

BRIEF COMMUNICATION

Myeloid dendritic cells type 2 in allergic asthma

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Keywords

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Abstract

Background: Myeloid dendritic cells type 2 (mDC2s) are a new subtype of DCs identified in both the circulation and the lung and suggested to have a role in allergic asthma.

Methods: Circulating mDC2s were enumerated in 19 healthy, 18 atopic nonasthmatic, 18 mild atopic asthmatic, and 16 moderate/severe atopic asthmatic subjects using flow cytometry.

Results: The number of circulating mDC2s was significantly lower in atopic subjects compared with healthy controls and in asthmatic subjects compared with nonasthmatic subjects. There was a trend toward lower levels of circulating mDC2s with increasing allergy and asthma severity. The largest differences were seen in moderate/severe atopic asthmatics being $430.78 \pm 48.91/\text{ml}$ compared with healthy controls being $767.05 \pm 101.64/\text{ml}$ ($P < 0.05$).

Conclusions: Circulating mDC2s are lower in atopic and asthmatic subjects, which suggests that these cells efflux from the blood into the airways in patients with allergic disease.

Two main subpopulations of dendritic cells (DCs) exist: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) (1). Another subpopulation of DCs has been identified – mDCs type 2 (mDC2s) (2, 3), identified by BDCA-3 (CD141, thrombomodulin) expression. In circulation, DCs represent 1% of PBMCs, of which 0.60% are mDC1s, 0.37% are pDCs, and 0.03% are mDC2s. mDC2s share many characteristics with mDC1s, including morphology, endocytic capacity, and maturation (2); however, mDC2s do not express FcεRI (2), and stimulate T-cell proliferation less efficiently than mDC1s, albeit better than pDCs (4).

Yerkovich et al. (5) have suggested a role for mDC2s in allergic disease. After stimulation with house dust mite (HDM), BDCA-3 expression on blood DCs was higher in atopics compared with nonatopics, and these mDC2s induced a strong Th2-polarized response compared with DCs lacking BDCA-3 (5). Furthermore, circulating mDC2s were increased in allergic and asthmatic subjects compared with healthy individuals (5, 6). mDC2s are found in airway tissue (7) and lumen (8) and are higher in atopic asthmatics compared with healthy individuals (9, 10).

Much less is known about the role of mDC2s in allergic disease, when compared to mDC1s or pDCs. As such, we examined circulating mDC2s across population groups with allergy and asthma. We hypothesized that, consistent with the results of Yerkovich and coworkers (5), circulating

mDC2s are increased in subjects with allergy and asthma compared with healthy individuals.

Methods

Subjects

A total of 19 healthy, 18 atopic nonasthmatic, 18 mild atopic asthmatic, and 16 atopic moderate/severe asthmatic subjects were enrolled (Table 1). Atopy was defined by ≥ 1 positive skin wheel responses to common aeroallergens. Asthma was defined by a $PC_{20} < 16$ mg/ml, along with a positive clinical history. Moderate/severe asthmatics did not undergo a methacholine challenge. Mild asthmatics were distinguished from moderate/severe asthmatics by a $FEV_1 \geq 80\%$ predicted and without the need for an inhaled corticosteroid (ICS) with a long-acting β_2 -agonist as maintenance treatment. This study was approved by the Hamilton Research Ethics Board.

Dendritic cell staining, acquisition, and enumeration

A commercial DC enumeration kit was used to phenotype circulating DCs, including mDC1s, mDC2s, and pDCs (Miltenyi Biotec, Auburn, CA, USA) (Fig. 1). One million cells were acquired with a 15-color LSRII flow cytometer equipped with three lasers (Becton Dickinson Biosciences,

