

## Peripheral blood dendritic cell subtypes are significantly elevated in subjects with asthma

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### Clinical & Experimental Allergy

#### Summary

**Background** Dendritic cells (DCs) are crucial for the processing of antigens, T lymphocyte priming and the development of asthma and allergy. Smokers with asthma display altered therapeutic behaviour and a reduction in endobronchial DC CD83 expression compared with non-smokers with asthma. No information is available on the impact of smoking on peripheral blood DC profiles.

**Objective** Determine peripheral blood DC profiles in subjects with and without asthma with differing smoking histories.

**Methods** Forty-three asthmatics (17 smokers, nine ex-smokers and 17 never-smokers) and 16 healthy volunteers (nine smokers and seven never-smokers) were recruited. Spirometry, exhaled nitric oxide and venesection was performed. DC elution was by flow cytometry via the expression of DC surface markers [plasmacytoid (pDC) (BDCA-2, CD303), type 1 conventional (cDC) (BDCA-1, CD1c), and type 2 cDC (BDCA-3, CD141)].

**Results** Subjects with asthma displayed increases in all DC subtypes compared with normal never-smokers: [type 1 cDCs – asthma [median% (IQR)]: 0.59% (0.41, 0.74), normal never-smokers: 0.35% (0.26, 0.43),  $P=0.013$ ]; type 2 cDCs – asthma: 0.04% (0.02, 0.06), normal never-smokers: 0.02% (0.01, 0.03),  $P=0.008$  and pDCs – asthma: 0.32% (0.27, 0.46), normal never-smokers: 0.22% (0.17, 0.31),  $P=0.043$ , and increased pDC and type 1 cDCs compared with normal smokers. Smoking did not affect DC proportions in asthma. Cigarette smoking reduced pDC proportions in normal subjects [normal never-smokers: 0.22% (0.17, 0.31); normal smokers: 0.09% (0.08, 0.15),  $P=0.003$ ].

**Conclusions and Clinical Relevance** This study shows for the first time that subjects with asthma display a large increase in peripheral blood DC proportions. Cigarette smoking in asthma did not affect the peripheral blood DC profile but did suppress pDC proportions in non-asthmatic subjects. Asthma is associated with a significant increase in circulating DCs, reflecting increased endobronchial levels and the importance of DCs to the development and maintenance of asthma. (Clinical trials.gov identifier: NCT00411320)

**Keywords** asthma, classical, dendritic cells, myeloid, plasmacytoid, smoking

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#### Introduction

Dendritic cells (DCs) process and display antigens and therefore have a crucial role in determining T lymphocyte responses [1]. Pulmonary DCs, which continually sample inhaled antigens directly from the airway, are increased in number in asthma and are thought to play an important role in the development and persistence of this common condition [1–4]. DCs are also known to display marked

heterogeneity [1], and a commonly employed sub-classification is into conventional (also known as myeloid) and plasmacytoid cells. Conventional DCs (cDCs) have a crucial role in the development of Th2 responses and allergic airways inflammation [1, 5], while plasmacytoid DCs (pDCs) have a role in response to viral infections, development of tolerance and control of allergic airways inflammation [1].

Cigarette smoking is known to impact on a number of allergic and autoimmune diseases [6, 7]. *In vitro* research

has demonstrated that cigarette smoke alters DC function with effects on the development of Th2 responses [8]. Smokers with asthma display altered disease characteristics when compared with never-smokers with asthma including increased symptoms, acceleration of lung function decline, a reduction in airway eosinophilia and attenuated short-term airway responses to both inhaled and oral corticosteroids [9]. Endobronchial biopsies from smokers with asthma also contain fewer CD83-expressing DCs, suggesting changes to airway DC maturation may be of relevance to the altered responses of this group [10].

The majority of pulmonary DCs are derived from a pool of DCs that traffic from peripheral blood rapidly in response to antigen [11, 12]. However, despite evidence of alteration of pulmonary DCs, blood DC profiles in smokers with asthma have not been studied. Therefore the aim of this pilot study was to describe peripheral blood DC proportions in a group of smokers with asthma in comparison to other groups with differing asthma and smoking histories. The study was driven by the hypothesis that DC profiles in subjects with asthma would be altered by current smoking.

## Methods

### Subjects

Subjects were recruited from the community, hospital clinics and following participation in previous clinical trials. All subjects were free of respiratory tract infection for at least 6 weeks and provided informed consent before participation. Ethical approval for the study was provided by West Glasgow Ethics Committee. The study is registered with Clinicaltrials.gov (identifier; NCT00411320).

