

Original article

Circulating myeloid and plasmacytoid dendritic cells after allergen inhalation in asthmatic subjects

Background: Dendritic cells are key contributors to initiation and maintenance of T-cell immunity to inhaled allergen. The purpose of this study was to enumerate the changes in peripheral blood myeloid (mDCs) and plasmacytoid dendritic cells (pDCs), the DCs expressing chemokine receptor 6 (CCR6) and chemokine receptor 7 (CCR7), following diluent and allergen inhalation in asthmatic subjects.

Methods: Peripheral blood was obtained from 16 allergic asthmatic subjects before and at 0.5, 1, 2, 3, 4, 6, 24, and 48 h after inhaled diluent and allergen challenges. Dendritic cells were enumerated using flow cytometry.

Results: Allergen inhalation significantly reduced mDCs at 6 h (21.3 ± 2.0 vs $15.0 \pm 1.8/\mu\text{l}$ blood; $P < 0.05$) and 24 h (21.5 ± 3.4 vs $16.4 \pm 2.4/\mu\text{l}$ blood; $P < 0.05$) after challenge. Circulating pDCs were significantly lower than baseline up to 24 h after both allergen and diluent challenges. There was a significant efflux of CCR6⁺ mDCs from peripheral blood at 6 h and CCR6⁺ pDCs at 4 h after allergen challenge, when compared with diluent. There was no difference in the number of circulating CCR7⁺ mDCs or pDCs after diluent or allergen challenges.

Conclusions: Peripheral blood mDCs and CCR6⁺ mDCs, but not pDCs, are reduced up to 24 h after allergen inhalation. Thus, allergen inhalation causes trafficking of immature CCR6⁺ DCs from blood into the airway, while that of the trafficking of the mature CCR7⁺ DCs from the airways into the regional lymph nodes probably occurs through the lymphatic system.

**E. Farrell, T. M. O'Connor, M. Duong,
R. M. Watson, T. Strinich,
G. M. Gauvreau, P. M. O'Byrne**

Department of Medicine, McMaster University,
Hamilton, ON, Canada

Key words: allergic asthma; antigen presenting cells; cell trafficking; myeloid dendritic cells; plasmacytoid dendritic cells; chemokine receptor 6; chemokine receptor 7.

Dr Paul O'Byrne
Department of Medicine
McMaster University
HSC 3 W10
1200 Main St West
Hamilton
ON L8 N 3Z5
Canada

Accepted for publication 15 May 2007

Allergic asthma is a chronic inflammatory disease of the lower airways characterized by bronchoconstriction, airway smooth muscle contraction, mucus hypersecretion, mucosal edema, and structural remodeling of the airway wall (1). Allergen inhalation challenge is a useful clinical model to study the mechanisms of allergic asthma. Allergen inhalation causes acute bronchoconstriction in sensitized asthmatic subjects, and in up to 70% of subjects, bronchoconstriction recurs 3–4 h following allergen inhalation, peaking at 8–12 h and lasting up to 24 h. This response is known as the late asthmatic response (LAR) (2). Subjects who develop an LAR usually have more marked allergen-induced airway inflammation and airway hyperresponsiveness (3, 4). The LAR has been suggested to occur as a result of allergen processing and presentation by airway dendritic cells (DCs) (5, 6) and an associated T-helper (Th2) response.

In humans, two subsets of DCs have been recognized. These are myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs). Myeloid DCs typically represent between 0.5% and 1.0% of circulating mononuclear cells (7). In recent years, it has become evident from animal models of allergic asthma, that mDCs are

crucial contributors to allergic Th2 sensitization and are responsible for the development of secondary immune responses to inhaled allergen (8–10). In contrast, pDCs which typically represent <0.3% of circulating blood mononuclear cells may play a role in providing tolerance to inhaled allergen (11).

The homing properties of DCs are regulated by the profile of chemokine receptors that they express. Chemokine ligand (CCL) 20 (MIP3 α) functions as important chemokine for the recruitment of chemokine receptor 6 (CCR6)-expressing immature DCs to tissues for subsequent antigen presentation (12, 13). Once DCs have entered the airways and captured antigen, maturation commences. Dendritic cells then begin traffic to regional lymph nodes to present antigen and initiate a T-cell response. A major event in maturation is the downregulation of CCR6 and the subsequent up-regulation of the chemokine receptors, CCR7 and CXCR4. The CCR7 has been documented only on DCs (7). Chemokine ligand 19 (MIP3 β) and CCL 21 are known ligands for the CCR7 receptor (14).