Table 1 Subject characteristics

	Healthy	Atopic nonasthmatic	Mild atopic asthmatic	Moderate/severe atopic asthmatic
Sample size (n)	19	18	18	16
Sex, male/female	6/13	7/11	6/12	3/13
Age	24.32 ± 2.31*	26.50 ± 1.76*	33.11 ± 3.59*	49.19 ± 3.84
FEV ₁ % predicted	94.96 ± 1.73*	93.14 ± 3.10*	95.43 ± 2.65*	62.10 ± 3.23
Methacholine PC ₂₀ (mg/ml)	>16	>16	2.38 ± 0.30	–
ICS/LABA use	0	0	0	16
HDM DP Allergy	0	11	8	6
Total serum IgE (KU/l)	23.05 ± 5.89	139.83 ± 24.99†	299.94 ± 94.25†	663.75 ± 487.71†

FEV₁, forced expiratory volume in 1s; PC₂₀, provocative concentration causing a 20% fall in FEV₁; ICS, inhaled corticosteroid steroid; LABA, long-acting β_2 -agonist.

Data are presented as means ± SEMs.

*Difference to moderate/severe atopic asthmatic group. $P < 0.05$.

†Difference to healthy group. $P < 0.05$.

Mississauga, ON, Canada). DCs were expressed as total numbers per milliliter of blood.

Statistical analysis

Statistics are expressed as means ± SEMs. Statistical analyses were performed using Prism software (La Jolla, CA, USA). Dunn's post-tests were performed to compare individual groups using a one-way ANOVA. Significance was accepted at $P < 0.05$.

Results

Moderate/severe atopic asthmatics were significantly older and, as expected from the enrollment criteria, had a lower resting FEV₁% predicted compared with each of the other three groups. Also, as expected, serum IgE levels were significantly lower in healthy subjects, when compared to each of the other three groups ($P < 0.05$) (Table 1).

Healthy subjects had the highest number of circulating mDC2s, followed by atopic nonasthmatics, mild atopic asthmatics, and finally moderate/severe atopic asthmatics (Fig. 2). When subjects were divided based on atopy and asthma, circulating mDC2s were lower in the atopic ($524.66 \pm 43.05/\text{ml}$) and asthma ($476.91 \pm 44.53/\text{ml}$) groups, compared with the nonatopic ($767.05 \pm 101.64/\text{ml}$) and nonasthmatic ($693.00 \pm 68.30/\text{ml}$) groups ($P = 0.03$ and $P = 0.02$, respectively). There was a trend toward lower mDC2 levels with increasing allergy and asthma severity ($P = 0.066$). Furthermore, there was a significant difference in number of circulating mDC2s between healthy subjects ($767.05 \pm 101.64/\text{ml}$) compared with moderate/severe atopic asthmatics ($430.78 \pm 48.91/\text{ml}$) ($P < 0.05$) (Fig. 2). This was also true for mDC2 percentage (Fig. 2). A trend toward a positive correlation between FEV₁% predicted and number of mDC2s existed among all subjects ($r = 0.23$, $P = 0.053$).

Discussion

Unexpectedly, our results differed from Yerkovich et al. (5). Using the same commercial kit, these investigators measured

the percentage of circulating mDC2s among similar groups, but did not include moderate/severe atopic asthmatics. These subjects were adolescents, and a minority of asthmatics were taking ICS. Circulating mDC2s were higher in atopic asthmatics compared with healthy subjects (5), and when subjects were further divided into atopic and asthmatic groups, blood mDC2s were higher in these groups compared with nonatopics and nonasthmatics, respectively (5). The authors illustrated a relationship between HDM allergen and mDC2s, demonstrating *in vitro* HDM exposure up-regulates BDCA-3 on DCs – the mDC2 marker (5). It is plausible that the increase in circulating mDC2s is a result of the perennial exposure to HDM, as supported by the fact that all their atopic subjects were HDM positive. A minority of subjects in our study were HDM-sensitized; therefore, it is possible that our results may more accurately reflect the relative number of circulating mDC2s in atopic asthmatics, without concomitant allergen exposure.

Asthma treatment may have influenced number of mDC2s, as moderate/severe asthmatics required regular treatment with ICS. However, mDC2s declined with increasing asthma and allergy, and there was not a difference in mDC1s among groups (data not shown), which argues against an effect of ICS treatment lowering DCs. It is also possible that age may have affected the number of mDC2, as the moderate/severe asthmatics were older. However, there was no correlation between age and mDC2s, and when a subgroup of moderate/severe asthmatics was compared with a subgroup of healthy subjects whose ages were similar, the number of mDC2s between these groups remained significantly different. As such, we believe neither ICS treatment nor age had an effect on the differences observed in number of mDC2s.