The criteria for inclusion of subjects with asthma was a history of asthma for more than 6 months and demonstration of either reversible airflow obstruction ( $\geq 12\%$  and 200 mL increase in FEV<sub>1</sub>), peak expiratory flow (PEF) lability (20% change in PEF in 3 days) or a positive methacholine challenge test result [13]. Asthma symptoms were assessed using the Juniper Asthma Control Questionnaire (ACQ) [14]. Healthy control subjects without asthma were included if they had no history of respiratory or inflammatory conditions and no evidence of airflow obstruction on spirometry.

Current smoking was defined as regular smoking of more than five cigarettes per day and more than five pack years smoking history, ex-smoking as no smoking for at least 2 years and more than five pack years smoking history. Never-smokers had no history of previous smoking.

### Measurements

Smoking history was confirmed by measurement of urine cotinine (SmokeScreen<sup>TM</sup> sampling system, GFC Diagnos-

tics, Bicester, UK) and exhaled carbon monoxide (Pico Smokerlyzer<sup>®</sup>, Bedford Scientific, Harrietsham, UK). The fractional concentration of nitric oxide in exhaled breath (F<sub>ENO</sub>) was assessed in subjects with asthma at the flow rate of 50 mL/s (Niox Flex<sup>®</sup>, Aerocrine AB, Solna, Sweden) following international standards [15].

### Dendritic cell elution

Venous heparinized blood samples were used to identify and quantify peripheral blood DC proportions and subtypes by flow cytometry (FACScalibur, Becton Dickinson UK Ltd, Abingdon, UK) using a commercial kit (Blood Dendritic Cell Enumeration kit, Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The procedure used a range of markers including CD303 and CD304 for pDC, CD1c for subset 1 of conventional (myeloid) dendritic cells (type 1 cDC), and CD141 for subset 2 of conventional (myeloid) dendritic cells (type 2 cDC). Some of these markers are also expressed by other cell types that might interfere with the analysis. In blood, co-expression of CD303 and CD304 is restricted to pDCs, however CD1c is also expressed on B-lymphocytes, and CD141 is also expressed at low levels on pDCs, on type 1 cDCs, on subsets of monocytes and on granulocytes. For this reason a cocktail of fluorochrome-labelled antibodies and a sophisticated sequential gating strategy was applied. This included using anti-CD303-FITC, anti-CD1c-PE and anti-CD141-APC to identify the DCs, and anti-CD19-PE-Cy<sup>TM</sup>5 and anti-CD14-PECy5 to identify and to exclude B lymphocytes and monocytes, respectively. Dead cells in the preparation were labelled with a fluorescent dye that is permeable only to those cells which have a disrupted cell membrane. This dye is activated by strong light, binds to nucleic acid and thereby labels dead cells for exclusion.

A 300  $\mu$ L sample of whole anti-coagulated blood was incubated with 20  $\mu$ L of the antibody cocktail and a separate 300  $\mu$ L of blood incubated with 20  $\mu$ L of the appropriate labelled isotype control antibody cocktail. This included mouse IgG2a-PE, mouse IgG1-FITC, mouse IgG1-APC, anti-CD19-PE-Cy5 (isotype: mouse IgG1) and anti-CD19-PE-Cy5 (isotype: mouse IgG1). The two samples were archived and analysed similarly in parallel. After cell staining by incubation for 30 min in the dark, erythrocytes were lysed using a red cell lysis solution supplied with the assay kit. Cells were then washed and fixed with formaldehyde, and acquired using four-colour flow-cytometry (dual laser FACScalibur, Becton Dickinson UK Ltd). Debris was excluded using an appropriate value for the threshold on forward scatter. The frequency of DCs in blood is low therefore at least  $1 \times 10^6$  events (cells) were acquired.

The data were analysed using a sequential gating strategy. Firstly, a size (forward scatter) and refractive

index (side-scatter) dot-plot was generated and a region (P1) was drawn to include the main cell clusters and to exclude platelets and debris. Gating on P1, a second plot was generated of refractive index on *x*-axis and PE-Cy5 on the *y*-axis. The positive PE-Cy5 clusters included B lymphocytes, monocytes and the dead cells using their dye emission at 625 nm. DCs can be intermediate in size between lymphocytes and granulocytes, and can express low levels of CD14; therefore a region (P2) was drawn around the lymphocyte cell cluster close to but excluding the PE-Cy5-positive cluster, and close to but excluding the large granulocyte cluster. Type 1 cDCs and pDCs were then identified, respectively, by gating on P1/P2 and creating a FITC-CD303 and PE-CD1c plot. The specificity of this labelling was confirmed on the control labelled preparation and being guided by this, appropriate regions were drawn around these DC clusters to quantify them. Quantifying the type 2 cDC fraction required creating a FITC/APC plot on P1/P2 thereby revealing type 2 cDCs by their strong CD141-APC label. Again, the control labelled cell plot provided a context to create an optimum region for a sample and control background count.

