

Aberrant dendritic cell function conditions Th2-cell polarization in allergic rhinitis

C. Pilette¹, M. R. Jacobson², C. Ratajczak¹, B. Detry¹, G. Banfield², J. VanSnick³, S. R. Durham² & K. T. Nouri-Aria²

¹Institute of Experimental & Clinical Research (pole of Pneumology – immunobiology group), Cliniques universitaires St-Luc, Université catholique de Louvain, Brussels, Belgium; ²Section Allergy & Clinical Immunology, National Heart & Lung Institute at Imperial College London, London, UK; ³C. de Duve Institute of Cellular Pathology & Ludwig Institute for Cancer Research, Université catholique de Louvain, Brussels, Belgium

To cite this article: Pilette C, Jacobson MR, Ratajczak C, Detry B, Banfield G, VanSnick J, Durham SR, Nouri-Aria KT. Aberrant dendritic cell function conditions Th2-cell polarization in allergic rhinitis. *Allergy* 2013; DOI: 10.1111/all.12090.

Keywords

allergens; dendritic cells; IL-10; IL-12; Th2 cytokines.

Correspondence

Pr. Charles Pilette, Institute of Experimental & Clinical Research (pole of Pneumology – immunobiology group), and Cliniques universitaires St-Luc, Université catholique de Louvain (UCL), Avenue Hippocrate 54/5490, B-1200 Brussels, Belgium.
Tel.: 0032 2764 2832
Fax: 0032 2764 2831
E-mail: charles.pilette@uclouvain.be

Accepted for publication 3 November 2012

DOI:10.1111/all.12090

Edited by: Hans-Uwe Simon

Abstract

Background: Myeloid (m) and plasmacytoid (p) dendritic cells (DCs) regulate immune responses to allergens, whereas it remains unclear whether abnormal DC function characterizes patients with airway allergy and whether putative dysfunction exists only in target organs. To evaluate DC function from patients with allergic rhinitis (AR), we assessed nasal, cutaneous as well as blood DCs after *in vivo* and *in vitro* allergen challenge, respectively.

Methods: DCs were immunostained in nasal and skin tissues, and cytokine expression was assessed by dual immunofluorescence. Cytokine production and regulation of cocultured peripheral CD4⁺ T cells were assayed by ELISA.

Results: In AR patients, local allergen challenge resulted in increases in pDC and mDC numbers at 8 h in the nasal mucosa and at 8–48 h in the skin. Defects in IL-10 and IFN- α were observed in both organs from AR. Blood mDCs from AR exhibited reduced IL-10 and IL-12 expression. The capacity of activated pDCs from AR to produce IFN- α and to trigger IL-10 by allogeneic CD4⁺ T cells was diminished, whereas mDCs from these patients supported Th2- and Th17-cell differentiation.

Conclusion: In allergic rhinitis, DCs are altered not only locally but also in the systemic circulation. mDCs and pDCs increased in airway and skin tissues exposed to the allergen and displayed reduced production of IL-10 and 'type 1 signals' (IL-12, IFN- α) both locally and in blood. Functional studies showed that this results in preferential Th2/Th17-cell polarization and impaired generation by blood DCs of IL-10⁺ T cells, linking systemic DC dysfunction and biased T-cell responses.

Dendritic cells (DCs) are specialized antigen-presenting cells that are strategically located in the skin and the mucosal system. Their roles include recognition and capturing of invading foreign antigens and pathogens and elicitation of immune responses through activation of effector T cells or the induction of tolerance through regulatory T cells (1). Maturation

status of DCs is believed to play a decisive role in the stimulation of immune responses, immature IL-10-expressing DCs favouring tolerance (2). In mice, at least five subpopulations of DCs have been described including myeloid DCs, plasmacytoid DCs and CD8⁺CD11c⁺CD11b⁺ DCs (3). In human peripheral blood, two major populations of DCs, CD11c⁺CD123⁺ pDCs and CD11c⁺CD123⁺ mDCs, have been identified (4). In normal human airways, there exist resident conventional DCs, including Langerhans cells as an intraepithelial subpopulation, and pDCs that are presumed to play a homeostatic role, whereas in inflamed airways, inflammatory DCs and IFN- α -producing pDCs accumulate during viral infection and chronic inflammation (5).

Abbreviations

DC, dendritic cells; Der p, *Dermatophagoides pteronyssinus*; FITC, fluorescein isothiocyanate; IQ, interquartile range; mDCs, myeloid dendritic cells; pDCs, plasmacytoid dendritic cells; PBS, phosphate buffer saline; TLR, toll like receptor; RT, room temperature; TRITC, tetramethylrhodamine isothiocyanate.

Studies using murine models of ovalbumin-induced asthma demonstrated a decisive role of myeloid dendritic cells (mDCs) in the induction of airway allergic inflammation (6). In contrast, pDCs showed suppressive functions, after transfer either before (7) or after allergen sensitization (8). In contrast to murine models, the role of DC subtypes in man is more controversial. Lower numbers of pDCs have been observed in blood from asthmatic children (9–11) and this reduction accompanied recurrent respiratory infections associated with wheezing (10). In adult asthmatics, higher numbers of pDCs have been reported, both in blood (12) and in the target organ compared with healthy controls (13), as well as increased mDC and pDC numbers after lung allergen challenge (14).

Allergic rhinitis (AR) and asthma are classically characterized by the production of allergen-specific IgE and tissue eosinophilia, events under the regulation of Th2 cells. A defect in IL-10-producing T cells has been implicated in the immunopathogenesis of airway allergy (15–17), but it remains elusive whether this stems from abnormalities in the frequency and/or function of DCs recruited to the site of allergic reactions. It remains also unclear whether putative DC defects could be observed only in the target organ. In the present study, we hypothesized that in allergic rhinitis, DCs in both the target organ and the 'periphery' (skin and blood) are functionally altered particularly in terms of IL-10 expression and regulation of T-cell responses. We investigated patients with allergic rhinitis (18), as compared to healthy nonatopic controls, the kinetics and phenotypic and functional characteristics of DCs in the nasal mucosa and skin following *in vivo* local allergen challenge. Blood DCs were also studied for their capacity to secrete cytokines and to drive allogeneic CD4⁺ T-cell responses.

Methods

Patients

Atopic patients with a clinical history of AR and positive immediate skin test responses (>3 mm more than a negative diluent control) and elevated serum-specific IgE responses to grass pollen [*Phleum pratense* (*Phl p*)] or house dust mite [*Dermatophagoides pteronyssinus* (*Der p*)] were recruited for nasal and skin allergen challenges and for biopsy sampling. Nonatopic (skin prick test negative) individuals without history of respiratory symptoms served as controls. All subjects were nonsmokers. The study was approved by the local Ethical Committee.