Previous studies have suggested that, in contrast to blood, mDC2s are at comparable or even higher levels than mDC1s and pDCs in the lung (7, 8), suggesting a preferential localization to this compartment. Furthermore, mDC2s were increased in the BALF and sputum of atopic asthmatics compared with healthy subjects (9, 10). The relevance of the increased localization and presence of mDC2s in the lung is uncertain, but may account for the decreased the number of circulating mDC2 we observed in our study. We have

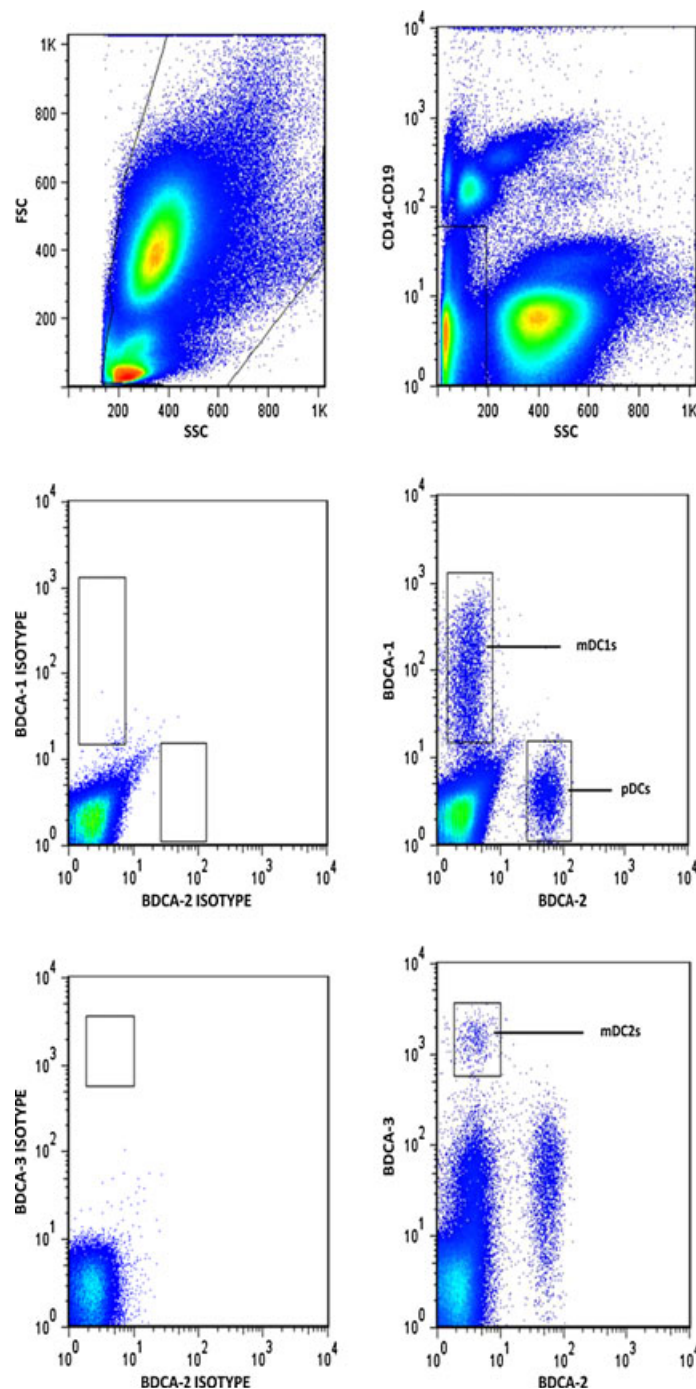


Figure 1 Flow cytometric gating strategy for DC phenotyping. An initial gate was set up to capture leukocytes, excluding debris and platelets on the FSC vs SSC plot. A subsequent gate was made to exclude B cells, monocytes, granulocytes, and dead cells on the

previously shown that both mDCs and pDCs increase in the sputum of asthmatics following allergen challenge (11). Sputum mDC2s were not quantified in this study; however, it is possible that mDC2s are increased in the airways of subjects with allergy and asthma.