The DC subset frequencies were calculated for each patient sample by subtracting the non-specific stained counts from the sample counts and then dividing this by the cell counts for the events (cells) acquired, and expressing this as a percentage. DC populations are also presented as a ratio of cDC/pDC, given previous research suggesting a reduction in the ratio indicates increased Th2 inflammation [16].

### Statistical analysis

Data were stored and analysed using Minitab 15 software (Minitab Inc., State College, PA, USA). Data were summarized as mean and standard deviation or median and interquartile range depending on distribution. Between groups comparison was made using the Mann-Whitney

*U*-test and correlations were examined using the Pearson product-moment correlation coefficient after log transformation of non-normal distributed data when required. Statistical significance was accepted at  $P < 0.05$ . Adjustment for multiple comparisons has not been performed in this study given its exploratory nature.

## Results

### Baseline characteristics

The groups were well matched at baseline (Table 1). Smokers with asthma had higher ACQ scores [smokers median ACQ: 2.1 (IQR 1.1, 3.0); never-smokers: 1.0 (0.8, 1.7),  $P = 0.004$ ] and lower levels of exhaled nitric oxide (NO) compared with never-smokers with asthma [smokers NO: 11.4 p.p.b. (4.1, 13.3); never-smokers NO: 30.0 p.p.b. (16.3, 63.4),  $P < 0.001$ ] but were prescribed equivalent doses of inhaled corticosteroids. Ex-smokers with asthma were prescribed significantly higher doses of inhaled corticosteroid and also had higher ACQ scores compared with never-smokers with asthma. There were no significant differences between pack year histories of smokers with asthma and ex-smokers with asthma and when compared with normal smokers.

### Blood dendritic cell profiles

#### Normal subjects

Comparison of the peripheral DC profiles of the smoking and never-smoking healthy control subjects revealed:

- a significant reduction in pDCs in smokers [normal never-smokers median: 0.22% (IQR 0.17, 0.31); normal smokers: 0.09% (0.08, 0.15),  $P = 0.003$ ]
- a tendency towards a reduction in type 1 cDCs in smokers [normal never-smokers: 0.35% (0.26, 0.43); normal smokers: 0.22% (0.16, 0.30),  $P = 0.056$ ]

Table 1. Baseline characteristics

|  | Subjects with asthma        |                               |                                   | Normal subjects            |                                  |
|--|-----------------------------|-------------------------------|-----------------------------------|----------------------------|----------------------------------|
|  | Smokers<br>( <i>n</i> = 17) | Ex-smokers<br>( <i>n</i> = 9) | Never-Smokers<br>( <i>n</i> = 17) | Smokers<br>( <i>n</i> = 9) | Never-smokers<br>( <i>n</i> = 7) |
| Age (years)  | 46.9 (6.8)                  | 49.1 (9.3)                    | 41.4 (9.1)                        | 47.8 (7.3)                 | 36.6 (9.0)                       |
| Sex (F : M)  | 10 : 7                      | 5 : 4                         | 10 : 7                            | 8 : 1                      | 5 : 2                            |
| Pack years   | 29.9 (16.0)                 | 23.8 (6.6)                    |                                   | 26.0 (9.4)                 |                                  |
| Asthma duration (years)                            | 21.7 (15.8)                 | 22.2 (4.9)                    | 27.9 (14.9)                       |                            |                                  |
| Inhaled steroid (Beclometasone equivalent; µg/day) | 929 (140)                   | 1244 (573)*                   | 709 (421)                         |                            |                                  |
| Pre BD FEV <sub>1</sub> (% predicted)              | 75.1 (19.9)                 | 79.4 (25.6)                   | 74.5 (16.4)                       | 100.0 (16.0)*              | 104.5 (12.3)*                    |
| ACQ score (0–6)                                    | 2.1 (0.9)*                  | 2.3 (0.3)*                    | 1.2 (0.7)                         |                            |                                  |

Data presented as mean ± SD.

\* $P < 0.05$  compared with never-smokers with asthma.

BD, bronchodilator; FEV<sub>1</sub>, forced expiratory volume in 1 s; ACQ, asthma control questionnaire; PEF, peak expiratory flow.