Studies in allergic asthmatics have shown that circulating mDCs are reduced between 3 and 24 h after allergen

inhalation (6), and start appearing in the airways 5–6 h after allergen (5). There are no studies that have evaluated the kinetics of pDCs, or of DCs expressing CCR6 and CCR7. This objectives of the current study were to enumerate the changes in the numbers of peripheral blood mDCs and pDCs, and the number of DCs expressing CCR6 and CCR7 following diluent and allergen inhalation challenges in asthmatic subjects. We hypothesized that CCR6 mDCs and CCR6 pDCs would decline after inhaled allergen, as these more immature DCs were recruited into the airways, while CCR7 mDCs and pDCs would not increase after inhaled allergen, as their trafficking into regional lymph nodes would occur through the lymphatic system.

Methods

Subjects

Sixteen subjects with allergic asthma, between the ages of 18 and 60 years, with previously documented allergen-induced early and late phase airway responses were included in the study. Subjects were considered atopic, if they had at least one wheal > 2 mm to common aeroallergens. All subjects were nonsmokers with stable, mild asthma, with a provocative concentration (PC₂₀) of methacholine < 16 mg/ml. Subjects were excluded if pregnant, or using any asthma medication other than short acting bronchodilators as required. This study was approved by the Hamilton Health Science Research Ethics Board.

Study design

All subjects underwent a screening allergen inhalation challenge (Fig. 1) and were enrolled if they had both an early asthmatic re-

sponse (EAR) [$\geq 20\%$ fall in forced expiratory volume for the first second (FEV₁) from baseline within 3 h after allergen, EAR] and a LAR [$\geq 15\%$ fall in FEV₁ between 3 and 7 h after allergen, LAR]. Following screening, subjects had a washout of 4 weeks, after which each of them underwent a diluent followed by an allergen challenge separated by 2 weeks. Prior to each challenge, a methacholine test was performed (within 2 weeks) to ensure the return of PC₂₀ to within one doubling dose of baseline values (Fig. 1). Blood samples were taken before and at 0.5, 1, 2, 3, 4, 6, 24, and 48 h after each challenge.

Allergen inhalation challenge

The baseline forced FEV₁ was $\geq 70\%$ before each inhalation challenge in all subjects. Allergen was inhaled via a Wright nebulizer (connected to a two-way valve mouthpiece and an exhalation filter). The flow rate through the nebulizer was determined to give an output of 0.13 ml/min. The starting allergen concentration was two doubling concentrations below that predicted to cause a 20% fall in FEV₁ (allergen PC₂₀) for the early response. This was calculated using a method previously described (15).

Allergen was diluted with normal saline. Doubling increasing concentrations of allergen were inhaled for 2 min each at tidal breathing with a nose clip and separated by intervals of 12 min. One technically acceptable FEV₁ was measured at 10 min after each inhalation. If it had fallen < 10%, the next concentration was administered. If it had fallen between 10% and 20% the FEV₁ was measured again after a further 10 min. If it was the same or started to increase, the next concentration was administered. Inhalations were stopped when FEV₁ had fallen by 20% or more. After the last inhalation, the FEV₁ was measured at 20, 30, 45, 60, 90, 120 min, and then at hourly intervals until 7 h after the last allergen inhalation.

The fall in FEV₁ was expressed as a percentage relative to the best preallergen baseline FEV₁. The greatest fall in FEV₁ between 0 and 3 h postallergen was taken as the EAR and between 3 and 7 h as the LAR. A dual response was determined by an EAR of $\geq 20\%$, and an LAR $\geq 15\%$. For the diluent inhalation challenge, the above steps were repeated, but rather than inhaled allergen, 1–3 ml of normal saline (0.9%) was utilized.

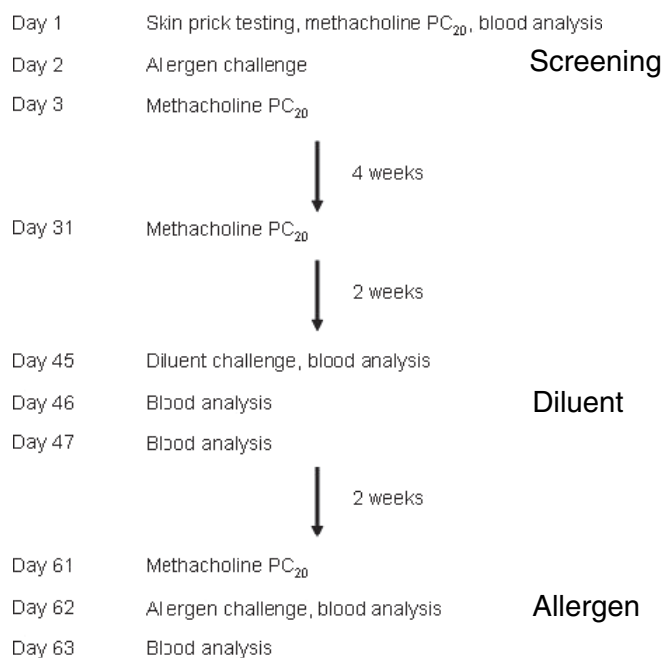


Figure 1. Study design.

