

Allergen-Induced Accumulation of CD68⁺, CD123⁺ Dendritic Cells in the Nasal Mucosa

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Key Words

Allergic inflammation • Allergic rhinitis • Dendritic cells • Nasal allergen challenge • Nasal mucosa • Plasmacytoid

Abstract

Background: Dendritic cells are antigen-presenting cells central to the immune system. They activate and orchestrate the innate and the adaptive immune systems. This phenotypically diverse group can be further divided into 2 subsets, the CD11c⁺ myeloid dendritic cells (mDCs) and the CD123⁺ plasmacytoid dendritic cells (pDCs). The aim of the study was to investigate the effect of allergen exposure on dendritic cells in subjects with allergic rhinitis. **Methods:** Atopic and non-atopic subjects were challenged intranasally with birch or timothy allergen. Nasal biopsies were taken before and 24 h after challenge, and were, after CD68 exclusion, stained for expression of CD11c and CD123 to identify dendritic cell subsets. The effect of allergic mediators on CD68⁺, CD123⁺ cells was studied with flow cytometry analysis in peripheral blood. **Results:** The amount of CD68⁺, CD123⁺ cells increased in the nasal sub-epithelium upon allergen challenge, whereas the number of CD68⁺, CD11c⁺ cells was unaffected. In vitro study of the effect of inflammatory mediators on pDC phenotype showed an increased activation in response to TNF- α , IL-4 and CpG. Furthermore, TNF- α caused a higher activation

among atopic than non-atopic subjects. **Conclusions:** An increased number of CD68⁺, CD123⁺ dendritic cells along with the positive pDC response following stimulation with inflammatory mediators suggest that the increased pDCs may be of an activated phenotype. It also suggests that the inflammatory response by pDCs to mediators such as TNF- α may be markedly higher in atopic subjects. These data support the notion of pDCs as important participants in allergic rhinitis.

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Introduction

The dendritic cells are central to both the innate and the adaptive immune systems. By acting as antigen-presenting cells, they promote activation of T and B cells. They can also orchestrate immunological responses by secretion of cytokines and chemokines, resulting in the recruitment of other cell types to the site of inflammation [1]. The dendritic cell population is phenotypically diverse, containing a large subset of cells, among them the CD11c⁺ myeloid dendritic cells (mDCs), and the CD123⁺ plasmacytoid dendritic cells (pDCs) [2]. The precursors of these cells are found in blood, lymphoid organs and in peripheral tissues. When these precursors are activated

Table 1. Clinical data on the subjects participating in the in vivo study

	Atopic	Non-atopic
Subjects, n	12	9
Sex (F/M), n	6/6	6/3
Age, years	28–54 (median 37)	25–42 (median 29)
History of allergic rhinitis	≥2 years	none
Allergy	birch (5), grass (2), both (5)	none
SPT (birch and/or timothy)	>3 mm wheal reaction diameter	negative
Symptoms during pollen season	moderate to severe rhinoconjunctivitis	none

SPT = Skin prick test.

by antigen encounter they mature and migrate to secondary lymphoid organs or to the site of inflammation [3, 4]. The activation causes the cells to undergo morphological changes, up-regulate cell surface co-stimulatory molecules, and alter their pattern of cytokine secretion [5]. Among the co-stimulatory molecules up-regulated upon cell activation and maturation is CD86, which is needed for the activation of T cells through antigen presentation [6, 7].

A role for dendritic cells in asthma and allergy has been advocated, not only due to their antigen-presenting abilities, but also due to their capacity as effector cells to alter T-cell polarization [8]. The main purpose of the present study was to visualize and explore 2 major populations of dendritic cells, the CD11c⁺ mDCs and the CD123⁺ pDCs, in the nasal mucosa of patients with allergic rhinitis.

Material and Methods

Subjects

The study was carried out as a parallel group study with patients suffering from seasonal allergic rhinitis (n = 12) and non-atopic volunteers (n = 9). All included subjects were healthy, with the exception of the allergy. The diagnosis of birch- and grass-pollen-induced allergic rhinitis was based on a positive history of seasonal allergic rhinitis for at least 2 years and a positive skin prick test to birch and/or timothy pollen, with a wheal reaction >3 mm. All patients were classified as having moderate to severe symptoms during birch and/or grass pollen season, and had in previous years medicated against their pollen allergy. The study was performed outside the allergen season, when the rhinitis patients lacked nasal symptoms. Exclusion criteria included upper airway infection within 2 weeks before the visits and treatment with local or systemic corticosteroids within 2 months before the visit. Control subjects were free of symptoms, had no history of allergic rhinitis, and displayed a negative skin prick test to the

standard panel of allergens. They had suffered no upper airway infection within 2 weeks before the time of inclusion and they were all free of medication. A detailed description of the subjects, including age, sex, diagnosis and symptoms, is presented in table 1. Before inclusion, all subjects (patients and controls) were evaluated by an ear, nose and throat consultant. Individuals with signs or symptoms of chronic rhinosinusitis, hypertrophy of turbinates, severe septum deviation or nasal polyposis were excluded from the study. None of the participants were subjected to any type of surgery other than the nasal biopsies described in the research protocol.