Nasal and cutaneous allergen challenge protocol and biopsy collection

After nasal or skin challenge, tissue biopsies were taken from the nasal mucosa (at 8 h, 2 mm) and skin (at 8 and 48 h, 3 mm), respectively, and processed for immunolocalization of DCs. No 48-h time-point could be performed in the nose, both for ethical reasons and practical reasons (biopsying the same turbinate twice could introduce the

confounding effect of a cellular response to the previous mucosal injury).

Immunofluorescence was used to visualize BDCA-1/CD1c+ mDCs or BDCA-2/CD303+ pDCs (20 µg/ml; Miltenyi Biotech, Surrey, UK) in 4-µm paraformaldehyde-fixed sections. Biotinylated secondary antibody (Strattech Scientific, Cambridge, UK) was followed by streptavidin-conjugated Alexa Fluor 594 or 488 (Invitrogen, Paisley, Scotland, UK). Dual immunofluorescence was used to colocalize mDCs or pDCs with cytokines, using anti-h IL-10 (rabbit), anti-h IL-12 p40 (rabbit) or anti-h IFN-α (goat) (40 µg/ml; Santa Cruz Biotech Heidelberg, Germany). DCs were detected as above, while cytokines were detected using either mouse anti-rabbit followed by fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse (Dako, Denmark) or rabbit anti-goat followed by tetramethylrhodamine isothiocyanate (TRITC)-conjugated swine anti-rabbit. Appropriate isotype-matched IgG negative control for DCs and IgG controls for cytokines were included. In addition, *in situ* hybridization was carried out for IL-4 mRNA as previously reported (19).

The stained sections were observed under Nikon Eclipse (E400) fluorescent microscope (Tokyo, Japan) and analysed immediately beneath the entire epithelium (nose i.e. submucosa) and epidermis (skin i.e. dermis) to two grids depth along the whole length of biopsy at ×400 magnification using appropriate absorption/emission wavelengths (590/617 nm for Alexa Fluor 594 or TRITC and 488/519 nm for FITC or Alexa fluor 488). The images were captured using Nikon Digital Still Camera DXM1200 (Tokyo, Japan) (×400 magnification) and analysed with LUCIA 4.80 software (system for Image processing and analysis; Prague, Czech Republic). The immune-stained cells were counted, and results were expressed as the number of positive cells/mm². On average, this involved counting cells in 15 fields for skin and eight fields for nasal biopsies, which were equivalent to 1.5 mm² (0.4–2.4 mm²) and 0.8 mm² (0.2–1.8 mm²) of sub-epithelial tissue per biopsy, respectively.

Peripheral dendritic cell purification and analysis

Blood mDCs and pDCs were purified from buffy coats, by immunomagnetic cell sorting (MACS; Miltenyi Biotech) using BDCA-1/CD1c (mDCs) and BDCA-4/CD304 abs (pDCs). DCs were either extensively phenotyped by 4-colour flow cytometry or pulsed with Der p allergen extract, in the presence or not of ligands for toll like receptor (TLR-4) (1 µg/ml LPS) on mDCs and TLR9 (500 ng/ml ODN2216 CpG-type A) on pDCs, for functional analyses of cytokine production and polarization of T-cell responses in 5 d cocultures with allogeneic CD4⁺ T cells (ratio 1 : 5, DC/T cell) purified by MACS from one nonatopic donor. In selected experiments, monocyte-derived mDCs (differentiated from monocytes with IL-4 and GM-CSF for 5 d) were also used and activated by IgE crosslinking [5 µg/ml human IgE followed by 100 µg/ml rabbit F(ab')₂]. IL-4, IL-5, IL-10, IFN-γ, IL-12(p70), IFN-α and IL-17 were determined by ELISA (R&D Systems, Abingdon, UK). IL-6 was determined by a bioassay using as IL-6-dependent B9

cells, one unit being defined as the concentration of IL-6 giving half-maximal stimulation.

Statistical analysis

Data were analysed using GraphPad (San Diego, CA, USA). Between-group comparisons were performed using the Mann-Whitney *U*-test, and within-group comparisons using the Wilcoxon matched pairs test. All tests were two-tailed, and *P*-values <0.05 were considered statistically significant.

Results

Clinical and inflammatory responses to allergen challenge

The response to allergen challenge included, as expected in AR, significant increases in immediate nasal symptoms or skin late-phase response as well as in tissue eosinophils and IL-4mRNA+ cells at 8–48 h (Table 1).

Recruitment of mDCs and pDCs during late cutaneous and nasal allergic responses

Numbers of mDCs and pDCs were significantly increased (by 1.5-fold and 1.6-fold, respectively, both *P* = 0.04) in the nasal mucosa from AR patients at 8 h after nasal allergen provocation, compared with diluent and in contrast with controls (Fig. 1A,B).

After intradermal challenge, there were no significant changes in mDC numbers at 8 h after allergen challenge either in AR or controls (Fig. 1C), whereas at 48 h, mDCs increased in AR by twofold. Increases in pDC numbers occurred earlier, at 8 h, in AR (2.6-fold compared with diluent, Fig. 1D), and subsequently decreased at 48 h. While no significant correlation was observed for pDCs, a negative correlation between mDC numbers at 8 h and the intensity of the late-phase response was noticed after challenge of the skin from AR patients (*r* = −0.71, *P* = 0.037).

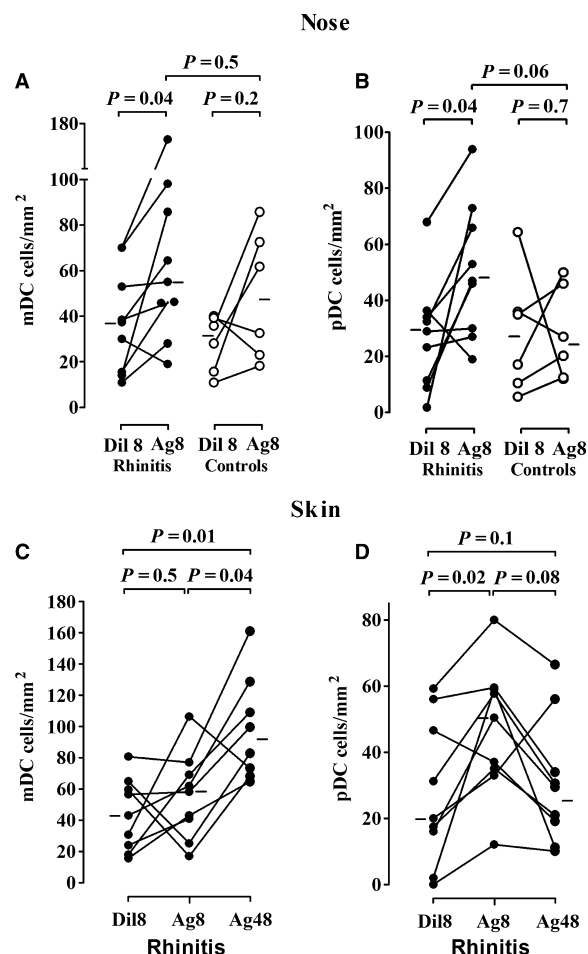


Figure 1 mDC (A) and pDC (B) numbers in nasal mucosa 8 h postdiluent and allergen provocation in allergic rhinitis and control subjects, and in the skin of rhinitis subjects 8 h postdiluent and at 8 and 48 h following allergen challenge.