Blood collection and processing of samples

For the inhalation challenge days, an intravenous catheter was inserted into the antecubital vein to allow repeated collection of blood (Becton-Dickinson Infusion Therapy Systems; Becton Dickinson, Franklin Lakes, NJ, USA). At each time point, blood was collected for routine blood count [2 mls in EDTA tube (Becton Dickinson)], flow cytometry analysis [5 mls in heparin tube (Becton Dickinson)] and serum for enzyme-linked immunosorbent assays (ELISA) [4 mls in unheparinized tube (Becton-Dickinson)]. These were repeated at 24 and 48 h postinhalation challenges. Blood samples for flow cytometry analysis contained between 2.2 and 12.3×10^6 leukocytes/ml and were incubated with BDIS fluorochrome-conjugated monoclonal antibodies (Becton-Dickinson Biosciences, Franklin Lakes, NJ, USA). Serum was separated by allowing blood to clot in unheparinized tube for 30 min centrifuged at 426 g for 10 min. Then 1 ml of serum was removed from the tubes, and stored in sterile Cryogenic Vials (Nalge Nunc International, New York, USA) at -78°C .

Antibodies

The following monoclonal antibodies were used: Lin1-FITC, CD3-PE, HLADR-PerCP, CD123-PE, CD11c-APC, CCR6-PeCy7,

CCR7-PeCy7, CD123-PE Isotype Control, CD11c-APC Isotype Control, CCR6-PeCy7 Isotype Control, CCR7-PeCy7 Isotype Control (Becton-Dickinson Biosciences).

Immunofluorescent cell staining of peripheral blood dendritic cells

Cell staining and multi-parameter sequential gating techniques for both mDCs and pDCs were performed according to manufacturer's protocol (Becton-Dickinson Biosciences). The additional staining of the CCR6 and CCR7 receptors on both mDCs and pDCs was developed in our laboratory. There were nine different time points stained: 0-h prechallenge, and 0.5, 1, 2, 3, 4, 6, 24, 48-h postchallenge. The only time points that had compensation tubes were the 0 and 24 h. Thus, 0 h compensation tubes were used as the compensation control for the 0–6 h samples. Furthermore, 24 h compensation tubes were used as the compensation control for the 24 and 48 h samples. The remaining time points only utilized four tubes.

Following the staining of peripheral blood with the appropriate antibodies, the samples were vortexed gently and incubated at

room temperature in the dark for 15 min. Samples were then treated with FACS Lysing Solution (Becton-Dickinson Biosciences), vortexed and incubated at room temperature for 10 min in the dark, to lyse the red blood cells. Samples were centrifuged at 1500 rpm for 5 min and supernatant was aspirated. Next, 1 ml of 10% wash buffer (Gibco; Burlington, ON, Canada) was added to the pellet and vortexed gently, using a vortex mixer. Samples were centrifuged at 1500 rpm for 5 min and supernatant was aspirated. Cells were then resuspended in 300 μ l of 1% paraformaldehyde (3 ml of 10% wash buffer with 1 ml of 4% paraformaldehyde). Stained cells were then stored in the dark at 4°C until flow cytometric acquisition which occurred within 24 h of staining. Following acquisition, the number of DCs was calculated. To accomplish this, the number of DC (either pDC or mDC) was obtained and divided by the corresponding number of mononuclear cells that were acquired in the sample. This value was then multiplied by the number of mononuclear cells from the complete blood count. The number was then converted to from DCs/l of blood to DCs/ μ l of blood $\pm$ SEM.