SSC vs CD14-CD19 plot. Finally, individual gates were created to enumerate mDC1s (BDCA-1+), pDCs (BDCA-2+), and mDC2s (BDCA-3+).

In summary, this study demonstrates, for the first time, that circulating mDC2s are lower in atopic and allergic asthmatics compared with healthy subjects. The relatively lower numbers of circulating mDC2s in atopic asthmatics might help explain their increased presence in the lung.

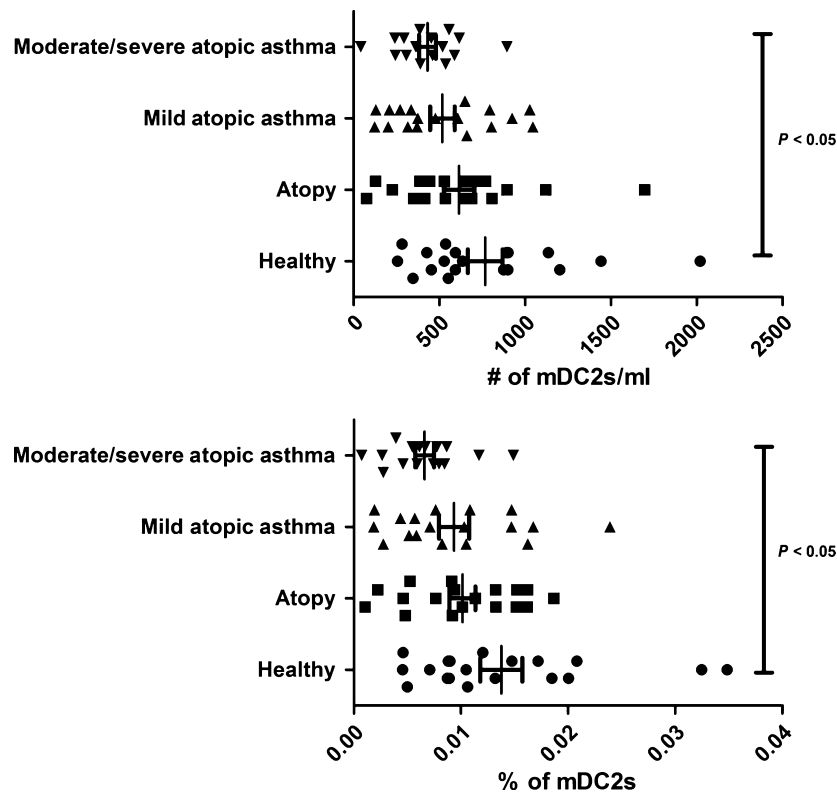


Figure 2 Comparison of number of mDC2s (top) and percentages (bottom) between healthy, atopic nonasthmatic, mild atopic asth-

matic, and moderate/severe atopic asthmatic subjects. Data are presented as means $\pm$ SEMs.

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Author contributions

Benny Dua is the primary author and has made significant contributions to the conception, and the acquisition, analysis and interpretation of the data. He has drafted, reviewed, and approved the manuscript for publication. Steven Smith has assisted with the acquisition and interpretation of the data. He has reviewed and approved the manuscript for publication. Takashi Kinoshita has assisted with the acquisition of the data. He has reviewed and approved the manuscript for

publication. Haruki Imaoka has assisted with the acquisition of the data. He has reviewed and approved the manuscript for publication. Gail Gauvreau has made significant contributions to the design and interpretation of the data. She has reviewed, revised, and approved the manuscript for publication. Paul O'Byrne has made significant and substantial contributions to the conception, design and interpretation of the data. He has reviewed, revised, and approved the manuscript for publication.

Conflict of interest

The authors declare no conflicts of interest as to the interpretation and presentation of this manuscript.

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