Study Design

The study was composed of 4 visits. At visit 1, all subjects underwent a health check that included a physical examination, laboratory screening, past and present medical/surgical history, vital signs and demography. At visit 2, the patients and non-atopic volunteers underwent invasive sampling where biopsies were taken for cryosectioning and subsequent immunohistochemical identification of CD123, CD11c and CD68. At visit 3, the rhinitis patients were challenged with 10,000 SQU per nostril of purified pollen extract diluted in saline (Aquagen, ALK, Copenhagen, Denmark) in order to provoke an allergic reaction. The allergen was delivered locally through a mechanical spray pump. At 24 h after the challenge (visit 4), additional nasal biopsies were obtained for immunohistochemistry.

The study was reviewed and approved by the local ethics committee, and informed consent was obtained from all subjects.

Nasal Biopsy Procedure

Biopsies were taken from the inferior turbinate after topical application of local anaesthesia containing lidocaine hydrochloride/nafazoline (34/0.17 mg/ml) for 20 min. The size of the nasal biopsies was approximately 2 × 2 × 2 mm.

Immunohistochemical Identification of Dendritic Cells in Nasal Biopsies

The biopsies used for immunohistochemistry were immediately frozen in Tissue Tek® O.C.T mounting media (Histolab, Gothenborg, Sweden). To identify myeloid and plasmacytoid dendritic cells, the following monoclonal antibodies were used: mouse anti-human CD11c (clone CBR-150/4G1, isotype IgG2a;

Chemicon, Temecula, Calif., USA; dilution 1:200) and mouse anti-human CD123 (clone 7G3, isotype IgG2a; BD Pharmingen, Franklin Lakes, N.J., USA; dilution 1:300) [9]. In the present study a standard IHC protocol was altered and optimized for identification of the dendritic cell type (CD11c⁺ or CD123⁺, respectively) without any confounding CD68⁺ macrophages. In this regard confounding staining in macrophages and monocytes were obscured by a prior staining of CD68⁺ macrophages/monocytes with a DAB complex (peroxidase, HRP-based protocol). In contrast to the minute CD68 expression potentially occurring in other cell types, the high CD68 expression in macrophages/monocytes yields extensive DAB-complex formation that renders the cell incapable of picking up any subsequent antibody staining. Briefly, after acclimatization to room temperature the sections were subjected to post-fixation with 2% buffered formaldehyde for 20 min, rinsed in phosphate buffered saline (PBS, pH 7.6, 3 × 5 min at room temperature). Next, they were subjected to mild permeabilization by immersion in 0.1% saponin in PBS for 20 min at room temperature. Non-specific binding sites were blocked with 5% normal serum (Dako, Glostrup, Denmark; dilution 1:10 in TBS) for 30 min. Avidin-binding sites were blocked with incubation in Avidin D solution (Vector Laboratories, Burlingame, Calif., USA) for 15 min. Thereafter, the sections were rinsed in PBS (3 × 5 min) before blocking of biotin-binding sites with biotin blocking solution (Vector Laboratories) for 15 min. After additional rinsing with PBS (3 × 5 min) sections were incubated with the primary antibody overnight at 4°C. In control sections the primary antibody was omitted. The primary antibody was diluted in PBS supplemented with 0.25% Triton X and 0.25% bovine serum albumin. After rinsing, the sections were incubated with a biotinylated secondary antibody (i.e. goat anti-mouse IgG2; Vector Laboratories; dilution 1:200) for 45 min at room temperature. After additional rinsing (3 × 5 min in PBS), the sections were incubated with alkaline phosphatase-labeled streptavidin (dilution 1:200) for 45 min and rinsed (3 × 5 min in PBS). Alkaline phosphate activity was developed for 6 min at room temperature using New Fuchsin (Dako) as enzyme substrate. Endogenous alkaline phosphatase activity was inhibited by levamisole. All sections were counter-stained with Harris's hematoxylin, coated with Aqua Perm mounting medium (Life Sciences International), dried overnight and mounted in DPX. Macrophages/monocytes were stained brown, whereas positive staining for CD123 or CD11c was identified by a bright red precipitate.

Evaluation of CD11c- and CD123-Positive Immunoreactivity

Only biopsies without severe damage due to mechanical stress or a variable degree of crush artifacts were analyzed (when taking the biopsies these types of changes occur to a certain degree). In the present study, the quantification was carried out in a blinded fashion. In randomly coded sections, CD123⁺ or CD11c⁺ cells lacking the CD68 marker were counted at ×200 magnification with a Nikon 90i microscope (Nikon, Japan). The entire tissue area was included in the cell quantification. The total analyzed area in each biopsy and the cell density were calculated by computerized image analysis (Image Pro-plus, Media Cybergenetics, Bethesda, Md., USA). Mann-Whitney test was used to determine statistical differences for unpaired comparisons and Wilcoxon signed rank test was used for paired comparisons. $p < 0.05$ was considered significant.