Table 1 Median (IQ range)/mm² of late-phase response (nasal VAS and skin LPR), tissue eosinophils and IL-4 mRNA-expressing cells in the skin and nasal mucosa from atopics and controls after challenge

	Nose		Skin		
	Dil8	Ag8	Dil8	Ag8	Ag48
VAS/LPR					
Rhinitis (<i>n</i> = 7)	0.8 (0, 8.2)	2.75 (0.75, 9.00) [#]	0 (0, 0)	4140 (3223, 5680) ^{###}	1239 (1122, 1578) ^{###}
Controls (<i>n</i> = 6)	0 (0, 5.43)	0 (0, 4.9)	0 (0, 0)	0 (0, 0) ^{***}	0 (0, 0) ^{***}
Eosinophils					
Rhinitis (<i>n</i> = 7)	7.5 (0, 42.8)	213.0 (60.6, 401.0) ^{###}	0 (0, 1.1)	224.0 (155.5, 346.5) ^{##}	78.1 (5, 261) ^{##}
Controls (<i>n</i> = 6)	1.9 (1.05, 19.93)	0.65 (0, 3.45) ^{**}	0 (0, 2.5)	2.2 (0, 13.23) ^{**}	0.65 (0, 1.75) ^{**}
IL-4mRNA					
Rhinitis (<i>n</i> = 7)	0 (0, 5)	20 (10.9, 38) ^{###}	0.4 (0, 1.38)	19.6 (15, 51.0) [*]	10.0 (6.6, 26.0) [*]
Controls (<i>n</i> = 6)	0 (0, 2)	0 (0, 0.6) ^{**}	0 (0, 0)	0 (0, 0) ^{###}	0 (0, 1.9) ^{###}

IQ, inter quartile range; VAS, visual analog score for nasal blockage.

****P* = 0.01, ***P* = 0.003, **P* = 0.03 when allergic rhinitis patients were compared with controls.

[#]*P* = 0.09, ^{##}*P* = 0.05, ^{###}*P* = 0.02 when diluent was compared with allergen.

Cytokine expression by DCs during cutaneous and nasal allergic responses

Table 2 represents the overall production of IL-10, IL-12p40 and IFN- α (irrespective of the cellular sources), while Figs 2 and 3 data illustrate DC expression of these cytokines, Fig. 2 A,B showing the distribution of mDCs and pDCs in the skin of AR at 8 h post challenge. In contrast to Langerhans cells, which are known to be located in the epidermis, CD1c+ mDCs and pDCs were both mainly located in the dermis of the skin from allergic rhinitis patients, where they were directly targeted by the intradermal allergen challenge.

IL-10 and IL-12 expression and IL-10/IL-12+ DCs in the nasal mucosa

Total numbers of IL-10+ cells increased by 2.7-fold after challenge in the nasal mucosa of controls, but not in AR (Table 2). Similarly, when localizing IL-10 to DCs, the percentage of IL-10+ mDCs tended to increase after allergen provocation in controls, but not in AR (Fig. 3A). A similar trend was observed for IL-12(p40), although both total IL-12+ cells (Table 2) and percentage of mDCs expressing IL-12 (Fig. 3C) in response to allergen provocation remained low in both groups.

IL-10 and IL-12 expression and IL-10/IL-12+ DCs in the skin

In the skin, overall expression of IL-10 – both total and mDC related – was globally lower in AR (Table 2, Figs 2C and 3B), and at 8 h post challenge, IL-10 and IL-10+ mDCs became significantly lower in AR as compared to controls. Numbers of dermal IL-12(p40)+ cells were also lower at 8 h post challenge in AR (Table 2, Fig. 2D). No significant differences in IL-12+ mDCs were noted in either group (Fig. 3D). The expression of IL-10 and IL-12 by cutaneous pDCs was very low in both groups (data not shown).

IFN- α expression and IFN- α + pDCs

Lower numbers of overall IFN- α -expressing cells were observed in the nasal mucosa of AR (Table 2). Co-localization of IFN- α to pDCs (illustrated in Fig. 2E, F, in subjects

with high pDC numbers) was not consistently observed, probably due to low frequency in most patients.

Cytokine responses of peripheral blood DCs from allergic rhinitis patients

The median percentage of circulating mDCs and pDCs was not significantly different between AR [0.53 (0.28–0.81), median (range) mDCs, 0.06 (0.02–0.14) pDCs; $n = 11$] and controls [0.56 (0.39–1.0 mDCs, 0.09 (0.001–0.18)) pDCs; $n = 16$].

As IL-10 was reduced in the nose and skin from AR patients in response to allergen exposure, we also assessed IL-10 in circulating DCs. IL-10 production was only marginally decreased in mDCs from AR *vs* controls, following Der p pulsing and LPS stimulation ($P = 0.07$, Fig. 4A), whereas no significant production of IL-10 protein was detected in pDCs. IL-12(p70) production was significantly lower in AR' mDCs and pDCs irrespectively of culture conditions (Fig. 4B), while p40 was not significantly different between groups (not shown). No IFN- α was detected in mDC supernatants (except in resting mDCs from two controls), while robust IFN- α production was observed in pDCs activated through TLR9. IFN- α production was strongly reduced in activated pDCs from AR (Fig. 4C). IL-6 was significantly increased in mDCs from AR patients following LPS activation, as compared to mDCs from controls (Fig. 4D), while pDCs from only two patients produced detectable levels of this cytokine (Fig. 4E).

In DCs differentiated *in vitro* from fresh monocytes (representing a more inflammatory phenotype), a clear defect in IL-10 and IL-12(p70) production was observed in AR upon LPS as well as upon IgE stimulation (Fig. 5). No effect of anti-IgE alone was observed (not shown).