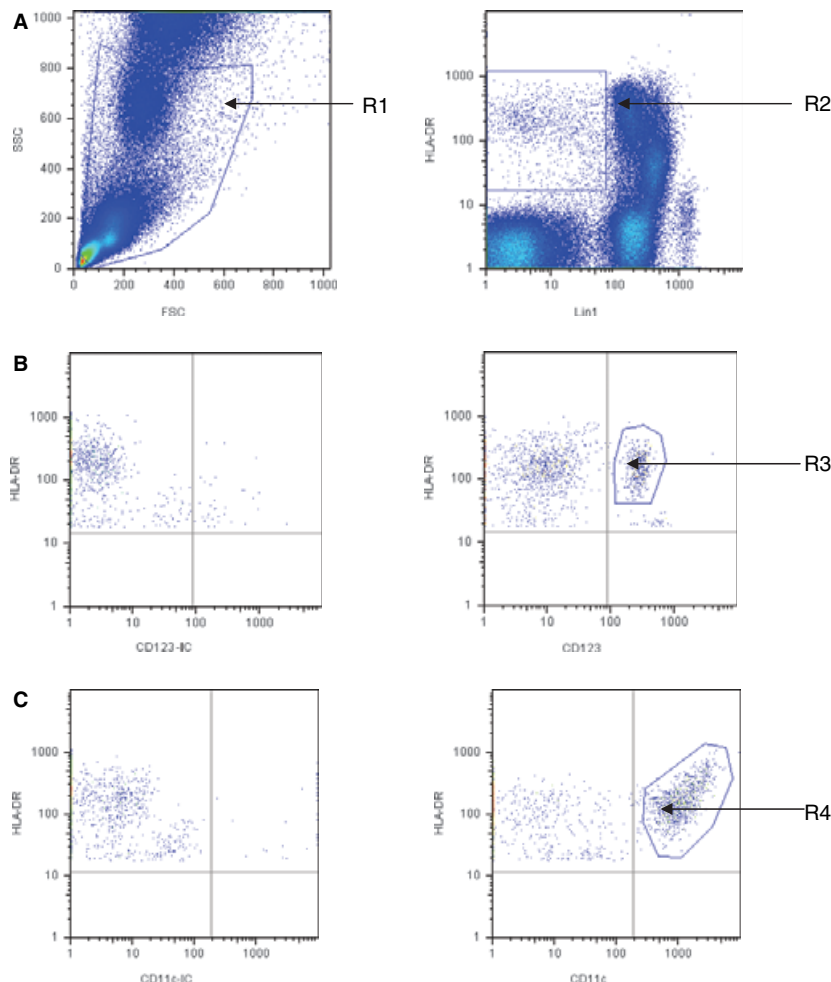


Figure 2. Gating strategy. (A) For enumeration of dendritic cell numbers. Regular R1 is a mononuclear gate made on mononuclear cell populations in the FSC vs SSC graph. This population was transferred to a Lin 1 vs HLA-DR to identify the dendritic cell populations (R2). (B) Gating strategy for enumeration of plasmacytoid dendritic cells (R3). (C) Gating strategy for enumeration of myeloid dendritic cells (R4).

Flow cytometric acquisition

The cells were acquired with a six color LSR II flow cytometer equipped with four lasers (Becton-Dickinson Instrument Systems; Becton-Dickinson) using the FACSDiva software program (Becton-Dickinson Biosciences, Mississauga, Canada). The following seven parameters were acquired: linear forward scatter (FSC), linear side-angle light scatter (SSC), log FITC, log PerCP, log PE, log APC, log PeCy7 fluorescence. Based upon preliminary experiments and work by Upham et al. (6) and Parameswaran et al. (16), it was determined that 200 000 mononuclear cells would be collected at flow cytometric acquisition, when CCR6⁺ and CCR7⁺ DCs are found at very low levels in peripheral blood.

Enumeration of myeloid and plasmacytoid dendritic cells via flow cytometry

Dendritic cells were enumerated as per manufacturer's procedures (Becton-Dickinson Biosciences). The primary plot set was FSC vs SSC and a mononuclear gate was set up to enumerate the number of mononuclear cells excluding as much debris as possible (Region R1, Fig. 2A).

Analysis of flow cytometric acquisition for myeloid and plasmacytoid DCs

Offline analyses of flow cytometric acquisition were performed using FlowJo (Tree Star Inc., Ashland, OR, USA). A mononuclear gate was made on the mononuclear cell population in the FSC vs SSC graph (Region R1, Fig. 2A). This population was then transferred to a Lin1 vs HLA-DR dot plot and the dendritic cell population which was Lin1⁻ and HLA-DR⁺ was gated around (Region R2, Fig. 2A). A CD123 vs HLA-DR plot was developed to obtain the number of pDC's. This graph was synthesized based upon its isotype control. Quadrant markers were set in such a way that cells stained with isotype control expressed 2% receptor expression. Plasmacytoid DC's are CD123⁺ and HLA-DR⁺ (Region R3, Fig. 2B). A CD11c vs HLA-DR plot was developed to obtain the number of mDC's. This graph was synthesized based upon its isotype control. Quadrant markers were set in such a way that cells stained with isotype control expressed 2% receptor expression. Myeloid DC's are CD11c⁺ and HLA-DR⁺ (Region R4, Fig. 2C).

Analysis of CCR6 and CCR7 expression on both myeloid and plasmacytoid dendritic cells

For analysis of CCR6 and CCR7 receptor expression on DC's, multiple plots were developed. To determine if the pDC's and mDC's expressed CCR6 or CCR7, cells from Region R3 (Fig. 2B) and Region R4 (Fig. 2C) were placed on a CCR6 or CCR7 plot. Quadrant markers were set in such a way that cells stained with isotype control expressed 2% receptor expression and the numbers of positive cells were enumerated.

