Rapid communication

Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge

F L Jahnsen, E D Moloney, T Hogan, J W Upham, C M Burke, P G Holt

Abstract

***Background*—Airway dendritic cells (DC) play an important role in chronic allergic airway inflammation in experimental animals, but a similar role for DC in human allergic asthma has been difficult to define. This pilot study was undertaken to elucidate the role of DC in allergic asthma by examining their potential to migrate to the lower airways in response to bronchial challenge with specific allergen.**

***Methods*—Bronchial biopsy specimens were obtained from seven patients with allergic asthma before and 4–5 hours after allergen challenge. Multicolour immunofluorescence staining was performed on mucosal cryosections to identify changes in the number and phenotypes of DC.**

***Results*—A dramatic increase in the number of CD1c+HLA-DR+ DC were observed in the lamina propria after challenge compared with baseline (22.4 *v* 7.8 cells/mm^2). The rapid accumulation (within 4–5 hours) of these cells strongly suggests that they were directly recruited from peripheral blood.**

***Conclusion*—We have shown for the first time that a specific DC subset rapidly emigrates into the human bronchial mucosa during allergic inflammation. While this study is based on relatively few patients, the consistency of the overall results strongly suggests that the rapid population dynamics of human airway DC closely parallel those in animal models of acute inflammation. These findings support suggestions that DC have an important role in human airway allergy.**

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Keywords: asthma; dendritic cells; allergen challenge

TVW Telethon Institute for Child Health Research and Centre for Child Health Research, University of Western Australia, Perth, Western Australia
F L Jahnsen
J W Upham
P G Holt

Department of Medicine, University of Western Australia
J W Upham

LIIPAT, Institute of Pathology, University of Oslo, Rikshospitalet, Oslo, Norway
F L Jahnsen

James Connolly Memorial Hospital, Dublin, Ireland
E D Moloney
T Hogan
C M Burke

Correspondence to:
Dr P G Holt, Division of Cell Biology, TVW Telethon Institute for Child Health Research, PO Box 855, West Perth, Western Australia 6872
patrick@ichr.uwa.edu.au

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Activated T cells producing Th2 cytokines are believed to orchestrate the inflammatory reaction in chronic allergic asthma in humans. Activation of T cells depends on antigens being presented by antigen presenting cells (APC) in the context of MHC molecules. Among APC, dendritic cells (DC) have the unique ability to activate naive T cells, and a dense network of DC has been identified in the airway mucosa of humans[1] and experimental animals.[2–4] Studies in experimental animals indicate that these DC populations can efficiently prime T cells in vivo for subsequent expression of Th2 responses.[5] It has also been shown that depletion of airway DC in sensitised mice abrogates chronic eosinophilic airway inflammation,[3] which suggests that airway DC not only activate naive T cells in the sensitisation phase but also play an important role in antigen dependent activation of the Th2 memory population. These findings strongly suggest that airway DC play a critical role in both the development and maintenance of chronic allergic airway inflammation in experimental animals, but a similar role for DC in human allergic asthma has not been demonstrated.

The dense network of intraepithelial DC in airways of experimental animals displays a high steady state turnover, and during acute inflammation this turnover rate appears to be markedly accelerated. For example, aerosol challenge with heat killed *Moraxella catarrhalis* induces a rapid accumulation (within 2 hours) of DC in the tracheal mucosa.[6] This accumulation is paralleled by increased DC migration from the airways and into the draining lymph nodes. A similar response has also been observed in OVA sensitised rats challenged with aerosolised OVA, suggesting that rapid recruitment of DC is a common feature of airway inflammation.[6]

In bronchial specimens from asthmatic patients it has been reported that the number of putative DC is increased compared with normal controls, and that the number of such cells was significantly lower in those receiving topical steroids.[7 8] Although these findings suggest that DC are involved in allergen induced airway inflammation, the dynamics of airway DC in allergic asthma has not been determined. This pilot study was therefore planned to further elucidate the role of DC in allergic asthma by examining their potential to migrate to the lower airways in response to bronchial challenge with specific allergen.