Aberrant regulation of T-cell responses by DCs from allergic rhinitis patients

The capacity to induce IL-10 production and polarization of allogeneic CD4⁺ T cells was evaluated in DCs from AR

Table 2 Median (IQ range)/mm² of IL-10-, IL-12-, IFN- α -expressing cells in the skin and nasal mucosa from allergic rhinitis patients and nonatopic controls, after diluent and allergen challenge

	Nose		Skin		
	Dil8	Ag8	Dil8	Ag8	Ag48
IL-10					
Rhinitis ($n = 7$)	12.4 (8, 21)	14.0 (5.7, 33)	15.1 (6, 29)	8.4 (2, 14)***	3.1 (1.8, 19)
Controls ($n = 6$)	10.1 (3.6, 13)	27.1 (17, 34)##	35.5 (25, 56)	28.6 (20, 57)	21.1 (14, 28)
IL-12					
Rhinitis ($n = 6$)	3.6 (1.6, 1.6)	2.8 (1.7, 7)	5.8 (2, 13)	10.4 (4, 18)***	7.0 (1, 9.8)
Controls ($n = 4$)	2.2 (0.95, 4.9)	6.3 (4.9, 7)	9.0 (6.3, 11.6)	20.8 (16, 28)	4.9 (1.9, 10.4)
IFN- α					
Rhinitis ($n = 7$)	23.3 (19, 29)**	6.2 (3.9, 28.5)*	50 (19, 72)	61.8 (43, 79)	40 (19, 62)
Controls ($n = 5$)	119 (57, 140)	33.6 (19, 63)##	19.7 (8.8, 34)	24.8 (24, 32)	17.1 (12, 20)

IQ, interquartile range.

*** $P = 0.01$, ** $P = 0.02$, * $P = 0.07$ when allergic rhinitis patients were compared with controls.

$P = 0.06$, ## $P = 0.03$ when diluent was compared with allergen.

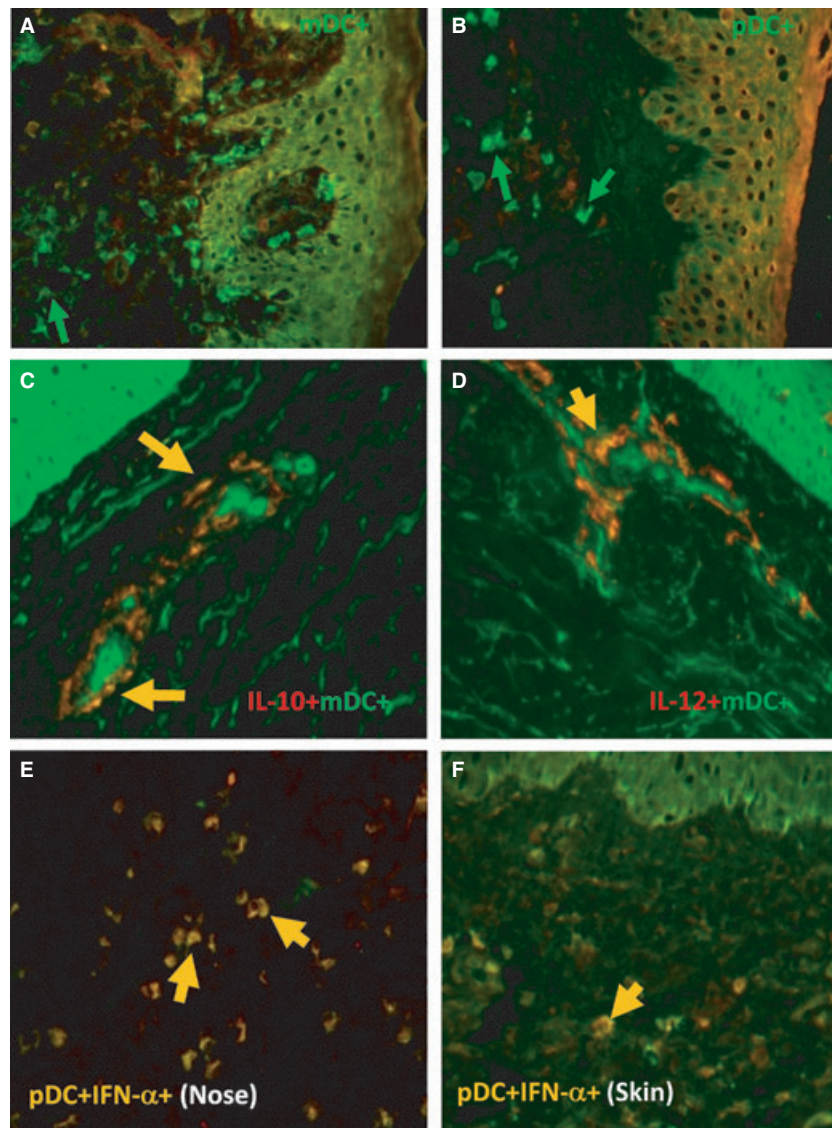


Figure 2 Immunolocalization of DCs. CD1c⁺ mDCs (A); CD303⁺ pDCs (B) after intradermal allergen challenge (Alexa Fluor 488-green); colocalization of mDCs (488-green) to IL-10 (C) or IL-12 (D) [tetramethylrhodamine isothiocyanate (TRITC) – red] in the skin of

control subjects postdiluent challenge; IFN- α (TRITC-red) pDCs (Alexa Fluor 488-green) in the nose (E) and skin (F) from one control subject and one allergic rhinitis patient, respectively, with high pDC numbers ($\times 400$ magnification).

patients compared with DCs from controls. Whereas IL-10 synthesis in coculture of T cells with AR⁺ mDCs was not significantly different from controls ($P = 0.09$), IL-10 production was strongly reduced in cocultures with activated pDCs from AR patients compared to controls (Fig. 6A).

Reduced IFN- γ responses were observed in cocultures of T cells with resting and allergen-pulsed mDCs from AR compared with controls (Fig. 6B). In contrast, only allergen-pulsed mDCs from AR could significantly elicit allergen-dependent IL-4 and IL-5 production (Fig. 6C,D), signature cytokines of Th2 responses. In line with IL-6 data, the capacity of mDCs to induce IL-17 secretion following LPS activation was also upregulated in AR patients (Fig. 7A), while in cocultures with

pDCs, IL-17 was not detected in most subjects (detected approximately 10 pg/ml in only two patients, after CpG activation; Fig. 7B).