Statistical analysis

Summary statistics are expressed as the mean $\pm$ SEM. All statistical analyses were performed with SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA). The effect of allergen was compared with diluent at various time points, using ANCOVA for repeated measures with prechallenge baseline values as covariance. Significance was assumed at $P < 0.05$.

Results

All subjects demonstrated a dual response to allergen inhalation, with a maximal fall in FEV₁ of $36.8 \pm 2.5\%$ for the EAR, and $19.7 \pm 3.48\%$ for the LAR. Diluent challenge did not result in a change in FEV₁ from baseline.

Following allergen inhalation, there was a significant fall in the number of circulating mDCs at 3 h, which remained significantly lower compared with baseline for all subsequent time points up to 48 h (Fig. 3A). When compared with diluent, circulating mDCs, after allergen challenge were significantly lower at 6 h (21.3 ± 2.0 cells/ μ l after diluent vs 15.0 ± 1.8 cells/ μ l after allergen, $P < 0.05$) and at 24 h (21.5 ± 3.4 cells/ μ l after diluent vs 16.4 ± 2.4 cells/ μ l after allergen, $P < 0.05$) (Fig. 3A). In addition, there were significantly lower numbers of mDCs expressing CCR6⁺ at 6 h after allergen, (7.11 ± 1.23 cells/ μ l) compared with diluent (9.63 ± 1.30 cells/ μ l) (Fig. 3), and in CCR7⁺ at 24-h postallergen inhalation, (0.15 ± 0.05 cells/ μ l) compared with diluent (0.45 ± 0.11 cells/ μ l) (Fig. 3B,C).

Overall, there were fewer numbers of circulating pDCs (9.76 ± 0.47 cells/ μ l) than mDCs (19.19 ± 0.66 cells/ μ l). Following allergen inhalation, there was a significant fall ($P < 0.05$) in the numbers of circulating pDCs between 0.5 and 24 h compared with baseline (Fig. 4A). A similar fall in pDCs numbers was observed after diluent challenge (Fig. 4A) with no significant differences detected between diluent and allergen challenge.

The number of circulating CCR6⁺ pDCs (1.66 ± 0.14 cells/ μ l) was comparatively smaller than the number of CCR6⁺ mDCs (8.35 ± 0.36 cells/ μ l). At 4-h postallergen inhalation, a significantly lower ($P < 0.05$) number of circulating CCR6⁺ pDCs was seen compared with diluent challenge (Fig. 4B). In contrast, no difference was observed between diluent and allergen challenge in CCR7⁺ pDCs numbers at any time point (Fig. 4C).

Discussion

The presentation of antigen to T cells by antigen presenting cells (APCs) is a hallmark of allergic disease. The most important APCs in the airways are DCs (7). In murine models of allergic asthma, mDCs are responsible for allergic Th2 sensitization and mediation of chronic inflammation (17), while pDCs were shown to induce tolerance to inhaled allergen {3908}. The role of both mDCs and pDCs in regulating allergen-induced airway responses in humans remains poorly understood.

This study examined the kinetics of peripheral blood mDCs and pDCs after inhaled allergen in allergic asthmatic subjects. The study demonstrated that levels