Flow Cytometry of pDCs in Peripheral Blood

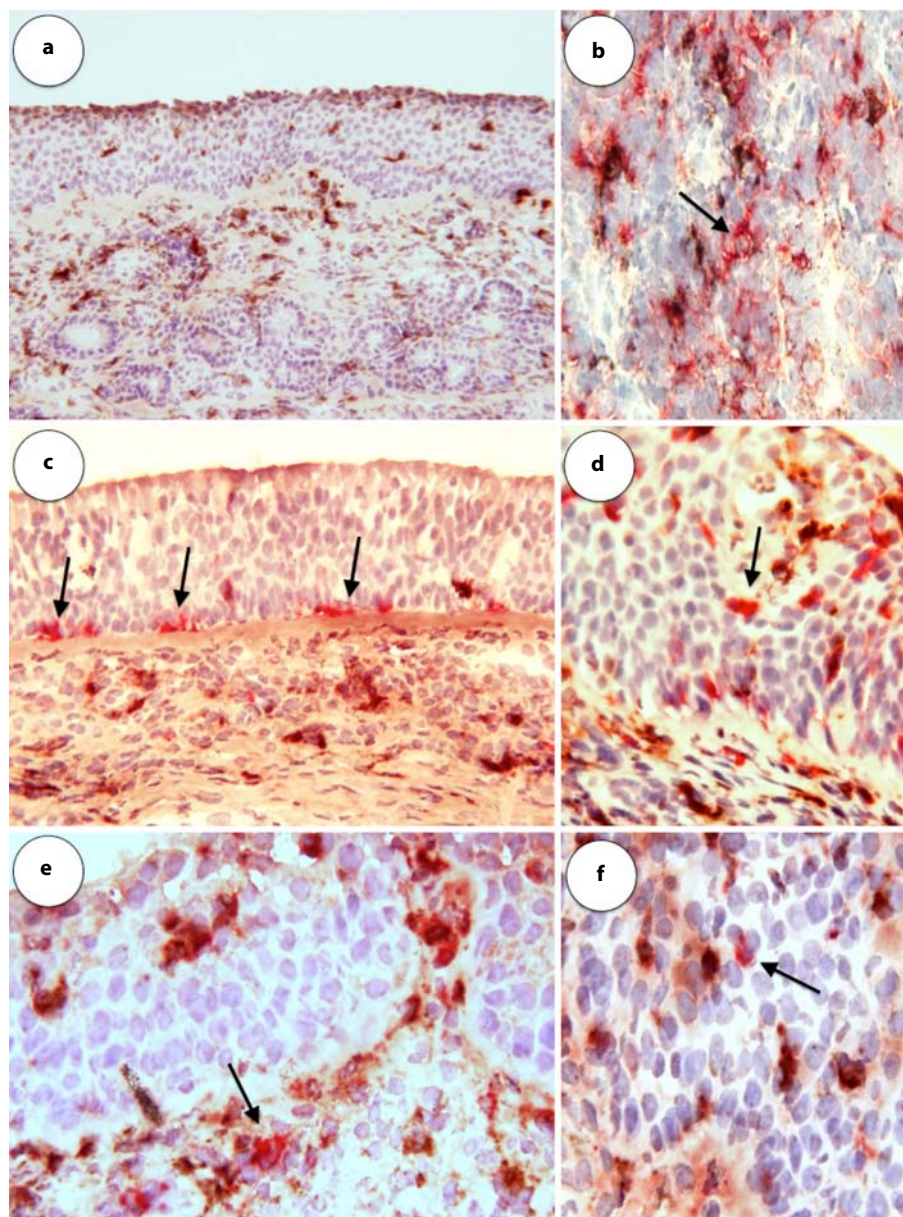
Samples of peripheral blood were collected in test tubes containing Heparin (Vacutainer®, Becton Dickinson, Plymouth, UK) from subjects with a history of seasonal allergic rhinitis caused by either birch or timothy pollen, or from non-atopic controls. A total of 200 µl whole blood was stimulated for 24 h with 10,000 SQU/ml of the relevant allergen (grass or birch; Aquagen, ALK), with TNF-α (10 ng/ml or 100 ng/ml; R&D Systems, Minneapolis, Minn., USA), histamine (100 µM or 1 mM; Sigma, St. Louis, Mo., USA), IL-4 (10 ng/ml or 100 ng/ml; R&D Systems), IL-5 (10 ng/ml or 100 ng/ml; R&D Systems) or CpG (0.1 or 1 µM; ODN 2006, DNA Technology, Risskov, Denmark). The blood was kept at 37°C in 5% CO₂. After stimulation, 50–100 µl of each sample was stained for intracellular and extracellular expression of CD123, CD68 and CD86 using Intraprep™ (Beckman Coulter, Marseille, France) according to the manufacturer's protocol. Briefly, staining of surface antigen was carried out. The cells were subsequently fixed and permeabilized, and antibody was once again added. All samples were labeled with mouse anti-human CD123-PE (Clone 9F5, Isotype IgG1; BD Bioscience, Erembodegem, Belgium), mouse anti-human CD68-FITC (Clone KP1, Isotype IgG1; Dako) and with mouse anti-human CD86-PECy5 (Clone IT2.2, Isotype IgG2b; eBioscience, San Diego, Calif., USA). To further ensure that the responses of the gated CD68⁺, CD123⁺ cells reflected pDC responses, a subset of experiments were conducted where samples were stained with antibodies for BDCA2-PE (Clone AC144, Isotype IgG1; Miltenyi Biotec, Bergisch Gladbach, Germany) and CD123-FITC (Clone 6H6, Isotype IgG1; eBioscience), along with CD86-PECy5. The samples were incubated for 20 min with the antibodies, and erythrocytes were subsequently lysed using formic acid (0.1% v/v). The ionic strength was rendered iso-osmotic by addition of 51 mM Na₂CO₃, 0.20 M Na₂SO₄, 0.22 M NaCl, and cells were washed in PBS. All samples were finally suspended in a solution of PBS containing 0.5% para-formaldehyde prior to analysis. A total of 200,000–300,000 events were collected with a Coulter Epics XL flow cytometer (Beckman Coulter) and gated based on forward- and side scatter properties, excluding dead cells and cell debris. Cells were gated on the lack of CD68, excluding monocytes, and further gated on positive expression of CD123. The expression of CD86 on these cells, as determined by mean fluorescent intensity (MFI), was investigated.