- no difference for type 2 cDCs [normal never-smokers: 0.02% (0.01, 0.03); normal smokers: 0.06% (0.02, 0.14),  $P = 0.114$ ].

This contributed to a significant difference in the cDC/pDC ratio when the groups were compared [normal never-smokers: 1.41 (1.29, 2.32); normal smokers: 2.38 (1.78, 4.99),  $P = 0.030$ ] (Figs 1–4).

*Subjects with asthma*

When examined as a whole (never-smokers, ex-smokers and smokers with asthma data combined) there was evidence of a significant increase in the proportion of peripheral blood DCs in the subjects with asthma. Subjects with asthma displayed increased type 1 cDCs (Fig. 1a), type 2 cDCs (Fig. 2a) and pDCs (Fig. 3a) compared with never-smoking normal subjects. No difference was evident in cDC/pDC ratio (Fig. 4a).

Compared with normal smokers, subjects with asthma displayed increased type 1 cDCs (Fig. 1a) and pDCs

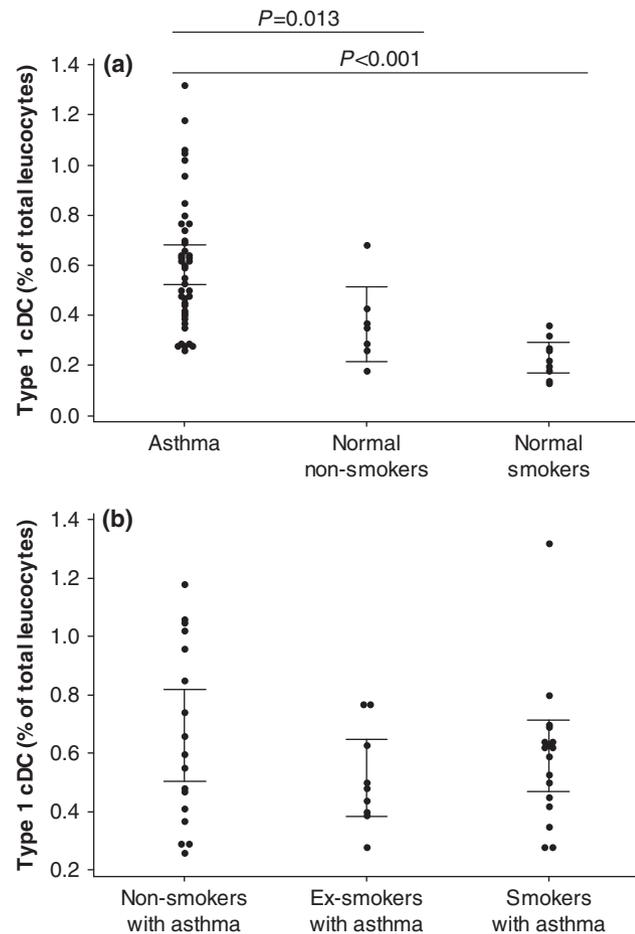


Fig. 1. (a) Type 1 cDC% in subjects with asthma compared with normal subjects. Interval bar 95% CI of mean. (b) Type 1 cDC% in subjects with asthma divided according to smoking history. cDC, conventional dendritic cell.

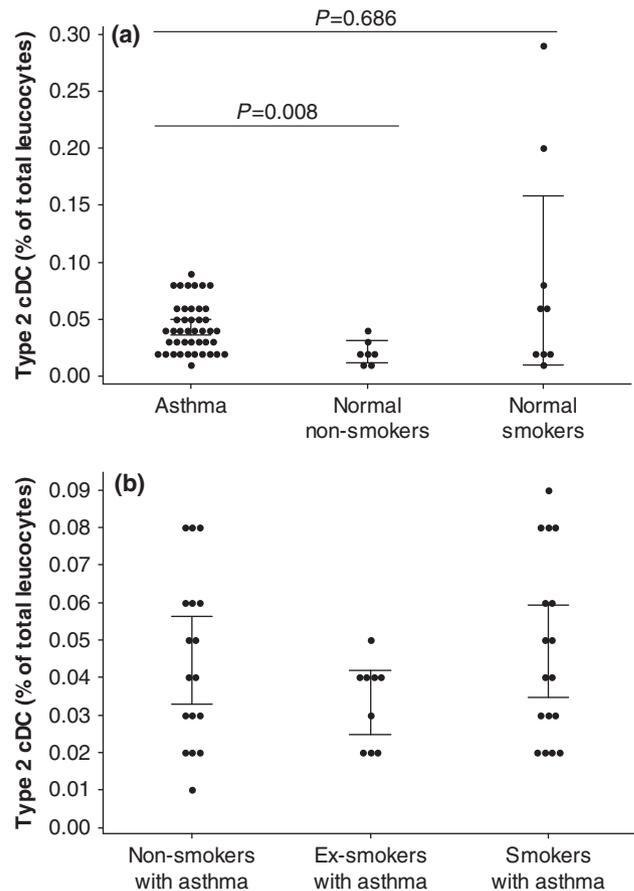


Fig. 2. (a) Type 2 cDC% in subjects with asthma compared with normal subjects. Interval bar 95% CI of mean. (b) Type 2 cDC% in subjects with asthma divided according to smoking history. cDC, conventional dendritic cell.