Methods

STUDY PATIENTS

Bronchial biopsy specimens were obtained from seven atopic subjects with a mean (SD)

Table 1 Subject details

Patient no	Sex	Age	Allergen*	% fall FEV_1 (EAR)	% fall FEV_1 (LAR)	Treatment
1	F	49	HDM 3+	22	0	
2	M	50	HDM 4+ Grass 2+	34	42	
3	M	21	HDM 3+	21	21	
4	M	40	HDM 4+ Grass 3+	32	29	
5	F	44	HDM 3+ Grass 2+	40	10	800 μg BD†
6	F	17	HDM 3+ Grass 2+	25	15	800 μg BD†
7	F	26	HDM 2+	11	2	1000 μg FP‡

HDM = house dust mite; BD = budesonide; FP = fluticasone propionate.
*Skin prick test graded on a scale from 0–4+.
†Duration of treatment 12 months.
‡Duration of treatment 6 months.

forced expiratory volume in 1 second (FEV_1) of 78 (11)%. The individual characteristics of the study subjects are shown in table 1. All met the ATS criteria for the diagnosis of asthma. Skin prick tests were performed with seven common aeroallergens and all patients reacted strongly to house dust mite (table 1). Three patients received topical steroids while the others used only short acting β agonists. None of the subjects had any intercurrent diseases and all were non-smokers. The study was approved by the ethics committee at James Connolly Memorial Hospital, Dublin, Ireland and each participant gave full written informed consent before entering the study.

STUDY DESIGN

Measurements of pre-challenge pulmonary function and allergen challenge were performed as described elsewhere.[9] Short and long acting β agonists were withheld for 8 and 24 hours, respectively, before challenge, and steroids were withheld for 24 hours. Known dilutions of house dust mite (*Dermatophagoides pteronyssinus*; ALK, Horsholm, Denmark) were made to give final concentrations of 250, 500, 1000, 2000, 4000, 8000, 16 000, and 32 000 SQU/ml. The patients were first challenged with the lowest dose from a breath activated dosimeter for five deep breaths with a pause time of 6 seconds and nebulisation of 1 second. FEV_1 was recorded as a single blow after 5 and 10 minutes. Increasing doses of allergen were inhaled until a 20% reduction in FEV_1 from the post-saline value was achieved. FEV_1 was then recorded at 5, 10, 20, 30, 45, 60 and thereafter at 30 minute intervals up to 10 hours. An early allergen response (EAR) was defined as a fall in FEV_1 of >20% from the diluent value within 2 hours, and a late allergen response (LAR) was defined as a fall in FEV_1 of 15% 4–10 hours after allergen challenge.

FIBREOPTIC BRONCHOSCOPY

Fibreoptic bronchoscopy was performed 1 week before (baseline) and 4–5 hours after the allergen challenge had commenced, as described in detail elsewhere.[10] At least three endobronchial biopsy samples were obtained at each time point through the bronchoscope with sterile cup and spike forceps from the second generation right upper lobe bronchus. The mucosal samples were immediately snap frozen as previously described.[10]

MULTICOLOUR IMMUNOFLUORESCENCE STAINING

To determine the density and phenotype of DC we applied a range of monoclonal antibodies specific for various DC or macrophage markers combined with anti-HLA-DR (pan-marker for APC) in a sequential multicolour immunostaining technique to acetone fixed serial cryosections (10 μm). Firstly, one of the following monoclonal antibodies was incubated for 1 hour: anti-CD1c (clone M241, IgG1; gift from Dr R Blumberg, Boston + clone F10/21A3.1, IgG1; gift from Dr S A Porcelli, New York, NY), anti-CD11c (clone KB90, IgG1; gift from Dr K Pulford, Oxford, UK), anti-CD123 (clone 9F5, IgG1; Pharmingen, San Diego, CA, USA), anti-CD68 (clone PG-M1, IgG3; Dako, Glostrup, Denmark), and clone RFD-7 directed against tissue macrophages (IgG1; gift from Dr L W Poulter, London, UK). Cy3-labelled goat anti-mouse IgG (Jackson Immuno Research, West Grove, PA, USA) was then applied for 1 hour, followed by a mixture of biotinylated monoclonal antibody specific for human HLA-DR (clone L243, IgG2a; Becton Dickinson, San Jose, CA, USA), rabbit antiserum to laminin (Dako), and 10% mouse serum to block free binding sites in the previous step. Finally, streptavidin-Alexa 488 (Molecular Probes, Eugene, OR, USA) combined with Cy5-labelled anti-rabbit (Amersham, Galesbury, UK) was applied for 30 minutes.