Data were analyzed using Expo32 ADC analysis software (Beckman Coulter). For statistical analysis, repeated measure paired or unpaired ANOVA with Dunnett's multiple comparison post-test, or paired or unpaired Student's *t* test was performed in GraphPad Prism 5 (San Diego, Calif., USA). $p < 0.05$ was considered significant. Data are displayed as mean ± SEM.

Results

Histological Evaluation of CD68⁺, CD11c⁺ and CD68⁺, CD123⁺ Cells in Nasal Tissues

Both CD68⁺, CD11c⁺ and CD68⁺, CD123⁺ cells were visible in the immunohistochemical sections (fig. 1), many of which had dendritic morphology. The amount of intra-epithelial CD68⁺, CD11c⁺ cells did not differ be-



Color version available online

Fig. 1. Immunohistochemical staining of CD123⁺ and CD11c⁺ dendritic cells. **a** Example of the CD68 single staining in the nasal mucosa that was used to mask any subsequent red staining in the double-staining experiments. After completed staining of CD68 cells, slides were stained for CD123 or CD11c with red detection substrate in order to detect CD68⁺,CD123⁺ and CD68⁺,CD11c⁺ cells, respectively. **b** A CD68-negative (no brown staining) but CD11c-positive (red) cell with dendritic morphology (arrow). **c, d** Immunohistochemical localization of CD11c and CD68. **e, f** Localization of CD123 and CD68. Color images published in online version only.

tween atopic and non-atopic individuals (atopic 5.8 ± 1.2 cells/area unit; non-atopic 5.7 ± 1.5 cells/area unit, $p > 0.05$, $n = 9-11$; fig. 2a). Neither was the intra-epithelial cell count of CD68⁺,CD11c⁺ cells in the atopic group affected by the allergen challenge (4.9 ± 1.0 cells/area unit after challenge, $p > 0.05$, $n = 8-11$; fig. 2a).

In the sub-epithelial tissue, no differences in the number of CD68⁺,CD11c⁺ cells were observed before challenge (atopic 4.8 ± 1.9 cells/area unit, non-atopic 4.2 ± 1.6 cells/area unit, $p > 0.05$, $n = 9-12$; fig. 2c), nor did the numbers of CD68⁺,CD11c⁺ cells in atopics increase after

challenge (3.7 ± 1.2 cells/area unit after challenge, $p > 0.05$, $n = 9-12$; fig. 2c).

The CD68⁺,CD123⁺ cells were before challenge found in a slightly higher amount in the epithelium in atopic subjects compared to non-atopic subjects, although this difference did not reach statistical significance (atopic 1.7 ± 0.5 cells/area unit, non-atopic 0.7 ± 0.3 cells/area unit, $n = 8-9$; fig. 2b). Allergen challenge did not affect the numbers of CD68⁺,CD123⁺ cells in the epithelium in the atopic group (1.4 ± 0.4 cells/area unit after challenge, $p > 0.05$, $n = 8-9$; fig. 2b). In the sub-epithelium, the num-

Fig. 2. Distribution of dendritic cells in the nasal mucosa. The figures show the number of intra-epithelial CD68⁺,CD11c⁺ (a) and CD68⁺,CD123⁺ (b), and the number of sub-epithelial CD68⁺,CD11c⁺ (c) and CD68⁺,CD123⁺ (d) cells in the atopic and non-atopic subjects examined before and after topical allergen challenge. Data are presented as mean $\pm$ SEM. n = 8–11 subjects in each data set. * p < 0.05.

