]. The population of mDCs showed more pronounced differences between allergic and control subjects. BDCA-1- and BDCA-3-expressing mDCs dominate the DC subpopulations in allergic patients (Fig. 4). Both CD16⁺ and CD16[−] BDCA-3⁺ mDCs are strikingly increased in the BALF of patients with allergy. The presence of BDCA-3⁺ cells has been previously demonstrated in lung tissue [4], but the predominance of this subset in the BALF of allergic patients was not yet shown and is of particular interest.

The BDCA-3 molecule is identical to thrombomodulin. In a recent study, thrombomodulin (BDCA-3, CD141) was identified by allergen-induced gene expression microarray performed on DCs as a gene that was differentially induced in allergen-stimulated DCs from atopic individuals [24]. Furthermore, sorted BDCA-3⁺ DCs induced a Th2-polarized response, suggesting that BDCA-3⁺ expression on DCs may have a significant role

in allergic diseases. BDCA-3⁺ DCs, in some reports also referred to as mDC2s, were showed to be more prevalent in the peripheral blood in atopic individuals (Fig. 2B) [24, 25]. The presence of this population in the BALF has not yet been tested. In this context, our results support the importance of BDCA-3⁺ DC subset in the immune reactions in asthmatic lungs.

Human BDCA-3⁺ DCs have also been suggested to be homologous to mouse CD8 α ⁺ DCs [26]. In mouse models, CD8 α ⁺ T cells are involved in allergic airway inflammation and induce the production of Th2 cytokines such as IL-4, IL-5 and IL-13 [27]. These reports further indicate the potential significance of BDCA-3⁺ DC subset in the BALF of asthmatic patients.

In addition to DC-specific lectins BDCA-1 and BDCA-3, we also analysed other lectin molecules on blood and lung DCs. Immature DCs express a plethora of specialized cell receptors for patterns associated with foreign antigens, such as the C-type lectin carbohydrate receptors (DC-SIGN, macrophage MR and DEC-205) [21, 28–30]. Their role in allergen uptake in DCs has already been described [31–33] and very recently confirmed for a larger set of allergens [34].

We describe here a significant increase in the expression of both MR and DC-SIGN in classical, CD16[−] mDCs in BALF of allergic patients, confirming thus the importance of DCs in antigen/allergen processing on a border with external environment. Lung DCs are in an immature state *in situ* and express the MR [28]. The MR expression on DCs is crucial for the Th2 polarization of an immune response [34], and DC-SIGN expression on DC surface is also important in DCs–T cells interaction [29]. These previous studies clearly demonstrate the importance of the expression of lectins on DCs in allergy; however, its presence and/or significance on DCs in the BALF of allergic patients have not yet been profoundly investigated. Our findings thus complement recently published data. Concerning other lectins, DEC-205, with repeatedly described role in antigen uptake [35], was also increased in blood CD16⁺ mDCs and BALF CD16[−] mDCs from allergic patients. Such finding might suggest a systemic nature of allergic response, with distinct roles for different subsets of DCs in different compartments.

In summary, we present a flow cytometry-based approach for the detection of DC subsets in the BALF. There was a striking difference in the proportion of BDCA-3-expressing mDCs in the BALF of allergic

Figure 5 Surface expression of C-type lectins on CD16⁺ myeloid DCs (mDCs) and CD16[−] mDCs in the peripheral blood and bronchoalveolar lavage fluid (BALF). Here, we show a detailed analysis of C-type lectin receptor expression on dendritic cell (DC) subsets in the peripheral blood and BALF using histograms (A). The grey histograms indicate unstained controls, and the white histograms indicate the expression of dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), dendritic and epithelial cells (DEC)-205 and mannose receptor (MR). The frequency of MR-positive mDCs (CD16⁺ mDCs and CD16[−] mDCs) and the expression of MR on mDCs (B) were investigated in the peripheral blood and BALF. Significant differences between the blood and BALF or between groups are indicated by horizontal bars: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. The results presented here are from one allergic patient and are representative of 20 blood and 10 BALF samples.

patients. The proportion of CD16[−]BDCA-3⁺ DCs in the BALF of allergic patients was tenfold higher than in the peripheral blood. Similarly, the frequency of CD16⁺BDCA-3⁺ DCs was four times higher in patients (Fig. 4A). Similar to the BDCA-3 molecule, there was a dramatic increase in MR⁺ CD16⁺ and CD16[−]mDCs in the BALF of allergic patients compared to the peripheral blood and the expression of MR on 16[−] mDCs in the BALF was significantly increased compared to the healthy controls (Fig. 5B). Similar results were found for other two C-type lectins. Specific increase in BDCA-3⁺ and/or MR⁺ DCs in allergic children, particularly in the BALF, brings new insight into the pathogenesis of bronchial asthma and together with the recently published data supports the role of these molecules in the allergen uptake and Th2 polarization. The importance of increased BDCA-3 and MR expression on lung mDCs for the pathogenesis of allergic reaction and bronchial asthma requires further studies.