(Fig. 3a) and equivalency for type 2 cDCs (Fig. 2a). A tendency to a difference was evident when cDC/pDC ratio for asthma was compared with normal smokers (Fig. 4a).

*Smoking*

Following division of the subjects with asthma according to smoking history, clear differences were still evident in peripheral blood DC subsets compared with normal subjects. When compared with normal smokers, smokers with asthma demonstrated increased type 1 cDCs [smokers with asthma: 0.62% (0.44, 0.67); normal smokers: 0.22% (0.16, 0.30),  $P < 0.001$ ] and pDCs [smokers with asthma: 0.40% (0.30, 0.57); normal smokers: 0.09% (0.08, 0.15),  $P < 0.001$ ]. The ratio of total cDCs/pDCs for smokers with asthma was significantly lower compared with normal smokers [smokers with asthma: 1.7 (1.1, 2.0); normal smokers: 2.4 (1.8, 5.0),  $P = 0.018$ ].

Smokers with asthma demonstrated higher proportions for all DC subtypes compared with normal never-smokers; type 1 cDCs [smokers with asthma: 0.62% (0.44, 0.67); normal never-smokers: 0.35% (0.26, 0.43),  $P = 0.028$ ];

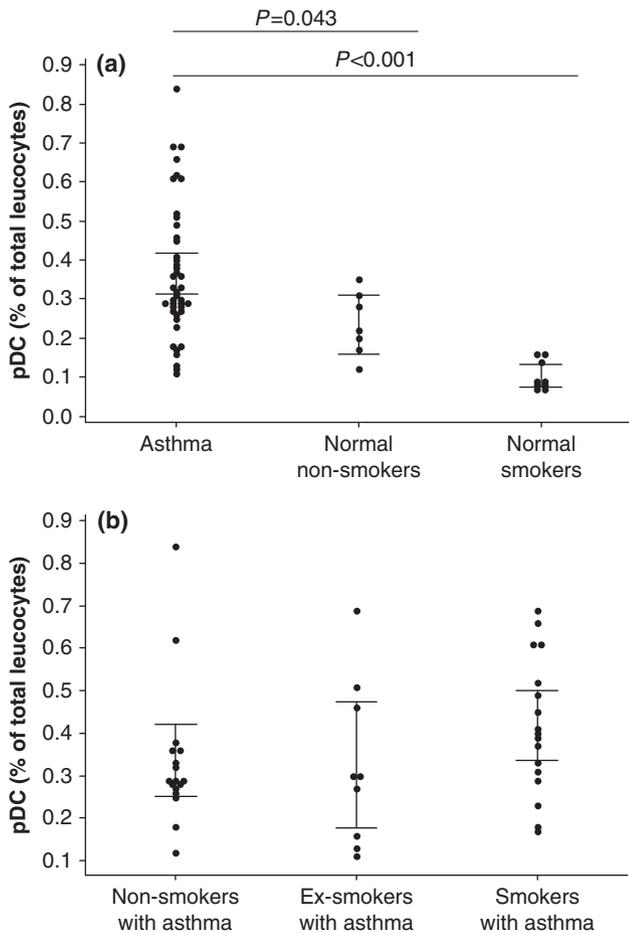


Fig. 3. (a) pDC% in subjects with asthma compared with normal subjects. Interval bar 95% CI of mean. (b) pDC% in subjects with asthma divided according to smoking history. pDC, plasmacytoid dendritic cell.

type 2 cDCs [smokers with asthma: 0.04% (0.03, 0.07); normal never-smokers: 0.02% (0.01, 0.03),  $P=0.011$ ] and pDCs [smokers with asthma: 0.40% (0.30, 0.57); normal never-smokers: 0.22% (0.17, 0.31),  $P=0.009$ ]. No difference was evident when cDCs/pDCs were compared.