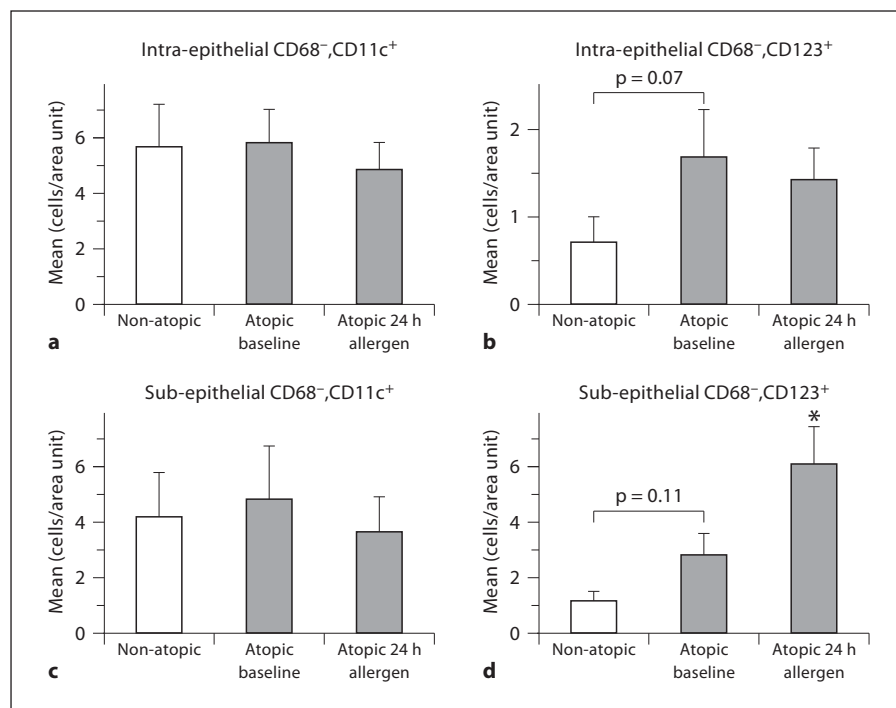


Table 2. Mean number of cells/area unit ($\pm$ SEM) in the nasal epithelium and subepithelium

	Intra-epithelial				Sub-epithelial			
	CD68 ⁺ ,CD11c ⁺	n	CD68 ⁺ ,CD123 ⁺	n	CD68 ⁺ ,CD11c ⁺	n	CD68 ⁺ ,CD123 ⁺	n
Non-atopic	5.68 $\pm$ 1.52	9	0.71 $\pm$ 0.29	8	4.19 $\pm$ 1.58	9	1.18 $\pm$ 0.36	8
Atopic	5.83 $\pm$ 1.18	11	1.67 $\pm$ 0.55	9	4.82 $\pm$ 1.94	12	2.84 $\pm$ 0.79	11
Atopic, 24 h	4.84 $\pm$ 0.96	8	1.42 $\pm$ 0.37	8	3.67 $\pm$ 1.25	9	6.11 $\pm$ 1.31*	10

* p < 0.05.

bers of CD68⁺,CD123⁺ cells did not differ between atopic and non-atopic subjects before allergen challenge (atopic 2.8 ± 0.8 cells/area unit, non-atopic 1.2 ± 0.4 cells/area unit, p = 0.11, n = 8–11; fig. 2d), but atopic individuals displayed an increase in CD68⁺,CD123⁺ cell numbers after challenge (6.1 ± 1.3 cells/area unit after challenge, p < 0.05, n = 10–11; fig. 2d and table 2).

In vitro Stimulation of Whole Blood

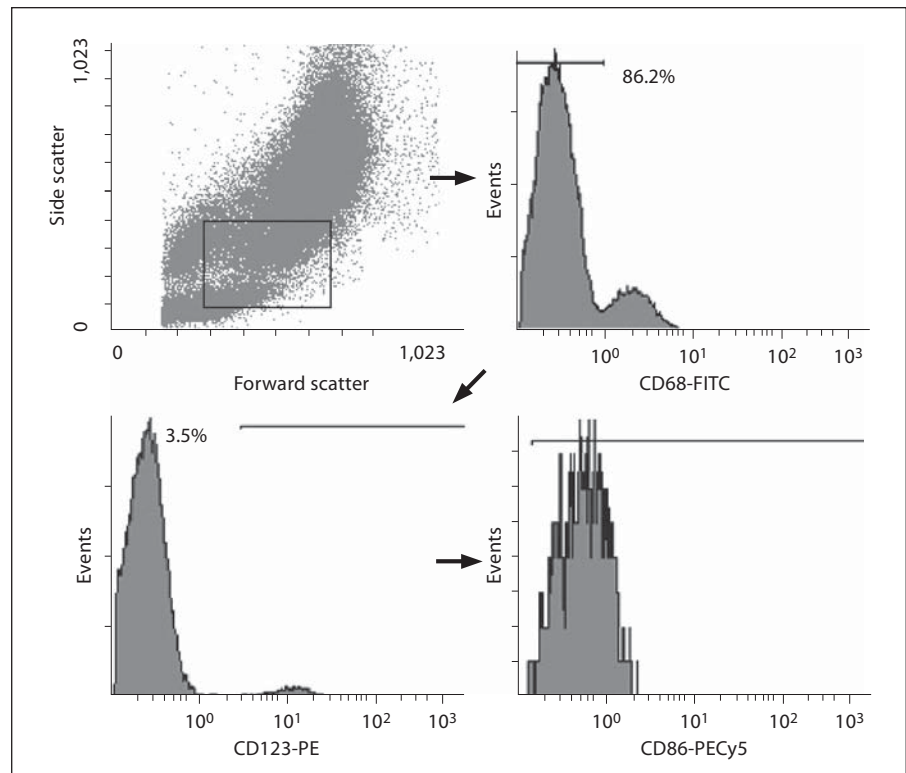
Allergen stimulation induced an increase in the number of CD68⁺,CD123⁺ cells in atopic subjects. In order to examine how these cells are affected by the milieu of an ongoing allergic inflammation, whole blood was ob-

tained from atopic subjects or non-atopic controls, and stimulated in vitro with allergen or inflammatory mediators. CD68⁺,CD123⁺ cells were gated with flow cytometry, and the expression of CD86 was taken as sign of cell activation and maturation (fig. 3).