In order to distinguish CD1c+ cells from other APC and to distinguish the myeloid and plasmacytoid DC subsets, monoclonal antibodies to CD1c were combined with monoclonal antibodies to either CD14 (clone RM052, IgG2a; Biosys, Compeigne, France), CD20 (IgG2a, Becton Dickinson), or CD68 (IgG3) and CD123 (clone 7G3, IgG2a; Pharmingen) combined with CD45RA (clone L48, IgG1; Becton Dickinson) as previously described.[11] Negative control experiments were obtained by omission of primary monoclonal antibodies and by incubation with irrelevant isotype and concentration matched primary monoclonal antibodies. Identification of eosinophils was performed using FITC-labelled BSA for 1 hour as detailed elsewhere.[12]

The immunostained tissue sections were examined blindly by the same investigator (FLJ) at ×400 magnification. To determine the relative cell densities, all immunostained cells were counted to a stromal depth of 400 μm parallel to the basement membrane of the surface epithelium. At least 2 mm^2 of lamina propria were examined at each time point to determine the number of CD1c+HLA-DR+ and CD123+HLA-DR+ cells. Selected specimens were examined with the other monoclonal antibody combinations. The density of eosinophils was scored on an arbitrary scale of 0–3.

DATA ANALYSIS

Paired and unpaired *t* tests were performed to compare the number of immunostained cells before and after challenge in all individuals and between steroid treated and steroid naive