Discussion

Our data show that following allergen challenge in the nose, both myeloid and plasmacytoid dendritic cells (pDCs) increased at 8 h, whereas in the skin, increase in pDCs occurred early and preceded mDCs. In allergic rhinitis compared with controls, a substantial reduction in DC expression of IL-10 was observed in the nose, the skin and in blood. In addition, a decrease in IFN- α expression was observed in the

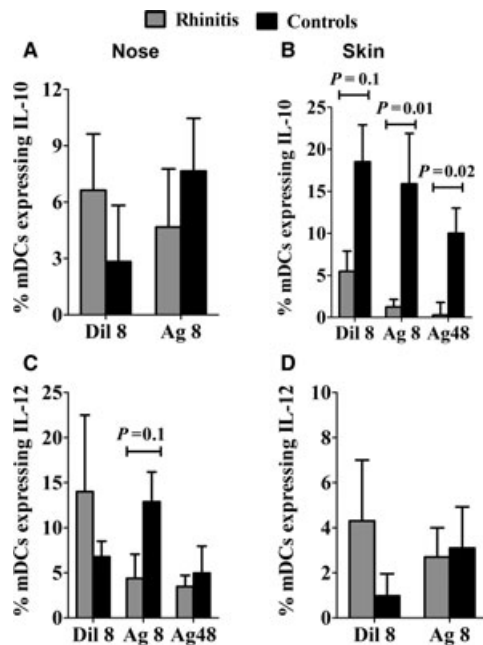


Figure 3 Colocalization of IL-10 (A, B) and IL-12p40 (C, D) to nasal and cutaneous mDCs, in allergic rhinitis ($n = 7$) and controls ($n = 6$) in response to allergen challenge vs diluent.

target organ (nasal mucosa) and in blood pDCs, but not in the skin. Moreover, reduction in IL-12 also characterized blood DCs from allergic rhinitis patients, which displayed aberrant capacity to induce Th2-type responses (mDCs) and impaired generation of IL-10+ T cells (pDCs).

The increases in pDC numbers at the site of allergen challenge during late responses in our study are in agreement with previous reports. Repetitive allergen provocation resulted in the recruitment of CD123^{high}CD45R⁺ cells in the nasal mucosa from atopic patients (13). A shift in the balance between blood mDC and pDC with a significant increase in pDCs was reported in asthma (12). Numbers of pDCs were also significantly increased in the skin from patients with allergic contact dermatitis (20) or psoriasis (21). Following allergen provocation, increases in numbers of both CD11c⁺ and CD123⁺ cells were observed in the nasal mucosa of allergic rhinitis patients (22), as well as in BAL (14) and sputum (23) from allergic asthmatics. In this study, we observed that, in the skin, increase in CD123⁺BDCA2⁺ pDC numbers precedes that of mDCs during allergic reactions.

Allergen-induced late skin responses represent a human model of Th2 responses. Inhibition of late responses has been observed following allergen-specific immunotherapy (24) reflecting immune tolerance that depends at least in part on IL-10 (15). Our findings of reduced IL-10 expression both at tissue sites of allergic inflammation and in blood could, at least in part, account for a systemic failure to regulate T-cell-mediated responses to allergens seen in atopic patients. Defective IL-10 responses to TLR or FcεR activation of DCs of the myeloid lineage (mDC and MD-DC) observed in our study are consistent with findings in circulating DCs and

LPS-stimulated monocytes from atopic dermatitis patients (25). In contrast to a previous observation where the ligation of FcεRI on CD123⁺BDCA2⁺ cells induced high IL-10 mRNA levels (26), we did not detect IL-10-producing pDCs within tissue or when isolated from peripheral blood and stimulated with allergen or CpG. These differences may relate to the tissue environment, as blood pDCs seem unable to robustly secrete IL-10, in contrast to DCs derived from the oral mucosa (27). The negative correlation observed in our study between mDCs and the late-phase skin response to allergen challenge is intriguing as it suggests that mDCs could play a protective role. This could also be viewed as a result of increased mDC recruitment in an attempt to control the local allergic reaction, although further evidence is required given their defect in IL-10 expression.

Impaired IL-12 expression in the skin and peripheral blood DCs from AR further suggest a systemic defect in DC programming in allergic rhinitis, as reported in atopic dermatitis (27). Aberrant programming of IL-10 and IL-12 expression in DCs from atopic patients could result in inappropriate T-cell responses to allergens. Thus, in our study, mDCs from AR displayed a reduced capacity to stimulate IFN-γ synthesis (consistently with reduced IL-12) and were capable of driving IL-4 and IL-5 production by CD4⁺ T cells. Charbonnier et al. reported that allergen (Der p)-pulsed mDCs induced IFN-γ, while pDCs induced IL-4 production by allogeneic T cells (28). This observation contrasted with another report where monocyte-derived DCs from Der p-allergic subjects induced IL-4 in autologous T cells (29). Differences between these studies could relate to several factors including patients' phenotypes and culture conditions, as well as subtypes of DCs. In addition, the defect in IL-12 production involving the p40 subunit in tissue and the p70 (and thereby the p35 subunit) in blood DCs, suggests that several mechanisms may underlie impairment in IL-12 biosynthesis. Whether this may also impact on other IL-12-related cytokines, such as IL-23, IL-27 or IL-35, remains to be evaluated.

In our allergic rhinitis patients compared with normal controls, lower numbers of IFN-α-expressing cells were observed in the nasal mucosa that further decreased upon allergen challenge. Although due to low cell numbers IFN-α could not be colocalized in tissue to pDCs, we observed decreased IFN-α production by blood pDCs from these patients upon *in vitro* TLR9 activation, confirming previous reports (26, 30). Although the precise mechanism remains unknown, defective response of pDCs to TLR9 ligands could relate to a cross-regulation by activated IgE signalling pathway (31). Our finding of blunted IFN-α production (and impaired IL-10 and/or IL-12) by DCs in response to IgE crosslinking by allergen *in vivo* (in the target organ) and following anti-IgE triggering *in vitro* (data not shown) is of interest and could underlie the increased susceptibility of atopic individuals to viral infections (30). This concept is supported by the recent demonstration that viral-induced seasonal exacerbations of asthma in children responded to anti-IgE treatment (32). However, in contrast to IL-10 and IL-12, this did not appear as a systemic defect of pDCs as IFN-α expression was main-