Never-smokers with asthma also demonstrated significant differences for peripheral blood DC subtypes. Compared with normal never-smokers, never-smokers with asthma displayed higher type 1 cDCs [never-smokers with asthma: 0.60% (0.39, 0.99); normal never-smokers: 0.35% (0.26, 0.43),  $P=0.026$ ] and type 2 cDCs [never-smokers with asthma: 0.04% (0.03, 0.06); normal never-smokers: 0.02% (0.01, 0.03),  $P=0.017$ ] but equivalency for pDCs [never-smokers with asthma: 0.29% (0.27, 0.36); normal never-smokers: 0.22% (0.17, 0.31),  $P=0.105$ ]. No significant differences were present when cDC/pDC for never-smokers with asthma was compared with normal never-smokers.

Compared with normal smokers, never-smokers with asthma demonstrated increased type 1 cDCs [never-smokers with asthma: 0.60% (0.39, 0.99); normal smokers:

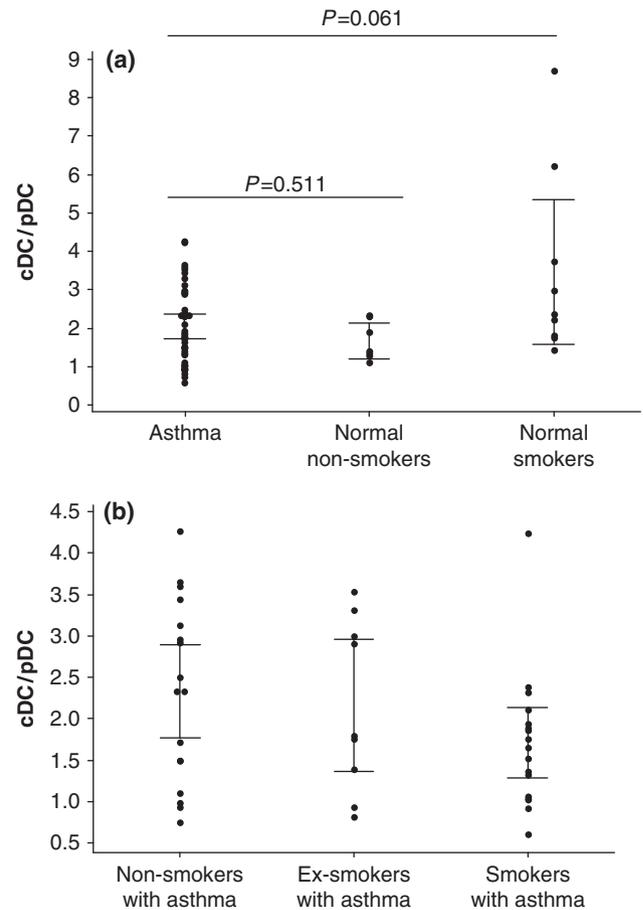


Fig. 4. (a) cDC/pDC ratio in subjects with asthma compared with normal subjects. Interval bar 95% CI of mean. (b) cDC/pDC ratio in subjects with asthma divided according to smoking history. cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell.

0.22% (0.16, 0.30),  $P<0.001$ ] and pDCs [never-smokers with asthma: 0.29% (0.27, 0.36); normal smokers: 0.09% (0.08, 0.15),  $P<0.001$ ] and no difference in the proportions of type 2 cDCs and cDC/pDC.

There were no differences evident between smokers with asthma and never-smokers with asthma for type 1 and 2 cDCs. There was evidence of a tendency to higher pDCs in smokers with asthma [smokers with asthma: 0.40% (0.30, 0.57); never-smokers with asthma: 0.29% (0.27, 0.36),  $P=0.056$ ] (Table 2 and Figs 1–3). Performing the same comparisons with ex-smokers with asthma demonstrated equivalency for all subgroups (Table 2). Smokers with asthma also demonstrated equivalency when cDC/pDC ratio was compared with never-smokers with asthma and ex-smokers with asthma (Table 2 and Fig. 4).

#### Correlation with clinical characteristics

When examined as a group subjects with asthma demonstrated correlations between asthma duration and pDCs