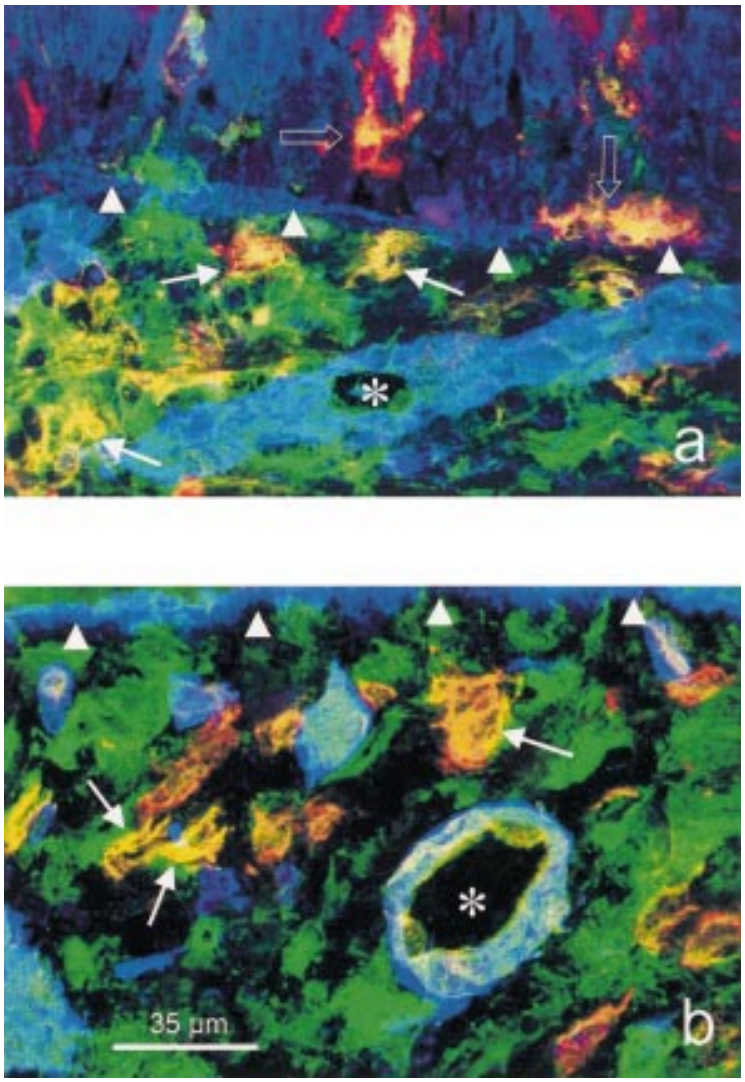

*Figure 1 Three-colour immunofluorescence staining for (A) CD1c and (B) CD123 (both Cy3, red) combined with HLA-DR (Alexa 488, green), and laminin (Cy5, blue) in cryosections of (A) challenged or (B) unchallenged bronchial mucosa, respectively. (A) CD1c+DR+ dendritic cells and (B) CD123+DR+ dendritic cells are shown by their mixed (yellow) colour. Some are indicated by filled and open arrows in lamina propria and epithelium, respectively. Epithelial basement membrane is indicated by arrow heads. (A) Note intraepithelial CD1c+HLA-DR+ cells displaying typical dendriform morphology. Most bronchial vessels are HLA-DR+ (mixed green and blue, some with luminal *). (B) Note one CD123+DR+ vessel (*).*

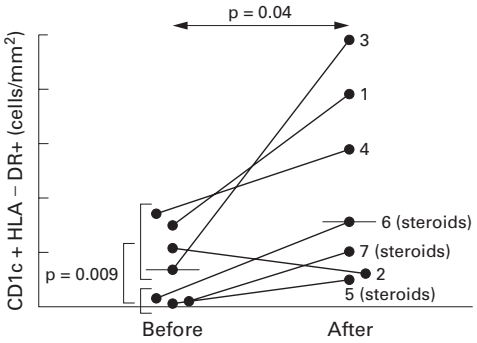

Figure 2 Density of lamina propria CD1c+HLA-DR+ dendritic cells (numbers/mm²) in cryosections of bronchial mucosa from patients with allergic asthma before and after challenge as indicated. Cell counts from the same individual are connected by lines. Medians are indicated by horizontal lines. Numbers refer to patient numbers in table 1. Patients receiving steroids (up to 24 hours before challenge) are indicated.

patients before challenge, respectively. Spearman's rank correlation coefficient was used to evaluate the relationship between change in DC numbers and LAR.

Results

DC in humans are a very heterogeneous population of cells, and their histological identification has to combine certain phenotypic characteristics with specific morphological criteria. In order to identify DC subsets in bronchial tissue we therefore applied a monoclonal antibody specific for HLA-DR to delineate all APC in combination with monoclonal antibodies to various DC and macrophage markers. HLA-DR+ cells were found in both the epithelium and lamina propria in all specimens examined. Most of these cells co-expressed CD11c, a marker for DC in the mouse. However, a large proportion of this HLA-DR+CD11c+ population also expressed macrophage markers CD68 and RFD-7, demonstrating that CD11c was not a suitable marker to identify DC in human bronchial tissue. However, a smaller proportion of the HLA-DR+ cells co-stained with monoclonal antibodies specific for CD1c. This marker is expressed on dermal DC and has previously been used to identify DC in bronchial tissue. Interestingly, CD1c is also expressed at high levels on most of the myeloid DC precursor subset in circulation characterised as lin^{neg}CD11c+ HLA-DR+.[13] In peripheral blood CD1c is also expressed on a subset of B cells. However, CD1c+ cells in bronchial tissue were invariably negative for the pan B cell marker CD20, and also for CD14 and CD68, confirming that they were distinct from B cells, monocytes, and macrophages. CD1c+HLA-DR+ cells were found scattered both in the epithelium and lamina propria. In the epithelium in particular, they often displayed typical pleomorphic dendriform morphology (fig 1a), confirming that this population was indeed DC. Interestingly, at baseline the number of CD1c+HLA-DR+ DC was significantly lower (p=0.009) in patients being treated with topical steroids (n=3) than in steroid naïve patients (fig 2), which agrees with previous human studies on allergic asthmatics[7] as well as similar studies in rats.[14]