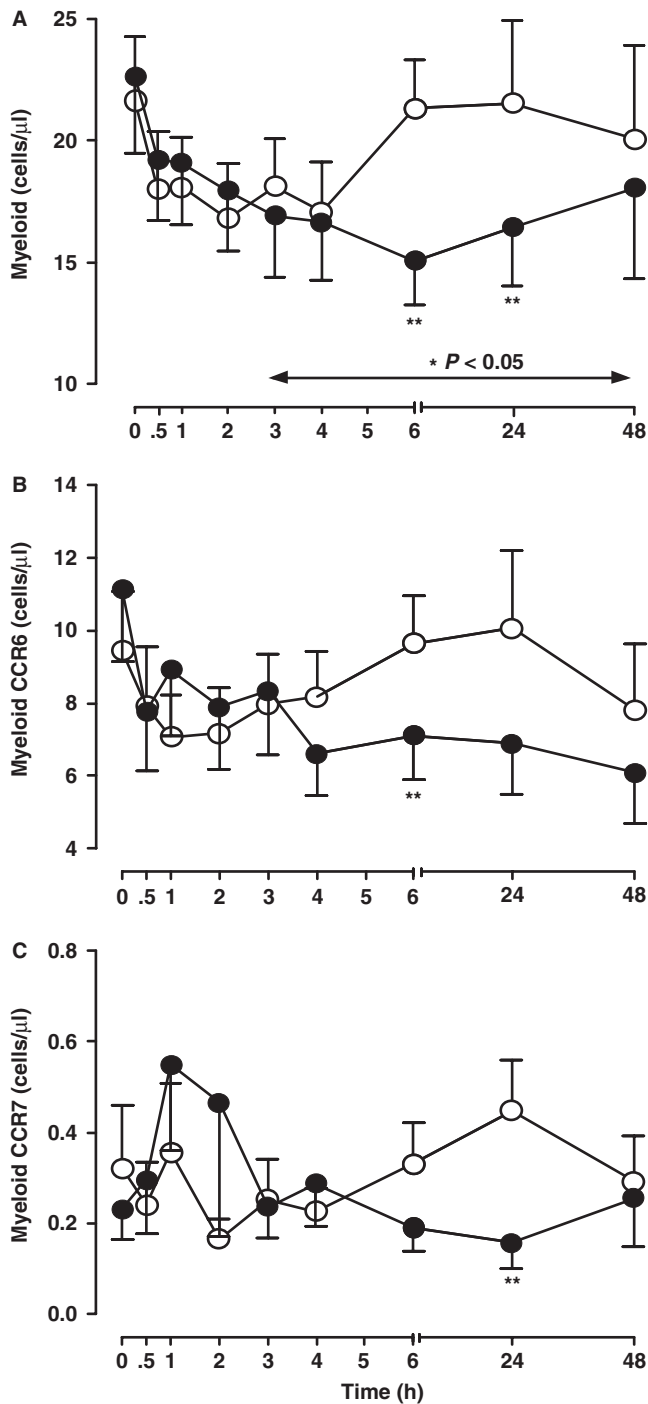


Figure 3. Dendritic myeloid cell counts in peripheral blood (A) total count (B) CCR6⁺ (C) CCR7⁺ at different time points following diluent (○) and allergen (●) inhalation challenge. *Difference to baseline at $P < 0.05$ for allergen challenge only; **difference between diluent and allergen at $P < 0.05$. Data are presented as mean $\pm$ SEM.

of mDCs were significantly lower than their baseline between 3 and 24-h postallergen inhalation and were significantly lower than the diluent control 6 and 24-h