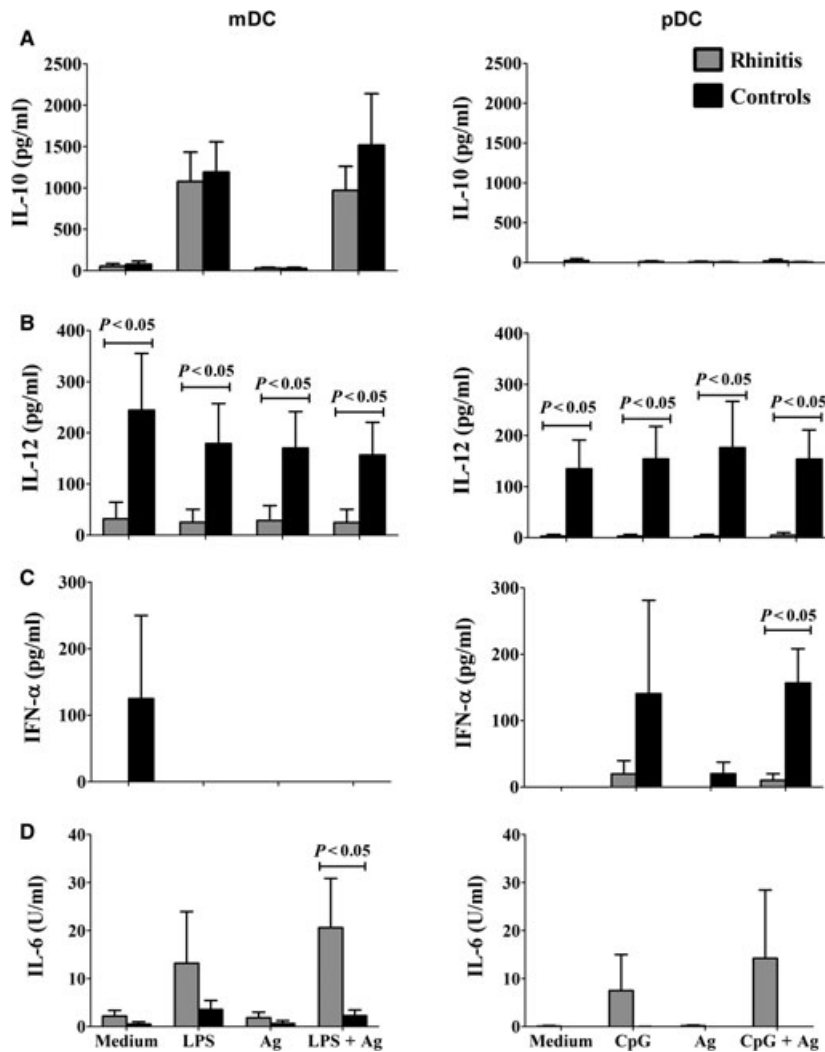


Figure 4 Cytokine profile of blood mDCs and pDCs following *in vitro* stimulation. IL-10 (A), IL-12p70 (B), IFN-α (C) and IL-6 (D) were assayed in mDCs and pDCs from allergic rhinitis or controls ($n = 6$

each) following 24-h pulsing with Der p allergen and/or stimulation by LPS (mDC) or CpG (pDC).

tained in the skin. These differences between the nose and the skin may be due, at least in part, to local (re)programming of immune cells (33), or possibly due to the 'dirty'

nature of the upper airway that is constantly exposed to environmental influences that could normally underlie a basal level of DC recruitment/activation.

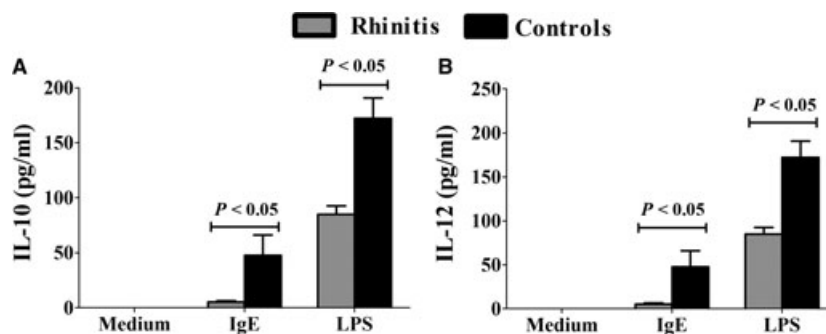


Figure 5 IL-10 (A) and IL-12p70 (B) production by DCs derived from monocytes of allergic rhinitis patients or controls ($n = 4$ each) and activated by LPS or IgE/anti-IgE.

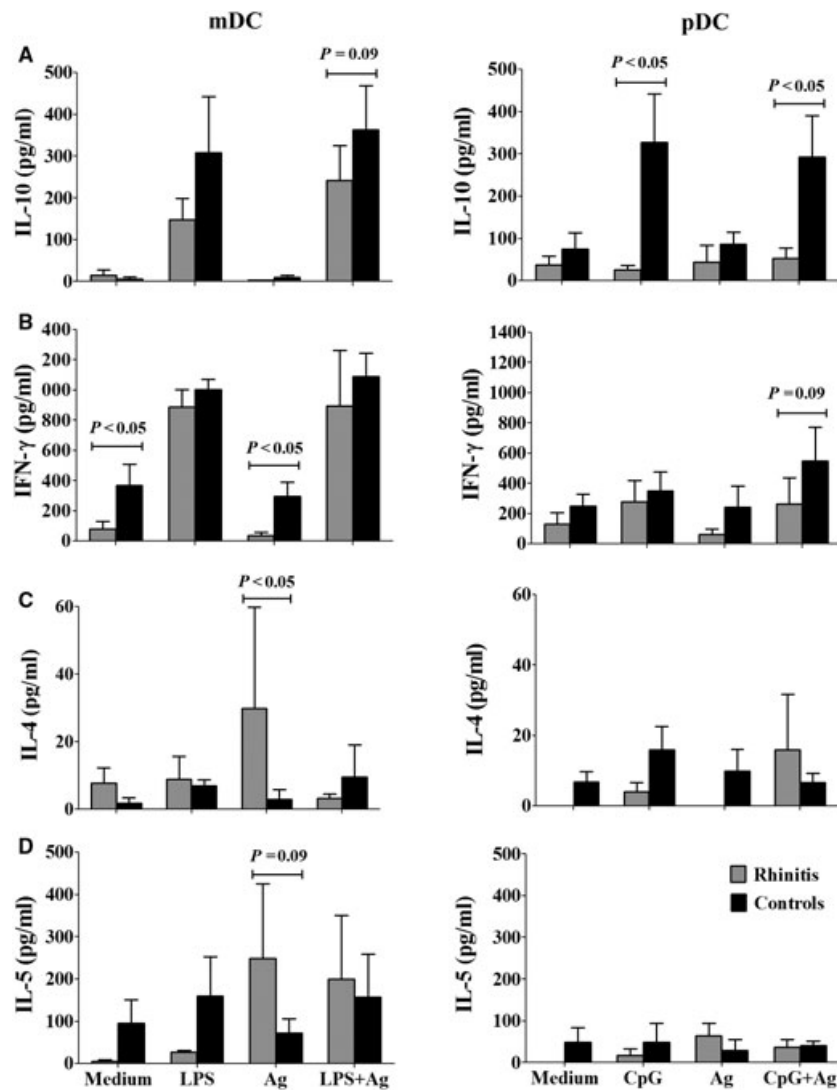


Figure 6 Th-cell polarization function of m and pDCs. Cytokine responses (IL-10, IL-4, IL-5 and IFN- γ) of CD4⁺ T cells (from one allogeneic nonatopic subject) cocultured for 5 days with mDCs

and pDCs from allergic rhinitis patients ($n = 10$) vs nonatopic controls ($n = 9$) pulsed with Der p allergen with/without LPS or CpG stimulation.