In vitro challenge of whole blood with allergen did not affect the expression of the activation marker CD86 on the CD68⁺,CD123⁺ cells [MFI allergen (10,000 SQU/ml) 1.5 ± 0.2 , untreated controls 1.3 ± 0.2 , n = 13; fig. 4a].

Upon treatment with TNF- α , CD86 was up-regulated at both tested concentrations compared to CD68⁺,CD123⁺ cells in untreated blood [MFI TNF- α (10 ng/ml) 1.8 ± 0.3 , TNF- α (100 ng/ml) 2.0 ± 0.3 , p < 0.05 and p < 0.01

Fig. 3. Gating strategy for the expression of CD86 by CD68⁻,CD123⁺ dendritic cells. A total of 200,000–300,000 events were collected. Monocyte/DC forward and side scatter were loosely gated, and cells were subsequently gated on the basis of lack of expression of macrophage/monocyte marker CD68 and a positive expression of CD123. The mean fluorescent intensity was obtained for CD86 expression by CD68⁻,CD123⁺ cells.



vs. untreated controls, respectively, $n = 13$; fig. 4b]. Stimulation of whole blood with IL-4 increased the expression of CD86 [MFI IL-4 (10 ng/ml) 2.5 ± 0.4 , IL-4 (100 ng/ml) 3.0 ± 0.5 , $p < 0.05$ and $p < 0.01$ vs. untreated controls respectively, $n = 13$; fig. 4c]. Similarly, treatment with CpG also altered the levels of CD86 expression [MFI CpG (0.1 μ M) 1.7 ± 0.2 , CpG (1 μ M) 2.2 ± 0.4 , $p < 0.01$ vs. untreated controls, $n = 13$; fig. 4d].

Treatment with histamine did not alter the expression [MFI histamine (100 μ M) 1.5 ± 0.2 , histamine (1 mM) 1.5 ± 0.2 , $n = 11$ –13, data not shown], neither did IL-5 [MFI IL-5 (10 ng/ml) 1.3 ± 0.1 , IL-5 (100 ng/ml) 1.3 ± 0.1 , $n = 13$, data not shown].

Staining for pDCs with BDCA-2-PE and CD123-FITC confirmed these results [MFI untreated controls 2.8 ± 0.4 ; TNF- α (100 ng/ml) 4.0 ± 0.5 , $p < 0.05$ vs. untreated control; IL-4 (100 ng/ml) 6.5 ± 1.4 , $p < 0.05$ vs. untreated control; CpG (1 μ M) 9.1 ± 2.0 , $p < 0.05$ vs. untreated control, $n = 6$ –7, data not shown].

Comparison of the group of atopic subjects against the group of non-atopics did not display any differences in baseline CD86 expression by CD68⁻,CD123⁺ cells (MFI atopic untreated controls 1.5 ± 0.2 , non-atopic untreated controls 1.0 ± 0.2 , $p = 0.11$, $n = 6$ –7; fig. 5a). Neither did

allergen alone induce any differences in response in the atopic subjects [MFI allergen (10,000 SQU/ml) 1.7 ± 0.2] compared to non-atopic subjects [MFI allergen (10,000 SQU/ml) 1.2 ± 0.3 , $n = 6$ –7; fig. 5a]. Nor did any of the groups increase their CD86 expression in response to allergen, compared to unstimulated controls.

Further comparison of the atopic group against the non-atopic revealed that both groups demonstrated an increase in CD86 after stimulation with TNF- α [MFI atopic: TNF- α (10 ng/ml) 2.2 ± 0.5 , TNF- α (100 ng/ml) 2.6 ± 0.3 , $p < 0.05$ for 100 ng/ml vs. untreated; MFI non-atopic: TNF- α (10 ng/ml) 1.5 ± 0.2 , TNF- α (100 ng/ml) 1.4 ± 0.3 , $p < 0.05$ for both concentrations vs. untreated, $n = 6$ and 7; fig. 5b]. Furthermore, the level of CD86 expression after stimulation with 100 ng/ml TNF- α was significantly higher in the allergic group compared to the non-atopic ($p < 0.05$, $n = 6$ and 7; fig. 5b).

Discussion

Dendritic cells are believed to be crucial for the allergic response, both in regulating the process of sensitization and in affecting the progression of allergic inflammation

Fig. 4. The influence of inflammatory mediators on the expression of CD86. The expression of CD86 by CD68⁺,CD123⁺ cells in peripheral blood as determined by flow cytometry after treatment of whole blood with allergen (10,000 SQU; **a**), TNF- α (10 ng/ml or 100 ng/ml; **b**), IL-4 (10 ng/ml or 100 ng/ml; **c**), CpG (10 or 100 μ M; **d**) or on untreated cells (Untreated). The figure shows mean fluorescent intensity for CD86 on CD68⁺,CD123⁺ cells. Data are presented as mean $\pm$ SEM. n = 13 subjects in each data set. * p < 0.05, ** p < 0.01.