To examine how DC subsets responded to local allergen challenge, all seven individuals underwent bronchial challenge with house dust mite allergen which resulted in an EAR in six patients, four of whom also experienced an LAR (table 1). There was a dramatic increase (almost threefold) in the number of CD1c+HLA-DR+ DC in the lamina propria 4–5 hours after the challenge (fig 1a and 2), while a nearly twofold increase (1.7 *v* 3.0 cells/mm basement membrane, fig 1a) occurred in the epithelium. The number increased in both the steroid naive patients and those treated with topical steroids, although the increase was more pronounced in the former (fig 2). There was no significant correlation between the magnitude of the LAR and the change in DC numbers.

We have recently shown that another DC subset, designated plasmacytoid DC (P-DC),

occurs in small numbers in patients with silent nasal allergy and increased dramatically in response to allergen challenge.[11] In this study, however, P-DC was almost absent from the bronchial mucosa both before and after challenge. Only specimens from one patient contained scattered P-DC with no difference between baseline values and those after challenge (fig 1b). To ascertain whether the recruitment of DC was accompanied by other leucocyte subsets we screened the sections for the presence of tissue eosinophils, a common feature of allergic airway inflammation. Interestingly, in those patients receiving steroids no eosinophils were detected before challenge, but they were invariably present after provocation; in one patient high numbers of eosinophils were found. In the steroid naive patients all specimens contained variable numbers of eosinophils, but there was no significant difference compared with baseline. This suggests that recruitment of DC and eosinophils may be regulated independently, and this possibility should be addressed in follow up studies.

Discussion

We have shown for the first time that CD1c+HLA-DR+ DC rapidly (within 4–5 hours) accumulate in human bronchial mucosa in response to allergic inflammation. This finding is in agreement with the kinetics of DC trafficking during the early phase of inflammatory reactions in rat airways.[6] It is likely that these incoming DC are derived from circulating Lin^{neg}CD11c+HLA-DR+ myeloid DC precursors. Freshly isolated Lin^{neg}CD11c+HLA-DR+ myeloid DC precursors from peripheral blood are potent stimulators of both CD4+ and CD8+ T cell proliferation, suggesting that this DC subset can act as a potent APC without prior activation signals.[15] It is therefore tempting to speculate that these DC newly recruited into the airways following bronchial challenge with allergen can participate directly in the late phase of local allergic reactions. The lack of correlation between the change in DC numbers and the magnitude of LAR in this study does not rule out this possibility, but rather emphasises the fact that allergic symptoms result from a complex series of interactions between various immune cells—for example, APC, T cells, mast cells, eosinophils—and other tissue elements.

The finding that P-DC were virtually absent from the lower airways contrasts with our recent findings in upper airway allergy.[11] This could imply that recruitment of this DC subset is restricted to certain regions within the airway tree. However, bronchial tissue from one patient contained some P-DC, which suggests that these cells also have the ability to traffic to the lower airways. It is therefore more likely that these cells have a delayed recruitment compared with CD1c+HLA-DR+ DC and, if so, this opens up the possibility that distinct DC subsets play different functional roles at different time points in the allergic process.

Ideally, a non-atopic control group should have been included in this study to confirm the immunological specificity of these responses. However, the inclusion of control subjects was not accepted by the ethical committee.

While this study is based on a relatively small number of patients, the consistency of the overall findings strongly suggests that the rapid population dynamics of human airway DC during inflammation closely parallels that previously described in animal models. Given the key role of these cells in regulating T cell activation to inhaled antigens in experimental animals, a more detailed study of their functional phenotype in human airways—particularly in asthmatics—would appear to be warranted.

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