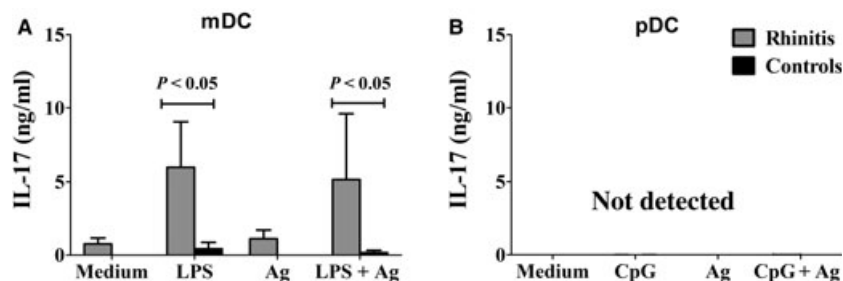


Figure 7 IL-17(A) production by CD4⁺ T cells (from one allogeneic nonatopic subject) cocultured for 5 days with mDCs and pDCs

from allergic rhinitis patients ($n = 6$) vs nonatopic controls ($n = 6$) pulsed with Der p allergen with/without LPS or CpG stimulation.

Our data show that DCs from allergic rhinitis patients are primed to drive not only Th2 but also IL-17(A)/Th17 responses, which are thought to underlie autoimmunity. IL-6 production was also upregulated in mDCs from these patients, consistent with the polarizing effect of this cytokine (in the presence of TGF- β) on Th17 differentiation (34). The nature and mechanisms of the relationship between allergy and autoimmunity remain debated (35), but our data suggest that aberrant function of mDCs from allergic rhinitis patients could integrate both Th2 and Th17 responses. The role of this IL-6/Th17 axis remains, however, to be explored *in vivo*, in these patients with allergic rhinitis. Of note, in contrast to Th2 cytokines, the production of IL-17 as well as IL-10 and IFN- γ was dependent on TLR activation and not on antigen pulsing of DCs, probably further highlighting that T-cell instruction by DCs depends not only on the allergen but also on the mucosal/cutaneous milieu. In addition, LPS at 1 μ g/ml could counteract in mDCs from AR patients the IL-4-inducing effect of the allergen, as observed with high-dose LPS *in vivo* (36).

Taken together, our data show that, in patients with allergic rhinitis, both mDCs and pDCs increase locally upon local allergen exposure of the target organ or of the skin. A systemic defect in IL-10 and IL-12 expression characterizes mDCs from these patients that presumably results in their aberrant capacity to trigger Th2-cell polarization. In contrast, impairment in IFN- α expression was observed in allergic rhinitis patients, in the target organ (not in the skin) from AR patients as well as *in vitro* in response to TLR9 ligation and resulted in impaired induction of IL-10+ T cells. Overall, the intrinsic dysfunction of DCs and their impaired responses to TLR stimulation could in turn favour Th2 reactions *in vivo*. Moreover, the IL-6/IL-17 pathway is also upregulated by blood mDCs from AR patients. Future research should focus on the molecular mechanisms that underlie these alterations in dendritic cells from patients with allergic respiratory disease.

References

1. Schuurhuis DH, Fu N, Ossendorp F, Melief CJM. Ins and outs of dendritic cells. *Int Arch Allergy Immunol* 2006;**140**:53–72.
2. Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH et al. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nat Med* 2002;**8**:1024–1032.
3. Masten BJ, Olson GK, Tarleton CA, Rund C, Schuyler M, Mehran R et al. Characterization of Myeloid and Plasmacytoid Dendritic Cells in Human Lung. *J Immunol* 2006;**177**:7784–7793.
4. Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. *Nat Rev Immunol* 2008;**8**:193–204.
5. GeurtsvanKessel CH, Lambrecht BN. Division of labor between dendritic cell subsets of the lung. *Mucosal Immunol* 2008;**1**:442–450.
6. Lambrecht BN, Salomon B, Klatzmann D, Pauwels RA. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J Immunol* 1998;**160**:4090–4097.
7. de Heer HJ, Hammad H, Soullié T, Hijdra D, Vos N, Willart MA et al. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004;**200**:89–98.
8. Kool M, van Nimwegen M, Willart MA, Muskens F, Boon L, Smit JJ et al. An anti-inflammatory role for plasmacytoid dendritic cells in allergic airway inflammation. *J Immunol* 2009;**183**:1074–1082.
9. Hagendorens MM, Ebo DG, Schuerwegh AJ, Huybrechts A, Van Bever HP, Bridts CH et al. Differences in circulating dendritic cell subtypes in cord blood and peripheral blood of healthy and allergic children. *Clin Exp Allergy* 2003;**33**:633–639.
10. Upham JW, Zhang G, Rate A, Yerkovich ST, Kusel M, Sly PD et al. Plasmacytoid dendritic cells during infancy are inversely associated with childhood respiratory tract infections and wheezing. *J Allergy Clin Immunol* 2009;**124**:707–713.
11. Silver E, Yin-DeClue H, Schechtman KB, Grayson MH, Bacharier LB, Castro M. Lower levels of plasmacytoid dendritic cells in peripheral blood are associated with a diagnosis of asthma 6 yr after severe respiratory syncytial virus bronchiolitis. *Pediatr Allergy Immunol* 2009;**20**:471–476.
12. Matsuda H, Suda T, Hashizume H, Yokomura K, Asada K, Suzuki K et al. Alteration of balance between myeloid dendritic cells and plasmacytoid dendritic cells in peripheral blood of patients with asthma. *Am J Respir Crit Care Med* 2002;**166**:1050–1054.