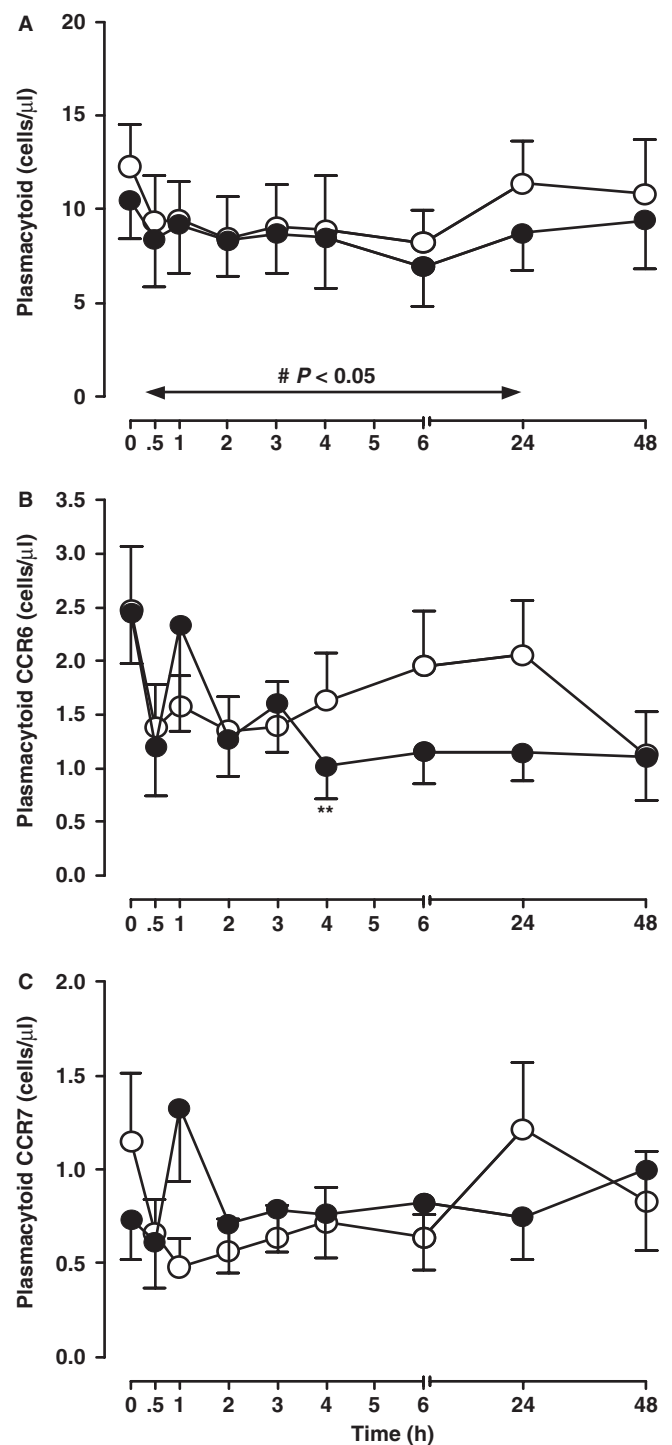


Figure 4. Dendritic plasmacytoid cell counts in peripheral blood (A) total count (B) CCR6⁺ (C) CCR7⁺ at different time points following diluent (○) and allergen (●) inhalation challenge. **Difference between diluent and allergen at $P < 0.05$; Difference to baseline at $P < 0.05$ for allergen and diluent challenge. Data are presented as mean $\pm$ SEM.

postallergen. By contrast, while the number of pDCs was also significantly lower after allergen between 0.5 and 6 h, when compared with baseline, there was no

significant difference in pDC numbers when compared with the diluent control. This suggests that there may be a diurnal variation in circulating pDCs over the course of the day and stresses the necessity of performing a diluent challenge to appropriately control for diurnal changes. These results also suggest that the trafficking of pDCs may follow a different kinetic pattern than mDCs.

A previous study has demonstrated that repeated allergen challenge to the nasal mucosa of patients with allergic rhinitis over the course of 7 days is associated with the recruitment of pDCs, (18). These studies, taken together, suggest that pDCs efflux from the blood at a later time point than mDCs, perhaps in an attempt to resolve inflammation and restore homeostasis. Previous work from our laboratory (6) examined the kinetics of circulating mDCs postallergen inhalation. This study demonstrated that when compared with baseline, there was a significant decrease in the number of circulating mDCs at 3, 6, and 24 h following allergen challenge. Our data complement these findings. However, a limitation to this study was the lack of a diluent control. The fate of mDCs leaving the circulation following allergen inhalation has not been determined; however, it is likely that the mDCs are trafficking into the airways to participate in the LAR. Supporting this is the observation of Jahnsen et al. (5), who demonstrated an increased number of mDCs in asthmatic airway mucosa 4–5 h following allergen challenge. This would suggest that the number of mDCs rapidly decrease (within 3 h) in the circulation and appear in the airways within the time frame of the LAR (3–7 h following allergen challenge).

The chemokine receptor, CCR6, is strongly expressed on immature DCs, which traffic from the bone marrow to the target tissue, via the blood (19). In order for chemotaxis to occur, MIP3 α , the major functional ligand for CCR6, must be expressed by the target tissue (12, 13). Thus, CCR6 is thought to be the receptor responsible for trafficking of immature DCs to the target tissue in response to proinflammatory stimuli (20). At baseline, CCR6⁺ mDCs accounted for 50% of the circulating mDCs (Fig. 3). After allergen inhalation, an efflux of CCR6⁺ mDCs from peripheral blood occurred between 4 h and 48 h, which was significantly different from diluent at the 6 h time point. These results are consistent with CCR6⁺ mDCs being attracted from the circulation in response to allergen inhalation, but the magnitude of the change, when related to the change in total mDCs, suggests that CCR6⁺ mDCs are also leaving the circulation.

Upon antigen capture, DCs begin a maturation process, with downregulation of CCR6 and upregulation of CCR7 expression. The CCR7⁺ DCs traffic towards

their ligand, CCL 19 (14). The CCL 19 is restricted to T-cell areas of regional lymph nodes (20). In a murine model, Hammad et al. (21) demonstrated that *in vivo* neutralization of CCL 19 reduced all the cardinal features of asthma via suppression of DC migration from the airways to regional lymph nodes. In the current study, the baseline levels of circulating CCR7⁺ mDCs were extremely low (<1% of the total mDCs). This implies that the predominant mechanism for CCR7⁺ mDCs to gain access to the lymph nodes is via the lymphatic system, not the blood. However, a small number of mDCs may gain access to regional lymph nodes via the blood as the numbers of CCR7⁺ mDCs were significantly different 24 h after allergen compared with diluent. Supporting this concept, Vermaelen et al. (22) have demonstrated that mDCs are capable of migrating to regional lymph nodes within 12 h of antigen challenge and peaked at 24 h.