Table 2. Peripheral blood DC proportions

|             | Subjects with asthma             |                             |                               |                                   | Normal subjects                  |                                  |                            |
|-------------|----------------------------------|-----------------------------|-------------------------------|-----------------------------------|----------------------------------|----------------------------------|----------------------------|
|             | All subjects<br>( <i>n</i> = 43) | Smokers<br>( <i>n</i> = 17) | Ex-smokers<br>( <i>n</i> = 9) | Never-smokers<br>( <i>n</i> = 17) | All subjects<br>( <i>n</i> = 16) | Never-smokers<br>( <i>n</i> = 7) | Smokers<br>( <i>n</i> = 9) |
| Type 1 cDCs | 0.60 (0.04) <sup>*†</sup>        | 0.59 (0.24) <sup>†</sup>    | 0.52 (0.17)                   | 0.66 (0.31) <sup>*</sup>          | 0.29 (0.04)                      | 0.37 (0.16)                      | 0.23 (0.09)                |
| Type 2 cDCs | 0.04 (0.02) <sup>*</sup>         | 0.05 (0.02)                 | 0.03 (0.01)                   | 0.04 (0.02) <sup>*</sup>          | 0.06 (0.02)                      | 0.02 (0.01)                      | 0.08 (0.10)                |
| pDC         | 0.37 (0.17) <sup>*†</sup>        | 0.42 (0.16) <sup>†</sup>    | 0.33 (0.19)                   | 0.34 (0.17)                       | 0.16 (0.02)                      | 0.24 (0.08) <sup>†</sup>         | 0.10 (0.04)                |
| cDC : pDC   | 2.05 (1.01) <sup>†</sup>         | 1.71 (0.83) <sup>†</sup>    | 2.17 (1.04)                   | 2.33 (1.10)                       | 2.69 (0.51)                      | 1.68 (0.51) <sup>†</sup>         | 3.48 (2.45)                |

Data expressed as mean (SD). Smokers with asthma are only compared with normal smokers and never smokers with asthma with normal never smokers.

<sup>\*</sup>*P* < 0.05 compared with normal non-smokers.

<sup>†</sup>*P* < 0.05 compared with normal smokers.

cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell.

(*r* = -0.3, *P* = 0.026) and cDC/pDC ratio (*r* = 0.4, *P* = 0.017) and a tendency to a correlation between subject age and cDC/pDC ratio (*r* = 0.3, *P* = 0.086) and between asthma duration and type 2 cDCs (*r* = 0.3, *P* = 0.054).

A number of correlations were present between clinical characteristics and peripheral blood DC proportions in smokers with asthma. Type 2 cDCs demonstrated a negative correlation with inhaled corticosteroid dose (*r* = -0.5, *P* = 0.038) while asthma duration correlated with both cDC/pDC ratio (*r* = 0.6, *P* = 0.011) and type 2 cDCs (*r* = 0.5, *P* = 0.045). A tendency to a relationship was evident between pack years and type 1 cDCs (*r* = 0.5, *P* = 0.062) and between age and ratio of cDC/pDC (*r* = 0.5, *P* = 0.056). No correlation was evident between DC proportions and either FEV<sub>1</sub>% predicted or ACQ score in smokers with asthma. No correlations were evident between clinical characteristics and peripheral DC subsets in never-smokers with asthma.

## Discussion

Atopic asthma is associated with an increase in airway Th2 lymphocytes, which secrete IL-4, IL-5 and IL-13 and other cytokines, driving airway hyper-reactivity, increased IgE production and eosinophilic inflammation. However a number of preceding steps are required to recruit and polarize airway Th2 cells. Pulmonary DCs, which are increased in asthma [2, 3], are believed to play a crucial role in this process [1], as they sample the local tissue and airway for antigens which they process and present to T lymphocytes, stimulating the development and proliferation of antigen-specific Th2 cells [1, 4]. Subsets of DCs are also believed to be capable of stimulating the development of tolerance to antigen by the induction of immunosuppressive regulatory T lymphocytes [17]. Given this degree of control over T cell determination, DCs are of considerable therapeutic interest [18]. Smokers with asthma display a different pattern of clinical and therapeutic behaviour compared with non-smokers with asthma and have also been demonstrated to have altered

endobronchial DC appearances, with reduced CD83 expression [10]. This background, coupled to the lack of information on peripheral blood DC profiles in subjects with asthma led us to undertake the presented study.

The most striking and novel observation is the demonstration of a large increase in the proportion of peripheral blood DCs in subjects with asthma. We also present the first description of peripheral DC subsets in subjects with asthma with differing smoking histories. The finding of increased peripheral blood DC levels, given the previous demonstration of increased endobronchial DCs in asthma, is not surprising. However, the lack of a clear difference between smokers and non-smokers with asthma was unexpected given that smokers with asthma display clinical characteristics that consistently differentiate them from non-smokers with asthma [19–23] and the previous finding of reduced CD83-positive DCs in endobronchial biopsies from smokers with asthma [10]. As our data are descriptive and limited to peripheral blood we are unable to provide a mechanism to explain this large increase in asthma and the lack of a difference between smokers and non-smokers with asthma at present.