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References

- Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007;449:419–26.
- Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. *Curr Top Microbiol Immunol* 2006;311:17–58.
- Bratke K, Klein C, Kuepper M, Lommatzsch M, Virchow JC. Differential development of plasmacytoid dendritic cells in Th1- and Th2-like cytokine milieus. *Allergy* 2011;66:386–95.
- Demedts IK, Brusselle GG, Vermaelen KY, Pauwels RA. Identification and characterization of human pulmonary dendritic cells. *Am J Respir Cell Mol Biol* 2005;32:177–84.
- Cook DN, Bottomly K. Innate immune control of pulmonary dendritic cell trafficking. *Proc Am Thorac Soc* 2007;4:234–9.
- van Haarst JM, de Wit HJ, Drexhage HA, Hoogsteden HC. Distribution and immunophenotype of mononuclear phagocytes and dendritic cells in the human lung. *Am J Respir Cell Mol Biol* 1994;10:487–92.
- Masten BJ, Olson GK, Tarleton CA *et al.* Characterization of myeloid and plasmacytoid dendritic cells in human lung. *J Immunol* 2006;177:7784–93.
- Autissier P, Soulas C, Burdo TH, Williams KC. Evaluation of a 12-color flow cytometry panel to study lymphocyte, monocyte, and dendritic cell subsets in humans. *Cytometry A* 2010;77:410–9.
- Ten Berge B, Muskens F, Kleinjan A *et al.* A novel method for isolating dendritic cells from human bronchoalveolar lavage fluid. *J Immunol Methods* 2009;351:13–23.
- von Garnier C, Nicod LP. Immunology taught by lung dendritic cells. *Swiss Med Wkly* 2009;139:186–92.
- Tsoumakidou M, Tzanakis N, Papadaki HA, Koutala H, Siafakas NM. Isolation of myeloid and plasmacytoid dendritic cells from human bronchoalveolar lavage fluid. *Immunol Cell Biol* 2006;84:267–73.
- GeurtsvanKessel CH, Lambrecht BN. Division of labor between dendritic cell subsets of the lung. *Mucosal Immunol* 2008;1:442–50.
- Hattori T, Konno S, Hizawa N *et al.* Genetic variants in the mannose receptor gene (MRC1) are associated with asthma in two independent populations. *Immunogenetics* 2009;61:731–8.
- Kassianos AJ, Jongbloed SL, Hart DN, Radford KJ. Isolation of human blood DC subtypes. *Methods Mol Biol* 2010;595:45–54.
- MacDonald KP, Munster DJ, Clark GJ, Dzionek A, Schmitz J, Hart DN. Characterization of human blood dendritic cell subsets. *Blood* 2002;100:4512–20.
- Inoue K, Takano H, Koike E *et al.* Candida soluble cell wall beta-glucan facilitates ovalbumin-induced allergic airway inflammation in mice: possible role of antigen-presenting cells. *Respir Res* 2009;10:68.
- Horvath R, Budinsky V, Kayserova J *et al.* Kinetics of dendritic cells reconstitution and costimulatory molecules expression after myeloablative allogeneic haematopoietic stem cell transplantation: implications for the development of acute graft-versus host disease. *Clin Immunol* 2009;131:60–9.
- Rozkova D, Horvath R, Bartunkova J, Spisek R. Glucocorticoids severely impair differentiation and antigen presenting function of dendritic cells despite upregulation of Toll-like receptors. *Clin Immunol* 2006;120:260–71.
- Sochorova K, Horvath R, Rozkova D *et al.* Impaired Toll-like receptor 8-mediated IL-6 and TNF-alpha production in antigen-presenting cells from patients with X-linked agammaglobulinemia. *Blood* 2007;109:2553–6.
- Dzionek A, Fuchs A, Schmidt P *et al.* BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol* 2000;165:6037–46.
- Heath WR, Carbone FR. Dendritic cell subsets in primary and secondary T cell responses at body surfaces. *Nat Immunol* 2009;10:1237–44.
- Piccoli D, Tavarini S, Borgogni E *et al.* Functional specialization of human circulating CD16 and CD1c myeloid dendritic-cell subsets. *Blood* 2007;109:5371–9.
- de Heer HJ, Hammad H, Soullie T *et al.* Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004;200:89–98.
- Yerkovich ST, Roponen M, Smith ME *et al.* Allergen-enhanced thrombomodulin (blood dendritic cell antigen 3, CD141) expression on dendritic cells is associated with a TH2-skewed immune response. *J Allergy Clin Immunol* 2009;123:209–16.e4.
- Spears M, McSharry C, Donnelly I *et al.* Peripheral blood dendritic cell subtypes are significantly elevated in subjects with asthma. *Clin Exp Allergy* 2011;41:665–72.
- Robbins SH, Walzer T, Dembele D *et al.* Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome Biol* 2008;9:R17.
- Agrawal DK, Shao Z. Pathogenesis of allergic airway inflammation. *Curr Allergy Asthma Rep* 2010;10:39–48.
- Cochand L, Isler P, Songeon F, Nicod LP. Human lung dendritic cells have an immature phenotype with efficient mannose receptors. *Am J Respir Cell Mol Biol* 1999;21:547–54.
- Geijtenbeek TB, Torensma R, van Vliet SJ *et al.* Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* 2000;100:575–85.
- Mahnke K, Guo M, Lee S *et al.* The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. *J Cell Biol* 2000;151:673–84.

- 31 Deslee G, Charbonnier AS, Hammad H *et al.* Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. *J Allergy Clin Immunol* 2002;110:763–70.
- 32 Emara M, Royer PJ, Abbas Z *et al.* Recognition of the major cat allergen Fel d 1 through the cysteine-rich domain of the mannose receptor determines its allergenicity. *J Biol Chem* 2011;286:13033–40.
- 33 Huang HJ, Lin YL, Liu CF, Kao HF, Wang JY. Mite allergen decreases DC-SIGN expression and modulates human dendritic cell differentiation and function in allergic asthma. *Mucosal Immunol* 2011;4:519–27.
- 34 Royer PJ, Emara M, Yang C *et al.* The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. *J Immunol* 2010;185:1522–31.
- 35 Birkholz K, Schwenkert M, Kellner C *et al.* Targeting of DEC-205 on human dendritic cells results in efficient MHC class II-restricted antigen presentation. *Blood* 2010;116:2277–85.