The kinetics of CCR6⁺ pDCs and CCR7⁺ pDCs was also evaluated. The baseline number of CCR6⁺ pDCs was low in comparison to mDCs and only 20–25% of total pDCs expressed CCR6, compared with 50% of mDCs. After allergen inhalation, there was a significant efflux of CCR6⁺ pDCs from peripheral blood at 4 h and trends to significance at 6 and 24 h. These results are somewhat surprisingly, as there was no change in the total number of pDCs from peripheral blood. A possible explanation for this is that by ‘default’, a small number of pDCs express CCR6, which are selectively targeted to the airways after allergen. Moreover, there was no difference in the number of circulating CCR7⁺ pDCs following diluent and allergen challenges. This is consistent with the view that the predominant mechanism for CCR7⁺ pDCs to gain access to the lymph nodes is via the lymphatic system, not the blood.

In summary, this study has demonstrated that allergen inhalation by allergic asthmatic subjects causes a reduction in mDCs and CCR6⁺ mDCs beginning at 4 h and persisting up to 24 h after challenge. The baseline levels of circulating pDCs were lower than mDCs and CCR6⁺ pDCs were reduced at 6 h after allergen. There were no significant changes in the mature CCR7⁺ mDCs or pDCs after allergen. The results suggest that trafficking of immature CCR6⁺ DCs from blood into the airway occurs after allergen inhalation and that the trafficking of the mature CCR7⁺ DCs from the airways into the regional lymph nodes occurs through the lymphatic system.

Acknowledgment

This study was funded by the Canadian Institutes for Health Research Grant #9362.

References

- Busse WW, Lemanske RF Jr. Asthma. *N Engl J Med* 2001;**344**:350–362.
- O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987;**136**:740–751.
- Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999;**160**:640–647.
- Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol* 1982;**70**:170–177.
- Jahnsen FL, Moloney ED, Hogan T, Upham JW, Burke CM, Holt PG. Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge. *Thorax* 2001;**56**:823–826.
- Upham JW, Denburg JA, O'Byrne PM. Rapid response of circulating myeloid dendritic cells to inhaled allergen in asthmatic subjects. *Clin Exp Allergy* 2002;**32**:818–823.
- Hammad H, Lambrecht BN. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. *J Allergy Clin Immunol* 2006;**118**:331–336.
- Lambrecht BN, Peleman RA, Bullock GR, Pauwels RA. Sensitization to inhaled antigen by intratracheal instillation of dendritic cells. *Clin Exp Allergy* 2000a;**30**:214–224.
- Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Theilemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000b;**106**:551–559.
- Hammad H, de Vries VC, Maldonado-Lopez R, Moser M, Maliszewski C, Hoogsteden HC et al. Differential capacity of CD8 alpha(+) or CD8 alpha(-) dendritic cell subsets to prime for eosinophilic airway inflammation in the T-helper type 2-prone milieu of the lung. *Clin Exp Allergy* 2004;**34**:1834–1840.
- de Heer HJ, Hammad H, Soullie 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.
- Greaves DR, Wang W, Dairaghi DJ, Dieu MC, Saint-Vis B, Franz-Bacon K et al. CCR6, a CC chemokine receptor that interacts with macrophage inflammatory protein 3alpha and is highly expressed in human dendritic cells. *J Exp Med* 1997;**186**:837–844.
- Power CA, Church DJ, Meyer A, Alouani S, Proudfoot AE, Clark-Lewis I et al. Cloning and characterization of a specific receptor for the novel CC chemokine MIP-3alpha from lung dendritic cells. *J Exp Med* 1997;**186**:825–835.
- Yoshida R, Nagira M, Kitaura M, Imagawa N, Imai T, Yoshie O. Secondary lymphoid-tissue chemokine is a functional ligand for the CC chemokine receptor CCR7. *J Biol Chem* 1998;**273**:7118–7122.
- Cockcroft DW, Davis BE, Boulet LP, Deschesnes F, Gauvreau GM, O'Byrne PM et al. The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen. *Allergy* 2005;**60**:56–59.
- Parameswaran K, Liang H, Fanat A, Watson RM, O'Byrne PM. Role for cysteinyl leukotrienes in allergen-induced change in circulating dendritic cell number in asthma. *J Allergy Clin Immunol* 2004;**114**:73–79.
- 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.
- Jahnsen FL, Lund-Johansen F, Dunne JF, Farkas L, Haye R, Brandtzaeg P. Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. *J Immunol* 2000;**165**:4062–4068.
- Dieu MC, Vanbervliet B, Vicari A, Bridon JM, Oldham E, it-Yahia S et al. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 1998;**188**:373–386.
- Dieu-Nosjean MC, Vicari A, Lebecque S, Caux C. Regulation of dendritic cell trafficking: a process that involves the participation of selective chemokines. *J Leukoc Biol* 1999;**66**:252–262.
- 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.
- Vermaelen KY, Carro-Muino I, Lambrecht BN, Pauwels RA. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J Exp Med* 2001;**193**:51–60.