Acknowledgements

The Authors thank Prof C. Hermans (Haematology department, Cliniques universitaires St-Luc, Brussels) for help with leukapheresis sampling. The Authors thank Stallergènes (Anthony, France) and ALK-Abello (Denmark) for their gifts of allergen extracts.

Funding

CP is clinicien chercheur of the Fonds National de la Recherche Scientifique, Belgium (grants FRSM 3.4565.06 and 3.4522.12). This study was in part supported by a grant awarded to SRD from the Academic Drug Discovery Initiative (ADDI) a joint initiative between GlaxoSmithKline and Imperial College London. KTNA was a recipient of support from the ADDI.

Authors' contributions

CP participated in the design of the study and recruitment of patients, supervised *in vitro* experiments and wrote the paper. These two authors (MRJ and CR) contributed equally to this work. MRJ carried out experiments and participated in the data analysis. CR and BD carried out experiments. GB participated in the patient recruitment and performed skin and nasal challenges. JVS participated in IL-6 bioassay and related data analysis. SRD participated in the design of the study, recruitment of patients, analysis of data and writing of the paper. KTNA contributed to the design of the study, carried out experiments and participated in the analysis of data and writing of the paper.

Conflict of interest

None.

13. Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123^{high}) dendritic cells in human nasal allergy. *J Immunol* 2000;**165**:4062–4068.
14. Bratke K, Lommatzsch M, Julius P, Kuemper M, Kleine HD, Luttmann W et al. Dendritic cell subsets in human bronchoalveolar lavage fluid after segmental allergen challenge. *Thorax* 2007;**62**:168–175.
15. Francis JN, James LK, Paraskevopoulos G, Wong C, Calderon MA, Durham SR et al. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. *J Allergy Clin Immunol* 2008;**121**:1120–1125.
16. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4⁺ CD25⁺ cells in the nasal mucosa. *J Allergy Clin Immunol* 2008;**121**:1467–1472.
17. Anderson AE, Mackerness KJ, Aizen M, Carr VA, Nguyen D, Du Pre F et al. Seasonal changes in suppressive capacity of CD4⁺ CD25⁺ T cells from patients with hayfever are allergen-specific and may result in part from expansion of effector T cells among the CD25⁺ population. *Clin Exp Allergy* 2009;**39**:1693–1699.
18. Banfield G, Watanabe H, Scadding G, Jacobson MR, Till SJ, Hall DA et al. CC chemokine receptor 4 (CCR4) in human allergen-induced late nasal responses. *Allergy* 2010;**65**:1126–1133.
19. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK et al. Grass Pollen Immunotherapy Induces Mucosal and Peripheral IL-10 Responses and Blocking IgG Activity. *J Immunol* 2004;**172**:3252–3259.
20. Wollenberg A, Wagner M, Günther S, Towarowski A, Tuma E, Moderer M et al. Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. *J Invest Dermatol* 2002;**119**:1096–1102.
21. Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O et al. Plasmacytoid predendritic cells initiate psoriasis through interferon- α production. *J Exp Med* 2005;**202**:135–143.
22. KleinJan A, Willart M, van Rijt LS, Braunstahl GJ, Leman K, Jung S et al. An essential role for dendritic cells in human and experimental allergic rhinitis. *J Allergy Clin Immunol* 2006;**118**:1117–1125.
23. Dua B, Watson RM, Gauvreau GM, O'Byrne PM. Myeloid and plasmacytoid dendritic cells in induced sputum after allergen inhalation in subjects with asthma. *J Allergy Clin Immunol* 2010;**126**:133–139.
24. Durham SR, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999;**341**:468–475.
25. Aiba S, Manome H, Yoshino Y, Tagami H. Alteration in the production of IL-10 and IL-12 and aberrant expression of CD23, CD83 and CD86 by monocytes or monocyte-derived dendritic cells from atopic dermatitis patients. *Exp Dermatol* 2003;**12**:86–95.
26. Novak N, Allam JP, Hagemann T, Jenneck C, Laffer S, Valenta R et al. Characterization of Fc ϵ s1RI-bearing CD123 blood dendritic cell antigen-2 plasmacytoid dendritic cells in atopic dermatitis. *J Allergy Clin Immunol* 2004;**114**:364–370.
27. Lebre MC, van Capel TM, Bos JD, Knol EF, Kapsenberg ML, de Jong EC. Aberrant function of peripheral blood myeloid and plasmacytoid dendritic cells in atopic dermatitis patients. *J Allergy Clin Immunol* 2008;**122**:969–976.
28. Charbonnier AS, Hammad H, Gosset P, Stewart GA, Alkan S, Tonnel AB et al. Der p 1-pulsed myeloid and plasmacytoid dendritic cells from house dust mite-sensitized allergic patients dysregulate the T cell response. *J Leukoc Biol* 2003;**73**:91–99.
29. Hammad H, Lambrecht BN, Pochard P, Gosset P, Marquillies P, Tonnel AB et al. Monocyte-derived dendritic cells induce a house dust mite-specific Th2 allergic inflammation in the lung of humanized SCID mice: involvement of CCR7. *J Immunol* 2002;**169**:1524–1534.
30. Tversky JR, Le TV, Bieneman AP, Chichester KL, Hamilton RG, Schroeder JT. Human blood dendritic cells from allergic subjects have impaired capacity to produce interferon- α via Toll-like receptor 9. *Clin Exp Allergy* 2008;**38**:781–788.
31. Schroeder JT, Bieneman AP, Xiao H, Chichester KL, Vasagar K, Saini S et al. TLR9- and Fc ϵ s1RI-mediated responses oppose one another in plasmacytoid dendritic cells by down-regulating receptor expression. *J Immunol* 2005;**175**:5724–5731.
32. Busse WW, Morgan WJ, Gergen PJ, Mitchell HE, Gern JE, Liu AH et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med* 2011;**364**:1005–1015.
33. Novak N, Allam JP. Mucosal dendritic cells in allergy and immunotherapy. *Allergy* 2011;**66**(Suppl 95):22–24.
34. Nakagiri T, Inoue M, Minami M, Shintani Y, Okumura M. Immunology mini-review: the basics of T(H)17 and interleukin-6 in transplantation. *Transplant Proc* 2012;**44**:1035–1040.
35. Bogiatzi SI, Guillot-Delost M, Cappuccio A, Bichet JC, Chouchane-Mlik O, Donnadieu MH et al. Multiple-checkpoint inhibition of thymic stromal lymphopoietin-induced TH2 response by TH17-related cytokines. *J Allergy Clin Immunol* 2012;**130**:233–240.
36. Ray P, Arora M, Poe SL, Ray A. Lung myeloid-derived suppressor cells and regulation of inflammation. *Immunol Res* 2011;**50**:153–158.