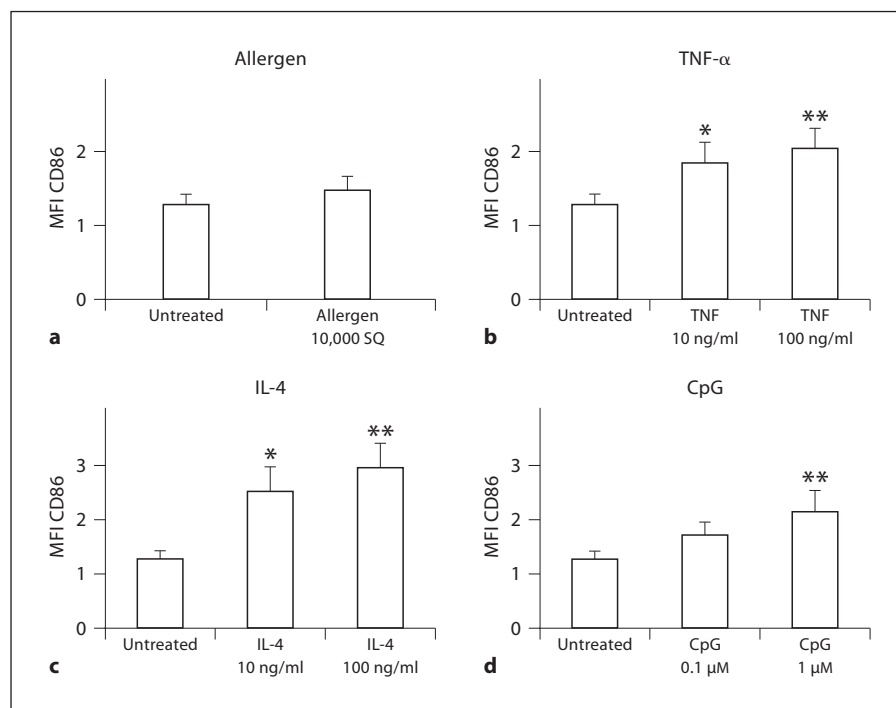
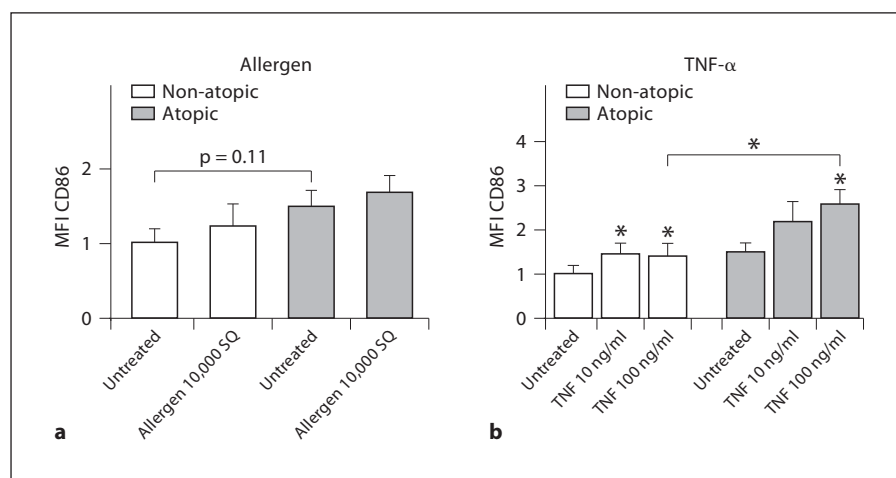


Fig. 5. Responses to allergen in atopic and non-atopic subjects. The expression of CD86 by CD68⁺,CD123⁺ cells in peripheral blood as determined by flow cytometry after treatment of whole blood with allergen (10,000 SQU/ml; **a**) or TNF- α (10 ng/ml or 100 ng/ml; **b**). The data are divided in atopic and non-atopic subjects. The figure shows mean fluorescent intensity for CD86 on CD68⁺,CD123⁺. Data are presented as mean $\pm$ SEM. n = 6–7 subjects in each data set. * p < 0.05.



[10]. Two distinct subsets of dendritic cells have been described in humans, the mDCs and the pDCs, which are identified on the basis of surface markers [11, 12]. The present study examined the effect of allergen exposure on dendritic cells in atopic and non-atopic subjects. In the epithelium, a tendency for a higher baseline amount of CD68⁺,CD123⁺ cells was found in atopic subjects, indicating elevated levels of pDCs, although this did not reach significance compared to non-atopic subjects. The

amount of CD68⁺,CD123⁺ cells was, however, increased in the sub-epithelial tissue of the nasal mucosa in atopic subjects. Stimulation of whole blood in vitro showed that inflammatory mediators such as TNF- α and IL-4 as well as Toll-like receptor 9 agonist CpG caused activation of CD68⁺,CD123⁺ cells and that CD68⁺,CD123⁺ cells from atopic subjects responded with a higher activation to TNF- α than CD68⁺,CD123⁺ cells from non-atopic subjects.