What could be driving this increase in circulating DCs in our subjects with asthma? Persistent inflammation, such as that observed in the airways of subjects with asthma, leading to increased cytokines and chemokines, promoting DC recruitment and enhanced survival is likely to be responsible. Asthma is characterized by impairment of epithelial repair and damage to the integrity of the epithelial layer [24, 25]. This phenomenon is associated with increases in cytokines such as thymic stromal lymphopoietin [26, 27] and chemokine ligand 20 (CCL20) [28], which are recognized to be important in DC priming and recruitment. Airway DCs are known to have a rapid turnover [29] and this combined with the epithelial damage evident in asthma could be driving an elevation in the proportion of cells trafficking via the bloodstream. However, if the relationship between airway inflammation alone and level of circulating DCs was strong we would expect smokers with asthma to display increased levels of

peripheral blood DCs reflecting their altered phenotype. Previous research in this group employing endobronchial biopsies has demonstrated that they display increased epithelial thickness and epithelial proliferation amongst other change but have similar proportions of intact epithelium compared with non-smokers with asthma [30]. Whether the key determinant of circulating DC levels in asthma is the degree of epithelial integrity is currently unknown but this is a tempting explanation for the lack of clear numerical differences in DC proportions between smokers and non-smokers with asthma.

Our findings also demonstrate that in healthy non-asthmatic subjects, smoking is associated with a reduction in pDCs, a trend towards a reduction in type 1 cDCs and an increase in the ratio of cDCs to pDCs. pDCs have an immunosuppressive effect in allergic airways disease models [17] and experimental arthritis [31] and are also thought to be important for induction of tolerance in the lung [32]. Therefore a reduction in circulating pDCs in smokers may reflect or actively contribute to some of the processes responsible for the increased inflammation present in smokers [7, 32]. However, an alternative viewpoint is that the observed reduction in pDC proportions may reflect an increase in airway pDC proportions in response to smoking. Unfortunately we do not have information on the airway DC profile in our groups to allow us to address this question.

Subdivision of the subjects with asthma into groups based on smoking history did not reveal any clear impact of smoking on DC profiles. Interestingly the only tendency observed was an increase in the proportion of pDCs in smokers compared with never-smokers with asthma which was not present when smokers with asthma were compared with ex-smokers with asthma. The tendency to a difference for pDC proportions and the clear difference related to smoking in normal subjects does suggest a potential role for this DC subtype in responses to smoking. Overall the absence of a clear difference in pDCs, coupled to the marked increase in peripheral blood DC proportions, suggests that the development of asthma overcomes the suppressive effect of smoking on peripheral blood pDCs proportions. Given the evidence linking pDCs to suppression of inflammation, the tendency to a higher proportion of this DC subtype in peripheral blood from smokers with asthma may reflect an attempt to reduce the greater level of endobronchial inflammation present in this group.

This study has some limitations that should be addressed by future studies. We did not examine the airway

DC profiles in our subjects and therefore lack information that would complement peripheral blood DC profiles. The subject's levels of circulating cytokines and chemokines, such as CCL2, 5 and 20, which could be important to the observed differences were not measured. Finally, DCs are known to display a high level of heterogeneity and the method employed in this study may not be able to detect other important differences in DC profiles between smokers with asthma and never-smokers with asthma. Future studies should attempt to characterize the *ex vivo* function of blood DCs from subjects with and without asthma, and differing smoking histories, including testing for stimulatory or inhibitory responses of syngenic lymphocytes to the subject's clinically relevant allergens and the relationship of airway DC profiles to these findings.

The large increase in circulating DCs in asthma relative to normal subjects displayed in this study suggest that measurement of peripheral blood DC proportions could provide an exploratory end-point in clinical trials examining new treatments in asthma. Reduction or normalization of DC levels could provide an interesting insight into biological mechanisms of new targeted therapies or existing approaches that may affect DCs such as anti-IgE therapy. Further examination in confirmatory trials will allow this issue to be addressed. A speculative point to consider is whether peripheral blood DC proportions could eventually aid clinicians with patients who are posing diagnostic dilemmas. Again further work would be required to clarify normal ranges for peripheral blood DC proportions in subjects with asthma, COPD and other obstructive airways diseases to facilitate this approach.

In conclusion, this report demonstrates the dramatic effect of asthma on the proportions of circulating DCs and the impact of smoking on pDC levels in normal subjects.

#### Clinical relevance

Asthma is associated with a large increase in circulating DCs relative to normal subjects. No clear impact from smoking is evident in asthma but current normal smokers display reduction of pDCs.

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