The fact that there was no observed increase in the amount of CD68⁺,CD11c⁺ cells in the nasal mucosa in response to allergen challenge suggests different modes of activation or recruitment for CD68⁺,CD11c⁺ and CD68⁺,CD123⁺ cells. Previous studies have described an influx of mDCs to the bronchial mucosa in response to allergen challenge. In a study of perennial rhinitis, an increase in mDCs, and a more activated phenotype in atopic subjects was described by KleinJan et al. [13]. Although we did not observe any corresponding increase of numbers of CD68⁺,CD11c⁺ cells in the nasal mucosa, it is likely that the mDC population is more activated in the allergic subjects. Differences in DC responses between the study by KleinJan et al. and the present study could be explained by alternate mechanisms in perennial allergic rhinitis in comparison with seasonal allergic rhinitis. Co-stimulation with CD86 has been demonstrated to be critically important for the allergen-induced Th2 cytokine production in atopic asthmatic subjects [14]. A difference in the expression of CD86 between atopic and non-atopic subjects may therefore have implications in regards to T cell activation and Th2 polarization.

Their ability to induce allergen-dependent Th2 memory responses has given the pDCs a role in the pathophysiology of allergic disease [8, 15]. Patients with asthma display a higher number of pDCs, and a shift in the mDC/pDC ratio towards a pDC balance [16]. Furthermore, an analysis of dendritic cell subsets in bronchoalveolar lavage fluid has demonstrated an accumulation of pDCs in the airway lumen after allergen challenge of patients with asthma [17]. A similar increase in pDCs after allergen challenge has been demonstrated in cutaneous biopsies by Nouri-Aria et al. [18]. Furthermore, a recruitment of pDCs to the nose upon allergen challenge after a daily allergen provocation for 6 days has been described by Jahnson et al. [19]. While they did not observe any increase in the number of pDCs in the nasal mucosa 1 day after challenge, we noticed an increase in CD68⁺,CD123⁺ cells as soon as 24 h after challenge. It is possible that different criteria used for identifying the pDCs may account for the differences between the results.

In the present study, we used CD68⁺,CD11c⁺ cells in order to assess mDCs. It should be noted that, as for most markers, some cautiousness is required. For example, although the major confounding CD11c-bearing cell (i.e. macrophages) were excluded from our analysis, and most of the CD68⁺,CD11c⁺ cells had a dendritic morphology, we cannot rule out a minor contribution of other CD11c-positive cell populations. A similar reasoning is also val-

id for CD68⁺,CD123⁺ cells since it has recently been shown that a small population of the mDC may also express CD123 [20].

The present study demonstrated an increase in the numbers of CD68⁺,CD123⁺ cells in the nasal mucosa upon allergen challenge. To further explore how these cells are affected by allergen exposure and the milieu of allergic inflammation, whole blood was stimulated with allergen and inflammatory mediators, CD68⁺,CD123⁺ cells were gated and investigated for signs of activation and maturation. The whole blood studies demonstrate how CD68⁺,CD123⁺ cells are affected by the local milieu of an ongoing allergic inflammation, where a mediator exerts its effect on not only 1 type of cell, but on many. The effects of TNF- α and IL-4 on CD68⁺,CD123⁺ cells in blood suggests that mediators present in the allergic inflammatory response [21, 22] may cause an activation of the CD68⁺,CD123⁺ cells localized to the nasal mucosa.

The lack of response to allergen alone suggests that activation of CD68⁺,CD123⁺ cells in the nasal mucosa would likely be secondary and induced by allergen effects on locally residing cell types. Furthermore, atopic subjects demonstrated a higher level of activation in response to TNF- α compared to non-atopic individuals.

While pDCs have been suggested to take part in the induction of an allergic response, other studies have suggested them to be vital in mediating tolerance to harmless, inhaled antigen [23]. A deficiency in the ability of pDCs to induce tolerance, or an erroneous response to the antigen, could be a reason for sensitization to allergen. While the present study demonstrates an increase in pDCs after allergen exposure and a more activated phenotype to stimulation with allergic mediators, the consequences of these findings on the activation of the adaptive immune system remain to be established.

In conclusion, we observed that allergen challenge increases the amount of CD68⁺,CD123⁺ cells in the nasal sub-epithelial tissue. No similar increase was seen in the number of CD68⁺,CD11c⁺ cells, suggesting that different subsets of dendritic cells may differ in the mechanism of activation and in their role in allergic rhinitis. This supports the notion of mDCs and pDCs as distinct populations with different roles in the allergic process. Furthermore, the positive response of the CD68⁺,CD123⁺ cells following stimulation with inflammatory mediators suggests that the increased number of CD68⁺,CD123⁺ cells may be of an activated phenotype. It also suggests that the inflammatory response by these cells to mediators such as TNF- α may be markedly higher in atopic sub-

jects. This, along with the greater extent of activation of CD68⁺,CD123⁺ cells in atopic subjects in response to TNF- α stimulation, suggests that atopic subjects may have a greater inflammatory load caused by pDCs than non-atopic subjects. These findings support the proposed role of pDCs in allergic rhinitis.

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